Telomere Length, Cigarette Smoking, and Bladder Cancer Risk in Men and Women

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Abstract

Truncated telomeres are among the defining characteristics of most carcinomas. Given the role of telomeres in tumorigenesis, we reasoned that constitutionally short telomeres might be associated with an increased risk of bladder cancer. Using quantitative real-time PCR, we measured relative telomere length in bladder cancer cases and healthy controls and evaluated the association between telomere length, cigarette smoking, and bladder cancer risk in a case-control study nested within the Health Professionals Follow-up Study and a case-control study nested within the Nurses' Health Study. Telomeres were significantly shorter in bladder cancer cases ($n = 184$) than in controls ($n = 192$). The mean relative telomere length in cases was 0.23 (SD, 0.16) versus 0.27 (SD, 0.15) in controls ($P = 0.001$). The adjusted odds ratio for bladder cancer was 1.88 (95% confidence interval, 1.05, 3.36) for individuals in the quartile with the shortest telomeres as compared with individuals in the quartile with the longest telomeres ($P_{trend} = 0.006$). We observed a statistically significant difference in telomere length among men and women ($P < 0.001$); however, the interaction between gender, telomere length, and bladder cancer risk was not significant. We also observed a significant difference in telomere length across categories of pack-years of smoking ($P = 0.01$). These findings suggest that truncated telomeres are associated with an increased risk of bladder cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(4):815–9)

Introduction

Telomeres are hexameric nucleotide $(TTAGGG)_n$ repeats on the distal ends of eukaryotic chromosomes that are critical in maintaining the structural integrity of the genome. Telomeres prevent fusion of chromosomal ends, nucleolytic decay, and atypical recombination (1). Telomeric repeats in normal somatic tissue shorten by ~30 to 200 bp after each mitotic division eroding chromosomal termini (2). The rate of telomere shortening per cell division is not constant and may be a function of oxidative stress and antioxidant defenses (3). Cells with critically truncated telomeres exhibit chromosomal rearrangements and undergo senescence and eventually apoptosis (4). Mouse models indicate an increased incidence of tumor formation with shorter telomeres (5-8). Telomere length is maintained by telomerase, a ribonucleoprotein reverse transcriptase not expressed in somatic tissue but highly expressed in germ cells (9). Telomerase is also expressed in neoplastic tissues and is a hallmark of most cancers (10). Cigarette smoking is a risk factor for bladder cancer (11) and may cause oxidative stress (12), which enhances telomere shortening (3). Smaller case-control studies observed shorter telomeres in peripheral blood leukocytes (PBL) and buccal cells from patients with bladder cancer than in controls (13, 14). We investigated the association between relative telomere length, cigarette smoking, and bladder cancer risk in a case-control study nested within the Health Professionals Follow-up Study (HPFS) and in a case-control study nested within the Nurses' Health Study (NHS).

Materials and Methods

The NHS originated in 1976 when 121,700 female U.S. nurses aged 30 to 55 years completed a self-administered questionnaire. Detailed information on individual characteristics and behaviors was obtained from questionnaires at baseline and biennially thereafter. Between 1989 and 1990, blood samples were collected from 32,826 women, and follow-up for this subcohort exceeds 96%. Eligible cases were women with confirmed bladder cancer diagnosed anytime after cohort inception and up to June 1, 2000, with no previously diagnosed cancer. We confirmed ~95% of self-reported bladder cancer cases. Controls were randomly selected participants who had provided a blood sample and were free of diagnosed cancer. Controls were matched to cases 1:1 by age and smoking status (current smoker versus not current smoker at the time of blood collection). This study includes 73 women with bladder cancer (median age, 59 years; range, 45-69) and 73 matched female controls (median age, 60 years; range, 45-69).

The HPFS is a prospective study of the causes of chronic diseases in men. The cohort originated in 1986 when 51,529 U.S. male health professionals aged 40 to 75 years responded to a mailed questionnaire (15). They provided information on medical histories and health-related exposures at baseline and biennial questionnaires thereafter. Follow-up is 93%. Blood samples were collected between 1993 and 1995 from 18,025 participants. Eligible cases were men with confirmed bladder cancer diagnosed anytime after cohort inception and up to June 1, 2000, with no previously diagnosed cancer; 96% of the cases were diagnosed with transitional cell carcinomas. Controls were randomly selected participants who had provided a blood sample and were free of diagnosed cancer (other than nonmelanoma skin cancer). Controls were matched to cases 1:1 by age and smoking status (never, former, and current smoking status at time of blood collection). This study consists of 136 men with bladder cancer (median age, 68; range, 49-81) and 136 matched male controls (median age, 68; range, 50-81).

Genomic DNA was extracted from buffy coat fractions using the QIAmp (Qiagen, Chatsworth, CA) 96-spin blood protocol.

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Table 1. Select population characteristics of bladder cancer cases and controls in the HPFS and NHS

Variable	HPFS			NHS		
	Cases, n = 123	Controls, n = 125	P value	Cases, n = 61	Controls, n = 67	P value
Age at blood draw*, mean (SD)	66.9 (8.1)	66.7 (8.0)	0.90	58.5 (7.3)	59.1 (6.9)	0.63
BMI at blood draw, mean (SD)	26.0 (3.0)	25.3 (3.2)	0.10	20.8 (10.7)	24.7 (8.2)	0.02
Relative telomere length,† mean (SD)	0.19 (0.14)	0.23 (0.13)	0.01	0.31 (0.17)	0.36 (0.14)	0.06
Relative telomere length, median	0.16	0.23		0.30	0.35	
Pack-years of smoking,‡ mean (SD)	35.0 (24.6)	26.5 (19.5)	0.02	29.7 (21.9)	27.8 (21.8)	0.70

*Age at blood draw was a matching factor.
† P value obtained from Wilcoxon rank sum test.
‡ Among cigarette smokers only.

We conducted a pico-green quantitation using a Molecular Devices 96-well spectrophotometer and confirmed results by using a Nanodrop SD-1000 spectrophotometer. Subsequent standardization by drying down the genomic DNA and resuspending ensured accurate and uniform DNA concentrations. Relative average telomere length was assessed by a modified version of the real-time PCR–based telomere assay described previously (16). Briefly, the telomere repeat copy number to single gene copy number (T/S) ratio was determined using an Applied Biosystems (Foster City, CA) 7300 thermocycler in a 96-well format. Twenty-five nanograms of buffy coat-derived genomic DNA was dried down in a 96-well plate and resuspended in 30 μL of either the telomere or β-globin PCR reaction mixture for 2 h at 4°C. The telomere reaction mixture consisted of 1× Qiagen Quantitect Sybr Green Master Mix, 2.5 mmol/L of DTT, 100 nmol/L of Tel-1b primer (CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT), and 900 nmol/L of Tel-2b primer (GGCTTGCCTTACCCT-TACCCTTACCCTTACCCTTACCCT). The reaction proceeded for 1 cycle at 95°C for 5 min, followed by 40 cycles at 95°C for 15 s, and 54°C for 1 min. The β-globin reaction consisted of 1× Qiagen Quantitect Sybr Green Master Mix, 300 nmol/L of hbg1 primer (GCTTCTGACACAACTGTGTTCACTAGC), and 700 nmol/L of hbg2 primer (CACCAACTTCATCCACGTT-CACC). The β-globin reaction proceeded for 1 cycle at 95°C for 5 min, followed by 40 cycles at 95°C for 15 s, 58°C for 20 s, and 72°C for 28 s. All samples for both the telomere and single-copy gene (human β-globin) reactions were done in triplicate, and the threshold value for both reactions was set to 0.5. In addition to the samples, each 96-well plate contained a six-point standard curve from 5 to 100 ng using genomic DNA derived from the T47D cell line. The purpose of the standard curve is to assess and compensate for interplate variations in PCR efficiency. The mean slope of the standard curve for both the telomere and β-globin reactions was −3.55 (range, −3.40 to −3.70), and the linear correlation coefficient (R^2) value for both reactions was 0.98 and 0.99, respectively. Blinded quality-control samples were interspersed throughout the data set to assess interplate and intraplate variability of threshold cycle (C_t) values. The T/S ratio ($-dC_t$) for each sample was calculated by subtracting the average β-globin C_t value from the average telomere C_t value. The relative T/S ratio ($-ddC_t$) was determined by subtracting the T/S ratio of the 25-ng standard curve point from the T/S ratio of each unknown sample (16).

Approximately 21% of NHS samples and 14% of HPFS samples were repeated on different plates to assess T/S reproducibility. For the NHS, inter-assay coefficients of variation (CV) of the telomere and single-gene assay were 2.46% and 2.26%, respectively, and the intra-assay CVs were 0.37% and 0.53%, respectively. For HPFS, inter-assay CVs were 2.27% and 2.22% for the telomere and single-gene assay, respectively, and intra-assay CVs were 0.55% and 0.54%, respectively.

We used the Wilcoxon rank sum test to compare the differences in telomere length and disease status. Spearman's partial correlation coefficients, adjusted for age, gender, and pack-years of smoking (when appropriate), were calculated to assess the correlations between age, body mass index (BMI), pack-years of smoking, and telomere length among controls only. The Kruskal-Wallis test was used to compare the differences in telomere length and smoking status. We categorized telomere length according to the cohort-specific distribution in the control population and compared the differences in categorical telomere length, case status, and smoking status with the χ^2 test. For NHS and HPFS, we examined the associations between telomere length and bladder cancer risk for each cohort separately using unconditional logistic regression to calculate odds ratios (OR) and 95% confidence intervals (95% CI) adjusted for the matching factors and a continuous term for pack-years of smoking (i.e., average reported number of cigarette packs smoked per day multiplied by the number of years of smoking). Median relative telomere lengths for each category were used to assess linear trends in ORs. To test statistical interactions between telomere length, smoking status, and bladder cancer risk in unconditional models, we used a likelihood-ratio test to compare nested models that included terms for all combinations of categorical telomere length and smoking status to models with indicator variables for main effects only. Interactions between telomere length, gender, and bladder cancer risk were evaluated using a likelihood-ratio test. We also did analyses combining the NHS and HPFS populations to increase our power to detect a meaningful association between telomere length and bladder cancer risk. We used unconditional logistic regression to calculate OR and 95% CI adjusting for age (continuous), smoking status (never, former, current), pack-years of smoking (continuous), and gender. We restricted our analyses to Caucasians. The P values are two-sided; P values <0.05 were

Table 2. Association between relative telomere length and female bladder cancer risk

Relative telomere length	Cases, n (%)	Controls, n (%)	OR (95% CI)*	OR (95% CI)†	OR (95% CI)‡
3rd tertile	16 (26.2)	23 (34.3)	1.00 ref	1.00 ref	1.00 ref
2nd tertile	11 (18.0)	23 (34.3)	0.69 (0.26, 1.80)	0.67 (0.25, 1.77)	0.78 (0.29, 2.11)
1st tertile	34 (55.7)	21 (31.3)	2.33 (1.01, 5.38)	2.34 (1.00, 5.49)	2.67 (1.11, 6.40)
			P_{trend} = 0.05	P_{trend} = 0.06	P_{trend} = 0.03

*Crude unconditional logistic regression.
†Unconditional logistic regression adjusted for the matching factors (age and smoking status).
‡Unconditional logistic regression adjusted for the matching factors (age, smoking status) and pack-years of smoking.

Table 3. Association between relative telomere length and male bladder cancer risk

Relative telomere length	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)*	OR (95% CI)†	OR (95% CI)‡
4th quartile	23 (18.7)	32 (25.6)	1.00 ref	1.00 ref	1.00 ref
3rd quartile	21 (17.1)	32 (25.6)	0.91 (0.42, 1.97)	0.88 (0.41, 1.91)	0.83 (0.37, 1.84)
2nd quartile	34 (27.6)	31 (24.8)	1.53 (0.74, 3.15)	1.47 (0.71, 3.05)	1.56 (0.74, 3.30)
1st quartile	45 (36.9)	30 (24.0)	2.09 (1.03, 4.23)	2.05 (1.00, 4.20)	1.94 (0.93, 4.05)
			P_{trend} = 0.02	P_{trend} = 0.02	P_{trend} = 0.03

*Crude unconditional logistic regression.
† Unconditional logistic regression adjusted for the matching factors (age and smoking status).
‡ Unconditional logistic regression adjusted for the matching factors (age, smoking status) and pack-years of smoking.

considered statistically significant. We used the SAS Version 9.1 software (SAS Institute, Cary, NC).

Results

Nurses' Health Study. For the NHS, analysis included 61 bladder cancer cases and 67 controls. Cases and controls had similar ages at blood draw and pack-years of smoking. Cases were leaner than controls, and their telomere lengths were shorter than those of controls, although this difference was only marginally statistically significant (Table 1).

We observed a statistically significant association between relative telomere length and risk of bladder cancer in women. Participants in the first tertile, those with the shortest telomere length, had an adjusted OR for bladder cancer of 2.67 (95% CI, 1.11, 6.40) compared with women in the third tertile (P_{trend} = 0.03; Table 2). We dichotomized telomere length at the median based on the distribution observed in the controls; the adjusted OR for bladder cancer for women with telomere lengths below the median compared with women with telomere lengths greater than or equal to the median relative telomere length was 2.17 (95% CI, 1.04, 4.52).

We also investigated the relation between cigarette smoking status, relative telomere length, and bladder cancer risk. We did not observe an association between smoking status (current versus not current; never versus ever) and telomere length among controls (all P values > 0.2). We also did not observe a significant interaction between median telomere length, risk of bladder cancer, and cigarette smoking status (ever versus never smoking; $P_{interaction}$ = 0.12; Table 6). We did not observe a correlation between telomere length and age, pack-years of smoking, and BMI at blood collection (P > 0.6), but we had limited power to detect such associations.

Health Professionals Follow-up Study. For HPFS, our analysis included 123 bladder cancer cases and 125 controls. Cases and controls had similar ages at blood draw and had similar BMI at blood draw. Cases smoked more than controls, and their telomere length was significantly shorter than that of controls (Table 1).

We observed a marginally significant association between relative telomere length and risk of bladder cancer in men. We categorized the participants into quartiles based on the relative telomere length distribution of the controls; the fourth quartile, the longest telomere length, served as the reference. Participants in the first quartile, those with the shortest telomere length, had an adjusted OR for bladder cancer of 2.55 (95% CI, 1.14, 5.83) compared with men in the fourth quartile (P_{trend} = 0.01; Table 3). Compared with men with relative telomere lengths greater than or equal to the median, men with relative telomere lengths below the median had an OR of 1.92 (95% CI, 1.14, 3.25).

We also investigated the relation between cigarette smoking status, relative telomere length, and bladder cancer risk among men. Among control smokers, we observed a significant correlation between relative telomere length and pack-years of smoking (r = −0.25, P = 0.02). We did not observe an association between smoking status (current versus not current; never versus ever) and telomere length among control subjects (all P values > 0.7). We also did not observe a significant interaction between median telomere length, risk of bladder cancer, and cigarette smoking status ($P_{interaction}$ = 0.32; Table 6). We did not observe a correlation between telomere length, age, and BMI (P > 0.2).

The combined analysis of the NHS and HPFS cohorts included 184 cases and 192 controls (Table 4). Among controls, the difference in the mean relative telomere length between women and men was statistically significant (P < 0.0001; age and smoking-adjusted means: women, 0.37; men, 0.22). Among cases, the difference in the mean relative telomere length between women and men was also statistically significant (P < 0.0001; age and smoking-adjusted means: women, 0.30; men, 0.19). However, we did not observe any interactions between gender, telomere length, and bladder cancer risk ($P_{interaction}$ = 0.53) and, therefore, did a combined analysis of NHS and HPFS adjusting for gender, age, pack-years of smoking, and smoking status.

We observed a statistically significant association between relative telomere length and bladder cancer. Telomeres were significantly shorter in cases than in controls. The mean

Table 4. Select population characteristics of bladder cancer cases and controls

Variable	Cases, *n* = 184	Controls, *n* = 192	*P* value
Age at blood draw,* mean (SD)	64.1 (8.8)	64.1 (8.4)	0.99
BMI at blood draw, mean (SD)	25.8 (3.6)	25.7 (4.2)	0.76
% male	67.2	65.5	0.72
Relative telomere length,† mean (SD)	0.23 (0.16)	0.27 (0.15)	0.002
Relative telomere length, median	0.22	0.26	
Pack-years of smoking,‡ mean (SD)	33.2 (23.8)	27.0 (20.2)	0.02
Cigarette smoking,§ *n* (%)			0.75
Never	58 (31.7)	60 (31.6)	
Former	92 (50.3)	101 (53.2)	
Current	33 (18.0)	29 (15.3)	

*Age at blood draw was a matching factor.
† *P* value obtained from Wilcoxon rank sum test.
‡ Among cigarette smokers only.
§ Smoking status was a matching factor. Note that for smoking in NHS, matching was current versus not current smoking.

Table 5. Association between relative telomere length and bladder cancer risk in men and women

Relative telomere length	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)*	OR (95% CI)†	OR (95% CI)‡
4th quartile	39 (21.2)	49 (25.5)	1.00 ref	1.00 ref	1.00 ref
3rd quartile	26 (14.1)	50 (26.0)	0.65 (0.35, 1.23)	0.64 (0.34, 1.21)	0.63 (0.33, 1.22)
2nd quartile	51 (27.7)	47 (24.5)	1.36 (0.77, 2.43)	1.32 (0.74, 2.35)	1.44 (0.80, 2.61)
1st quartile	68 (37.0)	46 (24.0)	1.86 (1.06, 3.26)	1.84 (1.04, 3.25)	1.88 (1.05, 3.36)
			P_{trend} = 0.01	P_{trend} = 0.01	P_{trend} = 0.006

*Crude unconditional logistic regression.
† Unconditional logistic regression adjusted for the matching factors (age and smoking status).
‡ Unconditional logistic regression adjusted for the matching factors (age, smoking status), gender, and pack-years of smoking.

telomere length in cases was 0.23 (SD, 0.16) versus 0.27 (SD, 0.14) in controls (P = 0.001). Participants in the lowest quartile (shortest telomeres) had an adjusted OR for bladder cancer of 1.88 (95% CI, 1.05, 3.36) compared with individuals in the top quartile (P_{trend} = 0.006; Table 5). The adjusted OR for bladder cancer was 2.00 (95% CI, 1.31, 3.06) for individuals with telomeres shorter than the median compared with those with telomeres equal to or greater than the median. These findings were robust to maximum control of cigarette smoking by both dose and duration as well as robust to the inclusion of interaction terms for age and gender, gender and pack-years of smoking, and age and pack-years of smoking.

We observed a significant difference in telomere length across categories of pack-years of smoking adjusted for age and gender (P = 0.01); the mean relative telomere length of controls who smoked ≥30 pack-years was 0.25 as compared with 0.29 for never smokers. We observed a marginally significant age and gender-adjusted correlation between relative telomere length and pack-years of smoking among control smokers (r = −0.17, P = 0.06) and a nonsignificant interaction between median telomere length, bladder cancer risk, and smoking status ($P_{interaction}$ = 0.08; Table 6). We did not observe an inverse significant correlation between relative telomere length and age and BMI (P > 0.8).

Discussion

To our knowledge, this is the largest population-based study addressing the potential contributions of relative telomere length to bladder cancer risk and the first large-scale study to use the real-time PCR method with PBLs to determine relative telomere length. We observed, in both men and women, that those with bladder cancer had shorter telomeres in PBLs than did controls. Our results are consistent with previously published studies on bladder cancer that observed an increased risk of bladder cancer in individuals with shorter telomeres (13, 14). Broberg et al. (13) assessed mean telomere length in buccal cells among 63 cases of bladder cancer (n = 9 women, 54 men) and 93 controls (n = 28 women, 65 men). They observed significantly shorter median telomere length (P = 0.001) in cases as compared with controls and an adjusted OR of 4.5 (95% CI, 1.7-12) for bladder cancer in the quartile with the shortest telomere length. Broberg et al. (13) did not report any differences observed in relative telomere lengths between men and women. Wu et al. (14) assessed PBL telomere length by quantitative fluorescence *in situ* hybridization (Q-FISH) in 135 cases of bladder cancer (n = 33 women, 102 men) and 135 controls (n = 33 women, 102 men) and observed a statistically significant difference between bladder cancer cases and controls.

In contrast to Wu et al. (14), we did observe a significant difference in relative telomere length between men and women. In our study, women had longer relative telomeres as compared with those in men, which is consistent with prior studies demonstrating a similar relation (17-22). Telomere length has been observed to be similar in male and female newborns (23), yet telomere lengths in adults may have potential differences due to gender differences and exposures to oxidative stress (21, 24). However, the gender differences we observed may be the result of true biological differences or may be the consequence of comparing the participants of the NHS and the HPFS cohorts, which have different characteristics and lifestyle behaviors (25). Our analyses are adjusted for the effect of cigarette smoking (pack-years of smoking and smoking status), a strong potential confounder for the association between telomere length and bladder cancer risk. Additional adjustment for BMI did not substantially change our point estimates and, therefore, was not included in the final models.

Telomere dysfunction is associated with an advanced age, and telomere length is considered to be a biomarker of biological age rather than chronological age (24). Because age-dependent shortening of telomeres in most somatic cells is thought to impair cellular function and viability (9), we evaluated telomere length with age and did not find a significant association. Our results do not support earlier findings by Valdes et al. (26) and Iwama et al. (27), who measured telomere length by assaying terminal restriction fragments. Valdes et al. (26) observed that telomeres shortened linearly with age by 27 bp per year in a study of 1,122 white women aged 18 to 76 years, and the correlation between age and telomere length was r = −0.46. Similarly, O'Sullivan et al. (28) observed that telomere length in PBLs as measured by Q-PCR decreased linearly with age (R^2 = 0.17; P = 0.01); however, there was no change in slope observed at older ages. In a smaller study of 80 healthy volunteers aged 4 to 95 years,

Table 6. Association between relative telomere length, cigarette smoking, and bladder cancer risk

Relative telomere length	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)*
NHS			
Never cigarette smoker			
≥ Median	8 (42.1)	9 (40.9)	1.00 ref
< Median	11 (57.9)	13 (59.1)	0.96 (0.28, 3.36)
Ever cigarette smoker			
≥ Median	13 (31.0)	25 (55.6)	1.00 ref
< Median	29 (69.1)	20 (44.4)	3.31 (1.33, 8.27)
HPFS			
Never cigarette smoker			
≥ Median	16 (41.0)	18 (47.4)	1.00 ref
< Median	23 (59.0)	20 (52.6)	1.30 (0.52, 3.21)
Ever cigarette smoker			
≥ Median	28 (33.7)	45 (52.9)	1.00 ref
< Median	55 (66.3)	40 (47.1)	2.31 (1.21, 4.41)
NHS + HPFS			
Never cigarette smoker			
≥ Median	24 (41.4)	27 (45.0)	1.00 ref
< Median	34 (58.6)	33 (55.0)	1.16 (0.56, 2.41)
Ever cigarette smoker			
≥ Median	41 (32.8)	70 (53.9)	1.00 ref
< Median	84 (67.2)	60 (46.2)	2.63 (1.56, 4.45)

*Unconditional logistic regression adjusted for the matching factors (age, smoking status), gender, and pack-years of smoking among smokers only.

Iwama et al. (27) observed that telomere length decreased by 84 bp per year in younger subjects (ages 4-39 years), and that the rate of shortening was slower, at 41 bp, in older (ages 40+ years) subjects. Although the inverse association between telomere length and age has previously been observed, telomere length has also been found to vary considerably in human PBLs from individuals of the same age (27, 29, 30). Furthermore, the age ranges in our studies were limited and constrained by the initial design of the cohorts, reducing our ability to observe a significant correlation with increasing age and decreasing relative telomere length. In addition, we had limited power to detect an association of such small magnitude.

We also evaluated the effect of smoking on telomere length and found significantly shorter telomeres in healthy individuals who smoked than in those who did not smoke. Age-adjusted telomere length was ~5 bp shorter for every pack-year smoked in the Valdes et al. (26) study, with 40 pack-years of smoking corresponding to 7.4 years of age-related shortening in telomere length. Similarly, Morla et al. (31) observed a dose-response relationship between cumulative lifetime exposure to tobacco smoking and telomere length.

The observed correlation with cigarette smoking provides further assurance that the real-time PCR method provides a biologically meaningful measure of telomere length. Measuring telomeres by real-time PCR is currently the most economical and versatile high-throughput method. It generates a T/S ratio that is proportional to a cell's average telomere length. Although the values are not absolute amounts, the relative T/S ratio has been confirmed to be highly consistent with the Southern blot assay, which measures telomere length as terminal restriction fragments (16). This technique also allows for the rapid and easy determination of relative telomere length in a closed-tube, fluorescence-based assay.

In summary, we observed a statistically significant association with relative telomere length and bladder cancer risk and with cigarette smoking. Our study, along with previously published studies (13, 14) investigating the relation between telomere length and bladder cancer risk, lend support to the idea that critically truncated telomeres may result in chromosomal rearrangements, and that telomere shortening, which causes chromosomal instability, occurs early in the multistep process of malignant transformation (4, 10, 32). Additional studies are needed to investigate the associations between relative telomere length and grade, stage, and type of bladder cancer.

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