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Leukocyte Telomere Length and Bladder Cancer Risk: A Large Case–Control Study and Mendelian Randomization Analysis

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ABSTRACT

Background: Leukocyte telomere length (LTL) has been associated with risk of several cancers. The association between LTL and bladder cancer is still inconsistent.

Methods: In this large case–control study consisting of 2,011 patients with bladder cancer and 2,259 healthy controls of European ancestry, we investigated the associations of real-time qPCR-measured LTL (a retrospective case–control study) and genetically predicted LTL [a Mendelian randomization (MR) study] with bladder cancer risk. Genotypes from 10 LTL-associated SNPs were used as instrumental variables to predict LTL. We used an individual level data–based weighted genetic risk score (GRS) and a summary statistics–based inverse-variance weighting (IVW) method in MR analyses.

Results: The qPCR-measured LTL was shorter in cases with muscle-invasive bladder cancer (MIBC) than those with non–muscle-invasive bladder cancer [NMIBC; ratio of telomere repeats copy number to single gene copy number (T/S): 1.19 ± 0.34 vs. 1.23 ± 0.36, $P = 0.081$]. Multivariable logistic regression analyses showed long qPCR-measured LTL was associated with a reduced risk of MIBC. In MR analyses, genetically predicted LTL was weakly associated with bladder cancer risk in both the GRS analysis [OR = 1.13, per SD increase; 95% confidence interval (CI), 0.73–1.75; $P = 0.595$] and the IVW analysis (OR = 1.14 per SD increase; 95% CI, 0.75–1.74; $P = 0.543$).

Conclusions: There was no strong evidence supporting an association between LTL and bladder cancer risk in European Americans.

Impact: This is the largest study of LTL and bladder cancer risk. The study showed that LTL does not play an important role in bladder cancer etiology.

Introduction

Bladder cancer is the fourth most common cancer in men in the United States with an estimated 62,100 new cases in men and 19,300 new cases in women in 2020 (1). Bladder cancer is an example of gene–environment interaction in cancer etiology. Smoking is the predominant risk factor for bladder cancer, accounting for approximately half of new cases in both men and women (2, 3). Occupational exposure to aromatic amines and other chemicals is the second most significant risk factor, responsible for approximately 20% of all bladder cancer cases (4, 5). Genetic predisposition also contributes to the etiology of bladder cancer. A large population-based classical twin study estimated that inherited genetic susceptibility contributes to 31% of bladder cancer risk (6). The risk of bladder cancer increases by 50% to 100% in first-degree relatives of patients with bladder cancer (7–10). Candidate gene and genome-wide association studies (GWAS) have identified at least 15 bladder cancer susceptibility loci in populations of European descent (11–18). These loci include three carcinogen-metabolizing genes GSTM1 (15, 17), NAT2 (15, 16), and UGT1A6 (14, 18). There were significant gene–smoking interactions in elevating bladder cancer risk, for instance, the NAT2 slow-acetylator genotype increases bladder cancer risk particularly among current smokers with a significant additive interaction (15, 16). Similar additive gene–smoking interactions were observed for UGT1A6 and four other individual loci, as well as a polygenic risk score consisting of 12 bladder cancer susceptibility loci (19). Interestingly, two of the GWAS-identified bladder cancer susceptibility loci are located at or near critical telomere maintenance genes: one is the telomerase reverse transcriptase (TERT) gene on chromosome 5p15.33 (20) and the other is the telomerase RNA component (TERC) gene on 3q26.2 (13), suggesting telomere dysfunction may play an important role in bladder cancer etiology.

Telomeres are hexametric DNA repeats and protein complex capping eukaryotic chromosome ends (21, 22). Telomeres prevent the termini of linear chromosomes from fusion and degradation (23, 24). Telomere dysfunction (e.g., shortened telomeres and disruption of telomere structure) causes chromosomal instability, cell-cycle arrest, and apoptosis, and plays critical roles in cancer development (24–26). Because telomere length is highly correlated between different tissues and blood cells among infants and adults (27–29) and the rates of telomere shortening are similar in proliferative (e.g., blood and skin) and minimally proliferative tissues (e.g., muscle and fat; ref. 29), telomere length in easily accessible tissues such as blood can serve as a surrogate for telomere length in other solid tissues. Leukocyte telomere length (LTL) is therefore often used in human population studies to investigate the relationship between a person's constitutive telomere length and disease risks. Classic twin studies estimated a heritability of up to 80% for LTL (30, 31). Although LTL shortens with increasing age, significant interindividual variation of LTL exists in people of the same ages (32, 33). This interindividual variation of LTL can contribute to genetic susceptibility to cancer and other diseases (34–39). Retrospective case–control studies have shown that short LTL was a risk factor for a few cancers, but prospective studies and recent Mendelian randomization (MR) studies using

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genetically predicted LTL have found that long LTL is associated with increased risks of many cancers (35–40).

There are a few notable limitations, such as reverse causation and environmental confounding, when using retrospective case–control study to assess the association of intermediate biomarkers like LTL with disease risks. In contrast, MR uses common genetic variants, mostly SNPs, as instruments to study the causal relations between risk factor/intermediate biomarkers and disease risks and is less affected by reverse causation, confounding, and technical variability (41). MR approach has three assumptions: (i) the selected SNPs are associated with the studied risk factor/intermediate biomarker; (ii) the selected SNPs are independent of confounding factors; and (iii) the selected SNPs only influence disease risk through their effects on the risk factor/biomarker. Large GWAS have identified 10 independent genomic regions associated with LTL (42–44). SNPs in these regions are believed to meet the assumptions of MR studies and have been widely used as genetic instruments to assess the causal relationship between LTL and risks of cancers and other diseases (35–40).

The associations of LTL with bladder cancer have been investigated in several small case–control studies (45–47), a prospective cohort study (36), and an MR study (39), but the results have been inconsistent with negative, null, and positive associations all being reported. In this study, we used a large retrospective case–control study and an MR study to evaluate the association of LTL with bladder cancer risk. This is by far the largest association study of measured LTL and bladder cancer risk and also the largest MR study of LTL and bladder cancer risk.

Material and Methods

Study population

A total of 2,011 bladder cancer cases and 2,259 healthy controls were included in this study. The cases were histologically confirmed transitional cell carcinoma of the bladder recruited from MD Anderson Cancer Center and Baylor College of Medicine. There were no restrictions on age, sex, ethnicity, and cancer stage in case recruitment. Controls were recruited from Kelsey Seybold Clinics, the largest multispecialty clinic system in the Houston metropolitan area. Controls went to Kelsey Seybold Clinics for annual health checkup and did not have a history of any cancer except for nonmelanoma skin cancer. The controls were frequency-matched to cases by age (±1 years) and gender. We restricted our analysis to participants of European ancestry, due to the small number of minority participants and the different genotype frequencies among different ethnic/racial groups. The demographic and basic epidemiologic information, including smoking, alcohol drinking, occupation, family history, and medical history, were obtained from the patient history database that all new patients filled when they registered into MD Anderson Cancer Center. The epidemiologic data of the controls were collected by trained interviewers using established questionnaires. The study was approved by the institutional review boards of MD Anderson Cancer Center, Baylor College of Medicine, and Kelsey Seybold Clinics. All participants signed an informed consent form.

LTL measurement by real-time qPCR

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp Blood DNA Extraction Kit (Qiagen). Relative LTL was measured using a modified real-time qPCR method (48). Detailed assay procedures and quality control steps were described previously (49–53). Briefly, two separate PCR reactions (telomere amplification and globulin amplification) were performed to determine the ratio of the telomere repeats copy number (T) to the single gene (human globulin) copy number (S; T/S), which was used as a parameter to represent relative LTL. The PCR for telomere amplification consisted of 1x SYBR Green Master Mix (Applied Biosystems), 200 nmol/L Tel-1 primer, 200 nmol/L Tel-2 primer, and 5 ng of genomic DNA. The PCR for human globulin (Hgb) amplification consisted of 1x SYBR Green Master Mix, 200 nmol/L Hgb-1, 200 nmol/L Hgb-2 primer, and 5 ng of genomic DNA. Each sample was done in duplicates. The intraassay and interassay coefficient of variation was <3% and <5%, respectively; the intraclass correlation coefficient was 0.959 [95% confidence interval (CI), 0.954–0.962] for telomere assay and 0.986 (95% CI, 0.985–0.988) for Hgb assay.

Genotyping and imputation

The genotyping was performed in the Genotyping Core of MD Anderson Cancer Center using Illumina's HumanHap610 chip or OncoArray-500K Beadchip. Genotype calling and quality control procedures for genotyping have been described previously (14). We randomly selected 2% of the samples for duplicate genotyping and the concordance of genotype calls was >99%. Individual SNPs with minor allele frequency <1% and call rate <90% were excluded for the final analysis. Imputation was performed using the Michigan Imputation Server (https://imputationserver.sph.umich.edu/; ref. 54). The mean R^2 of imputation was 0.96.

MR analysis and genetic risk score construction

We applied two MR methods based on individual level data and summary statistics, respectively. In the first individual level data–based method, we calculated weighted genetic risk score (GRS) using 10 LTL-associated SNPs according to the following formula.

$$\mathrm{GRS}_i = \sum_{j=1}^{10} w_j x_{ij}$$

where GRS_i is the risk score for individual *i*. x_{ij} ($x_{ij} = 0$, 1, or 2) is the number of telomere length increasing alleles for the *j*th SNP and w_j is the weight or effect coefficient (β estimate) for each SNP. The β estimate for each SNP (SNP–LTL effect) was derived from published GWAS (42–44). A higher GRS value for any study participant represents longer genetically predicted LTL. The second MR method was the commonly used summary data–based method called inverse-variance weighting (IVW; ref. 55), which give consistent estimates if all of the genetic variants in the analysis are valid instrumental variables. A fixed-effects IVW model was used. In addition, we performed sensitivity analyses to check the presence of horizontal pleiotropy (i.e., genetic variants influencing bladder cancer risk via a different biological pathway other than LTL) by MR-Egger regression approach (56) and a complementary weighted-median method that provided valid MR estimates if up to 50% of the information in an instrument come from SNPs that are valid (57). The IVW, MR-Egger, and weighted-median method were conducted using the Mendelian Randomization package (58) in R software. We also calculated the variance explained (%) and *F*-statistics (59) to evaluate whether the instrumental variable for LTL is well-powered for MR analysis. A threshold of *F*-statistics <10 has typically been used to define a "weak IV" (60).

Statistical analysis

We used χ^2 test or Fisher exact test to compare allele frequencies of each individual SNP between cases and controls. We then analyzed the association between qPCR-measured LTL, individual SNP, or GRS and

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Table 1. Selected characteristics of the study population.

Variables	Cases, *N* (%)	Controls, *N* (%)
Age, mean (SD)	65.00 (10.64)	65.43 (9.70)
Sex		
Male	1,596 (79.36)	1,748 (77.38)
Female	415 (20.64)	511 (22.62)
Smoking status		
Never	489 (28.61)	971 (42.98)
Former	879 (51.43)	985 (43.60)
Current	341 (19.95)	303 (13.41)

the risks of bladder cancer by calculating OR and corresponding 95% CI using an unconditional multivariable logistic regression model adjusting for age, gender, and smoking status. Real-time qPCR-measured LTL was analyzed as both a continuous and categorical variable. As a categorical variable, we dichotomized LTL at the median value in controls and used multivariable logistic regression model adjusting for age, gender and smoking status. GRS was analyzed as a continuous variable. In line with recommendations from the American Statistical Association (61, 62), we presented *P* values against null hypotheses as continuous variables and avoided using $P < 0.05$ as a threshold to interpret results as statistically "significant" or "not significant."

Results

Characteristics of the study population

The distribution of selected characteristics of the 2,011 patients with bladder cancer and 2,259 age and gender-matched controls are shown in **Table 1**. All the participants were of European ancestry. The average age at diagnosis for cases was 65 years (SD = 10.64) and average age for controls was 65.43 years (SD = 9.70). About 80% of patients were men, which is consistent with the overall gender distribution of bladder cancer worldwide. The distributions of never, former, and current smokers in cases were 28.61%, 51.43%, and 19.95%, respectively; the corresponding distributions in controls were 42.98%, 43.60, and 13.41, respectively.

Association between qPCR-measured LTL and bladder cancer risk

We first used real-time qPCR to measure LTL in cases and controls. We only included newly diagnosed and previously untreated patients for this assay because treatment has profound effect on LTL. The mean LTL was shorter in cases (mean ± SD, 1.20 ± 0.35) than in controls (1.25 ± 0.38; $P = 0.004$), and shorter in muscle-invasive bladder cancer (MIBC; 1.19 ± 0.34) than in non–muscle-invasive bladder cancer (NIMBC; 1.23 ± 0.36; $P = 0.081$) cases. Smokers had shorter LTL than nonsmokers (1.22 ± 0.39 vs. 1.29 ± 0.35; $P = 0.009$).

In multivariable logistic regression analysis adjusting for age, gender, and smoking status, when individuals were dichotomized into long and short LTL groups based on the median (50th percentile) LTL value in the controls, individuals with long LTL had a reduced risk of bladder cancer (OR = 0.82; 95% CI, 0.68–0.98; $P = 0.033$). In stratified analyses, the ORs between long LTL and bladder cancer risks were 0.72 (95% CI, 0.52–1.00; $P = 0.053$) in never-smokers and 0.89 (95% CI, 0.71–1.13; $P = 0.338$) in ever-smokers. The association was stronger in MIBC (OR = 0.78; 95% CI, 0.62–0.99; $P = 0.046$) than in NMIBC (OR = 0.88; 95% CI, 0.70–1.10; $P = 0.285$; **Table 2**).

MR analyses of LTL and bladder cancer risk

The association between each individual LTL-associated SNP and bladder cancer risk is shown in **Table 3**. Among the 10 SNPs, rs10936599 on *TERC* gene had the largest OR and smallest *P* value; individuals carrying the allele associated with long LTL exhibited an increased risk of bladder cancer (OR = 1.12; 95% CI = 1.01–1.24; $P = 0.027$).

We then constructed a genetic risk score (GRS) for LTL using the 10 SNPs and tested the association of this GRS with bladder cancer risk. There was a strong correlation between GRS and qPCR-measured LTL in both cases and controls with a correlation coefficient of 0.127 ($P = 0.0001$) and 0.125 ($P = 0.0003$), respectively, confirming

Table 2. Association of qPCR-measured LTL with the risk of bladder cancer overall and stratified by smoking status and disease severity.

LTL	Cases *N* (%)	Controls *N* (%)	OR[a] (95% CI)	*P* value
Overall				
Short	498 (54.13)	422 (45.87)	1 (reference)	
Long	454 (49.40)	465 (50.60)	0.82 (0.68–0.98)	0.033
Smoking status				
Never-smokers				
Short	126 (44.68)	156 (55.32)	1 (reference)	
Long	122 (36.64)	211 (63.36)	0.72 (0.52–1.00)	0.053
Ever-smokers				
Short	371 (58.24)	266 (41.76)	1 (reference)	
Long	331 (56.58)	254 (43.42)	0.89 (0.71–1.13)	0.338
Disease severity				
Superficial				
Short	244 (36.64)	422 (63.36)	1 (reference)	
Long	247 (34.69)	465 (65.31)	0.88 (0.70–1.10)	0.285
Muscle-invasive				
Short	215 (33.75)	422 (66.25)	1 (reference)	
Long	183 (28.24)	465 (71.76)	0.78 (0.62–0.99)	0.046

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

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Table 3. Individual LTL-associated SNPs and bladder cancer risk.

SNP ID	Chr.	Position	Gene	Allele[a]	β[a]	EAF case	EAF control	OR[b] (95% CI)	*P* value
rs11125529	2	54475866	ACYP2	A/C	0.065	0.135	0.134	1.00 (0.88–1.14)	0.960
rs6772228	3	58376019	PXK	T/A	0.041	0.947	0.952	0.89 (0.73–1.09)	0.270
rs10936599	3	169492101	TERC	C/T	0.100	0.776	0.755	1.12 (1.01–1.24)	0.027
rs7675998	4	164007820	NAF1	G/A	0.048	0.782	0.782	0.98 (0.88–1.08)	0.673
rs2736100	5	1286516	TERT	C/A	0.085	0.499	0.504	0.98 (0.89–1.06)	0.584
rs9420907	10	105676465	OBFC1	C/A	0.142	0.138	0.140	1.01 (0.89–1.15)	0.840
rs3027234	17	8136092	CTC1	C/T	0.103	0.767	0.775	0.95 (0.86–1.05)	0.314
rs8105767	19	22215441	ZNF208	G/A	0.064	0.302	0.285	1.05 (0.96–1.16)	0.292
rs6028466	20	38129002	DHX35	A/G	0.058	0.055	0.059	0.94 (0.78–1.13)	0.525
rs755017	20	62421622	ZBTB46	G/A	0.019	0.129	0.123	1.05 (0.93–1.20)	0.422

Abbreviation: EAF, effect allele frequency.
[a]Alleles are short allele/long allele. Short alleles are used as the reference allele and long alleles as effect allele. β estimates of SNP–LTL association were from published GWAS.
[b]Adjusted by age, gender, and smoking status.

the validity of our GRS as a genetic instrument to estimate LTL. In multivariable logistic regression analysis, no strong association was found between GRS and bladder cancer risk (OR = 1.13 per SD increase; 95% CI, 0.73–1.75; $P = 0.595$; **Table 4**).

Next, we applied a summary statistics–based MR approach (IVW) to assess the associations of genetically predicted LTL and bladder cancer risks. The IVW method produced an almost identical risk estimate as GRS analysis (OR = 1.14 per SD increase; 95% CI, 0.75–1.74; $P = 0.543$; **Table 4**). Similar results were found in the MR-Egger and the weighted-median sensitivity analyses (**Table 4**).

Discussion

In this study, using a retrospective case–control study design, we found a strong association between long LTL and reduced bladder cancer risk, which was more evident in MIBC than in NMIBC. In MR analysis, we did not observe a strong association between genetically predicted LTL and bladder cancer risk. Our data suggest that LTL is not a risk factor for bladder cancer.

The associations of LTL with bladder cancer have been investigated in several small case–control studies (45–47), a prospective cohort study (36), and an MR study (39), but the results have been inconsistent. The earliest retrospective (i.e., blood samples were collected after cancer diagnosis) case–control study of 135 cases and 135 controls found short LTL was associated with over four-fold increased risks of bladder cancer (45). A subsequent smaller retrospective case–control study with a sample size of 63 cases and 93 controls measured mean telomere length in buccal cells and found an adjusted OR of 4.5 (95% CI, 1.7–12) for bladder cancer in the quartile with the shortest telomere length as compared with individuals in the quartile with the longest telomeres (46). The third study was a nested case–control study with 184 cases and 192 controls that found an adjusted OR of 1.88 (95% CI, 1.05–3.36) for individuals in the quartile with the shortest telomeres as compared with individuals in the quartile with the longest telomeres (47). The only published prospective cohort study of 47,102 Danish general population participants with 131 bladder cancer cases diagnosed during a follow-up period of up to 20 years did not find any association between LTL and bladder cancer risk (HR = 1.00 per 1,000 bp increase; 95% CI, 0.82–1.21; ref. 36). In contrast, in a recently published MR study evaluating genetically predicted LTL and the risks of cancer and other diseases, long LTL was associated with significantly increased risks of nine cancers, including bladder cancer (OR = 2.19 per SD increase; 95% CI, 1.32–3.66; ref. 39).

Table 4. MR analyses of LTL and bladder cancer risk.

MR method	OR (95% CI)[a]	*P* value
Weighted genetic risk score (GRS)	1.13 (0.73–1.75)	0.595
Inverse-variance weighted (IVW)	1.14 (0.75–1.74)	0.543
MR-Egger	0.83 (0.19–3.58)	0.799
Weighted median	0.87 (0.48–1.55)	0.626

[a]OR per SD increase, adjusted by age, gender, and smoking status.

It is well known that retrospective case–control studies may produce spurious associations when assessing an intermediate biomarker such as LTL due to reverse causation, that is, disease state causes LTL attrition. Pooley and colleagues (63) compared retrospective and prospective studies of LTL and risks of colorectal and breast cancer and found the mean telomere length was substantially shorter in retrospectively collected cases than in controls but the equivalent association was markedly weaker in the prospective studies, suggesting telomere shortening largely occurs after diagnosis. In an earlier meta-analysis, Wentzensen and colleagues found short LTL was strongly associated with cancer risk in retrospective studies (pooled OR = 2.9; 95% CI, 1.75–4.8; $P < 0.0001$, for the shortest LTL quartile compared with the longest quartile); on the other hand, the pooled OR in prospective studies was much weaker (OR = 1.16; 95% CI, 0.87–1.54; $P = 0.32$; ref. 64). The results of our retrospective case–control study are consistent with these literature data. For the first time, we performed a stratified analysis based on bladder cancer severity and found LTL was shorter in MIBC than in NMIBC, and the association between short LTL and increased bladder cancer risk was stronger in MIBC than in NMIBC. This observation suggests that LTL shortens with increasing disease severity in bladder cancer and reverse causation is likely the major reason for the association between short LTL and increased risk of MIBC. However, we cannot rule out the possibility that residual confounding and/or other sources of bias may also contribute to the observed association between short LTL and increased risk of MIBC.

In addition, our MR study did not find a strong association between genetic predicted LTL and bladder cancer risk. A recently published MR study showed long LTL was associated with a significantly increased risk of bladder cancer (OR = 2.19 per SD increase; 95% CI, 1.32–3.66; ref. 39). The sample size for bladder cancer was 1,601

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cases and 1,819 controls from Iceland in that study, which did not specify disease severity (NMIBC and MIBC). Our sample size was slightly larger (2,011 bladder cancer cases and 2,259 controls). The inconsistent MR analysis results can be caused by multiple factors, such as population stratification, horizontal pleiotropy, trait heterogeneity, complexity of association, weak instrument bias, low statistical power, etc. (65). The *F*-statistics in our current study was 35.5, exceeding the Staiger-Stock rule-of-thumb threshold *F*-value of 10 (60). The previous study did not specify the *F*-statistics of bladder cancer MR analysis, but the range of *F*-statistics for different cancers was between 18 and 28 (39). Therefore, both of these two studies had relatively strong genetic instruments. Population substructure may have profound impact on MR analysis. A recent study found that individual SNPs and polygenic risk scores are associated with birth location within UK Biobank and that genetic substructure cannot be fully controlled for using routine adjustment and principal components derived from genotype data, thus yielding biased disease associations (66). Our study population is European Americans, who have higher genetic diversity than Icelanders. Population substructure may result in different allele frequencies. Among the 10 SNPs included in these two studies, the effect allele frequency (EAF) of several SNPs was quite different between our control population and the previous study population (e.g., rs11125529: 0.13 vs. 0.16; rs6772228: 0.95 vs. 0.87; rs3027234: 0.78 vs. 0.83; rs8105767: 0.29 vs. 0.25; and rs755017: 0.12 vs. 0.17). These EAF differences could contribute to inconsistent disease association results. Disease heterogeneity is another likely contributing factor. The etiology of bladder cancer in Iceland and our population may be slightly different due to different smoking prevalence and distinct occupational risk exposure profiles in Nordic countries and North America (67). Etiologic difference may lead to different biological alterations and disease heterogeneity, which could exhibit differential associations with specific SNPs. Finally, given the genetic substructure, disease heterogeneity, and biological complexity of SNP–LTL–bladder cancer associations, horizontal pleiotropy may not be completely mitigated by various sensitivity analyses. Horizontal pleiotropy can skew MR results toward null or produce false-positive associations. Finally, the sample sizes of both of these two studies were smaller than other common cancers in MR studies. Future larger MR studies in populations of European ancestry are needed to clarify the association between genetically predicted LTL and bladder cancer risk.

This is by far the largest case–control study evaluating LTL and bladder cancer risk. The large sample size allowed us to perform stratified analyses of NMIBC and MIBC. We reported for the first time that MIBC has shorter LTL than NMIBC and we observed an association between shorter LTL and higher risk of MIBC, which is most likely due to reverse causation. Combined with the only prospective cohort study reporting a HR of 1.00 for LTL and bladder cancer risk (36), and lack of a strong association in our MR analysis, these data provided compelling evidence that LTL does not play an important role in bladder cancer etiology in European Americans. There are a few limitations in this study. First, for qPCR-measured LTL, we do not have information on blood cell subtypes. Different blood cell types have slightly different telomere length, and different proportion of blood cell types could contribute to the observed difference in LTL between cases and controls. Controlling for blood cell subtype proportion or blood cell subtype-specific comparison of LTL would be more appropriate to assess the difference between cases and controls. Second, just like other MR studies using SNPs as genetic instruments, the SNPS used in this study only explain approximately 2% of the variability of LTL. Additional SNPs are needed to enhance instrument strength and increase statistical power in MR analyses. Third, although we performed sensitivity analyses, we could not rule out pleiotropic effects of these SNPs. But it is not likely that pleiotropic effects could explain the observed null associations. Fourth, we only included participants of European ancestry in this study due to the limited number of minorities. Future studies are needed to assess the associations between LTL and bladder cancer in other racial/ethnic groups.

In conclusion, in our retrospective case control analysis, short LTL, as measured by real-time qPCR, was a risk factor for MIBC, which was most likely due to reverse causation. In our MR analysis, genetically predicted LTL was not strongly associated with bladder cancer risk. We conclude LTL does not play an important role in bladder cancer etiology in European Americans.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

M. Chen: Conceptualization, data curation, investigation, methodology, writing–original draft, writing–review and editing. **Y. Xu:** Formal analysis, investigation, methodology, writing–review and editing. **J. Xu:** Investigation, methodology, writing–review and editing. **H. Chancoco:** Investigation, methodology, writing–review and editing. **J. Gu:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, writing–original draft, project administration, writing–review and editing.

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