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Telomere Length as a Risk Factor for Hereditary Prostate Cancer

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Abstract

BACKGROUND—Telomeres are repetitive nucleotide sequences that stabilize the ends of chromosomes. Critically short telomeres are thought to contribute to cancer development by increasing chromosomal instability. We hypothesized that shorter leukocyte telomere length, a surrogate for inherited prostate cell telomere length, would be associated with increased risk of prostate cancer in hereditary prostate cancer (HPC) families.

METHODS—One hundred twelve affected and 63 unaffected men from 28 families were drawn from the Johns Hopkins HPC family database. Relative mean telomere length was measured in isolated peripheral leukocyte DNA by quantitative PCR. Conditional logistic regression was used to estimate the association between quartile of age-adjusted telomere length and prostate cancer.

RESULTS—Men in the shortest quartile of telomere length did not have increased odds of prostate cancer compared to men in the other three quartiles (OR = 0.84, 95% CI: 0.32–2.20, *P* = 0.73). However, when the analysis was restricted to affected men with blood drawn before or within a year of diagnosis (N = 39) and all unaffected men, shorter telomere length was moderately associated with increased odds of prostate cancer (OR = 3.55, 95% CI: 0.82–15.43, *P* = 0.09).

CONCLUSIONS—Though we found no association overall, shorter leukocyte telomere length may be associated with increased odds of prostate cancer when measured in pre-diagnostic samples. Further prospective research is warranted exploring the utility of telomere length as a prostate cancer biomarker.

Keywords

HPC families; telomere biology; prostate cancer risk

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INTRODUCTION

Telomeres are repetitive nucleotide sequences that cap the ends of chromosomes and protect them from degradation, fusion, and undue recombination [1]. Telomeres shorten progressively throughout the lifespan due to the inability of DNA polymerases to copy the extreme 3′ ends of chromosomes during cell division (the “end replication problem”). Factors such as chronic inflammation and oxidative stress also increase the rate of telomere attrition [2]. Critically short telomeres lead to chromosomal instability and loss of cell viability [1]. By instigating chromosomal instability, critically short, dysfunctional telomeres are thought to contribute to the initiation of prostate and other cancers [3–5]. This hypothesis has been substantiated by the finding of widespread telomere shortening in prostate cancer precursor lesions [6].

Several large, population-based studies have examined the relationship between cancer risk and telomere length in peripheral leukocytes [7,8]. Since telomere lengths of different somatic tissues are correlated [9], leukocyte telomere length is considered a surrogate measure of telomere length in prostate tissue and other tissues of interest. Previous studies found no association between prostate cancer risk and leukocyte telomere length measured in pre-diagnostic DNA [8,10], but one study did observe shorter telomere length in cases when the analysis was restricted to men with a positive family history [10].

In the current study, we sought to investigate the relationship between telomere length and hereditary prostate cancer. Using a case–control design embedded in a family study, we aimed to test whether inherited short telomeres contribute to the development of prostate cancer in certain families, since mean telomere length is heritable and the heritability of prostate cancer is still largely unexplained. Estimates of the heritability of mean telomere length range from 36% to 90%, though the mode of inheritance is currently contested [11–13]. We measured telomere length in leukocytes and assumed that this measurement reflected inherited telomere length in both leukocytes and prostate cells.

MATERIALS ANDMETHODS

Study Population

The families in this study were drawn from the Johns Hopkins Hereditary Prostate Cancer (HPC) family database. All participants had provided informed consent. Hereditary prostate cancer families are defined as having three or more first-degree relatives with prostate cancer, prostate cancer cases in three successive generations of either the paternal or maternal lineages, and/or two or more relatives affected with prostate cancer before age 55 [14].

For this study on telomeres, families were chosen for inclusion if they exhibited an autosomal dominant mode of inheritance for prostate cancer, a low median age of onset, a high ratio of affected men to unaffected men at risk, and no observed linkage to familial prostate cancer genes known at the time. Within families, individuals were chosen for inclusion if they had available peripheral leukocyte DNA. These selection criteria were employed to enrich the study for prostate cancer cases with a strong hereditary component,

thus increasing the statistical power to detect genetic determinants of hereditary prostate cancer risk.

Based on these criteria, 39 HPC families were chosen for inclusion. Mean leukocyte telomere length was measured in 289 individuals, including 156 affected men, 64 unaffected men, and 69 women. The samples were assayed in two batches, with 128 individuals from 17 families in batch one and 161 individuals from 22 families in batch two. The primary statistical analysis was restricted to the 220 men, since women are not at risk for prostate cancer. Three outliers were excluded with mean leukocyte telomere length greater than three standard deviations away from the mean, and 11 uninformative families (with n = 42 affected men) dropped out of the analysis because blood samples from related unaffected men were not available. The primary analysis thus included 175 men (112 affected and 63 unaffected) from 28 HPC families.

Measurement of Telomere Length

Leukocyte DNA was isolated using the DNeasy Blood and Tissue kit (Qiagen). Quantitative PCR was used to estimate the ratio of telomeric DNA to that of a single copy gene (β-globin) as previously described [15], with the following modifications. Briefly, 5 ng of genomic DNA was used in a 25 μl volume for either the telomere or β-globin reactions; each sample was run in triplicate. The telomere reaction mixture consisted of 1 × PCR buffer, 1.5 mM $MgCl_2$, 100,000-fold dilution of SyberGreen, 200 nM dNTP mix, 1% DMSO, 100 nM forward telomere primer (CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT), 900 nM reverse telomere primer (GGCTGGCCTTACCCTTACCCTTACCCTTACCCTTACCCT), and 0.8 U of Platinum Taq polymerase. The reaction proceeded for one cycle at 95°C for 5 min, followed by 35 cycles at 95°C for 15 sec, and 54°C for 30 sec. The β-globin reaction mixture consisted of 1 × PCR buffer, 2.5 mM $MgCl_2$, 100,000-fold dilution of SyberGreen, 200 nM dNTP mix, 2% DMSO, 300 nM forward β-globin primer (CACATGGCAAGAAGGTGCTGA), 700 nM reverse β-globin primer (ACAGTGCAGTTCACTCAGCTG), and 0.5 U of Platinum Taq polymerase. The β-globin reaction proceeded for one cycle at 95°C for 5 min, followed by 35 cycles at 95°C for 30 sec, 58°C for 30 sec, and 72°C for 45 sec. Each 96-well plate contained a no template negative control and two separate five-point standard curves ranging from 0.04 to 25 ng using leukocyte DNA; these standard curves allowed the PCR efficiency to be determined for each experimental run. Each plate also included three samples isolated from a series of LNCaP prostate cancer cell lines with known telomere lengths, ranging from 3 to 15 Kb, as determined by terminal restriction fragment analysis. Inclusion of these samples provided an additional quality control check and allowed for direct conversion of the quantitative PCR measurements to actual average telomeric DNA lengths. The $-dC_t$ for each sample was calculated by subtracting the average β-globin C_t value from the average telomere C_t value. The $-ddC_t$ was determined by subtracting the $-dC_t$ of the 5 ng standard curve point of the LNCaP series from the $-dC_t$ of each unknown sample.

Statistical Analyses

Since telomere length shortens with age, and different generations within families were of different ages at the time of blood draw, the raw telomere length measurements were age-adjusted using a residuals method [16]. Telomere length was regressed on age at blood draw using a linear regression model that accounted for clustering by family. The residuals, defined as the variation in telomere length unexplained by the linear relationship between telomere length and age, were then centered to the mean telomere length and used as the age-adjusted telomere length values for all subsequent analyses.

Age-adjusted telomere length was divided into quartiles defined by the batch-specific distributions in the unaffected men. Odds ratios (OR) of prostate cancer and 95% confidence intervals (CI) were calculated through conditional logistic regression using quartile 4 (longest telomeres) as the referent category. The *P*-trend was calculated by treating the quartile variable as ordinal (values of 1, 2, 3, and 4). Age at blood draw was included in the model as a continuous variable to reduce residual confounding by age and generate correct estimates for the random error [16]. The ORs were also adjusted for year of birth, as categorized into three groups (1900–1919, 1920–1939, and 1940–1966), in order to reduce confounding by birth cohort effects. As a result of the adjustment for age (at blood draw) and birth cohort effects, the models were indirectly adjusted for period effects as well.

The data were analyzed by batch and overall, but only the combined results are reported as there were no known systematic differences between the batches. Separate models were run for cases with blood collected prior to or during the same calendar year as diagnosis and cases with blood collected one or more calendar years after diagnosis. Sensitivity analyses were conducted including the women along with the men; the patterns of association were similar and thus data are not provided.

RESULTS

As shown in Table I, the mean age at blood draw of the affected men was 65 years (range: 39–93) and the mean age of the unaffected men was 55 years (range: 32–104). The majority of affected men (59.8%) were born between 1920 and 1939, while the majority of unaffected men (63.5%) were born between 1940 and 1966. The affected men were primarily probands (15.2%) and their brothers (53.6%), while the unaffected men were primarily nephews (36.5%), maternal and paternal cousins (23.8%), brothers (19.1%), and sons (17.5%) of probands. Two of the 28 families were African-American, and the other 26 families were Caucasian.

For the 112 affected men, the mean age at diagnosis was 63 years (range: 39–84, data not shown). On average, blood samples were drawn from these men 2 years after diagnosis (range: 9 years prior to diagnosis to 15 years after diagnosis). Information on stage and grade at diagnosis was not available for most cases.

As expected, mean leukocyte telomere length decreased with increasing age among men in the study ($\beta = -0.02$, 95% CI: −0.05 to −0.001, $P = 0.04$). Men with the shortest leukocyte telomere lengths (quartile 1 [Q1]) had slightly reduced odds of prostate cancer compared to

men with the longest telomeres (quartile 4 [Q4]), but this association was not statistically significant (OR: 0.54, 95% CI: 0.18–1.68, *P* = 0.29; Table II). Men with intermediate telomere lengths (quartile 2 [Q2] and quartile 3 [Q3]) also had non-significant reduced odds of prostate cancer (*P*-trend = 0.18). When Q1 was compared to Q2–Q4 combined, the OR for prostate cancer was 0.84 (95% CI: 0.32–2.20, *P* = 0.73).

To explore whether the wide range of time between diagnosis and blood draw for the affected men could influence the results due to possible survival bias, we restricted the case group based on the timing of blood collection. In the analysis of affected men for whom blood was drawn prior to or during the same year as diagnosis (N = 39) and unaffected men, those with the shortest leukocyte telomere lengths (Q1) appeared to have increased odds of prostate cancer compared to those in the other three quartiles combined (OR = 3.55, 95% CI: 0.82–15.43, *P* = 0.09; Table III). In contrast, among affected men for whom blood was drawn after diagnosis (N = 79) and unaffected men, those with the shortest leukocyte telomere lengths appeared to have decreased odds of prostate cancer compared to those in the other three quartiles combined (OR = 0.44, 95% CI: 0.13–1.45, *P* = 0.17).

DISCUSSION

Overall, we did not observe an association between leukocyte telomere length and prostate cancer in men from hereditary prostate cancer families. However, in an analysis of affected men with blood drawn before or within a year of diagnosis and all unaffected men, men with shorter leukocyte telomeres appeared to have increased odds of prostate cancer. Our findings highlight the need to consider the possibility of survival bias in epidemiologic studies on telomere length, as survival bias could adversely influence inferences in retrospective case–control studies.

Because we used a family study in which eligible families had multiple men with prostate cancer at the time that blood was collected, and because shorter telomere length in epidemiologic studies is related to overall mortality [17–19], cancer-specific mortality [4], and prostate cancer-specific mortality [20], we were concerned about survival bias. The affected men in this study had blood drawn at vastly different times in relation to their prostate cancer diagnoses. Since only men who survived their prostate cancer and other competing causes of death could join the study after diagnosis, the affected men with blood drawn after diagnosis may have represented a healthier group of men, with longer telomeres, as compared to all men with prostate cancer. Our study shows evidence of survival bias in that affected men with telomere length measured after diagnosis tended to have longer telomeres than the unaffected men. In contrast, when the analysis was restricted to affected men with blood collected before or within a year of diagnosis in order to avoid survival bias, affected men tended to have shorter telomeres than unaffected men. This analysis, though limited in power, indicates that shorter leukocyte telomere lengths may be associated with hereditary prostate cancer when the effect of survival bias is removed.

Beyond true lack of association between telomere length and familial prostate cancer and possible survival bias, we sought other explanations for the overall null result. First, there may have been undetected prostate cancer among the unaffected men. Compared to the

general population, the majority of the unaffected men in this study were at an increased risk of prostate cancer due to their family histories, so it is possible that some of the unaffected men had undiagnosed prostate cancer at the time of blood draw. Some of the unaffected men may have also been too young to have developed prostate cancer at the time of inclusion into the study. Potential contamination among the unaffected men may have obscured true differences in telomere lengths in this study that, by design, did not account for time to event.

Second, all cases in this study were assumed to be hereditary prostate cancer, but some may have actually been sporadic prostate cancer (i.e., phenocopies). Phenocopies often plague genetic studies of prostate cancer as prostate cancer is common in the general population and there is no reliable way to distinguish the hereditary and sporadic forms of the disease [14]. Telomere dynamics may differ between hereditary and sporadic prostate cancer and inclusion of both types of cases in this study may have masked a true association between telomere length and hereditary prostate cancer risk.

Third, we measured telomere length in peripheral leukocytes as opposed to telomere length in the etiologically relevant prostate tissue. We assumed that inter-individual differences in leukocyte telomere length reflected differences in heritable determinants of telomere length, and that these determinants similarly influenced telomere length in the prostate. In support of this assumption, a recent study of telomeres in leukocyte, muscle, skin, and fat cells found that telomere lengths in these somatic cells were strongly correlated within individuals [9]. However, telomere length dynamics may differ between peripheral leukocytes and prostate cells due to differences in exposure to endogenous and exogenous factors that we were unable to measure, including proliferative factors and factors causing oxidative stress. Measuring telomere length in peripheral leukocytes is more useful from a clinical perspective, as peripheral leukocytes are easily accessible and could be used for screening and risk prediction purposes, but measuring telomere length in prostate tissue itself would be more useful in elucidating the role of telomeres in prostate cancer biology.

Finally, short telomeres may contribute to prostate cancer risk in only a subset of hereditary prostate cancer families. Prostate cancer is an extremely complex and heterogeneous disease, and the genetic factors that contribute to prostate cancer may vary widely from family to family [14]. If short telomeres contribute to prostate cancer in only a small subset of families, then larger studies will be needed to detect such an effect.

To address these challenges, future studies should collect prospective measurements on both telomere length and known determinants of telomere length, such as smoking status, obesity, and infection history. Adjustment for known determinants of telomere length will allow for better estimation of inherited telomere length, and measuring telomere length in samples obtained before or within a year of diagnosis will preclude survival bias and reverse causation. If future prospective studies find that shorter telomere length predicts risk of prostate cancer within certain families, telomere length measurements could help to determine which men within these families should be screened and actively treated.

CONCLUSIONS

Leukocyte telomere length was not associated with prostate cancer in men from hereditary prostate cancer families, although there was suggestive evidence that men with shorter telomere lengths were more likely to develop prostate cancer when the measurement of telomere length predated the prostate cancer diagnosis. Further research is needed to confirm these results and explore the utility of telomere length as a prostate cancer biomarker.

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TABLE I

Characteristics of the Affected and Unaffected Men, Johns Hopkins Hereditary Prostate Cancer Family Database

Total (N = 175)	Affected men (N = 112)	Unaffected men (N = 63)
Age at blood draw; mean (range)	65 (39–93)	55 (32–104)
Birth year; N (%)		
1900–1919	19 (17.0)	7 (11.1)
1920–1939	67 (59.8)	16 (25.4)
1940–1966	26 (23.2)	40 (63.5)
Race; N (%)		
Caucasian	106 (94.6)	59 (93.7)
African-American	6 (5.4)	4 (6.3)
Relation to proband; N (%)		
Proband	17 (15.2)	—
Father	2 (1.8)	0 (0.0)
Brother	60 (53.6)	12 (19.0)
Son	2 (1.8)	11 (17.5)
Paternal uncle	3 (2.7)	2 (3.2)
Nephew	8 (7.1)	23 (36.5)
Maternal Cousin	8 (7.1)	6 (9.5)
Paternal cousin	12 (10.7)	9 (14.3)

TABLE II

Associations Between Age-Adjusted Telomere Length and Prostate Cancer, Johns Hopkins Hereditary Prostate Cancer Family Database

TL quartile[a]	No. of affected men	No. of unaffected men	OR[b]	95% CI	*P*-value
Q1	38	16	0.54	(0.18, 1.68)	0.29
Q2	16	16	0.41	(0.14, 1.22)	0.11
Q3	21	15	0.66	(0.23, 1.91)	0.44
Q4	37	16	1.00	—	—
P-trend					0.18
Q1	38	16	0.84	(0.32, 2.20)	0.73
Q2–Q4	74	47	1.00	—	—

[a] Quartiles are based on batch-specific cut-points derived from the distribution of age-adjusted telomere length in unaffected men.

[b] ORs are adjusted for age and decade of birth and account for clustering by family.

TABLE III

Associations Between Age-Adjusted Telomere Length and Prostate Cancer, Stratified by Timing of Blood Draw Relative to Diagnosis, Johns Hopkins Hereditary Prostate Cancer Family Database

TL quartile[a]	No. of affected men	No. of unaffected men	OR[b]	95% CI	*P*-value
Affected men with blood drawn before or within a year of diagnosis and all unaffected men					
Q1	15	16	2.26	(0.40, 12.64)	0.35
Q2	2	16	0.38	(0.06, 2.57)	0.32
Q3	7	15	0.66	(0.12, 3.67)	0.63
Q4	15	16	1.00	—	—
P-trend					0.45
Q1	15	16	3.55	(0.82, 15.43)	0.09
Q2–Q4	24	47	1.00	—	—
Affected men with blood drawn after diagnosis and all unaffected men					
Q1	23	16	0.34	(0.09, 1.33)	0.12
Q2	14	16	0.64	(0.18, 2.28)	0.49
Q3	14	15	0.70	(0.20, 2.42)	0.57
Q4	22	16	1.00	—	—
P-trend					0.14
Q1	23	16	0.44	(0.13, 1.45)	0.17
Q2–Q4	50	47	1.00	—	—

[a] Quartiles are based on batch-specific cut-points derived from the distribution of age-adjusted telomere length in unaffected men.

[b] ORs are adjusted for age and decade of birth and account for clustering by family.