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Genetic variation in telomere maintenance genes, telomere length, and lung cancer susceptibility

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Abstract

Telomeres are responsible for the protection of the chromosome ends and shortened telomere length has been associated with risk of multiple cancers. Genetic variation in telomere related genes may alter cancer risk associated with telomere length. Using lung cancer cases (n = 120) and population-based controls (n = 110) from Xuanwei, China, we analyzed telomere length separately and in conjunction with single nucleotide polymorphisms in the telomere maintenance genes *POT1*, *TERT*, and *TERF2*, which we have previously reported were associated with risk of lung cancer in this study. *POT1* rs10244817, *TERT* rs2075786, and *TERF2* rs251796 were significantly associated with lung cancer ($p_{trend} \leq 0.05$). The shortest tertile of telomere length was not significantly associated with risk of lung cancer (OR = 1.58; 95% CI = 0.79 – 3.18) when compared to the longest tertile of telomere length. When stratified by genotype, there was a suggestion of a dose-response relationship between tertiles of telomere length and risk of lung cancer among the *POT1* rs10244817 common variant carriers (OR (95%CI) = 1.33 (0.47 – 3.75), 3.30 (1.14 – 9.56), respectively) but not among variant genotype carriers ($p_{interaction} = 0.05$). Our findings provide evidence that telomere length and genetic variation in telomere maintenance genes may be associated with risk of lung cancer susceptibility and warrant replication in larger studies.

Keywords

POT1; *TERT*; *TERF2*

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Introduction

Telomeres are located at the end of chromosomes and are responsible for the protection of the chromosome ends from nucleolytic degradation, end-to-end fusion, irregular recombination, and other lethal events to a cell (1). As such, telomeres are critical for genome stability and integrity. Telomeres consist of nucleotide repeats (TTAGGG) and the associated telomere protein complex, the shelterin (1;2). With each cell division, telomeres shorten by 50-200 bases due to DNA polymerase's inefficient replication of linear DNA ends (3). When telomeres reach a critically short length, the effected cells will undergo apoptosis, undergo senescence, or acquire chromosomal structural abnormalities (4). Therefore, short telomere length decreases the cell's integrity. Shortened telomere length has been associated in case-control studies, in a dose-dependent manner, with multiple cancers, including head and neck cancer (5), bladder cancer (5;6), lung cancer (5;7), and renal cell carcinoma (5).

Besides telomere shortening, telomeres may also malfunction due to genetic variation in telomere maintenance genes, such as protection of telomeres 1 (*POT1*) (8), telomerase (*TERT*) (3), telomeric repeat-binding factor 1 (*TERF1*) (9), and telomeric repeat-binding factor 2 (*TERF2*) (10). *POT1*, *TERT*, *TERF1*, and *TERF2* are highly conserved between species and ethnic groups, including non-Hispanic Caucasian Americans, African/African-Americans, Hispanics, and individuals with Pacific Rim heritage (11). We previously reported that genetic variation in *POT1*, *TERT*, and *TERF2* were associated with risk of lung cancer in this population (12).

Xuanwei Province in China poses a unique opportunity to assess lung cancer susceptibility in a population with substantial in-home coal smoke exposure, a classified human carcinogen (13). Xuanwei has the highest prevalence of lung cancer in China (14) and the age-adjusted lung cancer mortality rates for men and women are 27.7 and 25.3 per 100,000, respectively (15). The similarity of lung cancer rates in Xuanwei men and women is interesting given that nearly all women and few men cook, while most men and nearly no women smoke tobacco (16). The primary source of indoor air pollution in Xuanwei is smoke combustion from most residents burning smoky coal (bituminous coal) for heating and cooking. Smoky coal use in Xuanwei homes is associated with very high and comparable risks of lung cancer in both men and women (17;18).

We hypothesized that telomere length would be associated with risk of lung cancer, and that this relationship might be modified by single nucleotide polymorphisms (SNPs) in telomere-related genes.

Methods

The study population of this population-based case-control study has been previously described (19). Briefly, all residents of Xuanwei, China from March 1995 to March 1996 were eligible for inclusion. Lung cancer cases with clinical symptoms and X-ray confirmation were identified at one of five hospitals servicing Xuanwei County. Of the 135 eligible cases, 133 (99%) agreed to participate. To be enrolled, cases had to be histologically or cytologically confirmed (n = 105) or have died within one year of diagnosis (n = 17), since death within one year of clinical diagnosis of lung cancer is a strong indicator of lung cancer diagnosis in Xuanwei (20). Based on these criteria, 122 of the 133 consenting cases (92%) were enrolled into the study. Controls, individually matched by sex, age (± 2 years), village, and type of fuel used for in-home cooking and heating at time of interview, were selected from the Xuanwei general population. The participation rate for controls was 100%. A detailed questionnaire was administered by trained interviewers to cases and controls and informed consent was obtained

from all study subjects. This research protocol was approved by a United States Environmental Protection Agency Human Subjects Research Review Official.

First morning sputum samples were collected on five consecutive mornings from all cases and controls, as these expectorations contain more exfoliated cells for the assay due to overnight accumulation (21). All sputum samples from both cases and controls were collected by spontaneous expectoration, and for cases, before surgery or treatment. Subjects first rinsed their mouths with water to remove all extraneous material, and then took a deep breath, coughed deeply and expectorated into a plastic cup. Samples from both cases and controls were evaluated cytologically for the presence of macrophages to confirm that the samples were derived from the lower respiratory tracts, as previously described (22). This method was successful at collecting epithelial cells from the lung and bronchial. Genomic DNA was initially extracted from sputum samples for all 122 cases and 122 controls via phenol-chloroform extraction (23). Initially, 1442 candidate SNPs for cancer susceptibility were genotyped by an Illumina GoldenGate Assay and have been previously reported (12). Briefly, genotyping was successful for 122 (100%) cases and 111 (91%) controls. Ten controls did not have ample DNA for genotyping. Duplicate samples (n = 21) of both cases and controls were used determine the intra-subject concordance rate for all assays (>98%). This report includes SNPs that were significantly associated with risk of lung cancer in *POT1*, *TERF2*, and *TERT*, out of 17 SNPs tested. Results for all SNPs genotyped, including the 5 SNPs genotyped in *TERF1* that were not associated with risk of lung cancer, can be found in Supplementary Table 2 of our previous report (12).

Of the 122 cases and 111 controls that were genotyped, genomic DNA was available from 120 lung cancer cases and 110 controls to determine telomere length by quantitative PCR (24). In addition to the 10 controls that did not have ample DNA for genotyping, 2 cases and 1 control also did not have enough DNA for the telomere length assay. Blind duplicate samples (n = 15) were included with cases and controls to assess assay reproducibility. The overall coefficient of variation was 23% and the interclass coefficient was 0.87 for this assay. Telomere length was successfully determined for 111 cases and 99 controls. Unconditional logistic regression was used to estimate the odds ratio (OR) and 95% confidence intervals (CI) for the association between lung cancer risk and telomere length tertiles (2^{nd}: 1.88-2.22; 1^{st}: <1.88), using the longest tertile of telomere length as the reference group (3^{rd}: >2.22), while adjusting for age (<55 years, ≥55 years), sex, smoking (ever, never), and lifetime smoky coal exposure (<130 tons, ≥130 tons).

Unconditional logistic regression was used to estimate the OR and 95%CI for the association between lung cancer risk and each SNP, using the homozygote of the common allele as the reference group and adjusting for age, sex, smoking, and smoky coal exposure. Gene-dose effects for each SNP were estimated by a linear trend test of the number of variant alleles. Lung cancer susceptibility associated with telomere length was then stratified by genotype status based on the dominant model (wildtype vs. variant genotypes). Interactions between the dominant model and telomere length were tested on the multiplicative scale, adjusting for age, sex, smoking, and smoky coal exposure. Interactions between smoky coal use and telomere length, as well as smoky coal use and the dominant models were also tested on the multiplicative scale, adjusting for age, sex, smoking, and smoky coal exposure.

Results

Cases and controls were comparable in age, sex, and smoking status (Table I). As in previous reports of this study population (19), cases tended to use significantly more in-home smoky coal over the course of their lifetimes than controls ($p < 0.01$) and smoking status did not differ by case status ($p = 0.71$). Since few females smoked, the effect of smoking was evaluated in

only males. Males smoking ≥25 pack-years compared to smoking <25 pack-years had a 1.7-fold (95% CI = 0.85 – 3.27) increased risk of lung cancer. In both cases and controls, older individuals had shorter telomere lengths than younger individuals. Telomere length significantly decreased as age increased in a dose-dependent manner (p = 0.007). When evaluating telomere length by tertile, the shortest telomere length tertile (1st tertile) was not statistically associated with risk of lung cancer in all subjects (OR = 1.58; 95% CI = 0.79 – 3.18) when compared to the longest telomere length tertile (3rd tertile).

As we previously reported (12), three SNPs in telomere length maintenance genes were independently associated with lung cancer risk ($p_{trend} \leq 0.05$) (Table II). The *G* variant at *POT1* rs10244817 (OR_{GA+GG} = 0.52, 95%CI = 0.29 – 0.94) and the *A* variant at *TERT* rs2075786 (OR_{GA+AA} = 0.49, 95%CI = 0.26 – 0.90) were associated with a decreased risk of lung cancer, while the *G* variant at *TERF2* rs251796 (OR_{GA+GG} = 1.96, 95%CI = 1.07 – 3.59) was associated with an increased risk of lung cancer.

Although telomere length was not significantly associated with lung cancer risk, evaluating the association by genotype in SNPs involved in telomere maintenance yielded significant results (Table III). Of the three SNPs independently associated with lung cancer risk, *POT1* rs10244817 interacted significantly with telomere length and risk of lung cancer ($p_{interaction}$ = 0.05). There was suggestion of a dose-response relationship between tertiles of telomere length and risk of lung cancer among the *POT1* rs10244817 common variant carriers (OR (95%CI) = 1.33 (0.47 – 3.75), 3.30 (1.14 – 9.56), respectively) but not among variant genotype carriers.

Smoky coal use did not interact significantly with telomere length, *POT1* rs10244817, *TERT* rs2075786, or *TERF2* rs251796 (data not shown).

Discussion

To the best of our knowledge, this is the first study to evaluate lung cancer risk associated with telomere length and genetic variation in telomere maintenance genes. Even though we found a non-significant association between telomere length and lung cancer, further analysis of this association found that a SNP in *POT1* interacted significantly with telomere length to infer lung cancer risk.

Shortened telomere length has been associated with lung cancer case-control studies among Caucasians (5) and Asians (7). Jang *et al*. found that in 243 cases and 243 controls from Korea, the shortest quartile of telomere length had a significantly increased risk of lung cancer (OR = 8.73; 95%CI = 4.08 – 18.71) compared to the longest quartile of telomere length, when adjusting for age, sex, and smoking (7). Wu *et al*. found that in 54 cases and 54 controls among Caucasians in the United States, the risk of lung cancer associated with shortened telomere length increased in a dose dependent manner for the three shortest quartiles (p_{trend} = 0.002) compared to the longest quartile of telomere length (5). Telomere shortening has also been associated with decreased disease-free survival in non-small cell lung cancer patients in Spain (25). Our study found a non-significant increase in lung cancer risk in the shortest tertile of telomere length.

Telomere length was significantly associated with lung cancer risk in our study only when evaluating the association by genetic variation in *POT1*. In common variant carriers of *POT1* rs10244817, the shortest tertile of telomere length was associated with a significantly increased risk of lung cancer. POT1 is essential to telomere integrity because it is responsible for recruiting telomerase to the single-stranded 3’ telomeric overhang, and consequently limiting telomere elongation by telomerase (8). In 148 non-small cell lung cancer tissues, no significant difference in the mRNA expression of *POT1* was found compared to normal tissue samples (26). On the contrary, significantly reduced *POT1* expression has been reported in

chronic lymphocytic leukemia (27) and gastric carcinomas (28). An evaluation of genetic variation and breast cancer risk in a Polish population found no significant associations with *POT1* SNPs (29). No study, to the best of our knowledge, has previously reported the association between *POT1* SNPs and lung cancer risk or evaluated the functionality of *POT1* rs10244817. *POT1* rs10244817 is an intronic SNP, causing an *A* to *G* base pair change.

Recent evidence suggests that genomic variation at chromosome 5p, which contains *TERT*, maybe be associated with lung cancer (30;31). Two independent comparative genomic hybridization studies found increased copy numbers at chromosome 5p15.33 to be associated with non-small cell carcinomas (30) and adenocarcinomas (31). Other research suggests the clinical importance of *TERT* expression as a potential biomarker of lung cancer (32;33). Our study found carriers of the *A* allele at *TERT* rs2075786 to be associated with a decreased risk of lung cancer. Three correlated *TERT* SNPs (rs2735940, rs2853669, and rs2736098) were associated with breast cancer risk in individuals with a family history of breast cancer (29).

Smoky coal has been consistently associated with lung cancer in both case-control and cohort studies in Xuanwei (12;17-19;34). In-home exposure to smoky coal combustion contains known carcinogens such as polycyclic aromatic hydrocarbons (PAHs), benzene, arsenic, and formaldehyde, which induce oxidative stress (35;36). Theoretically, response to this oxidative stress should lead to increased cell replication, and subsequently, shortened telomere length. However, this phenomenon was not observed in our population-based study. Potential explanations for this may be that most cells are instead undergoing apoptosis in response to the oxidative stress, our study did not have adequate power to detect an association, or other factors not yet identified.

The high participation rate is a major strength of our population-based case-control study. While the telomere length assay may have contained some tumor cells for cases, potentially artificially inflating the association between telomere length and lung cancer risk, a histological review of the sputum samples determined the number of tumor cells to be minimal. Since moderate sample size may lead to both false positive and false negative findings (37), especially for sub-group analyses (38), our findings should be viewed as hypothesis-generating. Our results are biologically plausible, however, given that telomere length and variants in telomere maintenance genes could contribute to lung cancer risk. Further, telomere length was measured in cells from lung sputum in our study; therefore, our results describe biological markers in target tissue instead of surrogate tissue, such as blood. Associations with any specific SNP should be cautiously interpreted until these results are replicated, since observed associations with a particular SNP in this study may be due to another SNP in linkage disequilibrium.

In summary, our findings provide evidence that telomere length and genetic variation in telomere maintenance genes may be important to lung cancer susceptibility. Our results suggest that SNPs in *POT1* interact with telomere length and lung cancer risk, and should be viewed as exploratory until they are replicated in larger studies.

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Table I

Subject characteristics for case-control study, Xuanwei, China

	Cases		Controls		
	N	**%**	**N**	**%**	**p value***
Gender					1.00
Male	74	66.7	66	66.7	
Female	37	33.3	33	33.3	
Smoking Status					0.71
Never	41	36.9	39	39.4	
Ever	70	63.1	60	60.6	
Telomere Length					0.28
3rd tertile (longest)	31	27.9	32	32.3	
2nd tertile	30	27.0	33	33.3	
1st tertile (shortest)	50	45.1	34	34.3	
	Mean (std)		**Mean (std)**		**p value****
Age (at interview)	55.0 (11.9)		54.7 (13.5)		0.87
Lifetime Smoky Coal Use (Tons)	171.2 (110.3)		131.1 (79.7)		<0.01

* chi-squared test;

** ttest

Table II

Significant genotyped telomere related SNPs and lung cancer risk, Xuanwei, China*

Gene	SNP	Genotype	Controls		Cases		Odds Ratio* *	95% Confidence Interval**	p value
			n	%	n	%			
POT1	rs10244817	AA	42	45.2	66	61.1	0.538376094	0.30-0.98	0.041999
		AG	45	48.4	38	35.2	0.409596465	0.10-1.60	0.198832
		GG	6	6.5	4	3.7	0.523705729	0.29-0.94	0.029088
		dominant model trend	51	54.8	42	38.9	0.577959965	0.35-0.95	0.030347
TERF2	rs251796	GG	39	40.2	31	28.2	1.809855917	0.97-3.38	0.062908
		AG	49	50.5	63	57.3	2.876057816	1.07-7.71	0.035802
		AA	9	9.3	16	14.5	1.956827281	1.07-3.59	0.029847
		dominant model trend	58	59.8	79	71.8	1.730398255	1.10-2.73	0.018579
TERT	rs2075786	GG	54	57.4	76	71.7	0.579262932	0.31-1.09	0.089653
		AG	34	36.2	30	28.3	not applicable	not applicable	0.975647
		AA	6	6.4			0.485180447	0.26-0.90	0.021257
		dominant model trend	40	42.6	30	28.3	0.438440511	0.25-0.77	0.004

* Previously reported in Hosgood 2008, supplementary table 2; restricted to only subjects with telomere data

* Odds ratios (ORs) and 95% confidence intervals (CIs) are adjusted for age (<55 years, ≥55 years), sex, smoking (ever, never), and lifetime smoky coal exposure (<130 tons, ≥130 tons).

Table III

Telomere length and lung cancer susceptibility, by telomere maintenance polymorphisms

Gene	SNP	Genotype	3rd tertile OR (95% CI)*	2nd tertile OR (95% CI)*	1st tertile OR (95% CI)*	p-trend	p-interaction
POT1	rs10244817	AA	1.00 (reference)	1.33 (0.47-3.75)	3.30 (1.14-9.56)	0.07	
		AG+GG	1.00 (reference)	0.72 (0.24-2.10)	0.85 (0.30-2.38)	0.83	0.05
TERF2	rs251796	GG	1.00 (reference)	0.89 (0.24-3.22)	1.59 (0.48-5.28)	0.60	
		GA+AA	1.00 (reference)	0.83 (0.33-2.11)	1.39 (0.56-3.45)	0.48	0.95
TERT	rs2075786	GG	1.00 (reference)	0.55 (0.21-1.41)	1.39 (0.55-3.53)	0.11	
		GA+AA	1.00 (reference)	3.05 (0.74-12.49)	1.60 (0.45-5.67)	0.30	0.81

*Odds ratios (ORs) and 95% confidence intervals (CIs) are adjusted for age (<55 years, ≥55 years), sex, smoking (ever, never), and lifetime smoky coal exposure (<130 tons, ≥130 tons).