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Circulating leukocyte telomere length and risk of overall and aggressive prostate cancer

B Julin^{1,2,3}, I Shui⁴, C M Heaphy⁵, C E Joshu⁶, A K Meeker^{5,7,8}, E Giovannucci^{1,4,9}, I De Vivo^{*1,2,10} and E A Platz^{6,7,8,10}

¹Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; ²Department of Epidemiology, Program in Genetic Epidemiology and Statistical Genetics, Harvard School of Public Health, Boston, MA, USA; ³Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; ⁴Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA; ⁵Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA; ⁶Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; ⁷Department of Urology and the James Buchanan Brady Urological Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA; ⁸Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA and ⁹Department of Nutrition, Harvard School of Public Health, Boston, MA, USA

Background: Recent large-scale prospective studies suggest that long telomeres are associated with an increase cancer risk, counter to conventional wisdom.

Methods: To further clarify the association between leukocyte telomere length (LTL) and prostate cancer, and assess genetic variability in relation to both LTL and prostate cancer, we performed a nested case–control study (922 cases and 935 controls). The participants provided blood in 1993–1995 and were followed through August 2004 (prostate cancer incidence) or until 28 February 2013 (lethal or fatal prostate cancer). Relative LTL was measured by quantitative PCR and was calculated as the ratio of telomere repeat copy number to a single gene (36B4) copy number (T/S). Genotyping was performed using the TaqMan OpenArray SNP Genotyping Platform. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of all prostate cancer and subtypes defined by Gleason grade, stage and lethality (metastasis or death).

Results: We observed a positive association between each s.d. increase in LTL and all (multivariable-adjusted OR 1.11, 95% CI: 1.01–1.22), low-grade (OR 1.13, 95% CI: 1.01–1.27), and localised (OR 1.12, 95% CI: 1.01–1.24) prostate cancer. Associations for other subtypes were similar, but did not reach statistical significance. In subgroup analyses, associations for high grade and advanced stage (OR = 2.04, 95% CI 1.00–4.17; $P_{\text{interaction}} = 0.06$) or lethal disease (OR = 2.37, 95% CI 1.19–4.72; $P_{\text{interaction}} = 0.01$) were stronger in men with a family history of the disease compared with those without. The minor allele of SNP, rs7726159, which has previously been shown to be positively associated with LTL, showed an inverse association with all prostate cancer risk after correction for multiple testing ($P = 0.0005$).

Conclusion: In this prospective study, longer LTL was modestly associated with higher risk of prostate cancer. A stronger association for more aggressive cancer in men with a family history of the disease needs to be confirmed in larger studies.

Several studies have examined leukocyte telomere length (LTL) in relation to cancers, but with contrasting results (Hou *et al*, 2012). Initially, shorter telomeres were believed to be associated with an

increase in cancer risk, but recent large-scale prospective studies have observed null associations (De Vivo *et al*, 2009; Weischer *et al*, 2013) or showed that long telomeres are associated with an

*Correspondence: Dr I De Vivo; E-mail: nhidv@channing.harvard.edu

¹⁰Shared senior authorship.

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increased risk in cancer (Shen *et al*, 2011; Hou *et al*, 2012; Lan *et al*, 2013; Lynch *et al*, 2013). One prospective study on LTL and colorectal cancer observed a u-shaped association (Cui *et al*, 2012). Recently, Gu and Wu (2013) proposed that this inconsistency may be in part because the effect of LTL varies by specific cancer type. Another potential explanation is that non-prospective case-control studies were subject to reverse causation in which tumour carcinogenesis affected telomere length. In a meta-analysis stratified by study design, Wentzensen *et al* (2011) observed that increased risk in cancer associated with short telomeres was mainly driven by case-control studies (odds ratio (OR) in pooled analysis = 1.96; OR in case-control studies = 2.9; OR in prospective studies = 1.16), suggesting that telomere shortening occurs mainly after diagnosis, and therefore, might not be of value in cancer risk prediction (Pooley *et al*, 2010). Indirect evidence that both short and long LTL may contribute to the development of specific cancers comes from a recent genome wide association study (GWAS) that identified loci associated with LTL (Codd *et al*, 2013) and assessed their association with different cancer types. The authors found that alleles associated with LTL showed associations with specific cancers in both directions (Codd *et al*, 2013).

Currently, only two prospective studies have investigated circulating LTL and prostate cancer. In a nested case-control study in the Prostate, Lung, Colon and Ovarian Cancer Screening Trial (PLCO), men with shorter telomeres appeared to have a lower risk of advanced prostate cancer (OR = 0.81, 95% confidence interval (CI) 0.64–1.02, comparing the lowest quartile with the highest) (Mirabello *et al*, 2009). A Danish population-based cohort study of 47 102 individuals indicated an inverse association between shorter telomeres and prostate cancer incidence (hazard ratio (HR) = 0.94, 95% CI 0.85–1.04, cases $n = 418$), but not fatal prostate cancer (HR = 1.04, 95% CI 0.87–1.25; deaths $n = 157$) (Weischer *et al*, 2013).

In genetic studies, the telomerase reverse transcriptase (*TERT*) and the telomerase RNA component (*TERC*) genes, together comprising the most important unit of the telomerase complex, were identified as risk loci for prostate cancer (Rafnar *et al*, 2009; Kote-Jarai *et al*, 2011; Kote-Jarai *et al*, 2013). Variants in these genes have been associated with LTL in recent GWAS (Codd *et al*, 2010; Bojesen *et al*, 2013; Codd *et al*, 2013; Pooley *et al*, 2013). The mechanisms that link LTL with cancer is much more complex than the oversimplified view presented so far. To further clarify the association between LTL and risk of all prostate cancer as well as subtypes defined by Gleason grade, stage and progression, we performed a case-control study of 922 cases and 935 controls nested within the prospective Health Professionals Follow-up Study (HPFS). In addition, we evaluated the association of variation in genes related to telomere length as well as prostate cancer with both prostate cancer risk and telomere length.

MATERIALS AND METHODS

Study population. We ascertained incident prostate cancer cases and sampled controls from participants in the HPFS, a prospective cohort study of 51 529 US men aged 40–75 years who enrolled in 1986 (<https://www.hsph.harvard.edu/hpfs>). The men filled out mailed surveys on their demographics, lifestyle, and medical history at baseline and during follow-up every 2 years, and on their diet at baseline and every 4 years. Deaths in the participants are identified through the National Death Index (Stampfer *et al*, 1984), reports by family members or the postal system in response to the mailed surveys. A total of 18 018 of the participants provided a blood sample between 1993 and 1995, as previously described (Platz *et al*, 2008). Of these men, we excluded those who had a

cancer diagnosis (except non-melanoma skin cancer) before the date that they provided a blood sample. The majority (95%) of the men are white of European descent; since both telomere length and prostate cancer incidence differ by race, we restricted the analyses to white men ($n = 123$ non-whites were excluded).

Prostate cancers were first identified from self-reports on questionnaires or from death certificates, and then confirmed by medical record review. Study investigators reviewed medical and pathology records to extract data on stage (TNM staging system) at diagnosis and histological grade, assessed using Gleason scores. We used pathological stage and grade when available and clinical measures if pathological information was not available. Deaths were identified via repeated mailings, telephone calls, and searches of the National Death Index. Causes of deaths were confirmed through review of medical records and death certificates. Biennial follow-up surveys were mailed to those who reported prostate cancer to collect information on disease progression (e.g., metastases). We identified 922 eligible prostate cancer cases between the dates of blood draw through August 2004. Follow-up for progression to prostate cancer-specific death was complete through 28 February 2013; 96.1% of the prostate cancer cases were confirmed by medical record review.

In the original nested case-control design, for each case, we sampled a control that was alive and had not been diagnosed with cancer up to the date of the case's diagnosis. The cases and controls were matched on year of birth, ever having had a PSA test before the date of providing the blood sample, and the time of day, season, and year that the blood sample was provided. To be eligible, controls were required to have had a PSA test after the date they provided a blood sample.

The Human Subjects Committee of the Harvard School of Public Health approved the HPFS, and written informed consent was obtained from all participants. Both the Human Subjects Committee of the Harvard School of Public Health and the Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health approved the study on telomeres, genetic variability and prostate cancer.

Telomere length determination. Genomic DNA was extracted from peripheral blood leukocytes using the QIAmp 96-spin blood protocol (Qiagen, Chatsworth, CA, USA). Pico-Green quantification of genomic DNA was performed using a Molecular Devices 96-well spectrophotometer (Sunnyvale, CA, USA). Relative LTL was determined using a modified, high-throughput version of the quantitative PCR (qPCR)-based telomere assay (Cawthon, 2002; Wang *et al*, 2008). The qPCR telomere assay was run on Applied Biosystems 7900HT Sequence Detection System (Foster City, CA, USA). Laboratory personnel were blinded to participant characteristics and all assays were processed in triplicates by the same technician, and under identical conditions. The average relative LTL was calculated as the ratio of telomere repeat copy number to a single gene (36B4) copy number (T/S). Relative LTL is reported as the exponentiated T/S ratio corrected for a reference sample. The telomere and single-gene assay coefficients of variation (CVs) for triplicates were <0.8%. The CV for the mean exponential T/S ratio was 16.0%. Although this assay provides a relative measurement of telomere length, T/S ratios highly correlate with absolute telomere lengths determined by southern blot ($r = 0.82$; $P < 0.001$) (Cawthon, 2002).

Covariate assessment. We used information from the 1994 questionnaire or, if not available, the most recent before 1994 to calculate body mass index (BMI), smoking amount (indicated by pack-years), alcohol consumption (indicated by grams of ethanol) and vigorous physical activity (indicated by metabolic equivalent (MET) per week) as close to time of blood donation (1993–1995, with the majority donating blood in 1994) as possible.

Single-nucleotide polymorphism (SNP) selection and genotyping.

The main aim was to evaluate SNPs previously related to telomere length, but we also included SNPs that have been related to prostate cancer risk if they were located in or close to telomere maintenance genes (*TERC* or *TERT*). We identified 32 SNPs from GWA (Rafnar *et al*, 2009; Codd *et al*, 2010; Levy *et al*, 2010; Kote-Jarai *et al*, 2011; Prescott *et al*, 2011; Mangino *et al*, 2012; Bojesen *et al*, 2013; Codd *et al*, 2013; Pooley *et al*, 2013) or fine mapping studies (Kote-Jarai *et al*, 2013) that had minor allele frequencies >5% in whites. For SNPs that were in linkage disequilibrium with $R^2 > 0.80$, we selected the SNP with the stronger association from the literature. We were able to genotype 22 SNPs (see Supplementary Table 1), but 1 failed genotyping (rs6772228). Blood samples from matched case-control pairs were handled identically and assayed in the same batch in a blinded fashion. Genotyping was performed at the Dana Farber/Harvard Cancer Center High-Throughput Genotyping Core using the TaqMan OpenArray SNP Genotyping Platform (Applied Biosystems) according to the manufacturer's instructions. To validate genotyping procedures, 10% blinded quality control samples were inserted. All SNPs had >90% genotype completion, and the concordance was 100% for blinded quality control samples.

Statistical analysis. The final sample size consisted of 922 cases and 935 controls, after removal of failed qPCR samples (25%). To preserve sample size, we included all cases and controls in the analysis irrespective of whether the matched pair was present. We used unconditional logistic regression to estimate ORs and 95% CIs of prostate cancer, adjusting for age at blood draw (continuous, years) and matching factors (age at selection (continuous, years), PSA test before blood collection (yes/no/unknown) and year of blood collection). We did not adjust for the time of day and season that the blood sample was provided because these factors were not related to telomere length. In a second model, we additionally adjusted for smoking (0, 0.1–20, 20.1–40, >40 pack-years), BMI (<25, ≥25–29.9, ≥30–34.9, ≥35 kg m⁻²), and vigorous physical activity (quartiles, MET-hours per week), since these factors have been associated with telomere length as well as prostate cancer (Giovannucci and Michaud, 2007; Mirabello *et al*, 2009). We also estimated the ORs of (a) low grade ($n = 461$; Gleason sum <7), (b) Gleason sum = 7 ($n = 307$), (c) high grade ($n = 90$; Gleason sum >7), (d) lethal disease ($n = 81$; death by prostate cancer or metastasis in bone or other organs, except lymph nodes), (e) localised disease ($n = 774$; TNM stage T1b, T2b, T3a, and NOM0) and (f) advanced stage or lethal disease ($n = 103$) (≥T3b, N+, or M+ at diagnosis or progression to metastasis or prostate cancer death during follow-up).

We modelled LTL in two ways: (1) using indicator variables for quartiles of relative LTL with cut points based on the distribution among the controls and (2) using LTL as a continuous measure (per s.d.). We assessed effect modification by age at blood draw (dichotomised by the median; ≤64 or >64 years), cigarette smoking status (ever, never) in 1994 and family history of prostate cancer (yes/no). We present stratified effect estimates by each of these characteristics. We also assessed whether telomere length was associated with early-onset prostate cancer (≤age 65). The statistical significance of the interaction was assessed using a Wald test for the multiplicative interaction term of each of the characteristics and LTL (modelled continuously).

The additive genetic model was used for the SNP analyses, which assumes that the effect of the heterozygous genotype is intermediate between the two homozygous genotypes. The homozygous genotype of the major allele was coded as 0. Age-adjusted (age at blood draw) unconditional logistic regression between each individual SNP and prostate cancer or low and high LTL (dichotomised at the median) was performed and *P*-values were Bonferroni corrected, considering 21 independent tests.

All *P*-values were two sided and analyses were conducted using SAS release 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

Cases and controls were similar on demographic and lifestyle factors (Table 1). The mean age at prostate cancer diagnosis was 69.5 years and the mean time between blood draw and diagnosis was 5.5 years. As expected, a statistically significant inverse correlation was found between relative telomere length and age at blood draw ($r = -0.19$, $P < 0.0001$) in controls.

Leukocyte telomere length was not associated with all prostate cancer or any of the subtypes when comparing quartiles of LTL; neither in models adjusting for the matching factors or when additionally adjusting for BMI, smoking and physical activity (Table 2). When telomere length was modelled continuously, however, longer telomeres were modestly positively associated with all prostate cancer ($P = 0.03$), low-grade ($P = 0.04$) and localised ($P = 0.03$; Table 2) prostate cancer. Per each s.d. increase in telomere length, the OR was 1.11 for all prostate cancer, 1.13 for low-grade disease and 1.12 for localised disease. Results were similar for intermediate grade, high-grade, advanced and lethal disease, but the estimates were not statistically significant. Of note, 28 cases were overlapping between the high-grade ($n = 90$) and the advanced stage or lethal disease ($n = 103$) groups. With that in mind, these two outcomes should not be considered completely independent results.

As presented in Table 3, there was some evidence that men with a family history of prostate cancer had an increase in risk of

Table 1. Characteristics of prostate cancer cases and controls, Health Professionals Follow-up Study

Characteristics	Cases	Controls	<i>P</i>
<i>N</i>	922	935	
Age at blood draw (years), mean (s.d.)	63.6 (7.9)	63.5 (7.8)	0.73
Age at diagnosis (years), mean (s.d.)	69.5 (7.5)	—	
Year of diagnosis, mean (s.d.)	1999 (2.8)	—	
Stage^a			
Localised or limited extraprostatic extension ^b , <i>n</i> (%)	774 (88.3)	—	
Advanced stage or lethal ^c , <i>n</i> (%)	103 (11.7)	—	
Grade^d			
Gleason <7, <i>n</i> (%)	461 (53.7)	—	
Gleason = 7, <i>n</i> (%)	307 (35.8)	—	
Gleason >7, <i>n</i> (%)	90 (10.5)	—	
Lethal prostate cancer ^e , <i>n</i> (%)	81 (8.8)	—	
PSA test before blood draw			
Yes, <i>n</i> (%)	669 (72.6)	682 (72.9)	0.74
No, <i>n</i> (%)	202 (21.9)	195 (20.9)	
Unknown, <i>n</i> (%)	51 (5.5)	58 (6.2)	
Family history of prostate cancer, <i>n</i> (%)	135 (14.6)	120 (12.8)	0.26
Ever smoker, <i>n</i> (%)	467 (50.7)	504 (53.9)	0.16
Diabetes, <i>n</i> (%)	51 (7.2)	44 (6.8)	0.79
Body mass index (kg m ⁻²), mean (s.d.)	25.8 (3.3)	25.8 (3.6)	0.98
Vigorous physical activity (MET-hours per week), mean (s.d.)	13.0 (21.5)	12.7 (20.9)	0.73
Total energy (kcal per day), mean (s.d.)	2033 (587)	2045 (615)	0.68

Abbreviations: MET = metabolic equivalent; PSA = prostate-specific antigen.

^aNumber with missing stage = 45.

^bLocalised or limited extraprostatic extension (T1b, T2b, T3a, and NOM0).

^cAdvanced stage (≥T3b, N+, or M+ at diagnosis) or lethal (progression to metastasis or prostate cancer death during follow-up).

^dNumber with missing grade = 64.

^eProgression to metastasis (bone or other organ) or prostate cancer death during follow-up.

Table 2. Odds ratios (95% confidence intervals) for prostate cancer and subtypes by quartiles of leukocyte telomere length

Outcome	Leukocyte telomere length									
	Q1		Q2		Q3		Q4		OR (95% CI) per s.d.	P
Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)			
Total prostate cancer										
Model 1 ^a	215/228	1.00 (ref.)	205/235	0.93 (0.71, 1.21)	247/242	1.09 (0.84, 1.41)	255/230	1.19 (0.91, 1.54)	1.11 (1.01, 1.22)	0.03
Model 2 ^b		1.00 (ref.)		0.92 (0.70, 1.20)		1.09 (0.84, 1.41)		1.18 (0.91, 1.54)		
Low grade^c										
Model 1 ^a	102/228	1.00 (ref.)	114/235	1.07 (0.77, 1.49)	121/242	1.14 (0.82, 1.57)	124/230	1.23 (0.89, 1.71)	1.13 (1.01, 1.27)	0.04
Model 2 ^b		1.00 (ref.)		1.05 (0.75, 1.46)		1.13 (0.82, 1.57)		1.20 (0.86, 1.67)		
Gleason sum 7										
Model 1 ^a	70/228	1.00 (ref.)	62/235	0.83 (0.56, 1.23)	91/242	1.17 (0.81, 1.68)	84/230	1.09 (0.75, 1.58)	1.07 (0.93, 1.22)	0.34
Model 2 ^b		1.00 (ref.)		0.81 (0.55, 1.21)		1.18 (0.82, 1.70)		1.08 (0.74, 1.57)		
High grade^d										
Model 1 ^a	25/228	1.00 (ref.)	16/235	0.69 (0.35, 1.35)	19/242	0.80 (0.42, 1.51)	30/230	1.36 (0.76, 2.43)	1.15 (0.92, 1.44)	0.23
Model 2 ^b		1.00 (ref.)		0.68 (0.34, 1.33)		0.76 (0.40, 1.45)		1.35 (0.75, 2.44)		
Lethal^e										
Model 1 ^a	25/228	1.00 (ref.)	15/235	0.78 (0.40, 1.56)	23/242	1.10 (0.60, 2.03)	18/230	0.98 (0.51, 1.89)	1.09 (0.86, 1.37)	0.48
Model 2 ^b		1.00 (ref.)		0.83 (0.41, 1.66)		1.15 (0.62, 2.14)		1.00 (0.52, 1.95)		
Localised^f										
Model 1 ^a	170/228	1.00 (ref.)	178/235	1.00 (0.75, 1.32)	209/242	1.13 (0.86, 1.49)	217/230	1.24 (0.94, 1.63)	1.12 (1.01, 1.24)	0.03
Model 2 ^b		1.00 (ref.)		0.98 (0.74, 1.30)		1.13 (0.86, 1.50)		1.22 (0.93, 1.62)		
Advanced or lethal^g										
Model 1 ^a	31/228	1.00 (ref.)	18/235	0.69 (0.37, 1.28)	28/242	1.01 (0.58, 1.75)	26/230	1.05 (0.59, 1.85)	1.10 (0.89, 1.36)	0.36
Model 2 ^b		1.00 (ref.)		0.71 (0.38, 1.34)		1.03 (0.59, 1.80)		1.06 (0.60, 1.89)		

Abbreviations: BMI = body mass index; CI = confidence interval; MET = metabolic equivalent; OR = odds ratio; PSA = prostate-specific antigen.

^aAdjusted for age at blood collection (continuous, years), age at selection (continuous, years), PSA test before blood collection (yes/no/unknown) and year of blood collection.

^bAdditionally adjusted for smoking (0, 0.1–20, 20.1–40, >40 pack-years), BMI (<25, ≥25–29.9, ≥30–34.9, ≥35 kg m⁻²), and vigorous physical activity (quartiles, MET-hours per week).

^cGleason sum <7.

^dGleason sum >7.

^eDeath by prostate cancer or metastasis in bone or other organs, except lymph nodes.

^fLocalised or limited extraprostatic extension (T1b, T2b, T3a, and N0M0).

^gAdvanced stage (≥T3b, N+, or M+ at diagnosis) or lethal (progression to metastasis or prostate cancer death during follow-up).

high-grade (OR = 2.04, 95% CI 1.00–4.17) as well as advanced stage or lethal disease (OR = 2.37, 95% CI 1.19–4.72) per s.d. increase in telomere length, with *P* for interaction 0.06 and 0.01, respectively. Among men without a family history, telomere length was not associated with high-grade (OR = 1.07, 95% CI 0.84–1.36) or advanced stage or lethal disease (OR = 1.01, 95% CI 0.81–1.25). Consistent with our family-history-specific findings, the association of LTL and early-onset prostate cancer (≤age 65) for high-grade (13 cases/236 controls) and advanced stage or lethal disease (21 cases/236 controls) were stronger in this subgroup compared with those diagnosed at a later age (>65). However, precision of these estimates lacked due to the small number of cases; OR 1.62 (95% CI: 0.85–3.11) for high-grade tumours and OR 1.37 (95% CI: 0.84–2.25) for advanced stage or lethal tumours.

The minor allele (A) of SNP, rs7726159 (*TERT*), showed a statistically significant inverse association with all prostate cancer risk after correction for multiple testing (per-allele OR 0.78, 95% CI: 0.68–0.90, *P* = 0.0005; Supplementary Table 1). Association within subtypes of prostate cancer yielded similar results (data not shown). None of the SNPs showed corrected significant associations with telomere length.

DISCUSSION

In this prospective study, we found that longer circulating LTL may be moderately associated with a higher risk of prostate cancer. Longer telomere length was associated with a higher risk of

high-grade, advanced stage or lethal disease in men with a family history of prostate cancer. The minor allele of SNP (rs7726159) in the *TERT* gene showed a statistically significant inverse association with prostate cancer, but there was no evidence that this SNP was associated with telomere length in our study.

Telomeres are repetitive DNA sequences (TTAGGG) that protect the ends of linear chromosomes. In adult somatic cells telomeres shorten over time because standard DNA polymerase cannot replicate them during cell division, a phenomenon called the end-replication problem. The epidemiological evidence for associations between circulating LTL and cancer has been equivocal. Some studies support the hypothesis that shorter circulating LTL is associated with higher cancer risk (Wentzensen *et al*, 2011; Hou *et al*, 2012), although the associations tend to be stronger in retrospective studies and may differ by cancer type (Gu and Wu, 2013). In prospective studies, long telomeres have been associated with an increased risk of several cancers such as melanoma (Han *et al*, 2009), lung cancer (Shen *et al*, 2011), non-Hodgkin lymphoma (Lan *et al*, 2013) and pancreatic cancer (Lynch *et al*, 2013). There are plausible explanations also for a positive association between LTL and cancer. As short telomeres may induce cellular senescence, long telomeres are generally a marker for actively reproducing cells that are at higher risk of obtaining tumour-causing mutations (Jones *et al*, 2012). The importance of balance between elongation (by the telomerase enzyme) and telomere shortening to maintain a stable, 'optimal' length for cell cycle control has also been suggested (Ducray *et al*, 1999). For an accurate comparison between studies,

Table 3. Odds ratios^a (95% confidence intervals) for total prostate cancer by continuous relative leukocyte telomere length (LTL) within strata of age at blood draw, smoking status and family history of prostate cancer

Strata	Total prostate cancer			High grade ^b			Advanced or lethal ^c		
	Ca/Co	OR ^a (95% CI)	P	Ca/Co	OR ^a (95% CI)	P	Ca/Co	OR ^a (95% CI)	P
Age at blood draw^d									
≤64 years									
Per s.d. increase in LTL	486/492	1.17 (1.02–1.34)	0.02	39/492	1.26 (0.88–1.79)	0.20	41/492	1.05 (0.75–1.47)	0.79
>64 years									
Per s.d. increase in LTL	436/443	1.05 (0.92–1.20)	0.44	51/443	1.10 (0.82–1.47)	0.52	62/443	1.10 (0.84–1.43)	0.50
Smoking status^e									
Never smoker									
Per s.d. increase in LTL	455/431	1.06 (0.92, 1.22)	0.43	39/431	1.23 (0.87–1.74)	0.24	43/431	0.98 (0.71–1.36)	0.92
Ever smoker									
Per s.d. increase in LTL	467/504	1.15 (1.01–1.30)	0.03	51/504	1.10 (0.82–1.47)	0.53	60/504	1.17 (0.89–1.53)	0.26
No family history of prostate cancer^f									
Per s.d. increase in LTL	787/815	1.09 (0.98–1.21)	0.10	76/815	1.07 (0.84–1.36)	0.57	88/815	1.01 (0.81–1.25)	0.97
Family history of prostate cancer									
Per s.d. increase in LTL	135/120	1.29 (0.99–1.67)	0.06	14/120	2.04 (1.00–4.17)	0.05	15/120	2.37 (1.19, 4.72)	0.01

Abbreviations: CI = confidence interval; LTL = leukocyte telomere length; OR = odds ratio; PSA = prostate-specific antigen.
^aAdjusted for age at blood collection (continuous, years), age at selection (continuous, years), PSA test before blood collection (yes/no/unknown) and year of blood collection.
^bGleason sum > 7.
^cAdvanced stage (≥T3b, N+, or M+ at diagnosis) or lethal (progression to metastasis or prostate cancer death during follow-up).
^dP for interaction = 0.85 for total prostate cancer, 0.98 for high-grade and 0.89 for advanced stage or lethal disease.
^eP for interaction = 0.81 for total prostate cancer, 0.65 for high-grade and 0.48 for advanced stage or lethal disease.
^fP for interaction = 0.16 for total prostate cancer, 0.06 for high-grade and 0.01 for advanced stage or lethal disease.

consistent methodologies are needed. Most of the large epidemiological studies have used qPCR to estimate LTL, since this method enables high-throughput and low amounts of DNA (Cawthon, 2002). The DNA extraction method may also affect telomere length estimates (Cunningham *et al*, 2013). Thus, inter-laboratory variability and measurement error may also explain some of the inconsistency between studies (Savage *et al*, 2013).

For prostate cancer, two prior prospective studies indicated that shorter telomeres were associated with a lower risk of prostate cancer (Mirabello *et al*, 2009; Weischer *et al*, 2013). In a previous study, derived from a sub-sample of the HPFS cohort, the association between telomere length and variability in telomere length (measured by a FISH assay) in prostate cancer cells and surrounding stromal cells was evaluated (Heaphy *et al*, 2013). In this study, men whose prostate cancer cells had higher cell-to-cell variability in telomere length or who had shorter telomeres in prostate-cancer-associated stromal cells were more likely to have a worse prognosis than other men. Although telomere length in different tissues shows a high correlation (Daniali *et al*, 2013), there were several differences between this study and the current including, the telomere length assessment method (FISH assay), timing of telomere measurement (after disease diagnosis), and the study population (a subset of men who had undergone treatment for disease by radical prostatectomy).

The results from the two prospective studies appear to be consistent with regard to prostate cancer incidence (aside from a non-statistically significant association in the PLCO (Mirabello *et al*, 2009) study between shorter telomeres and increased risk of prostate cancer when restricting to men with a family history of prostate cancer). The PLCO study (Mirabello *et al*, 2009) focused on aggressive disease only—defined as advanced stage and Gleason sum ≥ 7. The Danish study (where PSA screening is not routine) also assessed death in men with prostate cancer, but in this group the associations were null (HR for each 1-kb decrease in telomere length 1.04, 95% CI: 0.87–1.25) (Weischer *et al*, 2013). We measured telomere length in the same laboratory as the PLCO study, and the Danish study used assays derived from the same method. The mean or median age at blood draw in all three studies

was in the early to the mid-60s. The results from the present study did not show a statistically significant association between longer telomere length and more aggressive prostate cancer (defined as high grade, lethal, advanced stage or lethal); however, we cannot exclude that modest associations exist. We observed a higher risk of more aggressive prostate cancer among men with longer telomeres who also had a family history of prostate cancer. These results are interesting given the finding that paternal age is a determinant of telomere length in offspring (Prescott *et al*, 2012). However, due to a small sample size and several stratifications, these results should be interpreted with caution.

The minor allele (A) of one individual SNP (rs7726159) in the *TERT* gene was modestly associated with a lower risk of prostate cancer after adjusting for multiple comparisons. Although this SNP has been shown to be associated with longer LTL in GWAS (Pooley *et al*, 2013), we did not observe that association in our study. Considering this, the present result should be interpreted with caution since we cannot exclude that the observed association is due to chance. The strengths of this study include its prospective design, rich covariate information, a relatively large number of prostate cases, detailed clinical information on the grade and stage of the cases, and long-term follow-up for progression. This study also had some limitations. We had a small number of high-grade, advanced stage or lethal cases, which reduced the precision of our estimates for these specific analyses. Our results, however, did not indicate any major differences in associations between subtypes.

In summary, our prospective findings suggest that longer circulating LTL may be associated with a higher risk of overall prostate cancer, including more aggressive disease, especially in men who have a family history of prostate cancer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Bojesen SE, Pooley KA, Johnatty SE, Beesley J, Michailidou K, Tyrer JP, Edwards SL, Pickett HA, Shen HC, Smart CE, Hillman KM, Mai PL, Lawrenson K, Stutz MD, Lu Y, Karevan R, Woods N, Johnston RL, French JD, Chen X, Weischer M, Nielsen SF, Maranian MJ, Ghousaini M, Ahmed S, Baynes C, Bolla MK, Wang Q, Dennis J, McGuffog L, Barrowdale D, Lee A, Healey S, Lush M, Tessier DC, Vincent D, Bacot F, Vergote I, Lambrechts S, Despierre E, Risch HA, Gonzalez-Neira A, Rossing MA, Pita G, Doherty JA, Alvarez N, Larson MC, Fridley BL, Schoof N, Chang-Claude J, Cicek MS, Peto J, Kalli KR, Broeks A, Armasu SM, Schmidt MK, Braaf LM, Winterhoff B, Nevanlinna H, Konecny GE, Lambrechts D, Rogmann L, Guenel P, Teoman A, Milne RL, Garcia JJ, Cox A, Shridhar V, Burwinkel B, Marme F, Hein R, Sawyer EJ, Haiman CA, Wang-Gohrke S, Andrulis IL, Moysich KB, Hopper JL, Odunsi K, Lindblom A, Giles GG, Brenner H, Simard J, Lurie G, Fasching PA, Carney ME, Radice P, Wilkens LR, Swerdlow A, Goodman MT, Brauch H, Garcia-Closas M, Hillemanns P, Winqvist R, Durst M, Devilee P, Runnebaum I, Jakubowska A, Lubinski J, Mannermaa A, Butzow R, Bogdanova NV, Dork T, Peltari LM, Zheng W, Leminen A, Anton-Culver H, Bunker CH, Kristensen V, Ness RB, Muir K, Edwards R, Meindl A, Heitz F, Matsuo K, du Bois A, Wu AH, Harter P, Teo SH, Schwaab I, Shu XO, Blot W, Hosono S, Kang D, Nakanishi T, Hartman M, Yatabe Y, Hamann U, Karlan BY, Sangrajrang S, Kjaer SK, Gaborieau V, Jensen A, Eccles D, Hogdall E, Shen CY, Brown J, Woo YL, Shah M, Azmi MA, Luben R, Omar SZ, Czene K, Vierkant RA, Nordestgaard BG, Flyger H, Vachon C, Olson JE, Wang X, Levine DA, Rudolph A, Weber RP, Fleisch-Janys D, Iversen E, Nickels S, Schildkraut JM, Silva Idos S, Cramer DW, Gibson L, Terry KL, Fletcher O, Vitonis AF, van der Schoot CE, Poole EM, Hogervorst FB, Tworoger SS, Liu J, Bandera EV, Li J, Olson SH, Humphreys K, Orlov I, Blomqvist C, Rodriguez-Rodriguez L, Aittomaki K, Salvesen HB, Muranen TA, Wik E, Brouwers B, Krakstad C, Wauters E, Halle MK, Wildiers H, Kiemeny LA, Mulot C, Aben KK, Laurent-Puig P, Altuna AM, Truong T, Massuger LF, Benitez J, Pejovic T, Perez JJ, Hoatlin M, Zamora MP, Cook LS, Balasubramanian SP, Kelemen LE, Schneeweiss A, Le ND, Sohn C, Brooks-Wilson A, Tomlinson I, Kerin MJ, Miller N, Cybulski C, Henderson BE, Menkiszak J, Schumacher F, Wentzensen N, Le Marchand L, Yang HP, Mulligan AM, Glendon G, Engelholm SA, Knight JA, Hogdall CK, Apicella C, Gore M, Tsimiklis H, Song H, Southey MC, Jager A, den Ouweland AM, Brown R, Martens JW, Flanagan JM, Kriege M, Paul J, Margolin S, Siddiqui N, Severi G, Whittemore AS, Baglietto L, McGuire V, Stegmaier C, Sieh W, Muller H, Arndt V, Labreche F, Gao YT, Goldberg MS, Yang G, Dumont M, McLaughlin JR, Hartmann A, Ekici AB, Beckmann MW, Phelan CM, Lux MP, Permuth-Wey J, Peissel B, Sellers TA, Ficarazzi F, Barile M, Ziogas A, Ashworth A, Gentry-Maharaj A, Jones M, Ramus SJ, Orr N, Menon U, Pearce CL, Bruning T, Pike MC, Ko YD, Lissowska J, Figueroa J, Kupryjanczyk J, Chanock SJ, Dansonka-Mieszkowska A, Jukkola-Vuorinen A, Rzepecka IK, Pylkas K, Bidzinski M, Kauppila S, Hollestelle A, Seynaeve C, Tollenaar RA, Durda K, Jaworska K, Hartikainen JM, Kosma VM, Kataja V, Antonenkova NN, Long J, Shrubsole M, Deming-Halverson S, Lophatananon A, Siriwanarangsang P, Stewart-Brown S, Ditsch N, Lichtner P, Schmutzler RK, Ito H, Iwata H, Tajima K, Tseng CC, Stram DO, van den Berg D, Yip CH, Ikram MK, Teh YC, Cai H, Lu W, Signorello LB, Cai Q, Noh DY, Yoo KY, Miao H, Iau PT, Teo YY, McKay J, Shapiro C, Ademuyiwa F, Fountzilas G, Hsiung CN, Yu JC, Hou MF, Healey CS, Luccarini C, Peock S, Stoppa-Lyonnet D, Peterlongo P, Rebbeck TR, Piedmonte M, Singer CF, Friedman E, Thomassen M, Offit K, Hansen TV, Neuhausen SL, Szabo CI, Blanco I, Garber J, Narod SA, Weitzel JN, Montagna M, Olah E, Godwin AK, Yannoukacos D, Goldgar DE, Caldes T, Imyanitov EN, Tihomirova L, Arun BK, Campbell I, Mensenkamp AR, van Asperen CJ, van Roozendaal KE, Meijers-Heijboer H, Collee JM, Oosterwijk JC, Hooning MJ, Rookus MA, van der Luijt RB, Os TA, Evans DG, Frost D, Fineberg E, Barwell J, Walker L, Kennedy MJ, Platte R, Davidson R, Ellis SD, Cole T, Bressac-de Paillerets B, Buecher B, Damiola F, Faivre L, Frenay M, Sinilnikova OM, Caron O, Giraud S, Mazoyer S, Bonadona V, Caux-Moncoutier V, Toloczko-Grabarek A, Gronwald J, Byrski T, Spurdle AB, Bonanni B, Zaffaroni D, Giannini G, Bernard L, Dolcetti R, Manoukian S, Arnold N, Engel C, Deissler H, Rhiem K, Niederacher D, Plendl H, Sutter C, Wappenschmidt B, Borg A, Melin B, Rantala J, Soller M, Nathanson KL, Domchek SM, Rodriguez GC, Salani R, Kaulich DG, Tea MK, Paluch SS, Laitman Y, Skytte AB, Kruse TA, Jensen UB, Robson M, Gerdes AM, Ejlertsen B, Foretova L, Savage SA, Lester J, Soucy P, Kuchenbaecker KB, Olswold C, Cunningham JM, Slager S, Pankratz VS, Dicks E, Lakhani SR, Couch FJ, Hall P, Monteiro AN, Gayther SA, Pharoah PD, Reddel RR, Goode EL, Greene MH, Easton DF, Berchuck A, Antoniou AC, Chenevix-Trench G, Dunning AM (2013) Multiple independent variants at the TERT locus are associated with telomere length and risks of breast and ovarian cancer. *Nat Genet* 45(4): 371–384.
- Cawthon RM (2002) Telomere measurement by quantitative PCR. *Nucleic Acids Res* 30(10): e47.
- Codd V, Mangino M, van der Harst P, Braund PS, Kaiser M, Beveridge AJ, Rafelt S, Moore J, Nelson C, Soranzo N, Zhai G, Valdes AM, Blackburn H, Mateo Leach I, de Boer RA, Kimura M, Aviv A, Goodall AH, Ouwehand W, van Veldhuisen DJ, van Gilst WH, Navis G, Burton PR, Tobin MD, Hall AS, Thompson JR, Spector T, Samani NJ (2010) Common variants near TERC are associated with mean telomere length. *Nat Genet* 42(3): 197–199.
- Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, Hottenga JJ, Fischer K, Esko T, Surakka I, Broer L, Nyholt DR, Mateo Leach I, Salo P, Hagg S, Matthews MK, Palmén J, Norata GD, O'Reilly PF, Saleheen D, Amin N, Balmforth AJ, Beekman M, de Boer RA, Bohringer S, Braund PS, Burton PR, de Craen AJ, Denniff M, Dong Y, Douroudis K, Dubinina E, Eriksson JG, Garlaschelli K, Guo D, Hartikainen AL, Henders AK, Houwing-Duistermaat JJ, Kananen L, Karssen LC, Kettunen J, Klopp N, Lagou V, van Leeuwen EM, Madden PA, Magi R, Magnusson PK, Mannisto S, McCarthy MI, Medland SE, Mihailov E, Montgomery GW, Oostra BA, Palotie A, Peters A, Pollard H, Pouta A, Prokopenko I, Ripatti S, Salomaa V, Srichaman HE, Valdes AM, Verweij N, Vinuela A, Wang X, Wichmann HE, Widen E, Willemsen G, Wright MJ, Xia K, Xiao X, van Veldhuisen DJ, Catapano AL, Tobin MD, Hall AS, Blakemore AI, van Gilst WH, Zhu H, Consortium C, Erdmann J, Reilly MP, Kathiresan S, Schunkert H, Talmud PJ, Pedersen NL, Perola M, Ouwehand W, Kaprio J, Martin NG, van Duijn CM, Hovatta I, Gieger C, Metspalu A, Boomsma DI, Jarvelin MR, Slagboom PE, Thompson JR, Spector TD, van der Harst P, Samani NJ (2013) Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet* 45(4): 422–427.
- Cui Y, Cai Q, Qu S, Chow WH, Wen W, Xiang YB, Wu J, Rothman N, Yang G, Shu XO, Gao YT, Zheng W (2012) Association of leukocyte telomere length with colorectal cancer risk: nested case-control findings from the Shanghai Women's Health Study. *Cancer Epidemiol Biomarkers Prev* 21(10): 1807–1813.
- Cunningham JM, Johnson RA, Litzelman K, Skinner HG, Seo S, Engelman CD, Vanderboom RJ, Kimmel GW, Gangnon RE, Riegert-Johnson DL, Baron JA, Potter JD, Haile R, Buchanan DD, Jenkins MA, Rider DN, Thibodeau SN, Petersen GM, Boardman LA (2013) Telomere length varies by DNA extraction method: implications for epidemiologic research. *Cancer Epidemiol Biomarkers Prev* 22(11): 2047–2054.

- Daniali L, Benetos A, Susser E, Kark JD, Labat C, Kimura M, Desai K, Granick M, Aviv A (2013) Telomeres shorten at equivalent rates in somatic tissues of adults. *Nat Commun* 4: 1597.
- De Vivo I, Prescott J, Wong JY, Kraft P, Hankinson SE, Hunter DJ (2009) A prospective study of relative telomere length and postmenopausal breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 18(4): 1152–1156.
- Ducray C, Pommier JP, Martins L, Boussin FD, Sabatier L (1999) Telomere dynamics, end-to-end fusions and telomerase activation during the human fibroblast immortalization process. *Oncogene* 18(29): 4211–4223.
- Giovannucci E, Michaud D (2007) The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. *Gastroenterology* 132(6): 2208–2225.
- Gu J, Wu X (2013) Re: short telomere length, cancer survival, and cancer risk in 47102 individuals. *J Natl Cancer Inst* 105(15): 1157.
- Han J, Qureshi AA, Prescott J, Guo Q, Ye L, Hunter DJ, De Vivo I (2009) A prospective study of telomere length and the risk of skin cancer. *J Invest Dermatol* 129(2): 415–421.
- Heaphy CM, Yoon GS, Peskoe SB, Joshi CE, Lee TK, Giovannucci E, Mucci LA, Kenfield SA, Stampfer MJ, Hicks JL, De Marzo AM, Platz EA, Meeker AK (2013) Prostate cancer cell telomere length variability and stromal cell telomere length as prognostic markers for metastasis and death. *Cancer Discov* 3(10): 1130–1141.
- Hou L, Zhang X, Gawron AJ, Liu J (2012) Surrogate tissue telomere length and cancer risk: shorter or longer? *Cancer Lett* 319(2): 130–135.
- Jones AM, Beggs AD, Carvajal-Carmona L, Farrington S, Tenesa A, Walker M, Howarth K, Ballereau S, Hodgson SV, Zuber A, Bertagnoli M, Midgley R, Campbell H, Kerr D, Dunlop MG, Tomlinson IP (2012) TERC polymorphisms are associated both with susceptibility to colorectal cancer and with longer telomeres. *Gut* 61(2): 248–254.
- Kote-Jarai Z, Olama AA, Giles GG, Severi G, Schleutker J, Weischer M, Campa D, Riboli E, Key T, Gronberg H, Hunter DJ, Kraft P, Thun MJ, Ingles S, Chanock S, Albanes D, Hayes RB, Neal DE, Hamdy FC, Donovan JL, Pharoah P, Schumacher F, Henderson BE, Stanford JL, Ostrander EA, Sorensen KD, Dork T, Andriole G, Dickinson JL, Cybulski C, Lubinski J, Spurdle A, Clements JA, Chambers S, Aitken J, Gardiner RA, Thibodeau SN, Schaid D, John EM, Maier C, Vogel W, Cooney KA, Park JY, Cannon-Albright L, Brenner H, Habuchi T, Zhang HW, Lu YJ, Kaneva R, Muir K, Benlloch S, Leongamornlert DA, Saunders EJ, Tymrakiewicz M, Mahmud N, Guy M, O'Brien LT, Wilkinson RA, Hall AL, Sawyer EJ, Dadaev T, Morrison J, Dearnaley DP, Horwich A, Huddart RA, Khoo VS, Parker CC, Van As N, Woodhouse CJ, Thompson A, Christmas T, Ogden C, Cooper CS, Lophatonanon A, Southey MC, Hopper JL, English DR, Wahlfors T, Tammela TL, Klarskov P, Nordestgaard BG, Roder MA, Tybjaerg-Hansen A, Bojesen SE, Travis R, Canzian F, Kaaks R, Wiklund F, Aly M, Lindstrom S, Diver WR, Gapstur S, Stern MC, Corral R, Virtamo J, Cox A, Haiman CA, Le Marchand L, Fitzgerald L, Kolb S, Kwon EM, Karyadi DM, Orntoft TF, Borre M, Meyer A, Serth J, Yeager M, Berndt SI, Marthick JR, Patterson B, Wokolorczyk D, Batra J, Lose F, McDonnell SK, Joshi AD, Shahabi A, Rinckleb AE, Ray A, Sellers TA, Lin HY, Stephenson RA, Farnham J, Muller H, Rothenbacher D, Tsuchiya N, Narita S, Cao GW, Slavov C, Mitev V, Easton DF, Eeles RA (2011) Seven prostate cancer susceptibility loci identified by a multi-stage genome-wide association study. *Nat Genet* 43(8): 785–791.
- Kote-Jarai Z, Saunders EJ, Leongamornlert DA, Tymrakiewicz M, Dadaev T, Jugurnauth-Little S, Ross-Adams H, Al Olama AA, Benlloch S, Halim S, Russell R, Dunning AM, Luccarini C, Dennis J, Neal DE, Hamdy FC, Donovan JL, Muir K, Giles GG, Severi G, Wiklund F, Gronberg H, Haiman CA, Schumacher F, Henderson BE, Le Marchand L, Lindstrom S, Kraft P, Hunter DJ, Gapstur S, Chanock S, Berndt SI, Albanes D, Andriole G, Schleutker J, Weischer M, Canzian F, Riboli E, Key TJ, Travis RC, Campa D, Ingles SA, John EM, Hayes RB, Pharoah P, Khaw KT, Stanford JL, Ostrander EA, Signorello LB, Thibodeau SN, Schaid D, Maier C, Vogel W, Kibel AS, Cybulski C, Lubinski J, Cannon-Albright L, Brenner H, Park JY, Kaneva R, Batra J, Spurdle A, Clements JA, Teixeira MR, Govindasami K, Guy M, Wilkinson RA, Sawyer EJ, Morgan A, Dicks E, Baynes C, Conroy D, Bojesen SE, Kaaks R, Vincent D, Bacot F, Tessier DC, Easton DF, Eeles RA (2013) Fine-mapping identifies multiple prostate cancer risk loci at 5p15, one of which associates with TERT expression. *Hum Mol Genet* 22(12): 2520–2528.
- Lan Q, Cawthon R, Gao Y, Hu W, Hosgood 3rd HD, Barone-Adesi F, Ji BT, Bassig B, Chow WH, Shu X, Cai Q, Xiang Y, Berndt S, Kim C, Chanock S, Zheng W, Rothman N (2013) Longer telomere length in peripheral white blood cells is associated with risk of lung cancer and the rs2736100 (CLPTM1L-TERT) polymorphism in a prospective cohort study among women in China. *PLoS One* 8(3): e59230.
- Levy D, Neuhausen SL, Hunt SC, Kimura M, Hwang SJ, Chen W, Bis JC, Fitzpatrick AL, Smith E, Johnson AD, Gardner JP, Srinivasan SR, Schork N, Rotter JI, Herbig U, Psaty BM, Sastrasin M, Murray SS, Vasan RS, Province MA, Glazer NL, Lu X, Cao X, Kronmal R, Mangino M, Soranzo N, Spector TD, Berenson GS, Aviv A (2010) Genome-wide association identifies OBFC1 as a locus involved in human leukocyte telomere biology. *Proc Natl Acad Sci USA* 107(20): 9293–9298.
- Lynch SM, Major JM, Cawthon R, Weinstein SJ, Virtamo J, Lan Q, Rothman N, Albanes D, Stolzenberg-Solomon RZ (2013) A prospective analysis of telomere length and pancreatic cancer in the alpha-tocopherol beta-carotene cancer (ATBC) prevention study. *Int J Cancer* 133(11): 2672–2680.
- Mangino M, Hwang SJ, Spector TD, Hunt SC, Kimura M, Fitzpatrick AL, Christiansen L, Petersen I, Elbers CC, Harris T, Chen W, Srinivasan SR, Kark JD, Benetos A, El Shamieh S, Visvikis-Siest S, Christensen K, Berenson GS, Valdes AM, Vinuela A, Garcia M, Arnett DK, Broeckel U, Province MA, Pankow JS, Kammerer C, Liu Y, Nalls M, Tishkoff S, Thomas F, Ziv E, Psaty BM, Bis JC, Rotter JI, Taylor KD, Smith E, Schork NJ, Levy D, Aviv A (2012) Genome-wide meta-analysis points to CTC1 and ZNF676 as genes regulating telomere homeostasis in humans. *Hum Mol Genet* 21(24): 5385–5394.
- Mirabello L, Huang WY, Wong JY, Chatterjee N, Reding D, Crawford ED, De Vivo I, Hayes RB, Savage SA (2009) The association between leukocyte telomere length and cigarette smoking, dietary and physical variables, and risk of prostate cancer. *Aging Cell* 8(4): 405–413.
- Platz EA, Clinton SK, Giovannucci E (2008) Association between plasma cholesterol and prostate cancer in the PSA era. *Int J Cancer* 123(7): 1693–1698.
- Pooley KA, Bojesen SE, Weischer M, Nielsen SF, Thompson D, Amin Al Olama A, Michailidou K, Tyrer JP, Benlloch S, Brown J, Audley T, Luben R, Khaw KT, Neal DE, Hamdy FC, Donovan JL, Kote-Jarai Z, Baynes C, Shah M, Bolla MK, Wang Q, Dennis J, Dicks E, Yang R, Rudolph A, Schildkraut J, Chang-Claude J, Burwinkel B, Chenevix-Trench G, Pharoah PD, Berchuck A, Eeles RA, Easton DF, Dunning AM, Nordestgaard BG (2013) A genome-wide association scan (GWAS) for mean telomere length within the COGS project: identified loci show little association with hormone-related cancer risk. *Hum Mol Genet* 22(24): 5056–5064.
- Pooley KA, Sandhu MS, Tyrer J, Shah M, Driver KE, Luben RN, Bingham SA, Ponder BA, Pharoah PD, Khaw KT, Easton DF, Dunning AM (2010) Telomere length in prospective and retrospective cancer case-control studies. *Cancer Res* 70(8): 3170–3176.
- Prescott J, Du M, Wong JY, Han J, De Vivo I (2012) Paternal age at birth is associated with offspring leukocyte telomere length in the nurses' health study. *Hum Reprod* 27(12): 3622–3631.
- Prescott J, Kraft P, Chasman DI, Savage SA, Mirabello L, Berndt SI, Weissfeld JL, Han J, Hayes RB, Chanock SJ, Hunter DJ, De Vivo I (2011) Genome-wide association study of relative telomere length. *PLoS One* 6(5): e19635.
- Rafnar T, Sulem P, Stacey SN, Geller F, Gudmundsson J, Sigurdsson A, Jakobsdottir M, Helgadóttir H, Thorlacius S, Aben KK, Blondal T, Thorgeirsson TE, Thorleifsson G, Kristjansson K, Thorisdottir K, Ragnarsson R, Sigurgeirsson B, Skuladottir H, Gudbjartsson T, Isaksson HJ, Einarsson GV, Benediktsson KR, Agnarsson BA, Olafsson K, Salvarsdottir A, Bjarnason H, Asgeirsdottir M, Kristinsson KT, Matthiasdottir S, Sveinsdottir SG, Polidoro S, Hoimov V, Botella-Estrada R, Hemminki K, Rudnai P, Bishop DT, Campagna M, Kellen E, Zeegers MP, de Verdier P, Ferrer A, Isla D, Vidal MJ, Andres R, Saez B, Juberias P, Banzo J, Navarrete S, Tres A, Kan D, Lindblom A, Gurdau E, Koppova K, de Vegt F, Schalken JA, van der Heijden HF, Smit HJ, Termeer RA, Oosterwijk E, van Hooij O, Nagore E, Porru S, Steineck G, Hansson J, Buntinx F, Catalona WJ, Matullo G, Vineis P, Kiltie AE, Mayordomo JJ, Kumar R, Kiemeny LA, Frigge ML, Jonsson T, Saemundsson H, Barkardottir RB, Jonsson E, Jonsson S, Olafsson JH, Gulcher JR, Masson G, Gudbjartsson DF, Kong A, Thorsteinsdottir U, Stefansson K (2009) Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. *Nat Genet* 41(2): 221–227.
- Savage SA, Gadalla SM, Chanock SJ (2013) The long and short of telomeres and cancer association studies. *J Natl Cancer Inst* 105(7): 448–449.
- Shen M, Cawthon R, Rothman N, Weinstein SJ, Virtamo J, Hosgood 3rd HD, Hu W, Lim U, Albanes D, Lan Q (2011) A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of lung cancer. *Lung Cancer* 73(2): 133–137.

- Stampfer MJ, Willett WC, Speizer FE, Dysert DC, Lipnick R, Rosner B, Hennekens CH (1984) Test of the National Death Index. *Am J Epidemiol* **119**(5): 837–839.
- Wang H, Chen H, Gao X, McGrath M, Deer D, De Vivo I, Schwarzschild MA, Ascherio A (2008) Telomere length and risk of Parkinson's disease. *Mov Disord* **23**(2): 302–305.
- Weischer M, Nordestgaard BG, Cawthon RM, Freiberg JJ, Tybjaerg-Hansen A, Bojesen SE (2013) Short telomere length, cancer survival, and cancer risk in 47102 individuals. *J Natl Cancer Inst* **105**(7): 459–468.
- Wentzensen IM, Mirabello L, Pfeiffer RM, Savage SA (2011) The association of telomere length and cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* **20**(6): 1238–1250.

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