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Telomere Length in Peripheral Blood Leukocytes and Lung Cancer Risk: A Large Case-Control Study in Caucasians

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Abstract

Telomere dysfunction is a crucial event in malignant transformation and tumorigenesis. Telomere length in peripheral blood leukocytes has been associated with lung cancer risk, but the relationship has remained controversial. In this study, we investigated whether the association might be confounded by study of different histological subtypes of lung cancer. We measured relative telomere lengths in patients in a large case-control study of lung cancer and performed stratified analyses according to the two major histological subtypes (adenocarcinoma [AC] and squamous cell carcinoma [SCC]). Notably, AC patients had longer telomeres than controls, whereas SCC patients had shorter telomeres compared to controls. Long telomeres were associated with increased risk of AC, with the highest risk associated with female sex, younger age (<60 years) and lighter smoking (<30 pack-years). In contrast, long telomeres were protective against SCC, particularly in male patients. Our results extend the concept that telomere length affects risk of lung cancer in a manner that differs with histological subtype.

Keywords

Telomeres; peripheral blood leukocytes; adenocarcinoma; squamous cell carcinoma; lung cancer risk

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No potential conflicts of interest were disclosed.

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Introduction

Telomeres are dynamic nucleoprotein complexes located at the chromosome ends that are composed of TTAGGG repeats and telomere binding-associated proteins. They protect the chromosome ends against degradation, end-to-end fusion, and atypical recombination and thus play an important role in maintenance of chromosomal integrity (1). Telomeres are typically 10–15 Kilobases (kb) long and they shorten progressively with cell division owing to incomplete replication of linear DNA molecules. When they reach a critical length, they are recognized as double-strand breaks, resulting in cellular senescence or apoptosis mediated by the Rb and p53 signaling pathways (2). This progressive telomere shortening regulates cell proliferation and limits cell division to a finite number of cycles, thus acting as a “cellular mitotic clock” (3).

It has been proposed that telomere dysfunction plays a complex role in carcinogenesis. Telomere attrition may induce cells to undergo senescence or apoptosis, serving as a mechanism for tumor suppression. However, excessive telomere loss may lead to genomic instability that drives oncogenesis via both through the activation of telomerase and generation of other mutations necessary for tumor progression (4). Measurement of telomere length in surrogate tissue markers such as peripheral blood leukocytes has been used as a biomarker of telomere dysfunction and cancer risk. Previous reports have indicated that decreasing telomere length is accelerated by many factors, such us aging (5–7), smoking (8–10), obesity (11), oxidative stress (12), and socioeconomic and lifestyle factors (13, 14). Over the past few years, the number of epidemiological studies evaluating the association between telomere length and cancer risk has increased greatly; however, the findings have been inconsistent (15, 16). Most of the initial studies used case-control designs and found that short leukocyte telomeres were associated with increased risk of several cancers (17). However, recent publications have suggested that long telomeres are associated with increased risk of certain tumors, including pancreatic cancer, lymphoma, hepatocellular carcinoma, melanoma, and sarcoma (18–22). In a recent study evaluating the association of relative telomere length (RTL) with cancer risk and cancer survival in 47,102 Danish participants (23), the investigators concluded that short telomeres were associated with reduced survival after cancer but not with cancer risk. This work included a large sample size, prospective population-based study design, and long-term follow-up. However, a detailed analysis of the data from this study suggested an association of RTL with cancer risk in a cancer-site specific manner (24). When pooling data for all the cancer sites, opposite associations could cancel each other out, therefore resulting in the null finding. Results of previous studies of telomere length in lung cancer patients have been inconclusive (25–29). The initial studies reported that short telomeres were associated with increased lung cancer risk (25–27), whereas a later prospective study performed in male smokers suggested that long telomeres are associated with increased risk of lung cancer (28). In addition, another recent prospective study in a female nested case-control cohort has also reported longer telomeres in the lung cancer cases (29). Previous studies have shown that women have longer telomeres than men (8, 9, 30) and the association between telomere length and specific cancer risk may vary by sex, for example, as reported in bladder cancer (9).

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In the present study, we aimed to determine whether this inconsistent relationship between RTL and lung cancer risk might be caused by differences in histological subtype and different sexes. We examined the RTLs in a large case-control study comprising of 1385 lung cancer cases patients and their respectively matched controls and evaluated the association of telomere length with the risk of lung cancer stratifying by the two main histological subtypes of lung cancer: adenocarcinoma (AC) and squamous cell carcinoma (SCC). To our knowledge, this is the largest epidemiological study of constitutive RTL and lung cancer risk. Our data support the role of telomere dysfunction in lung carcinogenesis in a histology-specific manner.

Materials and Methods

Study population and epidemiological data

This case-control study included 1,385 lung cancer patients and 1,385 healthy control subjects. The patients were recruited at The University of Texas MD Anderson Cancer Center from September 1995 to March 2010 in a daily review of computerized appointment schedules. There was no sex, histological or disease stage restriction in this study. Control subjects with no prior history of cancer were identified at Kelsey-Seybold Clinic, the largest multispecialty physician group in the Houston metropolitan area. Cases and controls were limited to non-Hispanic white individuals (Caucasians) and equally matched to their corresponding controls with respect to age (± 1 year) and sex. Written informed consent to participate in the study was obtained from each participant. All participants were interviewed to collect information regarding demographics, smoking history, alcohol consumption, family cancer history, medical history, and working history. Blood samples (40mL each) were collected from the study participants in coded heparinized tubes after the interviews. This study was approved by the respective institutional review boards at MD Anderson and Kelsey-Seybold Clinic. A never-smoker was defined as an individual who had never smoked or had smoked fewer than 100 cigarettes in his or her lifetime. An ever-smoker was defined as an individual who was a smoker at the time of enrollment or had smoked 100 or more cigarettes in his or her lifetime. The cumulative cigarette dose (pack-years) was calculated using the following formula: pack-years = packs per day × years smoked.

Overall RTL measurement real-time PCR

Genomic DNA was extracted from peripheral blood lymphocytes (PBLs) using QIAamp Maxi DNA kit (QIAGEN) according to the manufacturer’s protocol. RTL was measured using quantitative polymerase chain reaction (Q-PCR) method as previously described by Cawthon (31). Briefly, the RTL was determined by PCR through two steps of relative quantification. First, the ratio of the telomere repeat copy number (T) to the single gene (human globulin) copy number (S) was determined for each sample using standard curves. The derived T/S ratio was proportional to the overall RTL length. Second, the ratio for each sample was then normalized according to that in a calibrator DNA sample to standardize different runs.

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The PCR (15μL) for telomere amplification consisted of 1x SYBR Green Master Mix (Applied Biosystems), 200nmol/L Tel-1 primer, 200nmol/L Tel-2 primer, and 5 ng of genomic DNA. In addition, the PCR for human globulin (Hgb) amplification consisted of 1x SYBR Green Master Mix, 200nmol/L Hgb-1, 200nmol/L Hgb-2 primer, and 5 ng of genomic DNA. The thermal cycling conditions were at 95°C for 10 minute followed by 40 cycles at 95°C for 15 seconds and at 56°C(for telomere amplification) or 58°C (for Hgb amplification) for 1 minute. The PCRs were done on separate 384-well plates including with the same samples in the same well positions. In each run, corresponding negative and positive controls, a calibrator DNA sample, and a standard curve were included. The positive controls contained a 1.2-kb telomere and a 3.9-kb telomere from a commercially available telomere length assay kit (Roche Applied Science). For each standard curve, 1 reference DNA sample (the same DNA sample for all runs) was diluted 2-fold serially to produce a 6-point standard curve between 20 ng and 0.625 ng of DNA in each reaction. The same reference DNA was used consistently for all plates in the present study and in our previous studies (32, 33). The coefficient of determination (R^2) for each standard curve was ≥0.99, with an acceptable standard deviation (SD) set at 0.25 (for the Ct values). If the result was outside the acceptable range, the sample was repeated. Each plate contained randomly selected samples to have equal representation of cases and controls. The adenocarcinoma and squamous cell carcinoma cases were intermixed on assays plates. The lab personal were blinded to case control status. Duplicates for each sample were done. The telomere and Hgb PCRs were done on separate 384-well plates, with the same samples in the same well positions. The intra assay coefficient of variation was<3% and the inter assay coefficient of variation was<5% for telomere length assay in our laboratory (22, 33). The intraclass correlation coefficient was 0.959 (95% CI 0.954–0.962) for telomere assay and 0.986 (95% CI 0.985–0.988) for Hgb assay.

Statistical analysis

All statistical analyses were performed using the Stata 10.1 statistical software program (version 10.1; StataCorp). The analyses were restricted to AC and SCC, the two main histological subtypes of lung cancer. Further analyses were stratified by histological subtype in which cases and controls were equally matched to their corresponding controls. Differences in the distribution of the host characteristics between cases and controls were evaluated by Pearson χ^2 test for categorical variables (sex, age, smoking status, and cumulative smoking [pack-years]), whereas the Student *t*-test was used to test differences for continuous variables. RTL was analyzed as both a continuous and categorical variable. The Wilcoxon rank sum test was used to evaluate the difference in telomere length as a continuous variable case-control status by sex, age (younger-age <60 years or older-age ≥60 years), smoking history (never- or ever-smoker), and cumulative smoking (light smokers (pack-years<30) or heavy smokers (pack years ≥30). Telomere length was also analyzed as a categorical variable by setting cutoff points at the median and quartile values in the overall control group. In addition, we performed decile analyses and generalized additive models to test for the potential non-linear relationships. The association between lung cancer risk and RTL was assessed using conditional multivariable logistic regression to determine the adjusted odds ratio (aOR) and 95% confidence interval (CI) adjusting for sex, age, smoking status, BMI and pack years (<30 pack-years vs ≥30 pack-years). Unconditional logistic

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regression analyses were also performed and the results were similar to conditional logistic regression. We only presented data of conditional logistic regression. In addition to the overall association analysis, stratified analyses of both histological subtypes of lung cancer according to sex, age, smoking status, and cumulative smoking were performed. Tests for trend were obtained for the quartile values of telomere length. Spearman's correlation test was used to examine the association of RTL with all of the confounding variables. All statistical tests were two-sided, and associations were considered statistically significant at *P* levels less than 0.05.

Results

Patient host characteristics

In total, 1385 patients with lung cancer and 1385 matched controls were included in this study. By study design cases and controls were all Caucasians and they were matched on age (±1 year) and sex. Further analyses were done stratifying by the two major histological subtypes (AC and SCC), final 706 AC and 320 SCC were matched their respective controls (Table 1). The mean (± SD) ages of the patients and controls were 62.49 ± 10.26 years and 62.38 ± 10.32 years, respectively, for AC and 65.00 ± 8.69 years and 64.82 ± 8.61 years, respectively, for SCC. There were not statistically significant differences in terms of age and sex between cases and controls for either subtype. However, there were significantly more ever-smokers among cases than among controls (82.44% in AC cases versus 58.49% in controls, P<0.001; and 97.81% in SCC cases versus 61.44% in controls, P<0.001). In addition, the number of pack-years in ever-smokers was significantly higher in the cases than in the controls (mean ± SD: 46.84 ± 32.09 in AC versus 40.15 ± 37.54 in controls, *P*=0.004; 62.02 ± 35.91 in SCC cases versus 42.13 ± 35.09 in controls, *P*<0.001). In addition, we observed the association of SCC with smoking since, as expected, more smokers was present in SCC cases in comparison to AC cases and the number of pack-years was higher in SCC cases than AC cases (Table 1).

The association between telomere length and lung cancer risk differs by histology

A real-time PCR method was used to measure the RTLs in all samples. When a correlation analysis was performed, we observed an inverse association between RTL and age, smoking status and pack-years (Supplementary Table 1). We then performed separate analyses of RTL in AC and SCC patients. We observed that AC lung cancer cases had significantly longer overall telomeres lengths in cases than did the controls (mean ± SD, 1.23 ± 0.38 versus 1.14 ± 0.37; *P*<0.001) (Table 2), which was consistent regardless of sex, age (<60 years versus≥60 years), smoking status, and cumulative smoking. Conditional logistic regression analysis showed that when we used the median telomere length in the controls as the cutoff point between long and short telomeres, individuals with long RTLs exhibited significantly increased risk of lung AC (aOR, 1.56 [95% CI, 1.23–1.98]; *P*<0.001). In contrast, we found the opposite effect in SCC patients. Overall, SCC cases had significantly shorter telomeres than did the controls (mean ± SD, 1.10 ± 0.44 versus 1.13 ± 0.33; *P* =0.015) (Table 2). When conditional logistic regression analysis was used, an overall borderline significant protective effect of long telomeres on SCC risk was observed (aOR, 0.66 [95% CI, 0.42–1.03]; *P*=0.068) (Table 3). When BMI was added in the logistic

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regression analysis, the adjusted OR (95% CI) were 1.70 (1.35–2.14), p<0.001 for AC and 0.71 (0.49–1.04), p =0.082 for SCC, compared to 1.56 (1.23–1.98), p<0.001 for AC and 0.66 (0.42–1.03), p=0.068 for SCC without BMI adjustment. The OR was comparable and therefore we did not include BMI in the final models. When adjusting the analyses for not only smoking status, but also smoking pack years (<30 pack-years vs. ≥30 pack-years), there were no significant differences in the risk estimates. In addition, we did not observe any pattern of non-linear relationship between telomere length and AC or SCC risk when decile analyses and generalized additive models were performed (data not shown).

Exploratory analyses of subgroups showed that in AC cases, the risk appeared to be higher in females (aOR, 1.83 [95% CI, 1.34–2.50]; *P*<0.001), younger-age individuals (age<60 years) (aOR, 2.01[95% CI, 1.34–3.01]; *P*<0.001), and light-smokers (pack-years<30) (aOR, 3.50 [95% CI, 1.18–10.390]; *P*=0.0241). In addition, we found a significant dose-response relationship between long RTLs and increased AC risk (Table 4). Compared to the individuals within the first (shortest) quartile of telomere length, the aORs for those in the second, third, and fourth quartiles were 1.20 (95% CI, 0.79–1.580), 1.44 (95% CI, 1.02–2.04), and 1.85 (95% CI, 1.33–2.57), respectively (*P* for trend <0.001). In contrast, in SCC cases, we observed a significant protective effect of long RTLs in males (aOR, 0.55[95% CI, 0.35–0.87]; *P* = 0.010) and in older-age individuals (age ≥60 years) (aOR, 0.64 [95% CI, 0.42–0.97]; *P*=0.037). We observed a non-significant association in ever smokers, although a trend between longer RTL and decreased risk was observed in this stratum (Table 4). In addition, when quartiles were used as cutoff points, we observed the opposite dose-response relationship between long RTLs and SCC risk in males and older-age individuals (≥60 years) (Table 5).

Discussion

In this study, we investigated the association between leukocyte telomere length and risk of lung cancer. This relationship has been controversial in previous studies, which led us to determine whether the inconsistency resulted from different histological subtypes of lung cancer. We focused our analyses on AC and SCC, the two major histological subtypes of lung cancer. We found that the relationship between telomere length and lung cancer risk was histology-dependent. Our novel findings indicated that AC cases had longer RTLs than did controls and that long RTLs were associated with increased AC lung cancer risk. In contrast, SCC cases had shorter RTLs than did controls.

The number of epidemiological studies evaluating the association between leukocyte telomere length and cancer risk has increased greatly in recent years. The majority of these studies showed significant associations between telomeres in PBLs and altered risks of different carcinomas, although the relationships seemed to be cancer type-specific (23, 24). The specific association of telomere length with risk of each cancer type may be attributed to the distinct biologies of the cancers and their different routes of tumorigenesis. Authors have reported association of short telomeres with increased risk of bladder, esophageal, gastric, head and neck, ovarian, renal cancer, oral premalignant lesions and oral squamous cell carcinoma (16, 25, 34, 35). Other studies have found that long telomeres were associated with increased risk of non-Hodgkin lymphoma (19) and sarcoma (22). In

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addition, researchers did not find significant associations of telomere length with cancer risk in several large prospective studies (17, 34, 36, 37). Thus, additional large studies using consistent methodologies are needed to clarify the association of leukocyte telomere length with cancer risk.

Several studies have examined the link between telomere length and lung cancer risk. The first epidemiological study showing the association between telomere shortening and lung cancer risk was done by our group (25), which reported a significant association between short telomere lengths and increased risk of lung cancer. However, only 40 NSCLC cases were included in that study and no further stratification by histology type was done. In another study of 243 lung cancer cases and matched controls, researchers found shorter telomeres in the cases than in the controls and reported a significant association between short telomere length and lung cancer risk (26). In contrast, two recent studies (28, 29) reported that long telomeres were associated with increased risk of lung cancer. These findings agree with those of a recent prospective study of two cohorts of male smokers in Finland and nonsmokers in China, respectively, demonstrating that long telomeres in peripheral white blood cell DNA were associated with increased risk of lung cancer.

These inconsistent findings prompted us to ask whether the association between telomere length and lung cancer risk is histology-specific. Some of the previous lung cancer studies looked at telomere length according to histology. However, the investigators did not further stratify the patients according to histological subtype. Although differences in the molecular, histological, and clinical characteristics of SCC and AC (the two major histological types of non-small cell lung cancer [NSCLC]) have been reported, no large detailed studies have looked at the leukocyte telomere lengths. A previous study (26) found that leukocyte telomere length differed according to lung cancer histology and that the effect of short leukocyte RTLs on the risk of lung cancer was more pronounced in SCC than in AC patients, suggesting a histology-specific association of telomere length with lung cancer risk. In the present study, we observed for the first time an apparently opposite association of leukocyte RTL with AC and SCC risk.

In contrast to AC, shorter leukocyte telomeres were found in SCC, particularly in males and older-individuals. Recent studies have suggested that telomere dysfunction has dual roles in cancer progression and carcinogenesis (38). In other cancer types including ovarian carcinoma and melanomas, the same histology-dependent relationship of leukocyte RTL and cancer risk has been observed (39, 40). The general perception has been that short RTLs confer increased risk of some cancers. However there are multiple lines of evidence supporting that short RTLs can confer reduced risk of other cancers. This includes indirect evidence, such as recent genome-wide association studies (41, 42) that identified loci affecting RTL and showed that alleles associated with both short and long RTLs may contribute to the development of specific cancers. In theory, either short or long telomeres can predispose individuals to development of cancer depending on the somatic mutation landscape of the cell’s history and their particular microenvironment context (38, 43). When the cell cycle checkpoint, cellular senescence, and apoptosis pathways are not altered, short telomeres are expected to protect against cancer. In contrast, long telomeres may increase cancer risk, due to the additional cell division rounds allowed by the longer telomeres that

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could lead to the accumulation of somatic mutations affecting apoptosis and senescence pathways, thus promoting tumorigenesis. Therefore, the importance of balance between elongation by telomerase and telomere shortening to produce a stabilized “optimal” length critical for cell proliferation, senescence and control has been suggested (44).

Furthermore, in our subgroup analyses, we observed an increased risk of AC in females (1.83-fold increased risk) and younger-age individuals (2.01-fold increased risk) and light - smokers (2.19-fold increased risk), consistent with pathological studies showing that AC was more prevalent in female and young-age onset than SCC (45–47). These studies also demonstrated a marked dose-response relationship. Moreover, our finding that long leukocyte telomeres are associated with AC risk is consistent with results of a recent prospective nested case-control study of 215 female lung cancer cases and 215 female controls, 94% of whom were never-smokers (29). In addition, subgroup analysis showed an inverse protective effect of long telomeres on SCC risk in males and older-age individuals in agreement with results of previous studies that have shown this histology-dependent relationship for other cancer types (40). To our knowledge, this is the largest epidemiological study to demonstrate a histology-dependent relationship between lung cancer risk and telomere length.

Telomere length in PBLs could be altered by the presence of malignant disease and by the chemotherapy of radiation therapy prior to blood collection (48, 49) and reverse causation in retrospective case-control study may impact the comparisons between cases and controls and produce spurious or over-estimated associations (50). In our study, all the cases were sampled at diagnosis before receiving chemotherapy and radiotherapy and 203 cases had surgery before sampling. No significant differences in telomere length were found between cases receiving surgery and cases without surgery (mean ± SD, 1.17 ± 0.38 vs 1.19 ± 0.40, P=0.500). Controls were recruited within the same time frame of case recruitment and blood was collected and processed into DNA generally within two hours of blood drawing. All controls were from Texas and cases included 858 cases from Texas and 147 cases from outside Texas. Telomere length in cases from Texas and cases from outside Texas were comparable (mean ± SD, 1.14 ± 0.39 vs 1.16 ± 0.31, P=0.432). While population-based studies are inarguably the gold standard, the practicality is sometimes questioned when conducting phenotypic assays that require previously untreated patients. These difficulties are magnified when the patients, as in this study, come from an urban cancer center that serves as a tertiary referral center. Because our research is driven by a genetic hypothesis, the use of population-based control is not as critical as it may have been in epidemiological studies of disease and exposure. We do not believe that the case control recruitment scheme and blood collection and processing protocol would bias our results.

A recent article indicated that Qiamp columns truncate telomeric DNA compared to other extraction methods (phenol-chloroform and PureGene method) (51). It would be important to confirm this observation, which may have important implications in epidemiological study of telomere length. However, we do not think that DNA extraction method biased the results of our study. The DNA extraction method was consistent throughout our study. All the DNA samples in this study and in the majority of published epidemiological studies of telomere length have been extracted by Qiamp method. In fact, we used Qiamp columns to

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clean previously phenol-chloroform-extracted DNAs. The RTLs for most samples in this current study were in the range of 1.0 to 1.3, comparable to literature reports using the same real-time PCR method. The cases and controls were recruited within the same time frame and their DNA samples were intermixed on each assay plate. The mean RTLs of samples from different time frame were similar (data not show). Therefore, the significant RTL differences between cases and controls observed in this study are not likely to be caused by DNA extraction methods.

Our data strongly support the role of telomere dysfunction in lung carcinogenesis, highlighting the differences between the two major histological subtypes of lung cancer. We found a differential association between relative telomere length and risk of AC and SCC, in which long telomeres were associated with increased risk of AC but decreased risk of SCC. Our findings provide strong evidence of a histology-specific association between telomere length and lung cancer risk. Additional studies are warranted to elucidate the mechanisms underlying this differential association.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Host Characteristic of cases and controls stratified by histology

Characteristic	AC, n (%)			SCC, n (%)		
	Cases	Controls	*P*[a]	Cases	Controls	*P*[a]
Mean age, years (SD)	62.49 (10.26)	62.38 (10.32)	0.836	65.00 (8.69)	64.82 (8.61)	0.791
Sex						
Male	328 (46.46)	328 (46.46)		210 (65.63)	210 (65.63)	
Female	378 (53.54)	378 (53.54)	1.000	110 (34.38)	110 (34.38)	1.000
Smoking status						
Never-smoker	124 (17.56)	291 (41.51)		7 (2.19)	123 (38.56)	
Ever-smoker	582 (82.44)	410 (58.49)	**<0.001**	313 (97.81)	196 (61.44)	**<0.001**
Mean number of pack-years (SD)[b]	46.84 (32.09)	40.15 (37.54)	**0.004**	62.02 (35.91)	42.13 (35.09)	**<0.001**

[a] All statistical tests were two-sided.

[b] Ever-smokers only.

AC- adenocarcinoma; SCC- squamous cell carcinoma.

Significant *P* values in bold font.

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Table 2

Effect ofcovariates on telomere length by case-control status stratified by histology

	AC					SCC				
	Cases		Controls			Cases		Controls		
Covariate	**N**	**Mean (± SD)**	**N**	**Mean (± SD)**	***P*[a]**	**N**	**Mean (± SD)**	**N**	**Mean (± SD)**	***P*[a]**
Sex										
Male	328	1.16 ± 0.35	328	1.12 ± 0.36	0.210	210	1.07 ± 0.46	210	1.13 ± 0.33	**0.006**
Female	378	1.28 ± 0.40	378	1.16 ± 0.39	**<0.001**	110	1.16 ± 0.40	110	1.14 ± 0.34	0.793
P[a]		**<0.001**		0.150			0.083		0.677	
Age, years										
<60	257	1.35 ± 0.39	259	1.18 ± 0.39	**<0.001**	82	1.23 ± 0.66	84	1.16 ± 0.30	0.725
≥60	449	1.15 ± 0.36	447	1.11 ± 0.36	0.129	238	1.06 ± 0.33	236	1.12 ± 0.34	**0.010**
P[a]		**<0.001**		**0.011**			**0.002**		0.358	
Smoking status										
Never-smoker	124	1.35 ± 0.42	291	1.15 ± 0.35	**<0.001**	7	1.35 ± 0.45	123	1.19 ± 0.31	0.263
Ever-smoker	582	1.20 ± 0.37	410	1.13 ± 0.39	**0.006**	313	1.10 ± 0.44	196	1.10 ± 0.34	0.280
P[a]		**<0.001**		0.401			0.143		**0.017**	
Cumulative smoking, pack-years[b]										
<30	192	1.26 ± 0.40	159	1.12 ± 0.39	**0.004**	41	1.14 ± 0.32	62	1.06 ± 0.30	0.249
≥30	386	1.17 ± 0.36	185	1.13 ± 0.42	0.300	270	1.09 ± 0.46	84	1.10 ± 0.39	0.405
P[a]		**0.007**		0.901			0.532		0.503	

[a] Wilcoxon ranksum test.

[b] Ever-smokers only.

AC- adenocarcinoma; SCC- squamous cell carcinoma.

Significant *P* values in bold font.

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Table 3

Association between RTL and lung cancer risk stratified according to histology

	AC				SCC			
	N (%)				N (%)			
RTL	**Cases**	**Controls**	**aOR (95% CI)[a]**	***P***	**Cases**	**Controls**	**aOR (95% CI)[a]**	***P***
Overall								
Short	299 (45.10)	364 (54.90)	1		195 (55.08)	159 (44.92)	1	
Long	407 (54.34)	342 (45.66)	1.56 (1.23–1.98)	**<0.001**	125 (43.71)	161 (56.29)	0.66 (0.42–1.03)	0.068
Male								
Short	158 (47.59)	174 (52.41)	1		138 (56.33)	107 (43.67)	1	
Long	170 (52.47)	154 (47.53)	1.25 (0.85–1.84)	0.251	72 (41.14)	103 (58.86)	0.47 (0.27–0.85)	**0.012**
Female								
Short	141 (42.60)	190 (57.40)	1		57 (52.29)	52 (47.71)	1	
Long	237 (55.76)	188 (44.24)	1.83 (1.34–2.50)	**<0.001**	53 (47.75)	58 (52.25)	1.18 (0.56–2.51)	0.661
Age <60 years								
Short	77 (39.49)	118 (60.51)	1		41 (53.25)	36 (46.75)	1	
Long	180 (56.07)	141 (43.93)	2.01 (1.34–3.01)	**<0.001**	41 (46.07)	48 (53.93)	0.92 (0.30–2.84)	0.890
Age≥60 years								
Short	222 (47.44)	246 (52.56)	1		154 (55.60)	123 (44.40)	1	
Long	227 (53.04)	201 (46.96)	1.30 (0.96–1.76)	0.091	84 (42.64)	113 (57.36)	0.64 (0.42–0.97)	0.083
Never-smokers								
Short	36 (20.00)	144 (80.00)	1		2 (3.77)	51 (96.23)	1	
Long	88 (37.45)	147 (62.55)	1.48 (0.68–3.24)	0.327	5 (6.49)	72 (93.51)	3.27 (0.29–35.98)	0.332
Ever-smokers								
Short	263 (54.56)	219 (45.44)	1		193 (64.33)	107 (35.67)	1	
Long	319 (62.55)	191 (37.45)	1.37 (0.99–1.91)	0.061	120 (57.42)	89 (42.58)	0.63 (0.40–1.02)	0.059
Cumulative smoking, pack-years <30[b]								
Short	79 (48.17)	85 (51.83)	1		20 (36.36)	35 (63.64)	1	
Long	113 (60.43)	74 (39.57)	3.50 (1.18–10.39)	**0.024**	21 (43.75)	27 (56.25)	NA	NA
Cumulative smoking, pack-years ≥30[b]								
Short	182 (65.00)	98 (35.00)	1		171 (78.44)	47 (21.56)	1	

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	AC				SCC			
	N (%)				N (%)			
RTL	Cases	Controls	aOR (95% CI)[a]	*P*	Cases	Controls	aOR (95% CI)[a]	*P*
Long	204 (70.10)	87 (29.90)	0.96 (0.51–1.82)	0.908	99 (72.79)	37 (27.21)	0.75 (0.35–1.61)	0.459

[a] Adjusted by age, sex, and smoking status.

[b] Ever-smokers only, adjusted by age and sex.

RTL- relative telomere length categorized by median value in overall controls as cut-off point: Short- <1.14; Long- ≥1.14.

AC- adenocarcinoma; SCC- squamous cell carcinoma. NA- not able to estimate by conditional logistic regression analyses.

Significant *P* values in bold font.

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Table 4

Association between RTL quartiles and lung cancer risk in AC patients and controls stratified according to selected characteristics

	N (%)			
RTL	**Cases**	**Controls**	**aOR (95% CI)[a]**	***P***
Overall				
1st	140 (44.30)	176 (55.70)	1	
2nd	159 (45.82)	188 (54.18)	1.12 (0.79–1.58)	0.519
3rd	179 (51.44)	169 (48.56)	1.44 (1.02–2.04)	**0.038**
4th	228 (56.86)	173 (43.14)	1.85 (1.33–2.57)	**<0.001**
P for trend[b]			**<0.001**	
Male				
1st	85 (49.42)	87 (50.58)	1	
2nd	73 (45.63)	87 (54.37)	0.98 (0.59–1.60)	0.922
3rd	80 (50.96)	77 (49.04)	1.06 (0.63–1.79)	0.820
4th	90 (53.89)	77 (46.11)	1.40 (0.85–2.31)	0.179
P for trend[b]			0.177	
Female				
1st	55 (38.19)	89 (61.81)	1	
2nd	86 (45.99)	101 (54.01)	1.37 (0.83–2.25)	0.210
3rd	99 (51.83)	92 (48.17)	1.89 (1.16–3.06)	**0.009**
4th	138 (58.97)	96 (41.03)	2.46 (1.55–3.89)	**<0.001**
P for trend[b]			**<0.001**	
Age <60 years				
1st	26 (33.33)	52 (66.67)	1	
2nd	51 (43.59)	66 (56.41)	1.38 (0.69–2.75)	0.356
3rd	65 (50.78)	63 (49.22)	2.03 (1.03–3.98)	**0.039**
4th	115 (59.59)	78 (40.41)	2.71 (1.47–4.99)	**<0.001**
P for trend[b]			**<0.001**	
Age ≥60 years				
1st	114 (47.90)	124 (52.10)	1	
2nd	108 (46.96)	122 (53.04)	1.15 (0.76–1.73)	0.507
3rd	114 (51.82)	106 (48.18)	1.23 (0.81–1.88)	0.333
4th	113 (54.33)	95 (45.67)	1.55 (1.02–2.33)	**0.038**
P for trend[b]			**0.040**	
Never-smokers				
1st	13 (15.66)	70 (84.34)	1	
2nd	23 (23.71)	74 (76.29)	0.79 (0.19–3.29)	0.745
3rd	33 (32.04)	70 (67.96)	1.18 (0.38–3.50)	0.776
4th	55 (41.67)	77 (58.33)	1.55 (0.54–4.45)	0.410
P for trend[b]			0.324	

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RTL	N (%) Cases	Controls	aOR (95% CI)[a]	*P*
Ever-smokers				
1st	127 (54.51)	106 (45.49)	1	
2nd	136 (54.62)	113 (45.38)	0.81 (0.50–1.30)	0.376
3rd	146 (60.33)	96 (39.67)	1.11 (0.69–1.77)	0.665
4th	173 (64.55)	95 (35.45)	1.35 (0.86–2.11)	0.188
P for trend[b]			0.089	
Cumulative smoking, pack-years <30[c]				
1st	34 (44.16)	43 (55.84)	1	
2nd	45 (51.72)	42 (48.28)	0.46 (0.10–2.10)	0.314
3rd	47 (58.02)	34 (41.98)	2.46 (0.58–10.49)	0.222
4th	66 (62.26)	40 (37.74)	2.46 (0.63–9.63)	0.195
P for trend[b]			0.072	
Cumulative smoking, pack-years ≥30[c]				
1st	92 (66.19)	47 (33.81)	1	
2nd	90 (63.83)	51 (36.17)	0.83 (0.37–1.83)	0.637
3rd	99 (69.23)	44 (30.77)	0.91 (0.38–2.14)	0.828
4th	105 (70.95)	43 (29.05)	0.85 (0.37–1.95)	0.697
P for trend[b]			0.743	

[a] Adjusted by age, gender and smoking status.

[b] P for trend for the quartile values of the telomere length.

[c] Ever-smokers only, adjusted by age and sex.

RTL- relative telomere length categorized by quartile values in overall controls: 1st- ≤0.93; 2nd- 0.94–1.14; 3rd- 1.15–1.33; 4th- >1.33.

Significant *P* values in bold font.

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Table 5

Association between RTL quartiles and lung cancer risk in SCC patients stratified according to selected characteristics

	N (%)			
RTL	**Cases**	**Controls**	**aOR (95% CI)[a]**	***P***
Overall				
1st	115 (58.38)	82 (41.62)	1	
2nd	80 (50.96)	77 (49.04)	0.70 (0.39–1.25)	0.230
3rd	61 (42.66)	82 (57.34)	0.47 (0.25–0.90)	0.023
4th	64 (44.76)	79 (55.24)	0.66 (0.35–1.24)	0.197
P for trend[b]			0.078	
Male				
1st	76 (57.58)	56 (42.42)	1	
2nd	62 (54.87)	51 (45.13)	0.83 (0.40–1.71)	0.615
3rd	40 (42.11)	55 (57.89)	0.46 (0.21–1.02)	0.057
4th	32 (40.00)	48 (60.00)	0.40 (0.17–0.96)	**0.041**
P for trend[b]			**0.012**	
Female				
1st	39 (60.00)	26 (40.00)	1	
2nd	18 (40.91)	26 (59.09)	0.33 (0.10–1.05)	0.061
3rd	21 (43.75)	27 (56.25)	0.45 (0.14–1.50)	0.195
4th	32 (50.79)	31 (49.21)	1.12 (0.42–2.96)	0.833
P for trend[b]			0.794	
Age <60 years				
1st	21 (55.26)	17 (44.74)	1	
2nd	20 (51.28)	19 (48.72)	0.75 (0.19–3.02)	0.686
3th	20 (44.44)	25 (55.56)	0.43 (0.10–1.82)	0.255
4th	21 (47.73)	23 (52.27)	1.81 (0.44–7.46)	0.409
P for trend[b]			0.615	
Age ≥60 years				
1st	94 (59.12)	65 (40.88)	1	
2nd	60 (50.85)	58 (49.15)	0.69 (0.36–1.35)	0.280
3rd	41 (41.84)	57 (58.16)	0.57 (0.27–1.21)	0.143
4th	43 (43.43)	56 (56.57)	0.49 (0.24–1.03)	0.061
P for trend[b]			**0.045**	
Never-smokers				
1st	2 (8.00)	23 (92.00)	1	
2nd	0 (0.00)	28 (100)	NA	NA
3rd	1 (2.78)	35 (97.22)	NA	NA
4th	4 (9.76)	37 (90.24)	NA	NA
P for trend[b]			NA	

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	N (%)			
RTL	**Cases**	**Controls**	**aOR (95% CI)[a]**	***P***
Ever-smokers				
1st	113 (66.08)	58 (33.92)	1	
2nd	80 (62.02)	49 (37.98)	0.73 (0.41–1.30)	0.287
3rd	60 (56.07)	47 (43.93)	0.47 (0.25–0.88)	0.020
4th	60 (58.82)	42 (41.18)	0.64 (0.34–1.20)	0.161
P for trend[b]			0.061	
Cumulative smoking, pack-years <30[c]				
1st	13 (37.14)	22 (62.86)	1	
2nd	7 (35.00)	13 (65.00)	NA	NA
3rd	9 (34.62)	17 (65.38)	NA	NA
4th	12 (54.55)	10 (45.45)	NA	NA
P for trend[b]			NA	
Cumulative smoking, pack-years ≥30[c]				
1st	99 (79.20)	26 (20.80)	1	
2nd	72 (77.42)	21 (22.58)	0.53 (0.18–1.62)	0.268
3rd	51 (76.12)	16 (23.88)	0.76 (0.25–2.33)	0.628
4th	48 (69.57)	21 (30.43)	0.41 (0.13–1.31)	0.131
P for trend[b]			0.211	

[a] Adjusted age, sex, and smoking status.

[b] P for trend for the quartile values of the telomere length

[c] Ever-smokers only; NA- Not available

NA-not able to estimate by conditional logistic regression analyses.

RTL- relative telomere length categorized by quartile values in overall controls as cut off points: 1st- ≤0.93; 2nd- 0.94–1.14; 3rd- 1.15–1.33; 4th- >1.33

Significant *P* values in bold font.