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Research paper

Leukocyte telomere length is associated with aggressive prostate cancer in localized prostate cancer patients

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ABSTRACT

Background: Telomeres play important roles in cancer initiation and progression. The aim of this study is to investigate whether leukocyte telomere length (LTL) is associated with aggressive prostate cancer (PCa).
Methods: We measured relative LTL in a cohort of 1,889 white PCa patients who were treated and followed up at the University of Texas MD Anderson Cancer Center and assessed its associations with aggressive disease characteristics at diagnosis and biochemical recurrence (BCR) after active treatments (radical prostatectomy and radiotherapy). We further used a Mendelian randomization (MR) approach to compute a weighted genetic risk score (GRS) predictive of LTL using 10 established LTL-associated genetic variants and determined whether this GRS is associated with aggressive PCa.
Findings: LTL was significantly shorter in patients with higher Gleason scores at diagnosis. Dichotomized at the median value of LTL, patients with short LTL exhibited a 2.74-fold (95% confidence interval, 1.79−4.18, $P = 3.11 \times 10^{-6}$) increased risk of presenting with GS≥8 disease than those with long LTL in multivariate logistic regression analysis. Moreover, shorter LTL was significantly associated with an increased risk of BCR (hazard ratio = 1.53, 95% confidence interval, 1.01−2.34) compared to longer LTL in localized patients receiving prostatectomy or radiotherapy with a significant dose-response association (P for trend = 0.017) in multivariate Cox proportional hazards regression analysis. In MR analysis, genetically predicted short LTL was also associated with an increased risk of BCR (HR=1.73, 95% CI, 1.08−2.78).
Interpretation: Our results showed for the first time that LTL was shorter in PCa patients with high Gleason scores and that short LTL and genetically predicted short LTL are associated with worse prognosis in PCa patients receiving prostatectomy or radiotherapy.
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1. Introduction

Prostate cancer (PCa) is the most prevalent cancer and the second leading cause of cancer death among US men [1]. Prostate-specific antigen (PSA) test is widely adopted as a PCa screening tool that resulted in the vast majority of patients being diagnosed at early, locoregional stages [2]. The five-year survival rate for locoregional PCa is nearly 100%, for distant stage is 30%, and for all stages combined is 98.2% in U. S. [2]. Most of the screening-detected PCa patients are indolent, but still receive aggressive treatment, including radical prostatectomy and radiotherapy, which could cause significant morbidity in patients [3]. Clinical variables such as blood PSA levels, Gleason Scores (GS), and clinical stages provide strong prognostic values but are not sufficient to discriminate between aggressive and indolent diseases in diagnosis [4–7]. Patients with similar clinical features at diagnosis often have quite heterogeneous prognoses. Independent biomarkers are needed to improve risk stratification of localized PCa at diagnosis before treatment and predict the risk of prognosis after treatment, and allow better-informed clinical decision-making.

Telomeres are protective nucleoprotein complexes with repetitive nucleotide sequences TTAGGG at each end of human chromosomes [8].

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Research in context

Evidence before this study

Prostate cancer (PCa) is the most prevalent cancer and the second leading cause of cancer death among US men. Clinical variables such as blood PSA levels, Gleason Scores (GS), and clinical stages are not sufficient to discriminate between aggressive and indolent diseases at diagnosis. Telomeres play multiple important cellular functions. Telomere length has been linked to cancer development and progression. No study has evaluated the role of leukocyte telomere length (LTL) as a predictor of aggressive PCa in localized patients receiving definitive therapy.

Added value of this study

Using one of the largest single center PCa patient cohorts, we measured LTL from 1,889 PCa patients and evaluated its associations with aggressive disease features at diagnosis and biochemical recurrence (BCR) after active treatments. We found that short LTL was associated with high-grade PCa at diagnosis, and was also an independent predictor of prognosis in localized PCa patients receiving active therapy (radical prostatectomy and radiotherapy). Furthermore, we applied a Mendelian randomization approach to show that genetically predicted short LTL was an independent predictor of worse prognosis in localized PCa patients receiving active therapy.

Implications of all the available evidence

Our results showed for the first time that LTL can predict aggressive PCa at diagnosis and prognosis in localized patients receiving definitive therapy. LTL may improve risk stratification of localized PCa patients for better-informed clinical decision making.

Telomeres play a critical role in maintaining chromosome's integrity and stability whereas telomere dysfunctions are frequent events during the process of age-related diseases including tumors [8–10]. Shortened telomeres are found in most tumor tissues and are one of the hallmarks of human cancers [10]. In addition, numerous studies have shown that leukocyte telomere length (LTL) is associated with the risks of different cancers. Earlier retrospective case control studies suggested that short LTL was a risk factor for a few cancers [11–16], but later prospective studies and recent Mendelian randomization studies using genetically predicted LTL have increasingly found that long LTL was a risk factor for a number of cancers, including B-cell lymphoma, melanoma, lung adenocarcinoma, neuroblastoma, adult glioma, meningioma, renal cell carcinoma, and osteosarcoma [17–31].

Mendelian randomization (MR) uses genetic determinants of an exposure or intermediate biomarker to investigate the potential causal relationship between the interested exposure or biomarker with a disease outcome [32]. Because genetic variants are distributed randomly at conception across the whole genome and precede both exposures and diseases, they are less susceptible to confounding, reverse causation and measurement errors [31,32]. There are three main assumptions for MR analysis: 1) the genetic variants are associated with the exposure/biomarker of interest; 2) the genetic variants are not related to other confounders of exposure-outcome relationship; and 3) the genetic variants affect outcome only through the exposure/biomarker of interest. Single nucleotide polymorphisms (SNPs) in 10 independent genomic regions have been unequivocally identified as genetic determinants of LTL by genome-wide association studies (GWAS) [33,34] and these SNPs have not been found to be related to other traits. Increasing number of MR studies are using these SNPs as instrumental variables for LTL to investigate the effects of LTL on disease risks and outcomes [26,27,31].

Prospective studies and large MR analysis did not observe significant associations between LTL and the risk of developing PCa [26, 35, 36]. There were a few small studies evaluating the association of LTL with the mortality of PCa, but the results were inconsistent [36–39]. No study has determined the prognostic role of LTL in localized PCa patients receiving definitive treatment. In this current study, we measured relative LTL in a large PCa patient cohort and analyzed the association of LTL with the risk of biochemical recurrence (BCR) in patients receiving radical prostatectomy or radiotherapy. Furthermore, we used a two sample Mendelian randomization design to assess genetically predicted LTL and the risk of BCR. We found consistent association of relative LTL and genetically predicted GRS with the risk of BCR in this patient population. Our study provides the first evidence that short LTL is associated with aggressive PCa in localized patients receiving radical prostatectomy or radiotherapy.

2. Materials and methods

2.1. Study population and data collection

This study consisted of a total of 1,889 non-Hispanic Caucasian men with histologically confirmed adenocarcinoma of prostate from the University of Texas MD Anderson Cancer Center. The patients were newly registered patients who were diagnosed and treated at MD Anderson for localized diseases between the years of 2003 and 2013. Patients were consecutively recruited and there were no selection bias. Standardized epidemiological information, including demographics, smoking, exposures, co-morbidities, family history, prior medical history and quality of life, were collected through the Patient History Database (PHDB), an electronic core institutional resource to collect data for all new patients at MD Anderson. Patients' blood specimens were collected before any treatments. Clinical and follow-up data, including date of diagnosis, performance status, clinical stage, histological grade and pathological stage, PSA measurements, treatment (active surveillance, prostatectomy, radiotherapy and hormone therapy) and progression (clinical recurrence and metastasis), were abstracted from patient medical records. MD Anderson Tumor Registry conducts annual vital status follow-ups for all cancer patients. The clinical endpoint of this study was the occurrence of BCR, which is the most commonly used clinical endpoint in localized PCa patients receiving radical prostatectomy or radiotherapy. Patients were followed up by PSA monitoring every 3–6 months. BCR was defined as a serum PSA level of at least 0.2 ng/ml with a second confirmatory PSA level of at least 0.2 ng/ml for patients who underwent a radical prostatectomy or with a rise in PSA level by at least 2 ng/mL above the nadir PSA for patients receiving external-beam radiotherapy. Imaging was performed if PSA arose to confirm clinical recurrence. This study was approved by the University of Texas MD Anderson Cancer Center Institutional Review Board (IRB), and written informed consent forms were obtained from each patient.

2.2. Relative LTL assessment by real-time quantitative polymerase chain reaction

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp blood DNA extraction kit (Qiagen, Valencia, CA). Relative LTL was measured using a modified real-time quantitative polymerase chain reaction (PCR) method originally developed by Cawthon [40]. Detailed descriptions of the laboratory procedures and quality controls have been published previously [12,41–43]. Briefly, the relative LTL for each sample was determined through two separate PCR reactions (telomere amplification and globulin amplification). The ratio of the telomere repeats 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 LTL. The PCR (15μL) for telomere amplification consisted of 1xSYBR 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. The thermal cycling conditions were at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and at 56 °C (for telomere amplification) or 58 °C (for Hgb amplification) for 1 min. 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. 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. Duplicates for each sample were done. The intra assay coefficient of variation was <3% and the inter assay coefficient of variation was <5% for telomere length assay in our laboratory. 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.

2.3. Genotyping and imputation

Custom Infinium OncoArray-500 K Beadchip was used to genotype all the samples on the Illumina iScan system in the Genotyping Core of MD Anderson Cancer Center. Genotyping data were analyzed and exported using the Genome Studio software (Illumina). We randomly selected 2% of samples for duplicate genotyping and the mean concordance rate of replicated samples was 99.2%. All patient samples had an overall SNP call rate >95%. Individual SNPs with minor allele frequency (MAF) <1% (n = 83,738) and call rate <90% (n = 2,945) were excluded for analysis. A total of 412,487 SNPs on the OncoArray-500 K Beadchip passed these strict quality control steps and were subjected to imputation. Imputation was performed using the Michigan Imputation Server (https://imputationserver.sph.umich.edu/), an online server that generates phased and imputed genotypes using the Haplotype Reference Consortium (HRC Version r1.1) reference panels [44]. The individual level data of the 10 LTL-associated SNPs were extracted from the genotyped and imputed dataset. Among these SNPs, four SNPs (rs10936599, rs2736100, rs9420907, and rs755017) were directly genotyped on OncoArray-500 K, and the other six were imputed with an imputation accuracy (mean R^2) of 0.96.

2.4. Mendelian randomization (MR) analysis and genetic risk scores for LTL

A two-sample MR design was used to assess the associations between genetically predicted LTL and the risk of BCR. The SNP-LTL effects (β estimate for each SNP) were derived from published GWAS of LTL [33, 34] and the SNP-PCa effects were estimated using individual-level genotype data from the patient population in this study. Genetic risk scores (GRS) calculation for 10 LTL- associated SNPs was done according to the following formula:

$$GRS_i = \sum_{j=1}^{10} = w_j x_{ij}$$

in which x_{ij} is the number of telomere-length associated risk alleles for the j-th SNP in the i th subject (x_{ij}=0, 1 or 2) and w_j is the weight or coefficient for the j-th SNP. Weighted GRS counted the number of alleles associated with longer LTL that an individual carried across all 10 SNPs, with the addition of published LTL-associated β estimates as w_j for each SNP. Weighted GRS produces higher specificity than unweighted GRS by assigning more weight to SNPs with stronger effects.

2.5. Statistical analysis

We first applied analysis of variance (ANOVA) to compare the mean LTL among patients with different clinical characteristics at baseline. We then analyzed the associations between LTL and presenting with high grade PCa (GS≥8) at diagnosis using a multivariate logistic regression model adjusting for age, smoking status, pack year, body mass index (BMI), clinical stage and PSA. Low-grade (GS=6) patients at diagnosis were used as the reference group for this logistic regression analysis. We also determined the association between LTL and the risk of BCR by calculating the hazard ratio (HR) and corresponding 95% confidence interval (95% CI) using multivariate Cox proportional hazards model, adjusting for age, smoking status, pack year, BMI, D'Amico risk groups and initial treatment. The proportional hazards assumption was verified by plotting and testing the Schoenfeld's residuals and through inclusion of time varying covariates in the models and no violation was identified. LTL was dichotomized at the median value of LTL or classified into three and four groups based on the tertile and quartile distributions of LTL. For each LTL-associated SNP, we evaluated its association with the risk of BCR using Cox analysis. To analyze the association between GRS and the risk of BCR, we dichotomized GRS at the median value or categorized into three and four groups based on the tertile and quartile distribution, and used multivariate Cox proportional hazards model to calculate HR and corresponding 95% CI, adjusting for age, smoking status, pack year, BMI, D'Amico risk groups and initial treatment. We used Kaplan−Meier survival function and log-rank test to compare BCR-free survival time among patients with different GRS. The missing data for each variable were grouped into one category in the regression models. All data were analyzed using R software (v3.4.1) and STATA (v13, STATA Corp). All P values were two-sided with P<0.05 considered statistically significant.

3. Results

3.1. Patient characteristics

The distribution of selected characteristics of the 1,889 PCa patients and the relative LTL stratified by their characteristics are shown in Table 1. Nearly 80% of patients were diagnosed at ages 55 and older. As expected, there was a strong inverse relationship between LTL and age (P < 0.001, ANOVA). There were 873 (46.5%) never-smokers, 846 (45.0%) former smokers and 159 (8.5%) current smokers. LTL was not different by smoking status (P = 0.818, ANOVA). The majority of patients were either overweight (45.9%) or obese (36.6%). It appeared that LTL was inversed associated with BMI (P = 0.08, ANOVA). According to the total Gleason score, 660 (35.1%) had GS of 6, 909 (48.4%) had GS of 7 and 309 (16.5%) had GS of 8 or above. Notably, LTL was significantly shorter in higher GS patients (P = 0.013, ANOVA). LTL was not associated with clinical stage (P = 0.672, ANOVA) or PSA at diagnosis (P = 0.432, ANOVA). For their initial primary treatments, 49.1% received definitive radical prostatectomy (RP) and 20.4% received definitive radiotherapy. LTL was significantly longer in patients receiving RP compared to other treatments (P = 0.010, ANOVA), which was due to the significantly younger age of patients receiving RP (mean age [SD]: 59.56 [7.00]) compared to other treatments (64.96 [7.70], P<0.001, t-test) because one the major criteria for selecting patients for RP is longer life expectancy.

3.2. Associations between LTL and high grade tumors at diagnosis

We then evaluated the association of LTL with the risk of presenting with high grade, aggressive disease at diagnosis using multivariate logistic regression analysis adjusting for age, smoking status, BMI, clinical stage, and PSA at diagnosis (Table 2). Dichotomized into low and high LTL groups by the median (50th percentile) value of LTL,

Table 1
LTL by selected characteristics of the study patients.

Characteristics	N (%)	Relative LTL Mean (SD)	*P* value
All, Mean (SD)	1889 (100)	0.93 (0.30)	N/A
All, Median (IQR*)	1889 (100)	0.89 (0.37)	N/A
Age at diagnosis, years			
< 55	425 (22.5)	0.98 (0.31)	
55–65	834 (44.2)	0.93 (0.30)	
> 65	630 (33.4)	0.89 (0.28)	4.18×10^{-7}
Smoking status at diagnosis			
Never-smoker	873 (46.2)	0.93 (0.29)	
Former smoker	846 (44.8)	0.93 (0.30)	
Current smoker	159 (8.4)	0.93 (0.30)	
Missing	11 (0.6)	0.93 (0.30)	0.818
BMI at diagnosis, kg/m^2			
< 25	273 (14.5)	0.96 (0.31)	
25–29.99 (overweight)	718 (38.0)	0.92 (0.29)	
≥ 30 (obese)	573 (30.3)	0.91 (0.29)	
Missing	325 (17.2)	0.93 (0.30)	0.081
Total Gleason score			
6	660 (34.9)	0.95 (0.31)	
7	909 (48.1)	0.92 (0.28)	
≥ 8	309 (16.4)	0.92 (0.32)	
Missing	11 (0.6)	0.82 (0.27)	**0.014**
Clinical tumor stage			
T1	1492 (79.0)	0.93 (0.29)	
T2	142 (7.5)	0.92 (0.29)	
T3-T4	255 (13.5)	0.95 (0.34)	0.672
PSA at diagnosis			
< 10 ng/ml	1589 (84.1)	0.93 (0.29)	
10–20 ng/ml	170 (9.0)	0.92 (0.29)	
>20 ng/ml	125 (6.6)	0.94 (0.38)	
Missing	5 (0.3)	1.08 (0.37)	0.433
D'Amico risk group			
Low	604 (32.0)	0.95 (0.32)	
Intermediate	847 (44.8)	0.92 (0.26)	
High	438 (23.2)	0.93 (0.33)	0.103
Initial primary treatment			
Radical prostatectomy	927 (49.1)	0.95 (0.31)	
Radiotherapy	386 (20.4)	0.91 (0.27)	
Surveillance or unknown	436 (23.1)	0.91 (0.29)	
Other treatment	140 (7.4)	0.91 (0.25)	0.011

* IQR: interquartile range.

patients with short LTL exhibited a 2.74-fold (95% CI, 1.79–4.18, $P = 3.11 \times 10^{-6}$, logistic regression analysis) increased risk of presenting with GS≥8 disease than those with long LTL. There was a significant dose-response relationship between LTL and presenting with aggressive diseases at diagnosis. In tertile analysis, compared to patients in the longest LTL tertile group, the ORs for patients in the medium and shortest tertile groups were 1.63 (95% CI, 0.97–2.74) and 2.79 (95% CI, 1.68–4.63), respectively (P for trend = 6.54×10^{-5}). In quartile analysis, compared to patients in the 4th quartile group (longest LTL), the ORs for patients in the 3rd, 2nd, and 1st (shortest) quartile groups were 0.71 (95% CI, 0.38–1.34), 2.09 (95% CI, 1.17–3.71), and 2.60 (95% CI, 1.47–4.59), respectively (P for trend = 2.82×10^{-5}) (Table 2).

3.3. Associations between LTL and prognosis of patients receiving radical prostatectomy or radiotherapy

We further assessed the association of LTL with the risk of BCR in patients receiving radical prostatectomy or radiotherapy using multivariate Cox proportional hazards model adjusting for age, smoking status, BMI, GS, stage, PSA and primary treatment (Table 3). Dichotomized into long and short LTL groups by the median value of LTL, patients with short LTL exhibited a 1.53-fold (95% CI, 1.01–2.34, $P = 0.044$, Cox analysis) increased risk of BCR compared to those with long LTL. In tertile analysis, compared to patients in the longest LTL tertile group, the HRs for patients in the medium and shortest tertile groups were 1.95 (95% CI, 1.13–1.37) and 1.70 (95% CI, 1.00–2.91), respectively. In quartile analysis, compared to patients in the 4th quartile group (longest LTL), the HRs for patients in the 3rd, 2nd, and 1st (shortest) quartile groups were 1.37 (95% CI, 0.72–2.64), 1.78 (95% CI, 0.97–3.31), and 1.84 (95% CI, 1.00–3.36), respectively (P for trend = 0.034) (Table 3).

3.4. Mendelian randomization analysis

To further investigate the associations of LTL with aggressive PCa, we applied a two-sample Mendelian randomization approach using 10 GWAS-identified SNPs predictive of LTL. Table 4 shows the features of these 10 SNPs and their individual associations with BCR of PCa patients receiving RP or radiotherapy. The β estimates (Table 4) for SNP-LTL association were obtained from published GWAS [33, 34]. Individually, one SNP, rs8105767 on ZNF208, was significantly associated with the risk of BCR, which remained significant after Bonferroni correction of multiple testing. Patients carrying the effect allele (longer LTL) exhibited a significantly reduced risk of BCR (HR=0.58, 95% CI, 0.42–0.80, $P = 0.001$, Cox analysis).

We then constructed a weighted genetic risk score (GRS) for each patient based on the direction and strength of association of these 10 SNPs with LTL. There was a significant correlation between GRS and measured LTL with a correlation coefficient of 0.112 ($P = 0.0002$, Spearman's correlation analysis), confirming the validity of this genetic instrument in estimating TLT. We subsequently tested the association between the GRS and risk of BCR. The GRS was significantly shorter in patients experiencing BCR than in patients who did not have BCR (mean ± SD, 0.69 ± 0.15 vs. 0.71± 0.14, $P = 0.0006$, *t*-test). In multivariate Cox analysis adjusting for age, smoking status, BMI, stage, GS, PSA, and primary treatment,

Table 2
Association of LTL with high-grade PCa at diagnosis.

LTL	GS=6N (%)	GS≥8N (%)	Crude OR(95% CI)	*P* value	Adjusted OR (95% CI)*	*P* value
Dichotomize						
Long	336 (50.91)	120 (38.83)	Reference	N/A	Reference	N/A
Short	324 (49.09)	189 (61.17)	1.63 (1.24–2.15)	0.0004	2.74 (1.79–4.18)	**3.11×10^{-6}**
Tertile						
3rd (longest)	227 (34.39)	90 (29.13)	Reference	N/A	Reference	N/A
2nd	218 (33.03)	89 (28.80)	1.02 (0.73–1.46)	0.869	1.63 (0.97–2.74)	0.063
1st (shortest)	215 (32.58)	130 (42.07)	1.53 (1.10–2.12)	0.011	2.79 (1.68–4.63)	**7.22×10^{-5}**
P for trend				0.010		**6.54×10^{-5}**
Quartile						
4th (longest)	167 (25.30)	67 (21.68)	Reference	N/A	Reference	N/A
3rd	169 (25.61)	53 (17.15)	0.78 (0.51–1.19)	0.249	0.71 (0.38–1.34)	0.291
2nd	161 (24.39)	84 (27.18)	1.30 (0.88–1.92)	0.183	2.09 (1.17–3.71)	**0.013**
1st (shortest)	163 (24.70)	105 (33.98)	1.61 (1.10–2.34)	0.013	2.60 (1.47–4.59)	**0.001**
P for trend				0.001		**2.82×10^{-5}**

* Adjusted by age, smoking status, BMI, clinical stage and PSA.

Table 3
Association of LTL with BCR in localized PCa patients receiving radical prostatectomy or radiotherapy.

LTL	No BCRN (%)	BCRN (%)	Crude HR(95% CI)	*P* value	Adjusted HR (95% CI)*	*P* value
Dichotomize						
Long	602 (91.77)	54 (8.23)	Reference	N/A	Reference	N/A
Short	719 (91.13)	70 (8.87)	1.41 (0.94−2.12)	0.093	1.54 (1.01−2.34)	**0.044**
Tertile						
3rd (long)	398 (91.92)	35 (8.08)	Reference	N/A	Reference	N/A
2nd	455 (91.55)	42 (8.45)	1.42 (0.85−2.37)	0.180	1.95 (1.13−3.37)	**0.016**
1st (short)	468 (90.87)	47 (9.13)	1.52 (0.91−2.52)	0.110	1.70 (1.00−2.91)	**0.048**
P for trend				0.117		0.065
Quartile						
4th (longest)	308 (93.05)	23 (6.95)	Reference	N/A	Reference	N/A
3rd	294 (90.46)	31 (9.54)	1.20 (0.65−2.24)	0.558	1.37 (0.72−2.64)	0.333
2nd	358 (91.79)	32 (8.21)	1.49 (0.83−2.66)	0.181	1.78 (0.97−3.31)	**0.064**
1st (shortest)	361 (90.48)	38 (9.52)	1.63 (0.91−2.91)	0.100	1.84 (1.00−3.36)	**0.049**
P for trend				0.074		**0.034**

* Adjusted by age, smoking status, BMI, stage, GS, PSA, and primary treatment.

Table 4
Individual LTL-associated SNPs and BCR in localized PCa receiving radical prostatectomy or radiotherapy.

SNP ID	Chr.	Position	Gene	Allele*	EAF*	β*	No BCR (n)	BCR (n)	HR** (95% CI)	P value
rs11125529	2	54475866	ACYP2	A/C	0.14	0.07	84\19\1	793\255\24	0.81(0.50−1.30)	0.385
rs6772228	3	58376019	PXK	T/A	0.94	0.04	0\7\98	4\120\951	1.70(0.79−3.67)	0.177
rs10936599	3	169492101	TERC	C/T	0.75	0.1	10\37\58	56\419\600	0.79(0.57−1.10)	0.163
rs7675998	4	164007820	NAF1	G/A	0.77	0.05	7\36\59	46\369\618	0.86(0.61−1.20)	0.372
rs2736100	5	1286516	TERT	C/A	0.51	0.09	34\50\21	254\569\251	0.86(0.63−1.18)	0.348
rs9420907	10	105676465	OBFC1	C/A	0.13	0.14	80\22\3	808\249\18	0.82(0.53−1.28)	0.384
rs3027234	17	8136092	CTC1	C/T	0.78	0.1	2\40\60	49\372\631	0.85(0.59−1.22)	0.377
rs8105767	19	22215441	ZNF208	G/A	0.29	0.06	13\39\44	81\414\531	**0.58(0.42−0.80)**	**0.001**
rs6028466	20	38129002	DHX35	A/G	0.06	0.06	93\12\0	947\125\3	0.93(0.47−1.83)	0.824
rs755017	20	62421622	ZBTB46	G/A	0.12	0.02	87\16\2	832\221\22	0.76(0.45−1.26)	0.279

* Alleles are short allele/long allele. Short alleles are used as the reference allele and long allele as effect allele. EAF: effect allele frequency; Beta estimates of SNP-LTL association were from published GWAS;.
** Adjusted by age, smoking status, BMI, stage, GS, PSA, and primary treatment.

when patients were dichotomized into low and high GRS groups by the median (50th percentile) value of GRS, patients with low GRS (i.e., short LTL) exhibited a 1.73-fold (95% CI, 1.08−2.78, *P* = 0.021, Cox analysis) increased risk of BCR compared to those with high GRS (long LTL). A significant dose-response relationship between lower GRS and higher risks of BCR was observed in fertile and quartile analysis (P for trend=0.011 and 0.004, respectively, Table 5). Patients with the lowest tertile and quartile GRS exhibited 2.13-fold (95% CI, 1.18−3.84) and 2.57-fold (95% CI, 1.31−5.09) increased risks of BCR, respectively, compared to those with the highest tertile and quartile GRS. In Kaplan−Meier survival analyses, patients with lower GRS exhibited significantly shorter BCR-free survival time than those with higher GRS in dichotomous, tertile and quartile analyses, with long-rank P values of 0.0006, 0.042, and 0.026, respectively (Fig. 1).

4. Discussion

In the present study, we investigated the association of LTL with aggressive PCa. The LTL was measured at baseline before any treatment. We found that short LTL was associated with high-grade PCa at diagnosis, and was also an independent predictor of prognosis in localized PCa patients receiving radical prostatectomy and radiotherapy. Furthermore, we applied a two-sample MR approach to show that genetically predicted short LTL was also an independent predictor of worse prognosis in localized PCa patients receiving radical

Table 5
GRS predictive of LTL is associated with BCR in localized PCa.

LTL	No BCRN (%)	BCRN (%)	Crude HR(95% CI)	*P* value	Adjusted HR (95% CI)*	*P* value
Dichotomize						
Long	531 (93.65)	36 (6.35)	Reference	N/A	Reference	N/A
Short	548 (90.13)	60 (9.87)	1.90 (1.19−3.01)	0.007	1.73 (1.08−2.78)	**0.021**
Tertile						
3rd (long)	368 (94.12)	23 (5.88)	Reference	N/A	Reference	N/A
2nd	359 (91.58)	33 (8.42)	1.66 (0.91−3.03)	0.099	1.54 (0.84−2.84)	0.162
1st (short)	352 (89.80)	40 (10.20)	2.08 (1.16−3.71)	0.013	2.13 (1.18−3.84)	**0.012**
P for trend				0.013		**0.011**
Quartile						
4th (longest)	277 (94.54)	16 (5.46)	Reference	N/A	Reference	N/A
3rd	254 (92.70)	20 (7.30)	1.29 (0.60−2.75)	0.513	1.40 (0.65−3.02)	0.386
2nd	288 (91.72)	26 (8.28)	1.86 (0.92−3.74)	0.081	1.63 (0.81−3.31)	0.172
1st (shortest)	260 (88.44)	34 (11.56)	2.48 (1.27−4.84)	0.008	2.57 (1.31−5.09)	**0.006**
P for trend				0.003		**0.004**

* Adjusted by age, smoking status, BMI, stage, GS, PSA, and primary treatment.

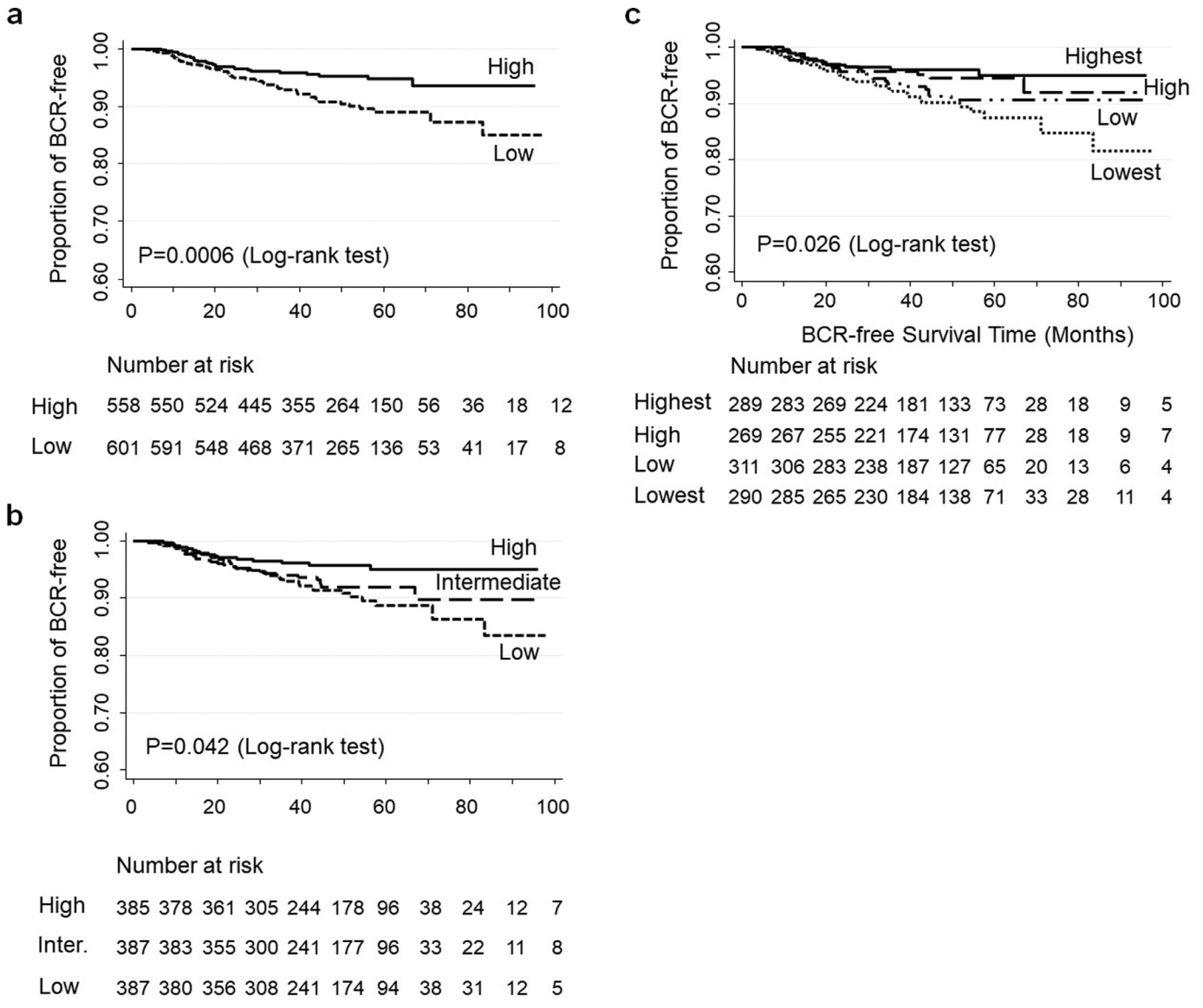

Fig. 1. Kaplan–Meier curve comparing the probability of the BCR-free survival in PCa patients based on genetic risk scores (GRS) that predict leukocyte telomere length. A. Dichotomized at the median value of GRS; B. Tertile analysis; C. Quartile analysis.

prostatectomy and radiotherapy. To our knowledge, this is the first study to evaluate LTL, either directly measured by real time quantitative PCR or genetically predicted, as a predictor of BCR in patients receiving radical prostatectomy and radiotherapy. Our data provided compelling evidence supporting that short LTL is a predictor of aggressive PCa.

A few small studies evaluated the association of LTL with overall mortality in PCa patients, but the results were inconsistent [36–39]. A large prospective study of 47,102 Danish general population participants with a follow-up of up to 20 years for cancer diagnosis and death found that short LTL was associated with increased risks of early death for all cancers, but not for PCa (418 patients, 157 deaths) with an HR of 1.04 (95% CI, 0.87, 1.25) [36]. One recent Australia study of 533 PCa patients (188 deaths) with a median follow-up of 149 months reported longer LTL were significantly associated with higher overall mortality (HR = 1.22; 95% CI, 1.07–1.39) [38]. The reasons for the heterogeneous results may be due to modest sample size, heterogeneity of patient population, technical variability of LTL measurement by real time PCR, and different causes of overall mortality. No study to date has specifically evaluated the association of LTL with BCR in localized PCa patients receiving radical prostatectomy or radiotherapy. The sample size of patients in our study more than doubled those of previous studies assessing LTL in the context of PCa risk and prognosis [36–39, 45]. The large sample size from a single institution with comprehensive clinical, treatment, and follow-up information allowed us to perform stratified analyses and observed significant associations between baseline LTL and clinically defined aggressive disease at diagnosis as well as BCR in localized PCa patients receiving definitive therapies.

MR utilizes genetic variants as a proxy for an exposure or an intermediate biomarker to investigate their potential to have a causal association with a disease [31]. Large MR studies did not find significant associations between genetically predicted LTL and the risk of PCa [21, 26]. Our study is the first to use an MR approach to assess LTL with the risk of BCR. Consistent with the results of real time PCR-measured LTL, genetically predicted short LTL was a significant predictor of worse prognosis in patients receiving radical prostatectomy and radiotherapy, providing compelling evidence for the causal relationship between short LTL and aggressive disease in localized PCa patients.

A number of studies have evaluated the association of LTL with the prognosis of other cancers and most showed that shorter LTL was associated with increased risks of death in cancer patients, including

bladder cancer [46], stage I and II cutaneous melanoma [47], gastric cancer [48], colorectal cancer [49], renal cell carcinoma [50], pancreatic cancer [51], and lung adenocarcinoma [52]. Interestingly, a recent large study of nasopharyngeal carcinoma in Hong Kong found that suboptimal LTL (both too short and too long) was associated with poor survival compared to patients with normal range LTL [53]. Only a couple of studies reported that shorter LTL increased risk of relapse [48, 49]. These literature reports are consistent with our observation of increased risk of BCR associated with short LTL.

Biologically, numerous studies have shown that telomere dysfunction plays a critical function in genetic instability and carcinogenesis [54–58]. Telomere shortening increases end-to-end chromosome fusion and genomic instability [54–58]. LTL may serve as a surrogate for telomere length in normal prostate tissues. LTL is under strong genetic control with an estimated heritability of up to 80% from classic twin studies [59, 60]. Telomere length is highly correlated between different tissues and blood cells among newborns [61]. For adults, previous studies also found a high correlation of telomere length between different tissues [62, 63]. Moreover, the rates of telomere shortening are similar in different tissues, such as proliferative (blood and skin) and minimally proliferative tissues (muscle and fat) [63]. These data suggest that telomere length is established during early life and maintained through adulthood, and the genetic determinants of telomere length are tissue-independent [62]. Therefore, relative telomere length in easily accessible tissues such as blood could serve as a surrogate for that in other tissues and the association of short LTL with aggressive PCa could infer the association of short telomere length in normal prostate tissues. Indeed, a previous study found that short telomere length in stromal cells of PCa tumor tissues was strongly associated with progression to metastasis and prostate cancer death [64]. The Mendelian randomization analysis of our study further supported the causal effect of short telomeres in malignant progression of PCa.

Another potential mechanism that may partially contribute to the association between short LTL and worse prognosis of PCa patients may be due to accelerated senescence of immune cells and altered immune functions. For instance, a previous study showed that colorectal patients with short LTL exhibited higher percentage of CD4(+) T cell and the lower percentage of B cell in peripheral blood mononuclear cells (PBMC), as well as lower concentration of plasma transforming growth factor-β1, suggesting reduced immune response [49]. Another study reported that gastric cancer patients with short LTL had a higher CD4(+) T cell percentage in PBMCs, CD19(+)IL-10(+) Breg percentage in B cells and plasma IL-10 concentration, indicating an enhanced immunosuppressive status with short LTL [48]. In both of these studies, short LTL was associated with poorer prognosis (recurrence and survival) of colorectal and gastric cancer patients, respectively [48, 49]. The exact mechanisms underlying the associations between short LTL and worse prognosis of PCa warrant further investigation.

There are several strengths for our study. This is the largest study of LTL in PCa patients and the first study to report significant associations between shorter LTL and increased risks of BCR in localized PCa patients using real-time PCR measured relative LTL and genetically predicted LTL. The reported association is biologically plausible. The blood samples were collected prior to any treatments. All patients were treated at MD Anderson Cancer Center with comprehensive clinical data and follow-up data. There are also a couple of limitations. First, we only investigated BCR, but not clinical recurrence mortality, as a prognosis endpoint due to the excellent prognosis and the small number of clinical recurrence and death events in our localized patients receiving definitive therapy. Second, all the patients were European Americans and the prognostic roles of LTL in PCa of other ethnicities warrant further study.

In summary, by measuring LTL using real time PCR or using a Mendelian randomization approach, our study reported for the first time that shorter LTL is associated with a significantly increased risk of biochemical recurrence in localized PCa patients receiving radical prostatectomy and radiotherapy. Future studies are warranted to confirm our observations in PCa patient cohorts of diverse ethnicities and investigate the underlying biological mechanisms of the association between short LTL and worse prognosis in PCa patients. LTL may have clinical utility in the risk stratification of localized prostate cancer.

Declaration of Competing Interest

The authors declare no conflict of interest.

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