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ARTICLE

Short Telomere Length, Cancer Survival, and Cancer Risk in 47 102 Individuals

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Background Recent meta-analyses have suggested that short telomere length was associated with increased risk of cancer. We therefore tested the hypotheses that short telomere length was associated with increased risk of cancer and with increased risk of early death after cancer.

Methods We measured leukocyte telomere length in a prospective study of 47 102 Danish general population participants from the Copenhagen City Heart Study and the Copenhagen General Population Study. Participants were followed for up to 20 years for cancer diagnosis and death. Follow-up was 100% complete. All statistical tests were two-sided.

Results Telomere length decreased linearly with increasing age (P <.001). During follow-up, we observed 3142 first cancers and, among these individuals, 1730 deaths. Decreasing quartiles of telomere length were associated with decreasing survival after cancer (log-rank P <.001). Multivariable-adjusted hazard ratios of early death were 1.31 (95% confidence interval [CI] = 1.14 to 1.52) in individuals in the quartile and 1.43 (95% CI = 1.13 to 1.80) in individuals in the decile with the shortest telomeres vs the longest. Unadjusted hazard ratios of cancer risk were 1.74 (95% CI = 1.58 to 1.93) and 2.00 (95% CI = 1.70 to 2.35) in individuals in the quartile and decile with the shortest vs longest telomeres; however, multivariable adjustment changed these hazard ratios to 0.98 (95% CI = 0.88 to 1.08) and 0.95 (95% CI = 0.80 to 1.11), mainly because of age adjustment.

Conclusions Short telomere length is associated with reduced survival after cancer but not with cancer risk. The latter contrasts with findings from recent meta-analyses.

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Telomeres are tandem repeats of TTAGGG nucleotides, 1500 to 15 000 base pairs long, which cap the end of linear chromosomes. On average, telomeres are shortened by 15 to 28 base pairs per year depending on mitotic activity of tissues and lifestyle (1–4). Among lifestyle factors, cancer risk factors such as smoking, adiposity, oxidative stress, ultraviolet irradiation, and low socioeconomic status have been associated with short telomere length (4–7). Short telomere length may thus, as a marker of cumulative cellular aging, be associated with increased risk of cancer and/or with increased risk of early death after cancer. Previous studies of risk of early death after cancer have been contradictive. The Bruneck Study examined 787 individuals followed for 15 years, observed 137 cancers and 62 cancer deaths, and reported a combined hazard ratio of 8.17 (95% confidence interval [CI] = 2.86 to 23.29) for individuals in the shortest vs the longest telomere tertile (8). Somewhat different were results from the Cardiovascular Health Study, which followed 1136 individuals for 6 years and reported 108 cancer deaths and a hazard ratio of death after cancer of 1.17 (95% CI = 0.65 to 2.10) for individuals in the shortest vs longest telomere quartile (9).

Measurement of telomere length is not trivial, and currently there is no agreement on which technique is the gold standard (10). Three fundamentally different groups of methods have evolved, each with its own scale of telomere length: fluorescence in situ hybridization–, Southern blot–, and quantitative polymerase chain reaction–based techniques. This technical obstacle has complicated meta-analyses approaches, and results about risk of many cancer types have so far been inconsistent (8,11–16). Nevertheless, recent meta-analyses suggested 1.4-fold to threefold increased risk of cancer in those with the shortest vs the longest telomeres (17,18). Furthermore, comparison between studies has also been limited by differences in study design, ethnicity, cancer types, and choice of tissue for telomere length measurement and by limited statistical power in previous studies. Thus, there is a need to examine the association between short vs long telomere length and risk of cancer and of cancer survival in a large prospective study.

We tested the hypotheses that short telomere length is associated with increased risk of cancer and with increased risk of early death after cancer in a prospective study of 47 102 individuals from the general population followed for up to 20 years.

Methods

Study Participants

Participants were included from two prospective studies of the Danish general population: the Copenhagen City Heart Study and the Copenhagen General Population Study (4,19,20). All participants were white and of Danish descent. None of the participants appeared in more than one study, which allowed us to combine the two similar general population studies to obtain maximal statistical power.

The Copenhagen City Heart Study was initiated in the period from 1976 to 1978 with follow-up examinations in the periods from 1981 to 1983, 1991 to 1994, and 2001 to 2003. Participants were randomly selected from the national Danish Civil Registration System to reflect the adult Danish population ranging from age 20 to 100 years. Participants in the present study are from the examinations performed during the periods from 1991 to 1994 and from 2001 to 2003, during which blood samples for DNA extraction were obtained and DNA samples for telomere length measurement were available for 9765 participants. Participation rate was 55%.

The Copenhagen General Population Study was initiated in 2003 with enrollment ongoing. Participants were selected and examined exactly as described for the Copenhagen City Heart Study. Blood samples for DNA extraction and telomere length measurement were available for 37 337 participants. Participation rate was 44%.

Both studies were approved by the institutional review boards and by Danish ethical committees (KF-V.100.2039/91, KF-01-144/01, H-KF-01-144/01) and were conducted according to the Declaration of Helsinki. Participants gave written informed consent.

Covariables

Before examination, participants filled in self-administered questionnaires about present and past lifestyle and health status. This questionnaire was completed together with an examiner at the day of examination and was followed by physical examination and blood sampling. The following covariables were obtained (21–24): age at examination in years, sex, current smoking (yes/no), cumulative smoking in pack-years (one pack-year was defined as 20 g of tobacco per day for a year), body mass index (measured weight in kilograms divided by squared measured height in meters), heavy alcohol intake (no/yes; yes if female and male participants reported a weekly alcohol intake >87.5 g and >175 g, respectively), and for women, also nulliparity (no/yes), postmenopausal status (no/yes), and use of hormone replacement therapy (no/yes). Follow up was 100% complete—that is, we did not lose track of even a single individual.

Ascertainment of Cancer Diagnosis and Deaths

Diagnoses of invasive cancer from January 1943 through December 2009 were obtained from the Danish Cancer Registry, which identifies 98% of all cancers diagnosed in Denmark (25,26). Cancer diagnoses were classified according to the World Health Organization International Classification of Diseases, Seventh Revision (ICD-7 codes 140–205) (27) and Tenth Revision (ICD-10 codes C00–D09), and grouped into 27 cancer standard types (20). The following covariables were obtained: cancer stage (localized, advanced, or unknown) and year at incident cancer diagnosis. Years from blood sampling to diagnosis of incident cancer and age at diagnosis were calculated. Information on deaths from the day of blood sampling until June 7, 2011, was obtained from the national Danish Civil Registration System.

Telomere Length

We measured telomere length in DNA extracted from leukocytes in peripheral blood. Telomere length was measured on a CFX384 real-time polymerase chain reaction detection system (Bio-Rad Laboratories, Copenhagen, Denmark), using a modified monochrome multiplex quantitative polymerase chain reaction method as previously described (4,28). Briefly, the telomere template was amplified simultaneously with the single-copy gene *ALB*, which encodes albumin, in the same well to adjust for DNA amount in the well. For each participant, quadruplicate reactions were carried out in 384-well plates. The telomere length was calculated from the mean of the four measurements using the ΔΔCt method, further normalized from plate to plate, and finally calibrated [for more details see Weischer et al. (4)] to absolute telomere length. Each lot of the calibrator lasted for approximately 10 000 measurements, and at change of calibrator lot, one 384-well plate with participant DNA was run twice (with old and new calibrator lot). Subsequently, the individual measurements were normalized across the five calibrator lots to obtain a functional single-calibrator measurement. Failed samples were measured a second round and a third if they failed again. Therefore, valid measurements of telomere lengths were available for more than 99.9% of participants. For all subsequent analyses, participants with telomere length longer than 15 000 basepairs (n = 6) were excluded as outliers. Measurements were blinded to cancer and vital status.

Assay Precision

The coefficient of variation was measured with the use of triplicates in each plate of DNA from the cell line NTERA-2, where ΔCt and the absolute telomere length were calculated exactly as for participant samples. Coefficients of variation and means were calculated for absolute telomere length. Coefficients of variation were 1.8% for Cttel (threshold cycle number for the telomere signal) at a mean level of 18.0, and 9.3% for absolute telomere length at a mean level of 2577 basepairs.

Statistical Analyses

We used the statistical software package STATA, version 11.1 (StataCorp, College Station, TX) for analysis. All statistical tests were two-sided. For trend test, participants were categorized according to decreasing telomere length in study-specific quartiles and deciles coded 1 to 4 and 1 to 10, with the first quartile or decile consisting of participants with the longest telomeres. The proportional hazards assumption was assessed visually by plotting –ln(–ln(survival)) vs ln(analysis time); no violations were observed. Information on age, sex, and year of birth was complete, whereas information on other covariables was more than 99% complete. Missing continuous covariables were imputed based on age and sex, whereas missing categorical values were assigned to a missing category.

For risk of early death after cancer, follow-up began at the day of incident cancer diagnosis and ended at death, emigration

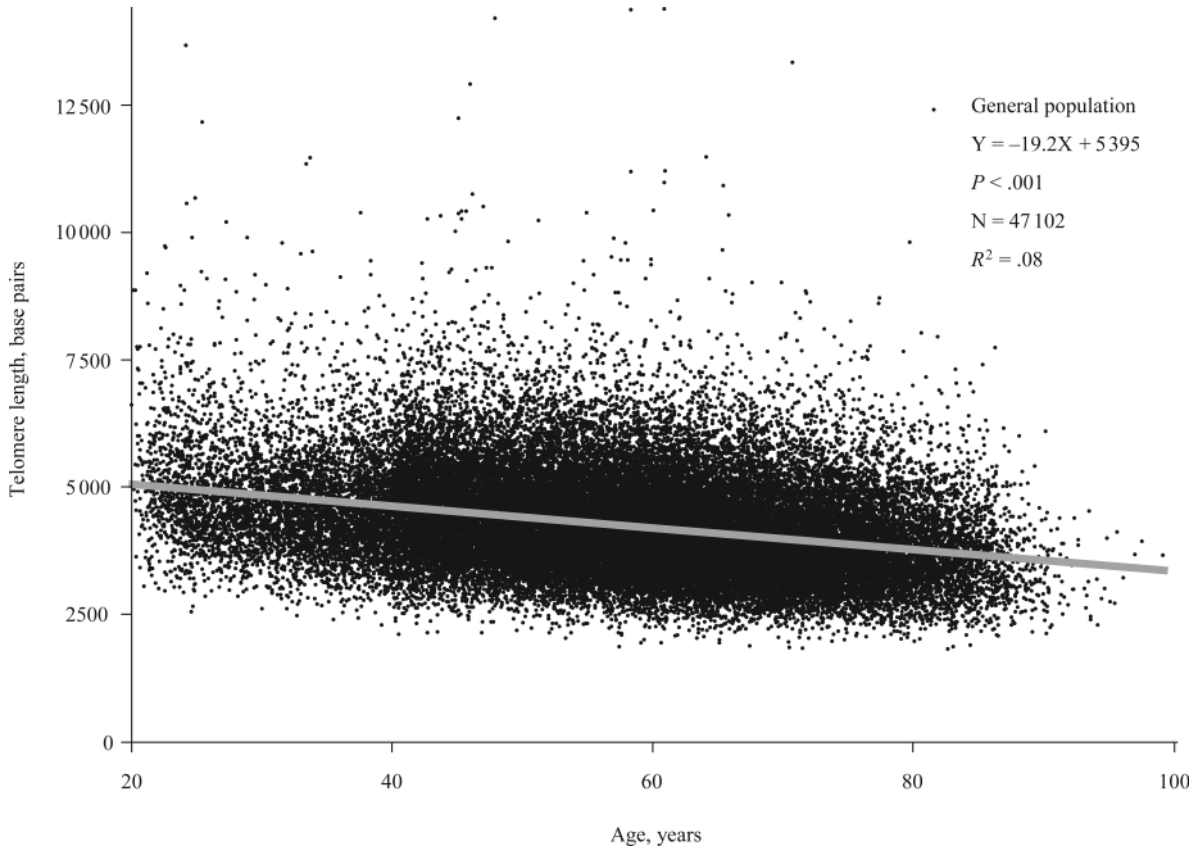

Figure 1. Telomere length in basepairs as a function of age in years at blood sampling. Linear regression is shown in equation and as a gray line. N = number of participants. *P* value and R^2 are for the correlation from the linear regression. Statistical tests were two-sided.

(n = 6 among cancer patients), or June 7, 2011, whichever came first. Participants diagnosed with cancer before study entry were excluded from analyses. Multivariable adjustment included age at incident cancer diagnosis (categorized in 5-year age intervals), sex, cancer stage, year of diagnosis, year of birth, and years from blood sampling to diagnosis of incident cancer. In analyses of sex-specific cancers, sex was not adjusted for.

For risk of cancer, follow-up began at the day of study entry and ended at death, emigration (n = 235 among all participants), or December 31, 2009, whichever came first. Thus for all cancer diagnoses, at least 18 months of follow-up were possible. Participants diagnosed with cancer before study entry were excluded from analyses, which is why the number of participants varies between cancer types. Multivariable adjustment included age at study entry (categorized in 5-year age-intervals), sex, year of birth, current smoking, cumulative smoking, body mass index, heavy alcohol intake, and, for female-specific cancers, postmenopausal status, use of hormone replacement therapy, and nulliparous state. In analyses of sex-specific cancers, sex was not adjusted for.

Results

Telomere Length

In 47 102 general population participants, we observed a 19–base pair decrease in telomere length per year of age (*P* <.001) (Figure 1). Variation in age explained 8% of the variation in telomere length (R^2 = 0.08). Characteristics of participants as a function of examination of specific telomere length quartiles are shown in Table 1. Besides age, short telomere length associated with male sex, current smoking, body mass index, heavy alcohol intake, and physical inactivity.

Telomere Length and Survival

During a maximum of 20 years of follow-up after blood sampling (median = 6 years), 3142 individuals from the general population were diagnosed with a first cancer and, among these, 1730 died. Decreasing quartiles of telomere length were associated with decreasing survival after cancer (log-rank *P* <.001) (Figure 2). Median times of survival after cancer were 9 years for individuals in the longest first quartile, 5 years in the second quartile, 4 years in the third quartile, and 2 years in the shortest fourth quartile.

Multivariable-adjusted hazard ratios of early death after cancer were 1.05 (95% CI = 0.90 to 1.23) for individuals in the second quartile, 1.13 (95% CI = 0.97 to 1.31) in the third quartile, and 1.31 (95% CI = 1.14 to 1.52) in the fourth quartile, compared with individuals in the first quartile of telomere length (P_{trend} <.001) (Table 2 and Figure 3). For individuals in the 10th decile, with the shortest telomere length, the multivariable-adjusted hazard ratio of early death was 1.43 (95% CI = 1.13 to 1.80) compared with individuals in the first decile of telomere length (Figure 3). In sex-stratified analysis, women and men in the fourth quartile had multivariable-adjusted hazard ratios of early death after cancer of 1.64 (95% CI = 1.12 to 1.74) and 1.07 (95% CI = 0.88 to 1.31) (Table 2). Sex interacted with quartile of telomere length on risk of early death

Table 1. Baseline characteristics of participants from the general population by quartiles of decreasing telomere length*

Characteristic	General population				P_{trend}†
	1st quartile	2nd quartile	3rd quartile	4th quartile	
Telomere length, kb	14.36–4.77	4.76–4.10	4.09–3.54	3.53–1.54	
No. of participants	11 751	11 754	11 820	11 777	
Age, median (range), years	56 (47–66)	59 (49–69)	63 (53–72)	68 (59–76)	<.001
Year of birth, median (range)	1951 (1941–1960)	1948 (1937–1958)	1943 (1933–1954)	1938 (1929–1948)	<.001
Men, No. (%)	5047 (43)	5232 (45)	5585 (47)	5792 (49)	<.001
Current smoking, No. (%)	3171 (27)	3203 (27)	3449 (29)	3409 (29)	<.001
Cumulative smoking, pack-years, median (range)	6 (0–25)	8 (0–27)	10 (0–31)	14 (0–35)	<.001
Body mass index, median (range), kg/m^2	25 (23–28)	25 (23–28)	26 (23–29)	26 (23–29)	<.001
Heavy alcohol intake, No. (%)‡	2507 (21)	2561 (22)	2646 (22)	2612 (22)	.003
Year of diagnosis of first cancer, median (range)	2002 (1995–2006)	2002 (1995–2006)	2001 (1995–2006)	2001 (1994–2006)	<.001
Women only, No. (%)					
Nulliparous	1172 (18)	1097 (17)	912 (15)	889 (15)	<.001
Postmenopausal	3634 (55)	4018 (62)	4499 (73)	5020 (85)	<.001
Hormone replacement therapy	856 (13)	947 (15)	964 (16)	1079 (19)	<.001

† *P* values <.001 are still statistically significant after adjusting for 12 comparisons.

* Covariables were more than 99% complete. Only measured values are shown. A *P* value for trend was calculated across the quartile categories treating them as an ordinal variable. Statistical tests were two-sided. kb = kilobasepairs.

‡ Weekly alcohol intake greater than 87.5 g in women and greater than 175 g in men.

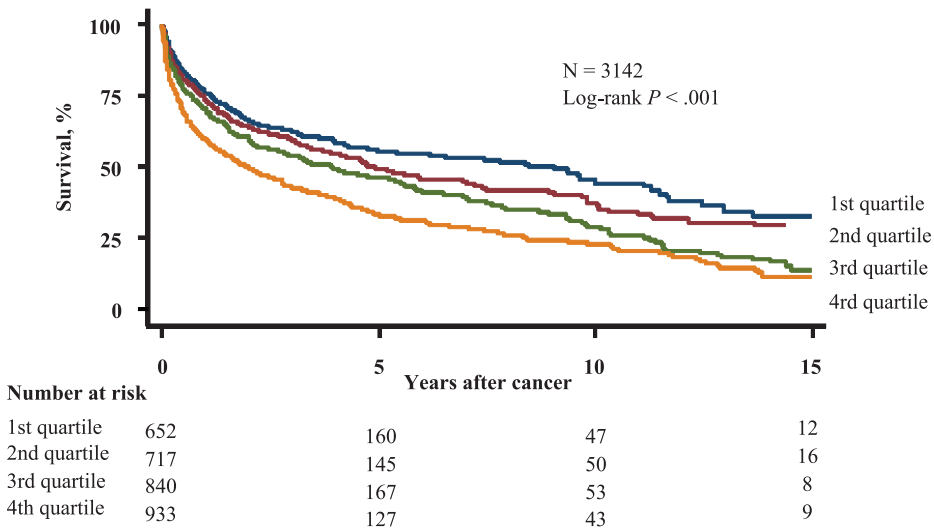

Figure 2. Survival in percent as a function of years after incident cancer by quartiles of telomere length. Individuals in the first quartile have the longest telomeres. Below the graph are numbers of individuals at risk. Log-rank test compared the survival function between the quartile categories and was two-sided.

after cancer at *P* equal to .01; however, if this was corrected for the four stratifications preformed using the Bonferroni method, the *P* value would be .01 × 4 = .04. In analysis stratified for cancer stage, individuals with localized and advanced cancer in the fourth quartile had multivariable-adjusted hazards ratios of early death after cancer of 1.86 (95% CI = 1.31 to 2.66) and 1.15 (95% CI = 0.89 to 1.47). Cancer stage interacted with quartile of telomere length on risk of early death after cancer at *P* equal to .003, which increased to *P* equal to.01 after correction for four multiple comparisons. Risks of early death across quartiles of telomere length remained roughly constant in different groups of age and time from blood sampling to cancer diagnosis.

Multivariable-adjusted hazard ratio of early death after cancer was 1.42 (95% CI = 1.13 to 1.80) in individuals in the decile with the shortest telomeres vs individuals in the decile with the longest telomeres (P_{trend} <.001) (Figure 3). The multivariable-adjusted hazard ratio of early death after cancer was 1.12 (95% CI = 1.06 to 1.18) per 1000 base pair decrease in telomere length (Figure 4). In analysis stratified for cancer type, multivariable-adjusted hazard ratios of early death were 1.27 (95% CI = 1.13 to 1.43) after lung cancer, 1.67 (95% CI = 1.12 to 2.48) after melanoma, 1.57 (95% CI = 1.07 to 2.32) after leukemia, and 0.42 (95% CI = 0.24 to 0.74) after esophagus cancer, whereas risk of early death after other individual cancers did not reach statistical significance.

Telomere Length and Risk of Cancer

Unadjusted hazard ratios of any cancer were 1.74 (95% CI = 1.58 to 1.93) for individuals in the quartile and 2.00 (95% CI = 1.70 to 2.35) for individuals in the decile with shortest telomere length, compared with individuals in the quartile and decile with the

Table 2. Risk of early death after cancer according to quartile of telomere length*

Characteristic	Cancer patients, No.	Deaths, No.	Multivariable adjusted hazard ratio (95% confidence interval) 1st quartile	2nd quartile	3rd quartile	4th quartile	P_{trend}	$P_{interaction}$
All	3142	1730	1.00	1.05 (0.90 to 1.23)	1.13 (0.97 to 1.31)	1.31 (1.14 to 1.52)	<.001	
Sex								
Men	1503	936	1.00	0.90 (0.72 to 1.12)	0.90 (0.74 to 1.11)	1.07 (0.88 to 1.31)	.25	.01
Women	1639	794	1.00	1.22 (0.98 to 1.53)	1.40 (1.12 to 1.74)	1.64 (1.12 to 1.74)	<.001	
Cancer stage								
Localized	633	355	1.00	1.32 (0.91 to 1.92)	1.59 (1.12 to 2.27)	1.86 (1.31 to 2.66)	.32	.003†
Advanced	701	624	1.00	1.13 (0.87 to 1.48)	1.18 (0.91 to 1.53)	1.15 (0.89 to 1.47)	.27	
Unknown	1808	751	1.00	0.99 (0.77 to 1.27)	1.03 (0.81 to 1.30)	1.36 (1.08 to 1.72)	.01	
Age at diagnosis								
≤70 years	1528	637	1.00	1.16 (0.92 to 1.46)	1.16 (0.92 to 1.46)	1.45 (1.16 to 1.81)	.002	.33
>70 years	1614	1093	1.00	1.05 (0.85 to 1.30)	1.18 (0.97 to 1.43)	1.39 (1.15 to 1.67)	<.001	
Time from blood sampling to cancer								
0–3 years	1331	622	1.00	0.97 (0.74 to 1.28)	1.16 (0.90 to 1.49)	1.44 (1.13 to 2.83)	<.001	.91
>3 to ≤ 6 years	826	429	1.00	0.93 (0.68 to 1.27)	0.89 (0.66 to 1.20)	1.16 (0.86 to 1.57)	.29	
>6 to ≤ 9 years	329	258	1.00	1.22 (0.80 to 1.85)	1.27 (0.85 to 1.89)	1.40 (0.96 to 2.06)	.09	
>9 years	656	421	1.00	1.20 (0.88 to 1.65)	1.28 (0.94 to 1.74)	1.31 (0.97 to 1.77)	.08	

* Individuals in the 1st quartile have the longest telomeres. A *P* value for trend was calculated across the quartile categories treating them as an ordinal variable. Statistical tests were two-sided.

† This test excluded those with unknown cancer stage.

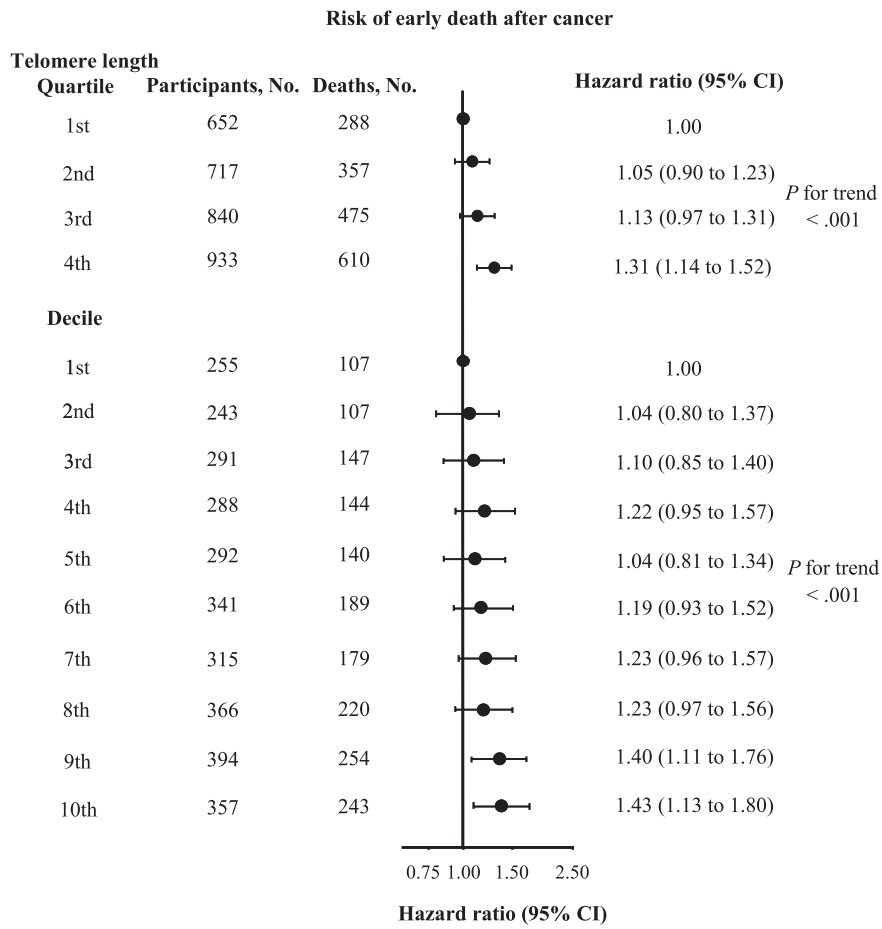

Figure 3. Risk of early death after incident cancer by quartiles and deciles of telomere length. Individuals in the first quartile or decile have the longest telomeres. Multivariable adjustment included age at incident cancer diagnosis, sex, cancer stage, year of diagnosis, year of birth, and years from blood sampling to diagnosis of incident cancer. A *P* value for trend was calculated across the quantile categories, treating them as an ordinal variable. Statistical tests were two-sided. CI = confidence interval.

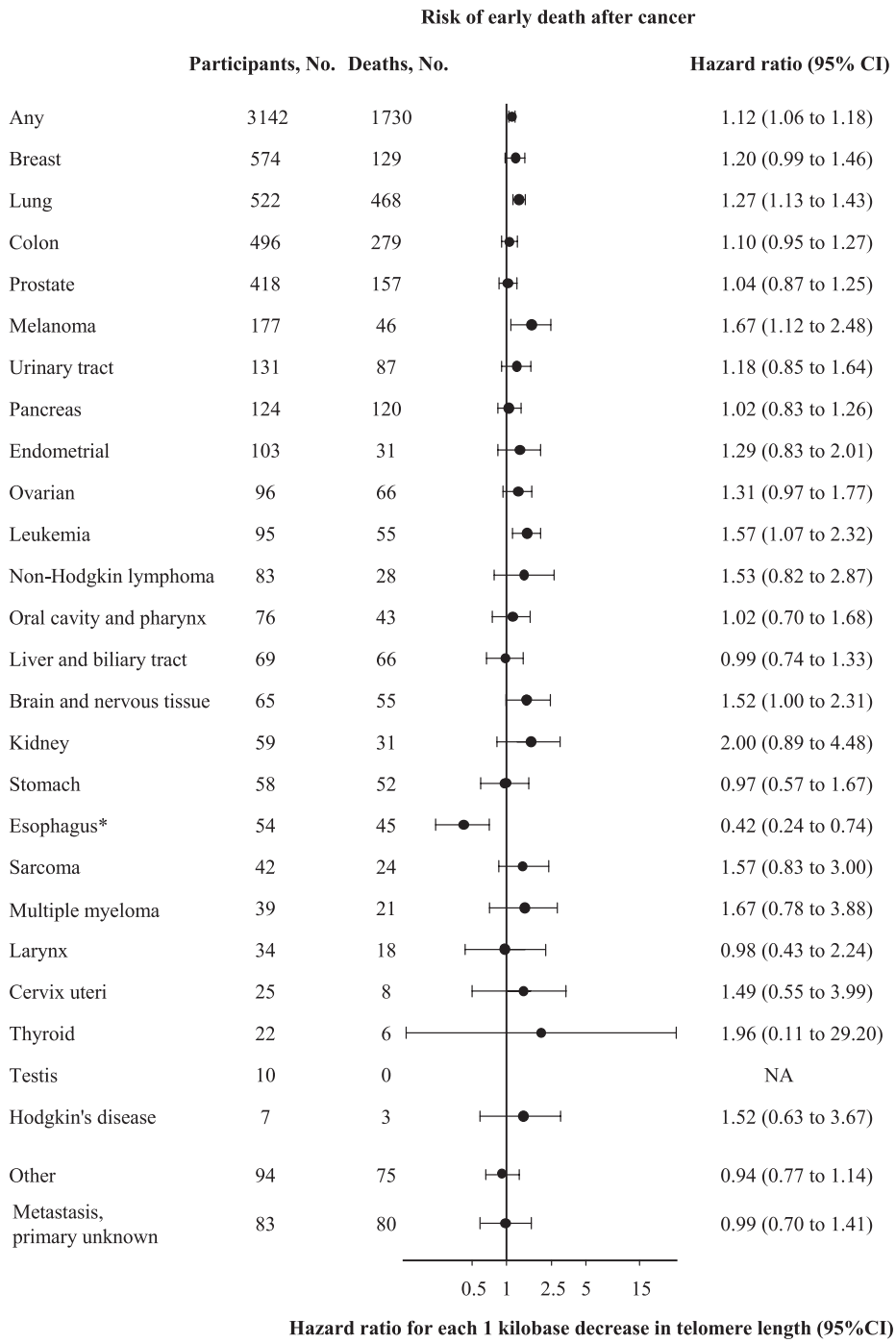

Figure 4. Risk of early death after any incident cancer and after individual incident cancers per 1000 base-pairs decrease in telomere length. Cancers are ranked by number of participants with the diagnosis, except for the groups "Other" and "Metastasis, primary unknown" listed at the bottom. Sum of deaths after individual cancers exceeds the number of deaths after any cancer because some participants had more than one cancer. Multivariable adjustment included age at incident cancer diagnosis, sex, cancer stage, year of diagnosis, year of birth, and years from blood sampling to diagnosis of incident cancer. In analyses of sex-specific cancers, sex was not adjusted for. Statistical tests were two-sided. CI = confidence interval; NA = not available. *P = .002.

longest telomeres (P_{trend} <.001 for both) (Figure 5). Multivariable adjustment changed these hazard ratios to 0.98 (95% CI = 0.88 to 1.08) and 0.95 (95% CI = 0.80 to 1.11) (P_{trend} = 0.63 and 0.69, respectively). Age adjustment was the main reason why the multivariable adjustment reduced the hazard ratio of cancer risk by quartiles of telomere length to a point of statistical nonsignificance (Supplementary Figure 1, available online). The association of cancer risk did not depend on the length of the follow-up time after blood sampling (Supplementary Table 1, available online).

Per 1000 base-pairs decrease in telomere length, the multivariable-adjusted hazard ratio of any first cancer was 0.99 (95% CI = 0.95 to 1.03) (Figure 6). We had 90% statistical power to

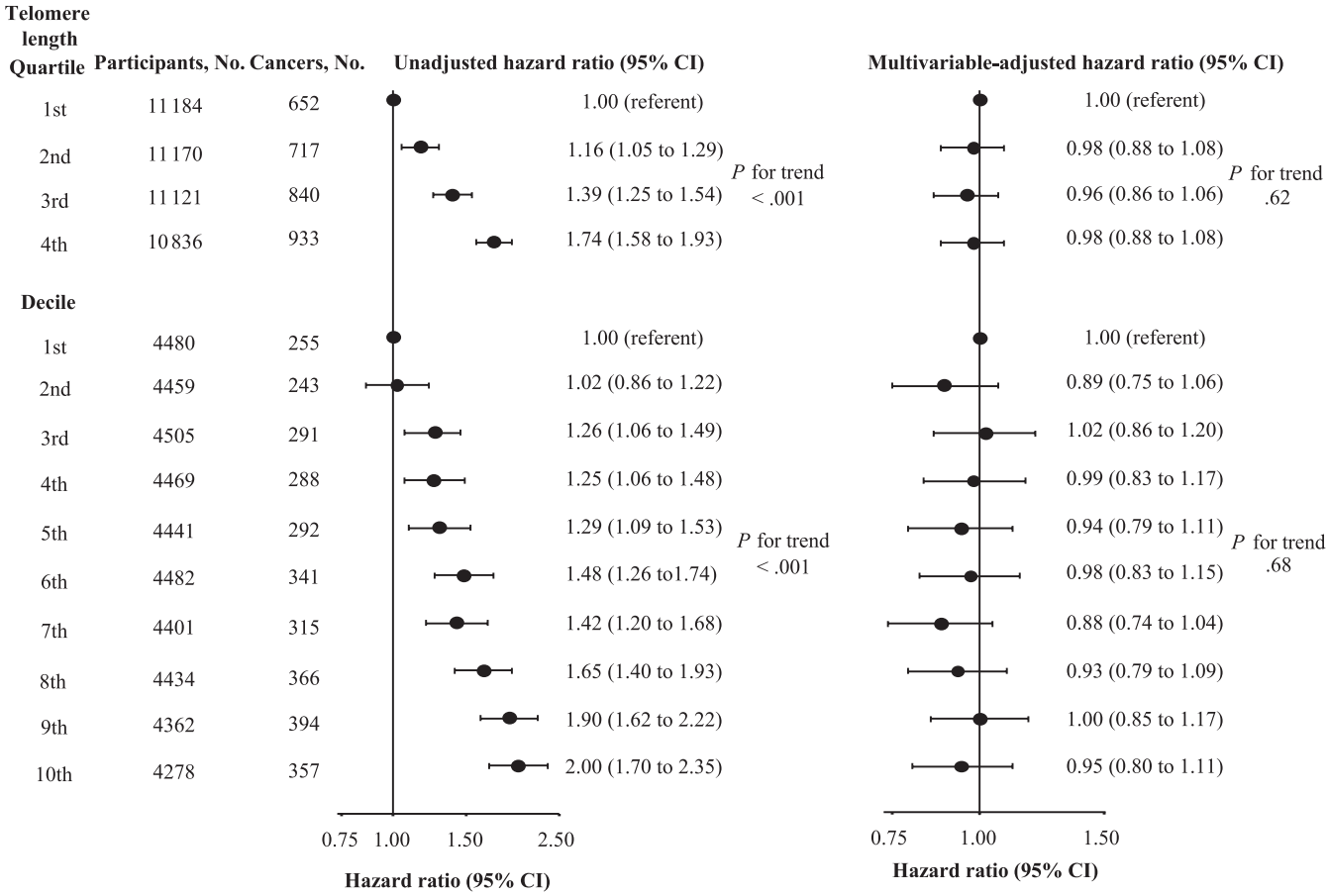

Figure 5. Risk of incident cancer by quartiles and deciles of telomere length. Individuals in the first quartile or decile have the longest telomeres. Numbers are smaller than the numbers in Table 1 because participants with any prevalent cancer were excluded from these analyses. Multivariable adjustment included age at blood sampling, sex, year of birth, current smoking, cumulative smoking, body mass index, and heavy alcohol intake. A *P* value for trend was calculated across the quantile categories, treating them as an ordinal variable. Statistical tests were two-sided. CI = confidence interval.

exclude corresponding hazard ratios at 0.97 or below and/or at 1.03 or above.

Discussion

After measuring telomere length in 47 102 individuals from the general population followed for up to 20 years, we observed that short telomere length was associated with increased risk of early death after cancer but not with cancer risk.

We confirmed a strong linear correlation between decreasing telomere length and increasing age, demonstrating the validity of our quantitative polymerase chain reaction assay. We found telomere length to decrease by 19 base pairs per year in the general population, which is similar to previous reports (1–3).

For risk of early death after cancer, the observed association between short telomere length and increased risk differed from that reported by one of two previous studies, both smaller than this study (8,9). Following 47 102 individuals prospectively for up to 20 years, we detected 3142 individuals with incident cancer; among these individuals, 1730 deaths after cancer; and a hazard ratio of early death after cancer of 1.31 (95% CI = 1.14 to 1.52) for individuals in the shortest vs longest telomere quartile. Somewhat different, the Bruneck Study, a prospective study of 787 individuals followed for 15 years, observed 137 cancers and 62 cancer deaths and reported a combined hazard ratio of 8.17 (95% CI = 2.86 to 23.29) for individuals in the shortest vs the longest telomere tertile (8). However, the Cardiovascular Health Study, a prospective study of 1136 individuals followed for 6 years, reported 108 cancer deaths and a hazard ratio of death after cancer of 1.17 (95% CI = 0.65 to 2.10) for individuals in the shortest vs longest telomere quartile (9). These differences in risk estimates may reflect differences in telomere measurement, differences in sex or cancer stage distribution, both of which we found possibly to interact with telomere length on risk of early death after cancer, or chance findings. In our study, telomere length was associated with early death after lung cancer, malignant melanoma, leukemia, and esophagus cancer in stratified analyses. However, numbers were low, and future studies may determine whether these associations are real or represent chance findings.

For risk of cancer, we observed a strong association with telomere length when not adjusting for covariables, with an up to twofold increased risk of cancer in individuals with the shortest telomere length decile compared with the longest. However, after adjustment for age and other cancer risk factors available, the association disappeared. We found that age adjustment was the main reason why the multivariable adjustment reduced the hazard ratio of cancer by

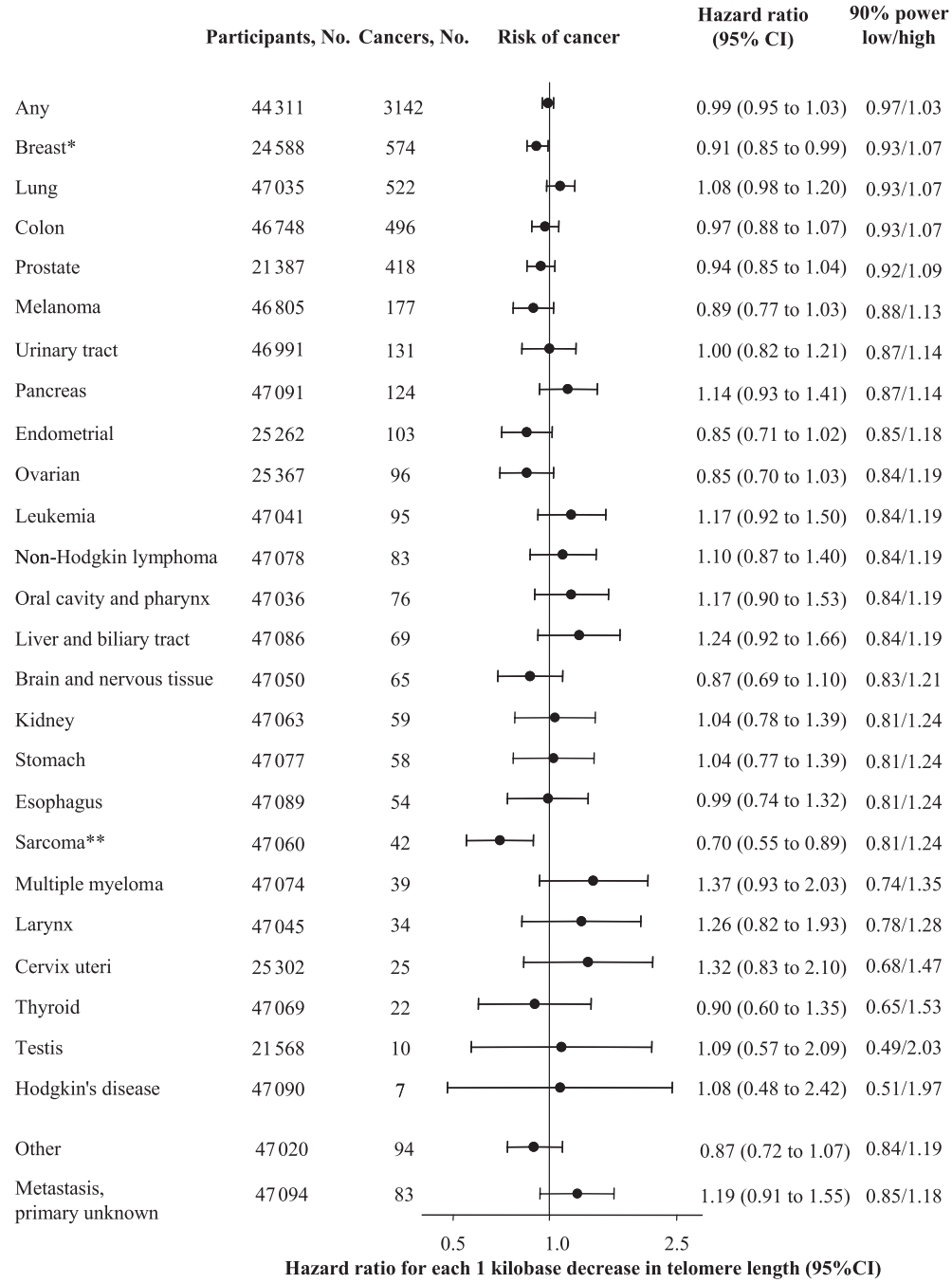

Figure 6. Risk of any incident cancer and individual incident cancers per 1000 base-pairs decrease in telomere length and the risk reduction (low) and risk increase (high) we had 90% power to detect. Cancers are ranked by number of participants with the diagnosis, except for the groups "Other" and "Metastasis, primary unknown" listed at the bottom. Sum of individual cancers exceeds the number of any cancer because some participants had more than one cancer. Multivariable adjustment included age at blood sampling, sex, year of birth, current smoking, cumulative smoking, body mass index, heavy alcohol intake, and, for female-specific cancers, nulliparous state, postmenopausal status, and use of hormone replacement therapy. In analyses of sex-specific cancers, sex was not adjusted for. Statistical tests were two-sided. CI = confidence interval. *P = .04. **P = .003.

quartile of telomere length to insignificance. This finding contrasts with the majority of previous reports, which recently were combined in two meta-analyses (17,18). The first combined data from 21 studies with a total of 11 255 case subjects and 13 101 control subjects and reported a pooled odds ratio of cancer for individuals with the shortest vs longest telomeres of 1.35 (95% CI = 1.14 to 1.60). The second meta-analysis (18) reported pooled odds ratios of cancer for individuals with the shortest telomeres vs the longest of 2.90 (95% CI = 1.75 to 4.80) in retrospective studies and of 1.16 (95% CI = 0.87 to 1.54) in prospective studies. Among prospective studies, follow-up ranged from 5 to 14 years, thus shorter than the maximal follow-up in this study of 20 years. Both meta-analyses noted considerable heterogeneity across studies. It is plausible that cancer itself and/or cancer treatment can reduce telomere length,

and thus the associations reported in both meta-analyses between short telomere length and cancer risk may be because of reverse causation. Alternatively, the differences in risk estimates between the two meta-analyses and this study may reflect interpopulation variation, differences in telomere length measurement, or chance findings in many previous studies because these studies were considerably smaller than this study. Accordingly, publication bias of positive results in smaller studies is a well-known phenomenon (29,30), as authors themselves, reviewers, and editors in general favor publication of positive rather than negative results. In our study, short telomere length was associated with reduced risk of breast cancer and sarcoma. However, numbers were low, and future studies may determine whether these associations are real or represent chance findings. In this study, we had 90% statistical power to exclude hazard ratios at 0.97 or below and/or at 1.03 or above per 1000 base-pairs decrease in telomere length for risk of any cancer.

Strengths of this study include the large study size of 47 102 participants from the general population followed prospectively for up to 20 years. Second, telomere length was measured with a very precise assay, and the measurements were validated through inverse association with age. Third, telomere length was measured on more than 99.9% of available participants because of several rounds of reruns. Fourth, because of the national Danish Civil Registration System and the national Danish Patient Registry, we had complete information on death and cancer diagnosis without loss to follow-up of even a single individual.

Limitations of this study include that we examined risk of early death after cancer and cancer risk in whites only, and our results may therefore not necessarily be applicable to other ethnicities. Also, we measured telomere length in leukocytes from peripheral blood only and not in all cell types in the body; however, leukocyte telomere length correlates highly with that in cells from other tissues (5,31–33).

In conclusion, short telomere length is associated with increased risk of early death after cancer but not with cancer risk. The latter contrasts with findings in recent meta-analyses.

References

1. Brouilette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol*. 2003;23(5):842–846.
2. Cherkas LF, Aviv A, Valdes AM, et al. The effects of social status on biological aging as measured by white-blood-cell telomere length. *Aging Cell*. 2006;5(5):361–365.
3. Maubaret CG, Salpea KD, Jain A, et al. Telomeres are shorter in myocardial infarction patients compared to healthy subjects: correlation with environmental risk factors. *J Mol Med*. 2010;88(8):785–94.
4. Weischer M, Bojesen SE, Cawthon RM, et al. Short telomere length, myocardial infarction, ischemic heart disease, and early death. *Arterioscler Thromb Vasc Biol*. 2012;32(3):822–829.
5. Butt HZ, Atturu G, London NJ, Sayers RD, Bown MJ. Telomere length dynamics in vascular disease: a review. *Eur J Vasc Endovasc Surg*. 2010;40(1):17–26.
6. Cherkas LF, Hunkin JL, Kato BS, et al. The association between physical activity in leisure time and leukocyte telomere length. *Arch Intern Med*. 2008;168(2):154–158.
7. Han J, Qureshi AA, Prescott J, et al. A prospective study of telomere length and the risk of skin cancer. *J Invest Dermatol*. 2009;129(2):415–421.
8. Willeit P, Willeit J, Kloss-Brandstatter A, Kronenberg F, Kiechl S. Fifteen-year follow-up of association between telomere length and incident cancer and cancer mortality. *JAMA*. 2011;306(1):42–44.
9. Fitzpatrick AL, Kronmal RA, Kimura M, et al. Leukocyte telomere length and mortality in the Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci*. 2011;66(4):421–429.
10. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res*. 2002;30(10):e47.
11. Hou L, Zhang X, Gawron AJ, Liu J. Surrogate tissue telomere length and cancer risk: shorter or Longer? *Cancer Lett*. 2012;319(2):130–135.
12. Liang G, Qureshi AA, Guo Q, De V, I, Han J. No association between telomere length in peripheral blood leukocytes and the risk of non-melanoma skin cancer. *Cancer Epidemiol Biomarkers Prev*. 2011;20(5):1043–1045.
13. Liu Z, Ma H, Wei S, Li G, Sturgis EM, Wei Q. Telomere length and TERT functional polymorphisms are not associated with risk of squamous cell carcinoma of the head and neck. *Cancer Epidemiol Biomarkers Prev*. 2011;20(12):2642–2645.
14. 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 Biomarkers Prev*. 2007;16(4):815–819.
15. Mirabello L, Huang WY, Wong JY, et al. The association between leukocyte telomere length and cigarette smoking, dietary and physical variables, and risk of prostate cancer. *Aging Cell*. 2009;8(4):405–413.
16. Willeit P, Willeit J, Mayr A, et al. Telomere length and risk of incident cancer and cancer mortality. *JAMA*. 2010;304(1):69–75.
17. Ma H, Zhou Z, Wei S, et al. Shortened telomere length is associated with increased risk of cancer: a meta-analysis. *PLoS One*. 2011;6(6):e20466.
18. Wentzensen IM, Mirabello L, Pfeiffer RM, Savage SA. The association of telomere length and cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2011;20(6):1238–1250.
19. Allin KH, Nordestgaard BG, Zacho J, Tybjaerg-Hansen A, Bojesen SE. C-reactive protein and the risk of cancer: a mendelian randomization study. *J Natl Cancer Inst*. 2010;102(3):202–206.
20. Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG. Integrin beta3 Leu33Pro homozygosity and risk of cancer. *J Natl Cancer Inst*. 2003;95(15):1150–1157.
21. Freiberg JJ, Tybjaerg-Hansen A, Jensen JS, Nordestgaard BG. Nonfasting triglycerides and risk of ischemic stroke in the general population. *JAMA*. 2008;300(18):2142–2152.
22. Kamstrup PR, Tybjaerg-Hansen A, Steffensen R, Nordestgaard BG. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. *JAMA*. 2009;301(22):2331–2339.
23. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA*. 2007;298(3):299–308.
24. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. *N Engl J Med*. 2008;359:(18)1897–1908.
25. Storm HH. Completeness of cancer registration in Denmark 1943–1966 and efficacy of record linkage procedures. *Int J Epidemiol*. 1988;17(1):44–49.
26. Storm HH, Michelsen EV, Clemmensen IH, Pihl J. The Danish Cancer Registry—history, content, quality and use. *Dan Med Bull*. 1997;44(5):535–539.
27. World Health Organization. *Third Report of the Expert Committee on Health Statistics*. Geneva, Switzerland: World Health Organization; 1952.
28. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res*. 2009;37(3):e21.
29. Ioannidis JP. Why most published research findings are false. *PLoS Med*. 2005;2(8):e214.
30. Kyzas PA, Loizou KT, Ioannidis JP. Selective reporting biases in cancer prognostic factor studies. *J Natl Cancer Inst*. 2005;97(14):1043–1055.
31. Friedrich U, Griese E, Schwab M, Fritz P, Thon K, Klotz U. Telomere length in different tissues of elderly patients. *Mech Ageing Dev*. 2000;119(3):89–99.
32. Okuda K, Bardeguez A, Gardner JP, et al. Telomere length in the newborn. *Pediatr Res*. 2002;52(3):377–381.
33. Wilson WR, Herbert KE, Mistry Y, et al. Blood leucocyte telomere DNA content predicts vascular telomere DNA content in humans with and without vascular disease. *Eur Heart J*. 2008;29(21):2689–2694.

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