Review

Cancer Epidemiology, Biomarkers & Prevention

The Association of Telomere Length in Peripheral Blood Cells with Cancer Risk: A Systematic Review and Meta-analysis of Prospective Studies

Xia Zhang[1], Qian Zhao[2], Wei Zhu[3], Tao Liu[4], Shao-Hua Xie[5], Li-Xin Zhong[1], Yuan-Yuan Cai[1], Xiao-Na Li[1], Mei Liang[1], Wen Chen[1], Qian-Sheng Hu[1], and Bo Zhang[1]

Abstract

The association between telomere length (TL) in peripheral blood cells and cancer risk remains inconclusive. We carried out a meta-analysis on prospective studies. The study-specific RR estimates were first transformed to a common comparable scale and then were pooled by a random-effects model. The dataset was composed of 13,894 cases and 71,672 controls from 28 studies in 25 articles. In the comparison of the longest versus shortest third of TL, we observed a marginally positive association between longer TL and higher risk of total cancers [OR = 1.086; 95% confidence interval (CI), 0.952–1.238]. Subgroup analyses showed that the association was stronger in lung cancer ($n = 3$; OR = 1.690; 95% CI, 1.253–2.280), in men ($n = 6$; OR = 1.302; 95% CI, 1.120–1.514) and in studies with more precise methods for DNA extraction (phenol–chloroform, salting-out or magnetic bead, $n = 6$, OR = 1.618; 95% CI, 1.320–1.985) and TL measurement (multiplex Q-PCR, $n = 8$; OR = 1.439; 95% CI, 1.118–1.852). Our meta-analysis suggested longer TL in peripheral blood cells is a likely risk factor for lung cancer or cancers in men. Accurate DNA extraction and TL measurement methods make it more liable to find significant associations between TL and cancer risk and thus should be taken into consideration in future epidemiologic studies. *Cancer Epidemiol Biomarkers Prev; 26(9); 1381–90. ©2017 AACR.*

Introduction

Telomeres, repetitive DNA sequences coated by capping proteins at the ends of linear chromosomes, play vital roles in the maintenance of chromosome integrity and stability during cell division. In human somatic cells without telomerase activity, telomere genetic material would lose approximately 30 to 200 bp after each mitotic cycle because of the asymmetric replication of DNA (1). With continuous shortening, when their length reaches a critical point, telomeres signal the arrest of cell proliferation, as well as senescence and apoptosis. Thus, telomere length (TL) has been suggested to be a "cellular mitotic clock" that records the number of cellular turnovers within an individual (2) and serves as a biomarker for biological age in epidemiologic studies. In addition to chronologic aging, TL is influenced by genetic factors (3) and varies considerably between individuals, even those with the same chronologic age. TL is also affected by numerous environmental factors (4); nutrients, foods, and dietary patterns (5); cigarette smoking (6); air pollution (7); physical activity (6, 8); and chronic inflammation (9).

The relationship between TL and cancer risk is debatable because of the dual role of telomeres in carcinogenesis (10, 11). In general, telomere attrition theoretically leads to genome instability, which is a fundamental event in the origin of tumors (12). However, long telomeres may allow for more cell divisions, promote immortality of the cells, and lead to aberrant cell proliferation and tumor formation (13, 14). The empirical evidence from epidemiologic studies also remains controversial. For example, some studies showed that short telomeres increased the risk of breast cancer (15, 16), whereas others revealed opposite or no associations (17, 18).

The true relationship between TL and cancer risk may be obscured by several population and study-specific characteristics. The first and foremost is age at TL measurement. For example, the prospective study of 47,102 Danish general population participants, with follow-up times of up to 20 years, showed a strong association between shorter TL and higher cancer risk in a crude model (HR, 1.74; 95% CI, 1.58–1.93, shortest vs. longest quartiles), but the association was attenuated to nonsignificance after adjusting for age at blood sampling (HR, 0.98; 95% CI, 0.88–1.08; ref. 19). The second is study design. Cancer *per se* and cancer treatment can shorten TL; therefore, the negative associations between TL and cancer risk reported in retrospective studies and prospective studies with short follow-up times may reflect reverse causation. The third is the accuracy of TL assessment; for example, TL measured by Q-PCR from QIAamp (column)-extracted DNA

[1]Guangzhou Key Laboratory of Environmental Pollution and Health Risk Assessment, Department of Preventive Medicine, School of Public Health, Sun Yat-Sen University, Guangzhou, China. [2]School of Public Health, Guangzhou Medical University, Guangzhou, China. [3]Department of Toxicology, Guangzhou Centre for Disease Control and Prevention, Guangzhou, China. [4]Guangdong Provincial Institute of Public Health, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou, China. [5]Upper Gastrointestinal Surgery, Department of Molecular Medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

X. Zhang, Q. Zhao, and W. Zhu contributed equally to this article.

Corresponding Author: Bo Zhang, Guangzhou Key Laboratory of Environmental Pollution and Health Risk Assessment, Department of Preventive Medicine, School of Public Health, Sun Yat-Sen University, #74, Zhongshan Road 2, Yuexiu District, Guangzhou 510080, China. Phone: 86-020-87332445; Fax: 86-020-87330446; E-mail: zhangb65@mail.sysu.edu.cn

doi: 10.1158/1055-9965.EPI-16-0968

was less than from either PureGene (Salting-out) or phenol–chloroform ($P < 0.001$), and the association was more evident in DNA samples extracted by the latter two methods (20). The fourth is that in statistical models TL has always been treated as a categorical variable (e.g., quantiles) according to its distribution in the control subjects, but the uncommon scales of categorization make the results not easily comparable. In addition, the relationship between TL and cancer risk are highly variable across cancer types (21). A recent meta-analysis pooling unadjusted risk estimates from both prospective and retrospective studies found no association between TL and overall cancer risk and the validity was further attenuated because the study-specific RR was not transformed to a common scale before combination (22).

Given the inconsistent results and the potential sources of heterogeneity from study-specific characteristics, we conducted a systematic review and meta-analysis on prospective studies to provide a precise assessment of the relationship between TL and cancer risk based on fully variable-adjusted models. We also aimed to compare these associations across a wide range of study-level characteristics.

Materials and Methods

Search strategy

To identify studies exploring the relationship between TL and cancer risk, we conducted a systematic literature search on PubMed and Web of Science. The search strategy used the following terms: "telomere" or "telomeres," "telomeric," "T/S ratio," or "T/C ratio"; "neoplasms," "neoplasm," "tumor," "tumors," "neoplasias," "cancer," "cancers," "benign neoplasms," "neoplasms, benign," "benign neoplasm" or "neoplasm, benign"; and "risk," "risks," "relative risk," "relative risks," "risk, relative" or "risks, relative." The search results were updated through April 2, 2017. Additional studies were sought by a manual search according to the list of references within eligible studies or reviews in the field.

Study selection

Studies eligible for inclusion in our meta-analysis had to meet the following criteria: (i) full reports in English, (ii) having evaluated the relationship between TL and cancer risk, (iii) prospective studies (e.g., nested case-control and prospective cohort studies in which DNA samples used for TL measurement were collected before diagnosis of cancer), (iv) presenting multivariate-adjusted risk estimates, that is, RR, OR, or HR across TL category groups or per unit change for cancer risk, (v) DNA extracted from peripheral blood, (vi) independent from other studies (if two or more reports used data from the same cohort, only the one with the largest sample size and/or longest follow-up time was included), and (vii) the reference group in the categorical analysis being the longest or shortest TL group. In addition, studies were excluded if participants had cancer susceptibility or had previously suffered from cancer. There were no restrictions on cancer types. Eligible studies were identified by two researchers (X. Zhang and W. Zhu) independently, and a third researcher (B. Zhang) dealt with discrepancies. The details of the process of study selection are shown in Fig. 1.

Data extraction and quality assessment

Two researchers (X. Zhang and W. Zhu) extracted data independently and reached a consensus on all projects. If two or more independent studies were reported in one article, they were treated separately in our meta-analysis unless the combined results were provided in the original study. A standardized information sheet was used to extract characteristics from eligible studies, including the first author, year of publication, geographic location, race, cancer type, population source, age at baseline or blood draw, age at diagnosis, time interval of follow-up, cohort time, number of participants (the number of cases and controls), gender, DNA extraction method, TL assay methods, number of experimental replicates, TL (mean or median), telomere units, RRs (HRs, risk ratios, and ORs) and corresponding 95% CI, and main findings. If the TL was divided into more than one category in one study, such as dichotomies, tertiles or quartiles, we used the results in tertiles. Study quality was evaluated by the Newcastle–Ottawa scale, and a study scoring equal to or greater than eight was regarded as high quality.

Statistical analysis

We made a plan for data analysis and performed analyses in strict accordance with the plan. Meta-analyses were performed with Stata version 12.0 (Stata Corp.). HRs and RR were assumed to have the same meaning as ORs. We calculated the summary OR for the association between TL and cancer risk by a random-effects model. We adopted methods previously described (23) to transform effect sizes, such as ORs, RRs, and risk ratios, into the same comparable values before meta-analysis, namely the longest versus shortest tertile of TL. Under the assumption that TL is normally distributed and has a log-linear relationship with cancer risk, the log OR for the longest versus shortest third of the distribution of TL is expected to be approximately 1.27 times the log OR for the top versus bottom half in TL, 0.86 times the longest versus shortest fourth, 0.78 times the longest versus shortest fifth and 0.62 times the longest versus shortest tenth (Supplementary Table S1). Data transformation was performed with Matlab version 7.0. The 95% CI of the transformed log OR was calculated by using the same multiplication coefficient as the originally reported 95% CI. When studies reported risk estimates with different degrees of statistical adjustment for covariates, we used the fully adjusted result.

A funnel plot and Egger's tests were used to investigate publication bias. The between-study heterogeneity was assessed by the Cochran Q test and I^2 statistic and considered significant if $P < 0.05$ or $I^2 > 50\%$. To evaluate the effect of a single study on the result, sensitivity analyses were performed to recalculate the OR and 95% CI after excluding each study individually. Sources of heterogeneity were assessed by subgroup analyses and meta-regression. The study-level characteristics tested as sources of heterogeneity included cancer types, geographical location of the study (Europe, North America, and Asia), study quality (Newcastle–Ottawa scale score), age at blood draw, time interval of follow-up, age at cancer diagnosis, gender, DNA extraction method (QIAamp vs. Non-QIAamp method, that is, phenol-chloroform, salting-out and magnetic bead method), TL detection method (singleplex or multiplex Q-PCR), number of experimental replicates, and sample size (<500, 500–1,000, or s,000).

Results

Characteristics of included studies

As shown in Fig. 1, 2,598 publications were originally identified. After duplicate checking and title or abstract reviewing, 63 remained. Thirty-eight publications were then removed after

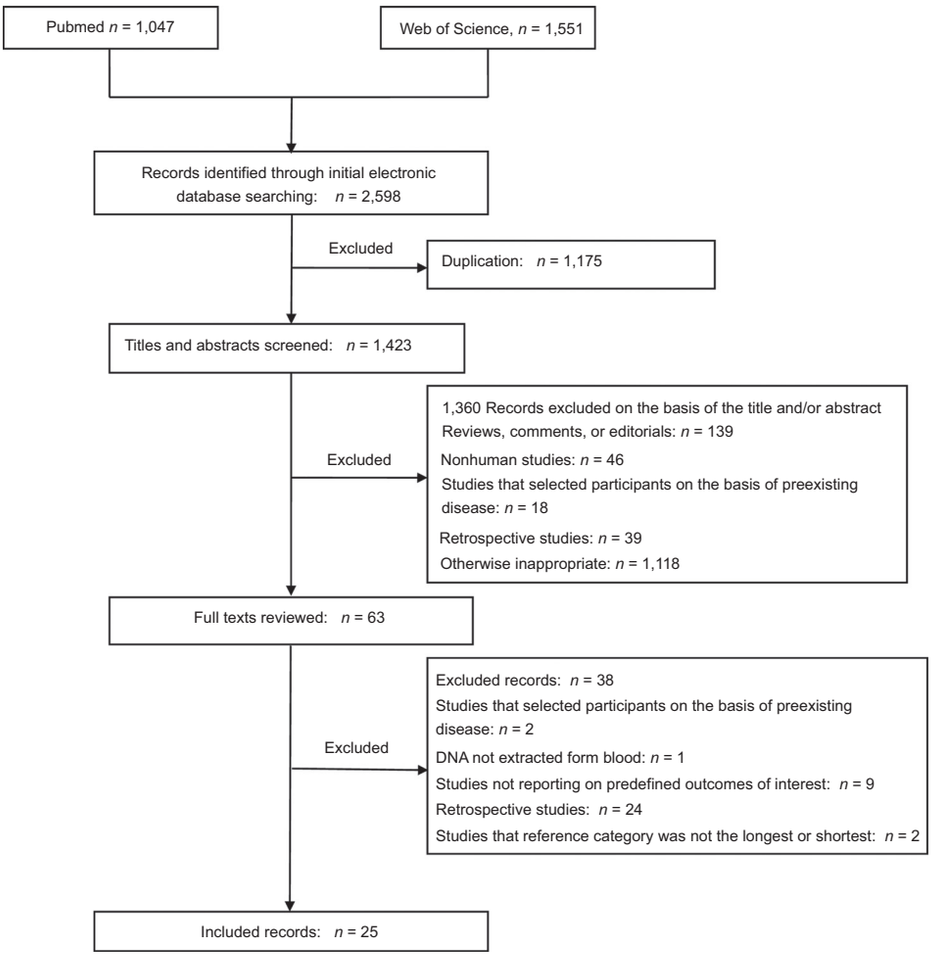

Figure 1.
Flow chart for the records selection.

reading full texts, resulting in 25 publications that were included in the meta-analysis (17, 18, 24–46). Liang and colleagues' study (24) included three independent nested case-control studies, skin squamous cell carcinoma (SCC) cases within the Health Professionals Follow-up Study (HPFS) and two groups of skin basal cell carcinoma (BCC) cases within the Nurses' Health Study (NHS), and Pooley and colleagues' study (18) reported results of breast cancer and colorectal cancer separately, so they were treated as separate entries, then there were totally 28 independent studies included in our meta-analysis. Han and colleagues' study (35) contained three types of skin cancer: melanoma, squamous cell carcinoma (SCC), and basal cell carcinoma (BCC), but melanoma and BCC cases were later reported in Liang and colleagues' study (24); therefore, only SCC cases were included.

The essential information of the included studies is listed in Table 1 and Supplementary Table S1. In total, there were five studies for skin cancer, three for pancreatic cancer, three for breast cancer, three for lung cancer, two for colorectal cancer, two for blood cancer, two for prostate cancer, two for all cancer risk, and six studies for other cancers (including renal cell carcinoma, glioma, endometrial, bladder, liver, and ovarian cancer). Ten studies were conducted in Europe, 15 in North America, and three in Asia. The population of the 28 studies were from 14 cohorts and eight cohorts were used at least twice and at most nine times to analyze different cancer types. We have seriously checked the data source to avoid repeated reports. We found that Seow and colleagues′ study (41) combined lung cancer cases from three cohorts: the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), the Alpha-Tocopherol, Beta-Carotene Cancer Prevention trial (ATBC), and the Shanghai Women's Health Study (SWHS), but the subjects in the latter two studies were reported separately in Shen and colleagues' (27) and Lan and colleagues' (28) studies. Thus, only PLCO subjects in Seow and colleagues' study were included. Regarding the gender of subjects, six studies were only composed of men (ATBC and HPFS were male cohorts), 10 only of women [NHS, WHS, SWHS, WHI-OS, Women's Health Initiative Observational Study (WHI-OS) were female cohorts], and 12 of both men and women. All studies provided results adjusting for either age at blood draw or age at cancer diagnosis or both. DNA extraction method was clearly illustrated in 22 studies, among which QIAamp method was used in 16 studies and non-QIAamp methods were used in six studies: four studies using phenol–chloroform (25, 27, 28, 30), one using PureGene (Salting-out; ref. 41) and one using magnetic bead (36). All studies measured TL using quantitative PCR (Q-PCR) method. Except Zeng and colleagues′ study (45), 27 studies used Cawthon method that was originally developed in 2002 (singleplex Q-PCR; ref. 47) and updated in 2009 (multiplex Q-PCR; ref. 48), expressing TL as the ratio (T/S) of the telomere repeat (T) copy number to the single copy gene (S) copy number. Among them 19 studies

Table 1. Characteristics of studies included in the meta-analysis

Author	Year	Location	Ethnic	Cancer location	Population source	Sex	Case/ control	Age at baseline or blood draw (mean± SD)		Age at diagnosis (mean or median)	Time intervals	DNA extraction method	TL detection method	Main find[f]	NOS quality score
								Case	Control						
McGrath et al. (33)	2007	USA	Caucasian	Bladder	HPFS, NHS	Mixed	184/192	64.1	64.1	NR	NR	QIAamp	Singleplex	Linear(-)	8
Lan et al. (30)	2009	Finland	European	Blood	ATBC	Male	107/107	58.3	57.5	NR	NR	P-C	Multiplex	Linear(+)	9
Mirabello et al. (34)	2009	USA	Caucasian	Prostate	PLCO	Male	612/1049	64	64	NR	NR	QIAamp	Singleplex	No	8
De Vivo et al. (17)	2009	USA	Caucasian	Breast	NHS	Female	1,122/1,147	58.4	58.4	65.7	NR	QIAamp	Singleplex	No	8
Han et al. (35)[a]	2009	USA	European	SCC	NHS	Female	285/273	59.3	58.8	64.7	NR	QIAamp	Singleplex	No	8
Prescott et al. (32)	2010	USA	Caucasian	Endometrial	NHS, WHS	Female	279/791	NR	NR	NR	5.9 years	QIAamp	Singleplex	No	8
Willeit et al. (31)	2010	Italy	European	Cancer risk	BS	Mixed	92/695	62.6	62.6	67	NR	NR	Singleplex	Linear(-)	9
Pooley et al. (18)[b]	2010	UK	European	Breast	EPIC	Female	199/420	64	64	NR	3.3 years	NR	Singleplex	No	8
Pooley et al. (18)[b]	2010	UK	European	Colorectal	EPIC	Mixed	185/406	64	64	NR	3.3 years	NR	Singleplex	No	8
Lee et al. (37)	2010	USA	White	Colorectal	WHS	Female	134/357	60.10	60.66	NR	5.80 years	QIAamp	Singleplex	No	8
Nan et al. (39)	2011	Europe	European	Melanoma	WHI-OS, HPFS, NHS	Mixed	557/579	60.7	60.7	65.9	NR	QIAamp	Singleplex	Linear(-)	9
Shen et al. (27)	2011	Finland	Finnish	Lung	ATBC	Male	229/229	59	58	NR	5.23 years	P-C	Multiplex	No	8
Liang et al. (24)[b]	2011	USA	Caucasian	SCC	HPFS	Male	241/241	63.1	63.1	69.0	NR	QIAamp	Singleplex	No	8
Liang et al. (24)[b]	2011	USA	Caucasian	BCC1	NHS	Female	260/260	56.3	56.3	66.1	NR	QIAamp	Singleplex	No	8
Liang et al. (24)[b]	2011	USA	Caucasian	BCC2	NHS	Female	363/1,683	61.0	59.5	69.3	NR	QIAamp	Singleplex	No	6
Walcott et al. (40)	2013	Europe	European	Brain	PLCO	Mixed	101/198	NR	NR	NR	NR	NR	Singleplex	No	8
Lynch et al. (25)	2013	Finland	Finnish	Pancreatic	ATBC	Male	193/660	58	58	NR	6.3 years	P-C	Multiplex	Linear(+)	8
Hofmann et al. (26)	2013	USA	Mixed	Renal	PLCO	Mixed	209/410	NR	NR	NR	NR	QIAamp	Multiplex	No	7
Qu et al. (38)	2013	China	Chinese	Breast	SWHS	Female	601/695	52.7	53.4	NR	NR	QIAamp	Multiplex	Linear(-)	9
Lan et al. (28)	2013	China	Chinese	Lung	SWHS	Female	215/215	NR	NR	NR	NR	P-C	Multiplex	Linear(+)	9
Hosnijeh et al. (29)	2014	USA	Caucasian	Blood	EPIC	Mixed	464/464	56.58	56.59	61.5	4.6 years	NR	Singleplex	Linear(+)	8
Campa et al. (36)	2014	Europe	Mixed	Pancreatic	EPIC	Mixed	331/331	57	57	63	5.3 years	Salting-out	Singleplex	No	8
Seow et al. (41)[c]	2014	USA	Caucasian	Lung	PLCO	Mixed	403/403	64.1	63.6	NR	7.41 years	Magnetic Bead	Multiplex	Linear(+)	8
Julin et al. (43)	2015	USA	Caucasian	Prostate	HPFS	Male	922/935	63.6	63.5	69.5	5.5 years	QIAamp	Singleplex	Linear(+)	8
Rode et al. (42)	2016	Denmark	Danish	Cancer risk	CCHS, CGPS	Mixed	4,510/56,773	NR	NR	NR	5 years	QIAamp	multiplex	No	8
Bao et al. (44)	2016	USA	European	Pancreatic	Five cohort[d]	Mixed	386/896	NR	NR	NR	6.7 years	QIAamp	Singleplex	Linear(-)	8
Zeng et al. (45)	2017	China	Chinese	Liver	CSP	Mixed	268/536	NR	NR	53.48	NR	NR	NR	No	8
Yang et al. (46)	2017	USA	Caucasian	Ovarian	NHS, NSHDS	Female	442/727	54.03	54.59	63.66	9.7 years	QIAamp	Singleplex	Linear(-)	8

Abbreviations: NR, not reported; P-C, phenol–chloroform method.

Study acronyms: HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; PLCO Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; PHS, Physicians' Health Study; WHS, Women's Health Study; BS, Bruneck Study; EPIC, European Prospective Investigation into Cancer and Nutrition; CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; CSP, Taiwan Cancer Screening Program cohort; NSHDS, the Northern Sweden Health and Disease Study.

[a]Han and colleagues' study included SCC, BCC, and melanoma. We speculated that BCC and melanoma cases were reanalyzed in Nan and colleagues' study and Liang and colleagues' study, respectively, so only SCC was included in the current meta-analysis.

[b]These data were reported separately in original article.

[c]Only samples from PLOC were included to avoid repeated reports.

[d]Including the NHS, HPFS, the PHS I, WHI-OS, the WHS.

[f]Linear(+) represent longer TL is a risk factor for cancer, Linear(−) represent shorter TL is a risk factor for cancer.

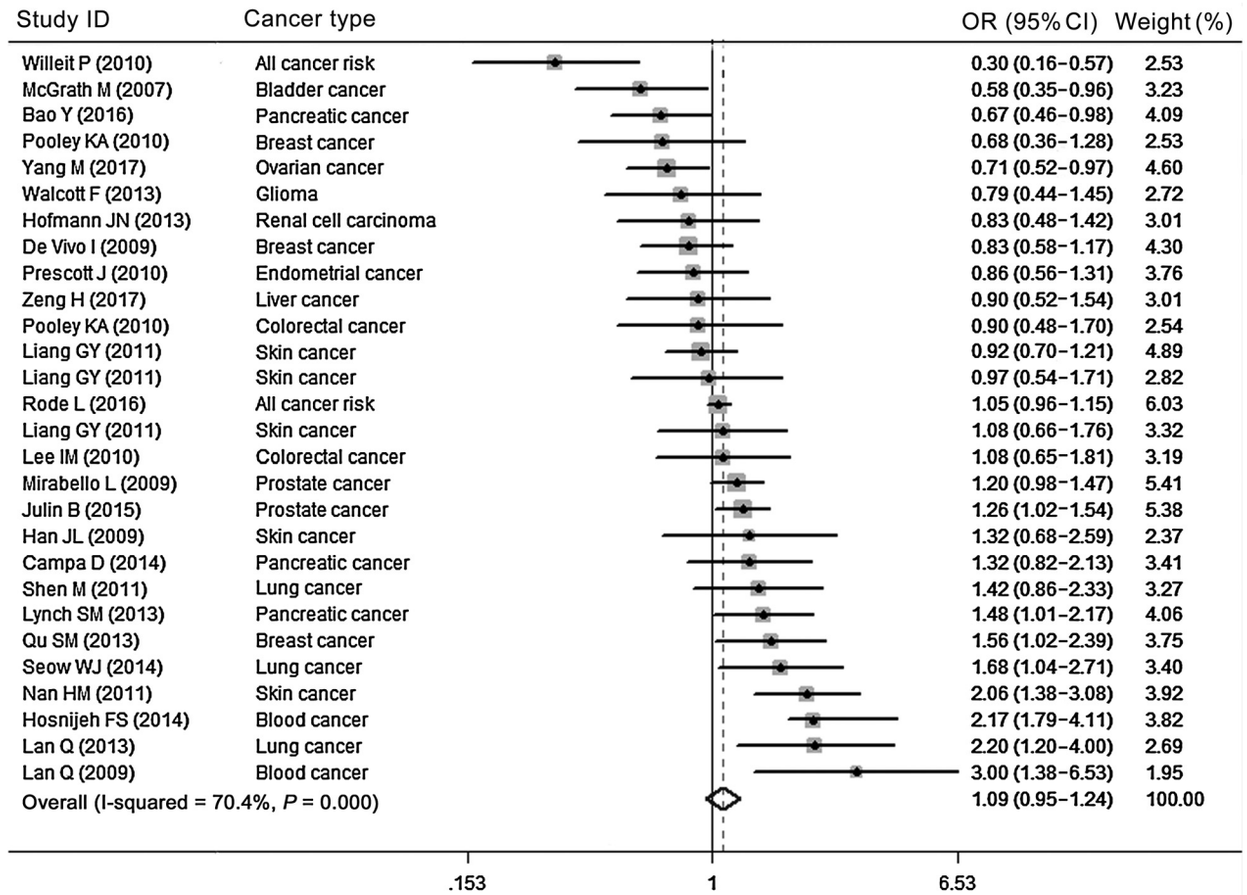

Figure 2.
Forest plot of pooled studies for the association between TL and cancer risk. NOTE: Weights are from random effects analysis.

used singleplex Q-PCR method (47) and eight used multiplex Q-PCR.

Meta-analysis results

When all eligible studies were combined, the pooled OR for cancer risk comparing the longest versus shortest third of TL was 1.086 (95% CI, 0.952–1.238) with moderate heterogeneity between studies (P for heterogeneity test <0.001, $I^2 = 70.4\%$), as shown in Fig. 2. Table 2 shows the results of subgroup analyses. Pooled ORs differed significantly by cancer types, gender, DNA extraction method, and TL measurement method. The associations between longer TL and higher cancer risk were statistically significant in lung cancer ($n = 3$; OR = 1.690; 95% CI, 1.253–2.280), in men ($n = 6$; OR = 1.302; 95% CI, 1.120–1.514), and in studies with more precise method for DNA extraction (non-QIAamp methods, i.e., phenol–chloroform, salting-out or magnetic bead, $n = 6$; OR = 1.618; 95% CI, 1.320–1.985) and TL measurement method (multiplex Q-PCR, $n = 8$; OR = 1.439; 95% CI, 1.118–1.852).

To explore the sources of heterogeneity, we conducted meta-regression by various clinically relevant characteristics and methodologic factors. DNA extraction method ($P = 0.021$), TL measurement method ($P = 0.083$), and study design ($P = 0.097$) might explain some source of heterogeneity for overall cancer risk. The sensitivity analysis indicated that no single study could change the pooled OR qualitatively.

Publication bias

A funnel plot was drawn for 28 studies, and results showed no obvious asymmetry (Fig. 3), which was consistent with Egger's test and Begg's test ($P = 0.897$; Supplementary Figs. S1 and S2), suggesting that there was no evidence of publication bias.

Discussion

We found a marginally positive association between longer TL and higher cancer risk in the present meta-analysis of 28 prospective studies in 25 articles. The associations were more significant in lung cancer, cancers in men, and in studies with more precise methods used in DNA extraction and TL measurement.

Comparison with previous meta-analysis and strengths

Our findings are not consistent with Zhu and colleagues' meta-analysis published recently (22), which found no statistically significant association between peripheral TL and overall cancer risk and short telomeres might be risk factors for the tumors of digestive system. Several factors may contribute to these disparities. First, we included 28 prospective studies in contrast to 16 prospective and 46 retrospective studies in Zhu and colleagues'

Table 2. Associations between TL and risk of cancer stratified by chosen factors

Variables	No. of studies	Sample case/control	Longest vs. shortest OR (95% CI)	$P_{heterogeneity}$		*P* for meta-regression
				P	I^2	
All	28	13,894/71,672	1.086 (0.952–1.238)	0.000	70.4%	
Cancer type						0.5540
Pancreatic cancer	3	910/1,887	1.086 (0.650–1.815)	0.009	78.7%	
Breast cancer	3	1,922/2,262	0.983 (0.607–1.592)	0.035	70.1%	
Lung cancer	3	847/847	1.690 (1.253–2.280)	0.547	0.0%	
Colorectal cancer	2	319/763	1.005 (0.675–1.496)	0.661	0.0%	
Skin cancer	5	1,706/3,036	1.208 (0.860–1.697)	0.024	64.3%	
Others	12	8,190/62,877	0.979 (0.799–1.200)	0.000	79.0%	
Location						0.4688
Europe	10	6,504/60,398	1.119 (0.843–1.484)	0.000	77.7%	
North America	15	6,306/9,828	1.013 (0.856–1.198)	0.000	66.4%	
Asia	3	1,084/1,446	1.445 (0.903–2.314)	0.085	59.3%	
Study quality						0.4870
<8	2	572/2,093	0.901 (0.706–1.150)	0.740	0.0%	
≥8	26	13,322/69,579	1.105 (0.960–1.271)	0.000	71.9%	
Study design						0.0970
Nested case–control study	27	9,292/14,204	1.128 (0.978–1.302)	0.000	66.4%	
Cohort	2	4,602/57,468	0.585 (0.172–1.991)	0.000	93.2%	
Age at blood draw						0.5136
<60	12	4,676/7,367	1.208 (0.963–1.514)	0.000	69.9%	
≥60	10	3,529/5,277	1.013 (0.777–1.322)	0.000	76.9%	
NR	6	5,689/59,028	0.965 (0.742–1.256)	0.025	61.1%	
Time interval of follow-up						0.9467
<5	3	848/1,290	1.134 (0.533–2.415)	0.004	81.9%	
≥5	10	7,829/62,102	1.081 (0.918–1.274)	0.004	62.9%	
NR	15	5,217/8,280	1.063 (0.847–1.334)	0.000	73.9%	
Age at cancer diagnosis						0.7206
<65	8	2,491/4,011	1.192 (0.892–1.592)	0.001	70.3%	
≥65	11	4,478/7,126	1.008 (0.790–1.287)	0.000	72.9%	
NR	9	6,925/60,535	1.082 (0.864–1.355)	0.000	73.4%	
Gender						0.2849
Men	6	2,304/3,221	1.302 (1.120–1.514)	0.287	19.4%	
Women	10	3,900/6,568	1.002 (0.817–1.229)	0.024	52.9%	
Mixed	12	7,690/61,883	0.997 (0.772–1.289)	0.000	80.0%	
DNA extraction method						0.0210
QIAamp	16	11,107/67,008	1.017 (0.891–1.161)	0.000	62.8%	
Non-QIAamp	6	1,478/1,945	1.618 (1.320–1.985)	0.464	0.0%	
NR	6	1,309/2,719	0.829 (0.474–1.449)	0.000	82.9%	
Sample size						0.8123
≤500	7	1,211/1,539	1.213 (0.828–1.776)	0.003	69.6%	
500–1,000	11	2,889/4,858	1.054 (0.778–1.429)	0.000	72.6%	
≥1,000	10	9,794/65,275	1.046 (0.892–1.226)	0.000	73.3%	
Method of TL measurement						0.0825
Singleplex qPCR	19	7,159/11,644	0.971 (0.813–1.160)	0.000	73.0%	
Multiplex qPCR	8	6,467/59,492	1.439 (1.118–1.852)	0.002	68.3%	
Others	1	268/536	0.900 (0.523–1.549)	–	–	

study. Thus, more information is available and reverse causation is expelled at most in our study. Second, our study primarily used adjusted risk estimates, which made the results more robust than Zhu and colleagues' study, in which crude data were combined. Third, we transformed risk estimates to a common scale of comparison before meta-analysis, which improved the power to detect the relationship between TL and cancer risk by reducing the probability of misclassification.

Causal inference

Although our meta-analysis was based on the results from prospective studies which are less prone to reverse causation bias than retrospective studies, the associations we found could be subject to residual confounding factors or other bias induced by meta-analysis itself. Thus, it is cautious about causal inference. Recently, Mendelian randomization studies, using germline genetic variants as instrumental variables for their random distribution in the general population, are performed to appraise the causal relevance of TL for cancer risk. Seven SNP robustly associated with white blood cell (WBC) TL were identified in a recent genome-wide association study (GWAS; ref. 49). Although these variants were believed to explain only a small proportion of the total variation in TL (0.08%–0.36%), a genetic risk score (GRS) based on them was used as an instrumental variable in Mendelian randomization studies to explore the causal relationship between TL and risk of cancer. Machiela and colleagues (50) showed that genetic variants related to a longer TL are associated with an increased lung cancer risk among never-smoking females in Asia (OR, 1.51; 95% CI, 1.34–1.69, comparing upper quartile to lower quartile of GRS predicting longer TL). Luu and colleagues (51) found short TL determined by six of the seven lead SNPs was associated with a decreased risk of breast cancer in Asian women

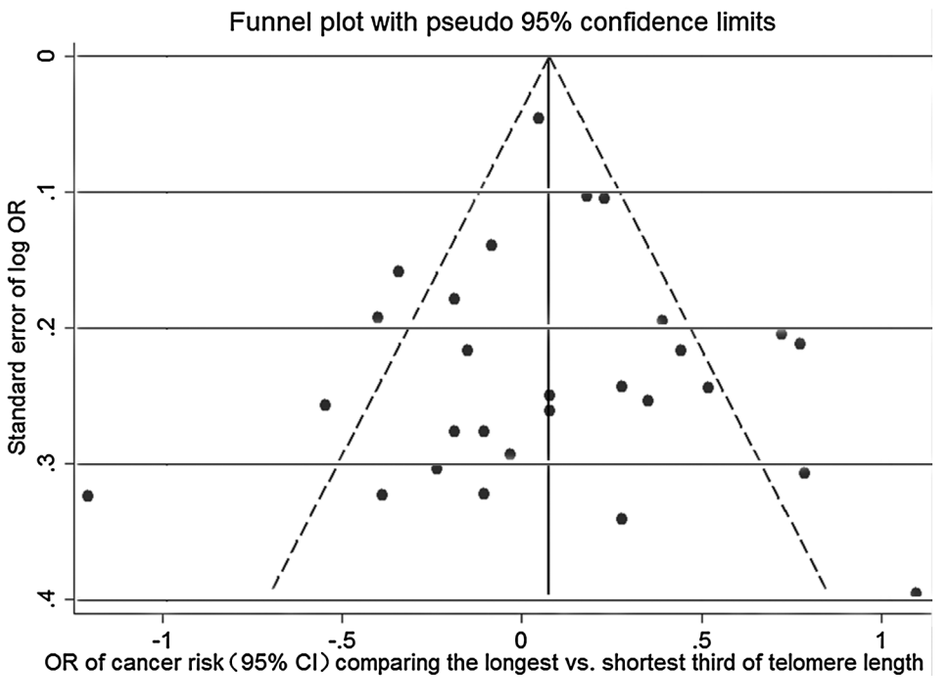

Figure 3.
Funnel plot of the association between TL and cancer risk.

(OR, 0.83; 95% CI, 0.72–0.95, for tertile 3 vs. tertile 1 of GRS predicting shorter TL). The telomeres Mendelian randomization collaboration recently reported that increased TL due to germline genetic variation was significantly associated with increased risk for nine site-specific cancers, that is, glioma, serous low-malignant-potential ovarian cancer, lung adenocarcinoma, neuroblastoma, bladder cancer, melanoma, testicular cancer, kidney cancer, and endometrial cancer (21). These estimates from genetic analyses, which in general avoid reverse causation bias and confounding factors in observational studies that directly build the relationship between TL and cancer risk, can be viewed as causal (52) and are consistent with our results, supporting that longer TL is a risk factor for cancer, at least for some types of cancers.

Implications of subgroup analyses

There are four study-specific characteristics, that is, cancer type, gender, DNA extraction method, and TL measurement method, that might modify the relationships between TL and cancer risk. Our findings of the tissue (lung)-specific association between longer TL and higher cancer risk are compatible with the results of the recent Mendelian randomization studies described previously: not every cancer risk increased with genetic determinants of long TL (21, 42). Such tissue-specific relationship need to be confirmed by future studies and the relevant mechanisms are also worthy of research.

In the stratification analysis of gender, we observed that longer TL was significantly related to higher cancer risk only in men. The underlying mechanism for such effect modification by gender is not fully known. Taking into account that women have longer TL than men (53) and that men have greater exposure to toxic factors that can shorten TL (e.g., smoking, alcohol, and stress) (54), the male-specific relationship needs further confirmation.

As for DNA extraction and TL measurement methods, Cunningham and colleagues (20) and Hofmann and colleagues (55) demonstrated that DNA extraction method is crucial to TL measurement and DNA extraction using phenol–chloroform, salting-out or magnetic bead methods produced more authentic and longer TL than QIAamp method which has more vigorous vortexing and multiple washes, and is prone to destroy DNA integrity, so the studies using QIAamp-extracted DNA are the least likely to find an association between TL and cancer risk (20). Similarly, multiplex Q-PCR method measured both *T* and *S* in each reaction well, providing improved consistency compared to singleplex Q-PCR which measured *T* and *S* in separate reaction wells, so the amount of DNA pipetted into the *T* and *S* reaction wells results in variation in *T*/*S*. Our meta-analysis indicates that accurate assessment of TL, from DNA extraction to Q-PCR, is critical to find true associations between TL and cancer risk. Thus, it is recommended that a standard protocol for DNA extraction (phenol–chloroform) and TL measurement (multiplex Q-PCR) should be made in further epidemiologic studies.

It is worth noting that cancer types, gender, DNA extraction method, and TL measure method are not randomly distributed across the included 28 studies. They are interrelated and coexist in some studies, so it is scarcely possible to disentangle these factors and identify the vital source of heterogeneity. For example, five of eight studies adopting multiplex Q-PCR used non-QIAamp method to extract DNA; all three lung cancer studies, as well as three male studies (half of the total six male studies) from ATBC cohort which is composed of male smokers, used both non-QIAamp and multiplex Q-PCR methods.

Biological mechanism of association

Our meta-analysis supports the viewpoint that longer TL is related to higher cancer risk, which is compatible with known biology. First, telomere elongation must rely on telomerase activity, but telomerase may have telomere-independent functions when reactivated in carcinogenesis. For example, Ghosh and colleagues (56) found that telomerase directly regulates NF-κB-dependent gene expression, such as that of IL6 and TNFα,

cytokines that are critical for inflammation and cancer progression. Telomerase could also regulate MYC-driven oncogenesis by stabilizing MYC levels on chromatin through regulating MYC ubiquitination and proteasomal degradation independent of its reverse transcriptase activity and role in telomere elongation (57), these results indicate TL is likely a surrogate of telomerase activity and telomerase may play a vital role in cell transformation. Second, although most studies have observed shorter TL in relation to environmental and occupational chemical exposures (4), some studies have reported carcinogen exposures [e.g., arsenic (58), persistent organic pollutants (59)], occupational exposure to benzene (60), and short-term exposure to particulate matter (61, 62) to be related to longer TL. These paradoxical phenomena reflect the complicated roles of TL and its regulatory signals during chemical carcinogenesis.

Limitations

Although our study has some advantages, as described previously, several limitations need to be mentioned. First, the method we used to harmonize the reported associations across studies to a common scale has three prerequisites: approximate normality of the distribution of TL, equal standard deviation of TL in cases and controls, and a log-linear association between TL and cancer risk (23). We have no original data to judge whether every study met these preconditions. Second, we were unable to test the nonlinear associations between TL and cancer risk. For example, Cui and colleagues' study (63) revealing a U-shaped association between TL in peripheral blood cells and colorectal cancer risk was excluded from the current meta-analysis. U-shaped or J-shaped associations indicate that either excessively shorter or longer TL may contribute to cancer development. The inflection point is unknown and the delicate TL balance in cells is worthy of detailed study. Third, average TL across all chromosomes was used in the included studies; however, it is not the average TL but the shortest telomeres that contribute to telomere dysfunction (64), and it is further hypothesized that chromosome-specific telomeres may be involved in specific cancer etiology (65). A technique for measuring chromosome-specific TL is available (66) but is less feasible for large scale epidemiologic studies. Fourth, meta-analyses of observational studies are subject to biases inherent in the original studies, and we could not access the individual data to adjust for the potential confounders across studies, for example, time interval from blood draw to cancer diagnosis. Thus, the heterogeneity among studies was not fully explored. Finally, 22 of the 28 included studies clearly described the average age at blood draw. All of them were older than 50 years. Given the negative association between TL and age and the individual difference of TL erosion dynamics, TL at different ages, especially at younger age, even at birth may serve as a predictive biomarker for cancer risk.

In conclusion, our meta-analysis including prospective studies showed a marginally positive association between TL and over all cancer risk. The association was stronger in lung cancer, cancers in men and in studies using precise method extracting DNA and measuring TL. More large-scale and well-designed prospective cohort studies are required to further explore the predictive values of TL on cancer risk and uncover the underlying biological mechanisms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported by the National Natural Science Foundation of China (to B. Zhang, 81273099).

Received December 1, 2016; revised April 27, 2017; accepted June 5, 2017; published OnlineFirst June 15, 2017.

References

1. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990;345:458–60.
2. Blackburn EH. Switching and signaling at the telomere. Cell 2001;106:661–73.
3. Slagboom PE, Droog S, Boomsma DI. Genetic determination of telomere size in humans: a twin study of three age groups. Am J Hum Genet 1994;55:876–82.
4. Zhang X, Lin S, Funk WE, Hou L. Environmental and occupational exposure to chemicals and telomere length in human studies. Occup Environ Med 2013;70:743–9.
5. Freitas-Simoes TM, Ros E, Sala-Vila A. Nutrients, foods, dietary patterns and telomere length: Update of epidemiological studies and randomized trials. Metabolism 2016;65:406–15.
6. Latifovic L, Peacock SD, Massey TE, King WD. The influence of alcohol consumption, cigarette smoking, and physical activity on leukocyte telomere length. Cancer Epidemiol Biomarkers Prev 2016;25:374–80.
7. Pieters N, Janssen BG, Dewitte H, Cox B, Cuypers A, Lefebvre W, et al. Biomolecular markers within the core axis of aging and particulate air pollution exposure in the elderly: a cross-sectional study. Environ Health Perspect 2016;124:943–50.
8. Du M, Prescott J, Kraft P, Han J, Giovannucci E, Hankinson SE, et al. Physical activity, sedentary behavior, and leukocyte telomere length in women. Am J Epidemiol 2012;175:414–22.
9. Jurk D, Wilson C, Passos JF, Oakley F, Correia-Melo C, Greaves L, et al. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nat Commun 2014;2:4172.
10. Hackett JA, Greider CW. Balancing instability: dual roles for telomerase and telomere dysfunction in tumorigenesis. Oncogene 2002;21:619–26.
11. Roos WP, Thomas AD, Kaina B. DNA damage and the balance between survival and death in cancer biology. Nat Rev Cancer 2016;16:20–33.
12. Boveri T. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. J Cell Sci 2008;121Suppl 1:1–84.
13. Bryan TM, Englezou A, Gupta J, Bacchetti S, Reddel RR. Telomere elongation in immortal human cells without detectable telomerase activity. EMBO J 1995;14:4240–8.
14. Mooi WJ, Peeper DS. Oncogene-induced cell senescence–halting on the road to cancer. N Engl J Med 2006;355:1037–46.
15. Shen J, Gammon MD, Terry MB, Wang Q, Bradshaw P, Teitelbaum SL, et al. Telomere length, oxidative damage, antioxidants and breast cancer risk. Int J Cancer. 2009;124:1637–43.
16. Zheng YL, Ambrosone C, Byrne C, Davis W, Nesline M, McCann SE. Telomere length in blood cells and breast cancer risk: investigations in two case-control studies. Br Cancer Res Treat 2010;120:769–75.
17. De Vivo I, Prescott J, Wong JY, Kraft P, Hankinson SE, Hunter DJ. A prospective study of relative telomere length and postmenopausal breast cancer risk. Cancer Epidemiol Biomark Prev 2009;18:1152–6.
18. Pooley KA, Sandhu MS, Tyrer J, Shah M, Driver KE, Luben RN, et al. Telomere length in prospective and retrospective cancer case-control studies. Cancer Res 2010;70:3170–6.
19. Weischer M, Nordestgaard BG, Cawthon RM, Freiberg JJ, Tybjaerg-Hansen A, Bojesen SE. Short telomere length, cancer survival, and cancer risk in 47102 individuals. J Natl Cancer Inst 2013;105:459–68.

20. Cunningham JM, Johnson RA, Litzelman K, Skinner HG, Seo S, Engelman CD, et al. Telomere length varies by DNA extraction method: implications for epidemiologic research. Cancer Epidemiol Biomark Prev 2013;22: 2047–54.
21. Haycock PC, Burgess S, Nounu A, Zheng J, Okoli GN, Bowden J, et al. Association between telomere length and risk of cancer and non-neoplastic diseases: a Mendelian randomization study. JAMA Oncol 2017;3:636–51.
22. Zhu X, Han W, Xue W, Zou Y, Xie C, Du J, et al. The association between telomere length and cancer risk in population studies. Sci Rep 2016;6: 22243.
23. Chene G, Thompson SG. Methods for summarizing the risk associations of quantitative variables in epidemiologic studies in a consistent form. Am J Epidemiol 1996;144:610–21.
24. Liang G, Qureshi AA, Guo Q, De Vivo I, Han J. No association between telomere length in peripheral blood leukocytes and the risk of non-melanoma skin cancer. Cancer Epidemiol Biomark Prev 2011;20: 1043–5.
25. Lynch SM, Major JM, Cawthon R, Weinstein SJ, Virtamo J, Lan Q, et al. A prospective analysis of telomere length and pancreatic cancer in the alpha-tocopherol beta-carotene cancer (ATBC) prevention study. Int J Cancer. 2013;133:2672–80.
26. Hofmann JN, Lan Q, Cawthon R, Hosgood HD III, Shuch B, Moore LE, et al. A prospective study of leukocyte telomere length and risk of renal cell carcinoma. Cancer Epidemiol Biomark Prev 2013;22:997–1000.
27. Shen M, Cawthon R, Rothman N, Weinstein SJ, Virtamo J, Hosgood HD III, et al. A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of lung cancer. Lung Cancer 2011;73: 133–7.
28. Lan Q, Cawthon R, Gao Y, Hu W, Hosgood HD III, Barone-Adesi F, et al. 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. 2013;8: e59230.
29. Hosnijeh FS, Matullo G, Russo A, Guarrera S, Modica F, Nieters A, et al. Prediagnostic telomere length and risk of B-cell lymphoma-Results from the EPIC cohort study. Int J Cancer 2014;135:2910–7.
30. Lan Q, Cawthon R, Shen M, Weinstein SJ, Virtamo J, Lim U, et al. A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of non-Hodgkin lymphoma. Clin Cancer Res 2009;15:7429–33.
31. Willeit P, Willeit J, Mayr A, Weger S, Oberhollenzer F, Brandstatter A, et al. Telomere length and risk of incident cancer and cancer mortality. JAMA. 2010;304:69–75.
32. Prescott J, McGrath M, Lee IM, Buring JE, De Vivo I. Telomere length and genetic analyses in population-based studies of endometrial cancer risk. Cancer 2010;116:4275–82.
33. McGrath M, Wong JY, Michaud D, Hunter DJ, De Vivo I. Telomere length, cigarette smoking, and bladder cancer risk in men and women. Cancer Epidemiol Biomark Prev 2007;16:815–9.
34. Mirabello L, Huang WY, Wong JY, Chatterjee N, Reding D, Crawford ED, et al. The association between leukocyte telomere length and cigarette smoking, dietary and physical variables, and risk of prostate cancer. Aging Cell 2009;8:405–13.
35. Han J, Qureshi AA, Prescott J, Guo Q, Ye L, Hunter DJ, et al. A prospective study of telomere length and the risk of skin cancer. J Invest Dermatol 2009;129:415–21.
36. Campa D, Mergarten B, De Vivo I, Boutron-Ruault MC, Racine A, Severi G, et al. Leukocyte telomere length in relation to pancreatic cancer risk: a prospective study. Cancer Epidemiol Biomark Prev 2014;23: 2447–54.
37. Lee IM, Lin J, Castonguay AJ, Barton NS, Buring JE, Zee RY. Mean leukocyte telomere length and risk of incident colorectal carcinoma in women: a prospective, nested case-control study. Clin Chem Lab Med 2010;48: 259–62.
38. Qu S, Wen W, Shu XO, Chow WH, Xiang YB, Wu J, et al. Association of leukocyte telomere length with breast cancer risk: nested case-control findings from the Shanghai Women's Health Study. Am J Epidemiol 2013;177:617–24.
39. Nan H, Du M, De Vivo I, Manson JE, Liu S, McTiernan A, et al. Shorter telomeres associate with a reduced risk of melanoma development. Cancer Res 2011;71:6758–63.
40. Walcott F, Rajaraman P, Gadalla SM, Inskip PD, Purdue MP, Albanes D, et al. Telomere length and risk of glioma. Cancer Epidemiol 2013;37: 935–8.
41. Seow WJ, Cawthon RM, Purdue MP, Hu W, Gao YT, Huang WY, et al. Telomere length in white blood cell DNA and lung cancer: a pooled analysis of three prospective cohorts. Cancer Res 2014;74:4090–8.
42. Rode L, Nordestgaard BG, Bojesen SE. Long telomeres and cancer risk among 95 568 individuals from the general population. Int J Epidemiol 2016;45:1634–43.
43. Julin B, Shui I, Heaphy CM, Joshu CE, Meeker AK, Giovannucci E, et al. Circulating leukocyte telomere length and risk of overall and aggressive prostate cancer. Br J Cancer 2015;112:769–76.
44. Bao Y, Prescott J, Yuan C, Zhang M, Kraft P, Babic A, et al. Leucocyte telomere length, genetic variants at the TERT gene region and risk of pancreatic cancer. Gut 2017;66:1116–22.
45. Zeng H, Wu HC, Wang Q, Yang HI, Chen CJ, Santella RM, et al. Telomere length and risk of hepatocellular carcinoma: a nested case-control study in Taiwan cancer screening program cohort. Anticancer Res 2017;37:637–44.
46. Yang M, Prescott J, Poole EM, Rice MS, Kubzansky LD, Idahl A, et al. Prediagnosis leukocyte telomere length and risk of ovarian cancer. Cancer Epidemiol Biomark Prev 2017;26:339–45.
47. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res 2002;30:e47.
48. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res 2009;37:e21.
49. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, et al. Identification of seven loci affecting mean telomere length and their association with disease. Nat Genet 2013;45:422–7, 7e1–2.
50. Machiela MJ, Hsiung CA, Shu XO, Seow WJ, Wang Z, Matsuo K, et al. Genetic variants associated with longer telomere length are associated with increased lung cancer risk among never-smoking women in Asia: a report from the female lung cancer consortium in Asia. Int J Cancer 2015; 137:311–9.
51. Luu HN, Long J, Wen W, Zheng Y, Cai Q, Gao YT, et al. Association between genetic risk score for telomere length and risk of breast cancer. Cancer Causes Control 2016;27:1219–28.
52. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003;32:1–22.
53. Gardner M, Bann D, Wiley L, Cooper R, Hardy R, Nitsch D, et al. Gender and telomere length: systematic review and meta-analysis. Exp Gerontol 2014;51:15–27.
54. Zhang C, Lauderdale DS, Pierce BL. Sex-Specific and Time-Varying Associations Between Cigarette Smoking and Telomere Length Among Older Adults. Am J Epidemiol 2016;184:922–32.
55. Hofmann JN, Hutchinson AA, Cawthon R, Liu CS, Lynch SM, Lan Q, et al. Telomere length varies by DNA extraction method: implications for epidemiologic research-letter. Cancer Epidemiol Biomark Prev 2014;23: 1129–30.
56. Ghosh A, Saginc G, Leow SC, Khattar E, Shin EM, Yan TD, et al. Telomerase directly regulates NF-kappaB-dependent transcription. Nat Cell Biol 2012; 14:1270–81.
57. Koh CM, Khattar E, Leow SC, Liu CY, Muller J, Ang WX, et al. Telomerase regulates MYC-driven oncogenesis independent of its reverse transcriptase activity. J Clin Invest 2015;125:2109–22.
58. Li H, Engstrom K, Vahter M, Broberg K. Arsenic exposure through drinking water is associated with longer telomeres in peripheral blood. Chem Res Toxicol 2012;25:2333–9.
59. Shin JY, Choi YY, Jeon HS, Hwang JH, Kim SA, Kang JH, et al. Low-dose persistent organic pollutants increased telomere length in peripheral leukocytes of healthy Koreans. Mutagenesis 2010;25:511–6.
60. Bassig BA, Zhang L, Cawthon RM, Smith MT, Yin S, Li G, et al. Alterations in leukocyte telomere length in workers occupationally exposed to benzene. Environ Mol Mutagen 2014;55:673–8.
61. Dioni L, Hoxha M, Nordio F, Bonzini M, Tarantini L, Albetti B, et al. Effects of short-term exposure to inhalable particulate matter on telomere length, telomerase expression, and telomerase methylation in steel workers. Environ Health Perspect 2011;119:622–7.
62. Hou L, Wang S, Dou C, Zhang X, Yu Y, Zheng Y, et al. Air pollution exposure and telomere length in highly exposed subjects in Beijing, China: a repeated-measure study. Environ Int 2012;48:71–7.

63. Cui Y, Cai Q, Qu S, Chow WH, Wen W, Xiang YB, et al. Association of leukocyte telomere length with colorectal cancer risk: nested case-control findings from the Shanghai Women's Health Study. Cancer Epidemiol Biomark Prev 2012;21:1807–13.
64. Hemann MT, Strong MA, Hao LY, Greider CW. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. Cell 2001;107:67–77.
65. Xing J, Ajani JA, Chen M, Izzo J, Lin J, Chen Z, et al. Constitutive short telomere length of chromosome 17p and 12q but not 11q and 2p is associated with an increased risk for esophageal cancer. Cancer Prevention Res 2009;2:459–65.
66. Britt-Compton B, Rowson J, Locke M, Mackenzie I, Kipling D, Baird DM. Structural stability and chromosome-specific telomere length is governed by cis-acting determinants in humans. Human Mol Genet 2006;15:725–33.