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# Telomere Length and Risk of Incident Cancer and Cancer Mortality

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**T**ELOMERES ARE NUCLEOPROTEIN complexes at the extreme ends of linear chromosomes implicit in the maintenance of chromosomal integrity. Telomeres shorten with each cell cycle and therefore reflect organism aging at a cellular level. At a critically short telomere length, cells experience replicative cell senescence. Malignant cells, in contrast, reactivate and overexpress the enzyme telomerase that lengthens telomeres and allows for plentiful divisions.

While telomere elongation is a common molecular feature of advanced malignancies, there are several lines of evidence from experimental animals, basic research, genome-wide association studies, and rare disorders of telomere maintenance<sup>1-5</sup> suggesting that short telomeres and concurrent chromosomal instability contribute to malignant cell transformation.<sup>3,6</sup> Proof of concept for this intriguing hypothesis remains to be established from an epidemiological perspective. Current evidence mainly derives from case-control studies and is inconsistent for various specific types of cancer.<sup>7-15</sup> Few evaluations have been prospective in design and have measured telomere length well in advance of cancer manifestation; all of the studies, to date, were conducted in selected patient groups or healthy volunteers re-

**Context** Telomeres are essential to preserve the integrity of the genome. Critically short telomeres lead to replicative cell senescence and chromosomal instability and may thereby increase cancer risk.

**Objective** To determine the association between baseline telomere length and incident cancer and cancer mortality.

**Design, Setting, and Participants** Leukocyte telomere length was measured by quantitative polymerase chain reaction in 787 participants free of cancer at baseline in 1995 from the prospective, population-based Bruneck Study in Italy.

**Main Outcome Measures** Incident cancer and cancer mortality over a follow-up period of 10 years (1995-2005 with a follow-up rate of 100%).

**Results** A total of 92 of 787 participants (11.7%) developed cancer (incidence rate, 13.3 per 1000 person-years). Short telomere length at baseline was associated with incident cancer independently of standard cancer risk factors (multivariable hazard ratio [HR] per 1-SD decrease in log<sub>e</sub>-transformed telomere length, 1.60; 95% confidence interval [CI], 1.30-1.98; *P* < .001). Compared with participants in the longest telomere length group, the multivariable HR for incident cancer was 2.15 (95% CI, 1.12-4.14) in the middle length group and 3.11 (95% CI, 1.65-5.84) in the shortest length group (*P* < .001). Incidence rates were 5.1 (95% CI, 2.9-8.7) per 1000 person-years in the longest telomere length group, 14.2 (95% CI, 10.0-20.1) per 1000 person-years in the middle length group, and 22.5 (95% CI, 16.9-29.9) per 1000 person-years in the shortest length group. The association equally applied to men and women and emerged as robust under a variety of circumstances. Furthermore, short telomere length was associated with cancer mortality (multivariable HR per 1-SD decrease in log<sub>e</sub>-transformed telomere length, 2.13; 95% CI, 1.58-2.86; *P* < .001) and individual cancer subtypes with a high fatality rate.

**Conclusion** In this study population, there was a statistically significant inverse relationship between telomere length and both cancer incidence and mortality.

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cruited among health professionals. Moreover, there is a considerable paucity of data regarding cancer mortality.

The aim of this study was to assess the association between leukocyte telomere length and risk of both cancer manifestation and cancer mortality in the prospective, population-based Bruneck Study with near complete participation and follow-up, and detailed information on all types of new-onset cancer available.

## METHODS

The Bruneck Study is a prospective, population-based survey conducted in 1000 individuals aged 40 to 79 years (125

per sex and decade of life), who were randomly selected from the inhabitants of the town of Bruneck (South Tyrol, Italy) and examined every 5 years starting from 1990.<sup>16-19</sup> Details are provided in the

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eMethods at <http://www.jama.com>. eFigure 1 shows the inclusion criteria. The 1995 evaluation served as the baseline for this study. Of the 826 men and women participating in 1995, 26 were excluded due to missing DNA and 13 were excluded due to presence of cancer. Complete follow-up data on clinical end points occurring between 1995 and 2005 were available for the remaining 787 participants. The study was approved by the ethics committee and all participants provided written informed consent.

All participants underwent a comprehensive clinical and laboratory examination as part of the study protocol. Standard risk factors were recorded by validated standard procedures (see eMethods at <http://www.jama.com>). Venous blood samples were taken after an overnight fast and 12 hours abstinence from smoking and were immediately frozen or processed. All laboratory parameters were quantified by standard methods.<sup>17</sup> Extraction of DNA was performed following standard procedures using the Invisorb Blood Universal Kit (Invitex, Berlin, Germany).

Leukocyte telomere length was measured using a quantitative polymerase chain reaction (PCR) technique developed by Cawthon<sup>20</sup> that compares signals from telomere repeat copy number, *T*, to a single-copy gene copy number, *S*, and allows calculation of a relative *T/S* ratio. Standard primers (tel1b: 5'-CGGTTT GTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3'; tel2b: 5'-GGCTTGCCCT ACCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3') and cycling conditions were applied, whereas protocols were modified with regard to control samples and data processing. Specifically, reaction efficiencies were determined using the steepness of the fluorescence increase during quantitative PCR cycles (efficiency =  $10^{\text{slope} - 1}$ ) and were used to calculate *T/S* ratios corrected for between-individual and between-gene differences in efficiency (corrected *T/S* ratio =  $[(1 + \text{efficiency}_{\text{tel, sample}})^{-\text{Ct}(\text{tel, sample})}] / [(1 + \text{Efficiency}_{\text{reference gene, sample}})^{-\text{Ct}(\text{reference gene, sample})}] / [(1 + \text{Efficiency}_{\text{tel, control}})^{-\text{Ct}(\text{tel, control})}] / [(1 + \text{Efficiency}_{\text{reference gene, control}})^{-\text{Ct}(\text{reference gene, control})}]$ ) (see

eMethods at <http://www.jama.com> for details). The reference gene used was 36B4.

Telomere length measurement was performed in quadruplicate for the 1995 and 2005 samples with the same individual's samples positioned directly next to each other. Standard DNA was located on each quantitative PCR plate to account for interplate measurement variation. As shown previously,<sup>21</sup> telomere length measurement by quantitative PCR was highly reproducible and correlated with Southern Blot–based mean telomere length restriction fragments. Both techniques produce comparable estimates of telomere length<sup>20</sup> (see eFigure 2 at <http://www.jama.com>), but Southern blot assessment includes the subtelomeric region. For practical reasons, quantitative PCR has become the favorite technique in epidemiological telomere research over the past year, but superiority in terms of risk prediction has not been established for either of these methods.

New-onset cancers were identified by patient self-report and screening of information provided by general practitioners, death certificates, and Bruneck Hospital files. Participation rates in the evaluations from 2000 and 2005 exceeded 90% (among survivors). Full medical information for screening was available in 100% of participants. Specifically, all reports from radiological and endoscopic examinations were searched as well as the hospital's medical records. A major strength of the Bruneck Study is that virtually all participants living in the survey area are referred to the same local hospital. The network that exists between the hospital and local general practitioners allows retrieval of practically all medical information ever taken on the study participants. Bruneck Hospital houses the only radiological and surgical department in the region and the only facility for ambulatory and in-hospital chemotherapy.

Ascertainment of cancer did not rely on hospital discharge codes or the patient self-report, but was obligatorily based on a careful review of the respective medical records containing infor-

mation on pathological and histological workup. All incident cancers were considered except nonmelanoma skin cancer.

Statistical calculations were performed using SPSS version 15.0 (SPSS Inc, Chicago, Illinois) and Stata version 10 (Stata Corp, College Station, Texas) and adhered to predefined protocols. Continuous variables are presented as means with 95% confidence intervals (CIs) or medians with interquartile ranges. Dichotomous variables are presented as numbers and percentages.

To facilitate comparison with previous reports, relative *T/S* ratios are depicted as means with 95% CIs despite a modest deviation from a normal distribution. Log-transformed *T/S* ratios were used in all analyses. The associations between telomere length and cancer risk factors, lifestyle characteristics, and other parameters were estimated by comparing variable levels across telomere length tertile groups. Cutoff values for telomere length tertile groups were defined based on the entire study population (*N* = 800).

Person-years of follow-up for each participant accrued from the 1995 baseline until cancer diagnosis, death, or October 1, 2005, whichever came first (median follow-up, 120 months). Cox proportional hazard models were used to assess the association between telomere length and both cancer incidence and mortality. Participants who experienced a cancer event were censored with respect to subsequent follow-up. The proportional hazard assumption was confirmed for telomere length by testing the interaction of telomere length with a function of survival time (Cox models with time-dependent covariates).

Separate analyses treated telomere length as a continuous variable or applied telomere length tertile groups. Separate models were fit for cancer incidence and mortality. To facilitate comparison with cancer mortality, analyses on mortality from cardiovascular and other causes were conducted in the same population free of baseline cancer (*n* = 787). Differential associations in sexes and other subgroups were ana-

lyzed by inclusion of appropriate interaction terms. All multivariable models included the variables of age, sex, social class, pack-years of smoking, alcohol consumption, diabetes, physical activity, body mass index (calculated as weight in kilograms divided by height in meters squared), log<sub>e</sub>-transformed high-sensitivity C-reactive protein, vitamin D, and low-density lipoprotein cholesterol (model 1) and interleukin 6 and chronic infection (model 2).

To compare the relevance of chronological age with biological age (reflected by telomere length), the number of years generating a hazard ratio (HR) of incident cancer identical to that assessed for a 1-SD decrease in telomere length were calculated by dividing the term  $\beta_{\text{telomere length}} \times \text{SD}_{\text{telomere length}}$  by  $\beta_{\text{age}}$  with both  $\beta$  coefficients derived from the same multivariable Cox regression equation. Supplementary analyses were fit after exclusion of participants with prior cancer (n=33) or had a focus on solid vs hematopoietic tumors and on individual cancers. To facilitate interpretation of the findings, power analyses for multivariable Cox proportional hazard models were performed. eFigure 3 (<http://www.jama.com>) displays the minimum HR detectable with a power of 80% ( $\alpha = .05$  for 1-sided hypothesis testing) as a function of the number of events. Finally, to rule out reverse causation (ie, effects of preclinical cancer on telomere length), separate Cox models were confined to cancers diagnosed more than 5 years after DNA sampling (2000-2005). A 2-sided *P* value of less than .05 was considered significant.

## RESULTS

During the 10-year follow-up, 92 of 787 participants (11.7%) developed cancer (incidence rate, 13.3 per 1000 person-years; 95% CI, 10.8-16.3); in 44 of whom it was fatal (mortality rate, 6.2 per 1000 person-years; 95% CI, 4.6-8.3). TABLE 1 shows the incidence of individual cancers with their corresponding fatality rates. Compared with participants who remained free of cancer, cases were older,

**Table 1.** Spectrum of Incident Cancers in the Bruneck Study (1995-2005)<sup>a</sup>

Type of Cancer	No. of Cases (n = 92)	Incidence/1000 Person-Years (95% CI)	Fatal Course, No. (95% CI) <sup>b</sup>
Breast cancer	12	1.74 (0.90-3.04)	1 (0.03-5.57)
Gastric cancer	11	1.59 (0.80-2.85)	6 (2.20-13.06)
Lung cancer	10	1.45 (0.69-2.66)	9 (4.12-17.08)
Prostate cancer	10	1.45 (0.69-2.66)	2 (0.24-7.22)
Colorectal carcinoma	8	1.16 (0.50-2.28)	3 (0.62-8.77)
Lymphoma	7	1.01 (0.41-2.09)	3 (0.62-8.77)
Uterine cancer	5	0.72 (0.24-1.69)	2 (0.24-7.22)
Gallbladder cancer	4	0.58 (0.16-1.48)	3 (0.62-8.77)
Ovarian cancer	4	0.58 (0.16-1.48)	4 (1.09-10.24)
Esophageal cancer	3	0.43 (0.09-1.27)	1 (0.03-5.57)
Melanoma	3	0.43 (0.09-1.27)	1 (0.03-5.57)
Pancreatic cancer	3	0.43 (0.09-1.27)	2 (0.24-7.22)
Renal cell carcinoma	3	0.43 (0.09-1.27)	2 (0.24-7.22)
Urothelial cell carcinoma	2	0.29 (0.04-1.05)	1 (0.03-5.57)
Glioblastoma	2	0.29 (0.04-1.05)	2 (0.24-7.22)
Hepatocellular carcinoma	2	0.29 (0.04-1.05)	0 (0-3.69)
Parotid cancer	1	0.14 (0-0.81)	0 (0-3.69)
Hypopharynx carcinoma	1	0.14 (0-0.81)	1 (0.03-5.57)
Meningiosarcoma	1	0.14 (0-0.81)	1 (0.03-5.57)

<sup>a</sup>For participants who developed more than 1 type of tumor during follow-up (n=4), the tumor that occurred first was considered in the analyses.

<sup>b</sup>Values presented are numbers (poisson 95% confidence intervals).

physically less active, and more likely to be current or ex-smokers (see eTable at <http://www.jama.com>). Moreover, they consumed more alcohol and had lower levels of low-density lipoprotein cholesterol and higher levels of C-reactive protein.

Baseline telomere length was substantially shorter in participants with incident cancer (mean telomere length, 1.12; 95% CI, 1.01-1.23) than in those who remained free of cancer (mean telomere length, 1.53 [95% CI, 1.47-1.59]; *P* < .001). Telomere length distribution is depicted in eFigure 2 at <http://www.jama.com>. A significant decrease in telomere length was seen with age (*r* = -0.223; *P* < .001). Men had shorter telomeres than women (1.41 [95% CI, 1.34-1.49] vs 1.55 [95% CI, 1.47-1.63], respectively; *P* = .02), and further associations were observed for the variables of diabetes, physical activity, vitamin D, presence of chronic infection, interleukin 6, and high level of C-reactive protein (TABLE 2). Ten-year changes in telomere length compared with baseline length are depicted in eFigure 5 at <http://www.jama.com>. Telomere attrition was most pro-

nounced in participants with the longest telomere length, whereas there was a paradoxical increase in telomere length among individuals with the shortest telomere length at baseline.

In Cox regression analyses, short telomere length at baseline emerged as a significant and independent predictor of new-onset cancer (FIGURE 1). The HR per 1-SD decrease in log<sub>e</sub>-transformed telomere length for incident cancer was 1.60 (95% CI, 1.30-1.98; multivariable model, *P* < .001), which equals the excess risk attributable to a 12.8-year difference in chronological age. Compared with participants in the longest telomere length group, the multivariable HR for incident cancer was 2.15 (95% CI, 1.12-4.14) in the middle length group and 3.11 (95% CI, 1.65-5.84) in the shortest length group (*P* < .001). Incidence rates were 5.1 per 1000 person-years in the longest telomere length group, 14.2 per 1000 person-years in the middle length group, and 22.5 per 1000 person-years in shortest length group. Of note, telomere length was preferentially associated with individual cancers characterized by a high fatality rate such as

gastric, lung, and ovarian cancer, but less so with tumors linked to better prognosis (see eFigure 4 at <http://www.jama.com>).

A total of 44 participants died of cancer (median survival time from diagnosis, 30 months). An association was seen between baseline telomere length and cancer mortality (multivariable HR per 1-SD decrease in log<sub>e</sub>-transformed telomere length, 2.13 [95% CI, 1.58-2.86]; *P* < .001) (Figure 1) that surpassed the associations obtained for cardiovascular mortality (HR, 1.41 [95% CI, 1.01-1.96]; *P* = .05) and mortality from other causes (HR, 1.57 [95% CI, 1.17-2.10]; *P* = .003). Compared with participants with the longest telomere lengths, the multivariable HR for cancer mortality was 5.63 (95% CI, 1.27-24.98) in the middle length group and 11.11 (95% CI, 2.61-47.36) in the shortest length group (*P* < .001). In absolute terms 2, 14, and 28 of individuals in the longest, middle, and short-

est telomere length groups, respectively, died of cancer, corresponding to mortality rates of 0.8, 6.0, and 12.9 per 1000 person-years, respectively. Cumulative hazard curves for both cancer incidence and cancer mortality are depicted in FIGURE 2.

Sensitivity analyses excluding participants with prior cancer or extending the list of covariates yielded similar results (Figure 1). Furthermore, an analysis revealed telomere length to have a similar predictive value for cancer in both men and women and in various age groups (FIGURE 3). The HR point estimate differed between groups for smoking quantity but the 95% CIs overlapped and the test for interaction was not significant. Findings were similar for solid and hematopoietic tumors and were consistent in the 5 years following telomere length assessment (1995-2000) and in the 5 years thereafter (2000-2005); thus, reverse causation was ruled out (Figure 3).

**COMMENT**

To our knowledge, this is the first prospective, population-based study to estimate the impact of telomere length on overall cancer manifestation and mortality. We demonstrated significant inverse correlations between baseline leukocyte telomere length and both cancer incidence and mortality, which emerged as independent of standard cancer risk factors (Figure 1). There was evidence of heterogeneous effects according to cancer type, whereby (1) tumors with a high fatality rate tended to exhibit more prominent relationships with telomere length (see eFigure 4 at <http://www.jama.com>) and (2) tumors with a more favorable prognosis showed modest or no associations.

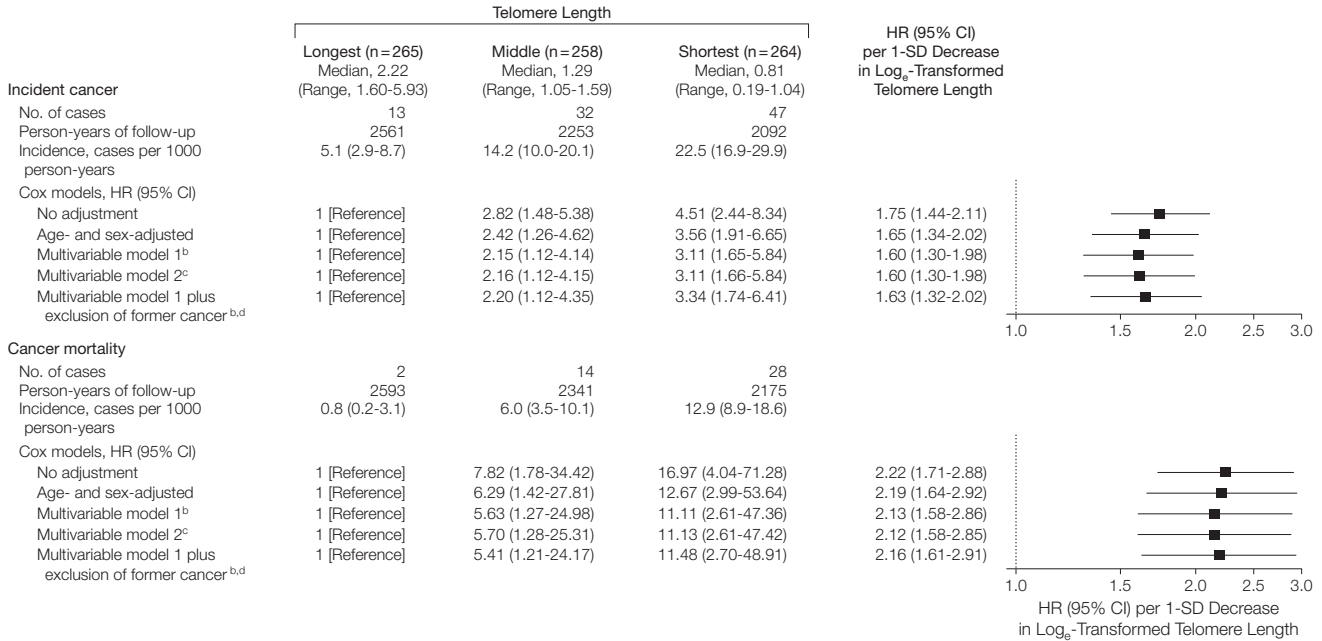
Our results corroborate well with previous evaluations demonstrating a link between short telomere length and bladder cancer,<sup>7,8,22</sup> renal cell carcinoma,<sup>8,9</sup> non-Hodgkin lymphoma,<sup>10</sup> lung cancer,<sup>11</sup> and head and neck tumors,<sup>8</sup> but failed to obtain significant correlations

**Table 2.** Baseline Characteristics

Characteristic	Total Participants (N = 787)	Telomere Length			P Value <sup>a</sup>
		Longest (n = 265)	Middle (n = 258)	Shortest (n = 264)	
Age, mean (95% CI), y	62.6 (61.8-63.3)	59.3 (58.1-60.5)	62.9 (61.5-64.2)	65.5 (64.2-66.9)	<.001
Male sex, No. (%)	388 (49.3)	116 (43.8)	133 (51.6)	139 (52.7)	.08
Social status, No. (%)					.41
Low	473 (60.1)	156 (58.9)	149 (57.7)	168 (63.6)	
Middle	177 (22.5)	67 (25.3)	58 (22.5)	52 (19.7)	
High	137 (17.4)	42 (15.8)	51 (19.8)	44 (16.7)	
		Mean (95% CI) <sup>b</sup>			
Telomere length <sup>c</sup>					
Relative T/S ratio assessed by quantitative PCR	1.48 (1.43-1.54)	2.36 (2.27-2.45)	1.30 (1.28-1.32)	0.78 (0.76-0.80)	<.001
Southern blot, kB	8.05 (7.97-8.12)	9.25 (9.12-9.37)	7.80 (7.77-7.83)	7.08 (7.05-7.11)	<.001
Lifestyle and cancer risk factors					
Alcohol consumption, g/d <sup>d</sup>	10.7 (0-33.5)	9.0 (0-30.3)	10.8 (0-30.1)	10.7 (0-41.0)	.43
Current or ex-smokers, No. (%)	353 (44.9)	112 (42.3)	123 (47.7)	118 (44.7)	.46
Pack-years of smoking <sup>d</sup>	0 (0-26)	0 (0-18)	0 (0-27)	0 (0-30.3)	.31
Physical activity via sport index	2.37 (2.31-2.43)	2.51 (2.41-2.61)	2.42 (2.32-2.53)	2.17 (2.06-2.29)	<.001
Body mass index <sup>e</sup>	25.7 (25.4-26.0)	25.8 (25.4-26.3)	25.6 (25.1-26.1)	25.7 (25.2-26.2)	.08
Diabetes, No. (%)	93 (11.8)	19 (7.2)	31 (12.0)	43 (16.3)	.005
LDL cholesterol, mmol/L	3.76 (3.69-3.83)	3.72 (3.61-3.83)	3.79 (3.67-3.92)	3.77 (3.65-3.90)	.67
High-sensitivity CRP, nmol/L <sup>d</sup>	16.2 (8.6-30.5)	13.3 (6.7-27.6)	16.7 (8.6-30.5)	17.1 (8.6-36.7)	.004
Chronic infection, No. (%)	242 (30.8)	54 (20.4)	87 (33.7)	101 (38.3)	<.001
Interleukin 6, pg/mL	8.2 (6.9-9.4)	7.0 (5.5-8.5)	7.2 (5.8-8.5)	10.3 (7.3-13.4)	.05
25-hydroxyvitamin D, nmol/L	79.7 (77.5-81.9)	83.5 (79.9-87.1)	79.3 (75.5-83.0)	76.4 (72.3-80.5)	.03

Abbreviations: CI, confidence interval; CRP, C-reactive protein; LDL, low-density lipoprotein; PCR, polymerase chain reaction. SI conversion factors: To convert CRP to mg/L, divide by 9.524; LDL cholesterol to mg/dL, divide by 0.0259; 25-hydroxyvitamin D to ng/mL, divide by 2.496. <sup>a</sup>Calculated with the  $\chi^2$  or Fisher exact test or with analysis of variance. <sup>b</sup>Unless otherwise indicated. <sup>c</sup>Transformation of relative T/S ratio (quantitative PCR) to telomere length in kilobases (kB) was performed with the formula: telomere length in kB = 6.016 + 1.365 × (relative T/S ratio). For further explanation, see eMethods at <http://www.jama.com>. <sup>d</sup>Values are expressed as median (interquartile range). <sup>e</sup>Calculated as weight in kilograms divided by height in meters squared.

**Figure 1.** Association of Telomere Length With Cancer Incidence and Mortality Between 1995 and 2005 in the Bruneck Study (N=787)<sup>a</sup>



<sup>a</sup>*P* < .001 for all comparisons of hazard ratios (HRs) and 95% confidence intervals (CIs) per 1-SD decrease in log<sub>e</sub>-transformed telomere length. In a separate Cox model, telomere length was treated as a categorical variable (tertile groups). Cancer incidence included any new cancer except nonmelanoma skin cancer. Only the first incidence of cancer was considered in the analysis.

<sup>b</sup>Adjusted for age, sex, social class, pack-years of smoking, alcohol consumption, diabetes, physical activity, body mass index, log<sub>e</sub>-transformed high-sensitivity C-reactive protein, vitamin D, and low-density lipoprotein cholesterol.

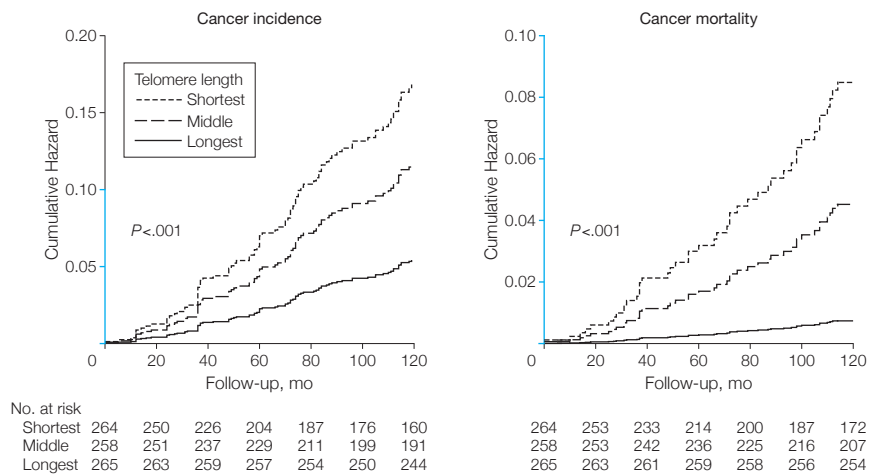
<sup>c</sup>Adjusted for everything in footnote "b" and for presence of chronic infection and level of interleukin 6.

<sup>d</sup>Participants with a history of prior cancer were excluded from this analysis (n=33). In the longest, middle, and shortest telomere length tertile groups, there were 12, 30, and 46 cases of incident cancer, respectively, of which 2, 13, and 28, respectively, were fatal. The corresponding incidence rates per 1000 person-years were 4.8, 14.0, and 22.9 for incident cancer and 0.8, 5.9, and 13.4 for cancer mortality.

between short telomere length and colorectal and breast cancer.<sup>12-14,23</sup> Several investigations suggested synergistic effects of short telomere length and risk conditions (eg, Barrett esophagus) on cancer manifestation,<sup>6,15</sup> and one study reported contradictory results.<sup>24</sup> Of particular note, only a minority of previous evaluations was prospective in design and measured telomere length well in advance of cancer manifestation. All of these evaluations were conducted in selected populations. Finally, short telomere length in tissue samples of patients with pancreatic ductal adenocarcinoma<sup>25</sup> and hepatocellular carcinoma<sup>26</sup> has been shown to predict survival.

A variety of experimental and genetic studies support the hypothesis that telomere attrition contributes to the manifestation and dissemination of malignancies. While fully functional telomeres confer protection of the genome, shortened telo-

**Figure 2.** Cumulative Cancer Incidence and Cancer Mortality Between 1995 and 2005 in the Bruneck Study



*P* values are for difference between telomere length tertile groups and were derived from the multivariable Cox models. The median for the shortest telomere length group is 0.81 (range, 0.19-1.04); middle length group, 1.29 (range, 1.05-1.59); and longest length group, 2.22 (range, 1.60-5.93). The y-axis regions shown in blue indicate cumulative hazard range from 0 to 0.10. There were 92 cases of cancer incidence and 44 cases of cancer mortality.

meres facilitate chromosomal instability.<sup>6</sup> In a mouse model, deficiency of the telomerase RNA component (the RNA template for telomere prolongation) caused a markedly enhanced frequency of chromosomal abnormalities and sporadic cancer,<sup>2</sup> particularly in the case of simultaneous inactivation of the tumor suppressor gene *p53*.<sup>1</sup> In fibroblast cultures, accruing senescent cells were shown to produce and release high amounts of growth factors,<sup>27</sup> causing an overwhelming proliferation of the surrounding tissue, and to secrete metalloproteinase, diminishing intercellular adhesions and potentially favoring metastatic spread of tumors.<sup>28</sup>

Moreover, a significantly higher production of vascular endothelial growth factor in senescent cells<sup>29,30</sup> may stimulate tumor growth and dissemination by promoting neovascularization. It is appealing to assume that the association between short

leukocyte telomere length and cancer formation is partially mediated by the aging of the immune system itself. Aging of leukocytes reflected by short telomere length may impair immune surveillance and reduce the clearance of tumor cells. The importance of an efficient immune system in tumor defense is substantiated by the excess tumor incidence among patients with AIDS<sup>31</sup> or undergoing immunosuppressive therapy.

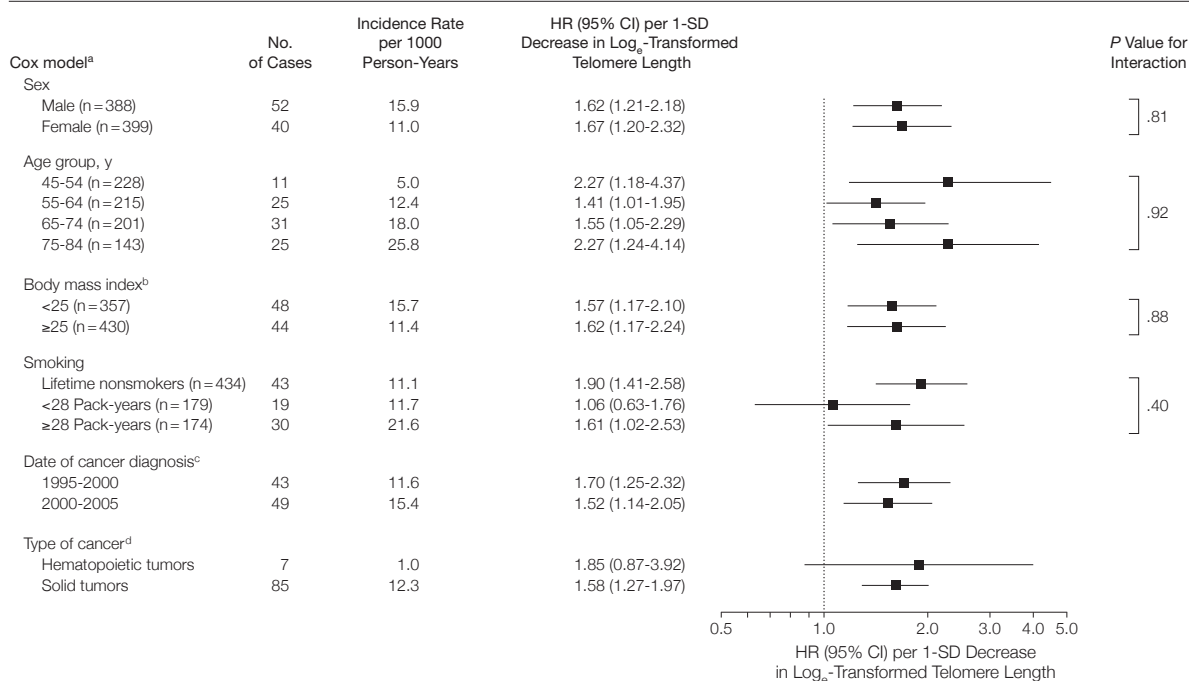
Further evidence for the significance of telomeres in carcinogenesis is provided by genome-wide association studies. Rafnar et al<sup>5</sup> demonstrated that polymorphisms in the gene encoding *TERT*, the catalytic subunit of telomerase, were associated with an increased risk for cancer of the lung, urinary bladder, prostate, and cervix. Dyskeratosis congenita, a rare disorder of telomere maintenance, was associated with visceral malignancies and

squamous cell carcinoma of the skin.<sup>32</sup> Finally, in the present study, a paradoxical increase in telomere length (1995-2005) was observed in participants with very short telomeres at baseline (see eFigure 5 at <http://www.jama.com>).

It is tempting to speculate that cells with a critically short telomere length may under certain circumstances reactivate the enzyme telomerase to escape from cell senescence and thereby facilitate malignant transformation. Telomerase expression has been documented in normal peripheral blood leukocytes.<sup>33</sup> T lymphocytes up-regulate *TERT* on activation by immunoinflammatory stimuli potentially linked to short telomere length.<sup>34</sup>

Our study has important merits and limitations. The Bruneck cohort is extremely well characterized with a 100% follow-up and high-quality ascertainment of clinical end points and potential con-

**Figure 3.** Association Between Telomere Length and Incident Cancer (N=787)



<sup>a</sup>All models were adjusted for age, sex, social class, pack-years of smoking, alcohol consumption, diabetes, physical activity, body mass index, log<sub>e</sub>-transformed high-sensitivity C-reactive protein, vitamin D, and low-density lipoprotein cholesterol. Cancer incidence included any new cancer except nonmelanoma skin cancer. Only the first incidence of cancer was considered in the analysis. Interactions were calculated by inclusion of interaction terms.

<sup>b</sup>Calculated as weight in kilograms divided by height in meters squared.

<sup>c</sup>The analysis labeled 1995-2000 considered the 5-year follow-up period between the 1995 and 2000 evaluation of the Bruneck Study, while in the analysis labeled 2000-2005, cancers manifesting in the first 5 years of follow-up were censored and the Cox model was confined to cancers diagnosed more than 5 years after DNA sampling in the follow-up period between 2000 and 2005. The latter analysis rules out inverse causation in a way that baseline leukocyte telomere length was affected by preclinical cancer disease.

<sup>d</sup>Separate Cox models were fit for hematopoietic tumors and solid tumors.

founders. Telomere length measurement was performed in quadruplicate, showed a high level of reproducibility, and was corrected for quantitative PCR efficiency. Moreover, the association was consistent in subgroups of men and women with considerable strength and dose-response type, and was robust under a variety of circumstances. However, the study population was entirely white and findings cannot necessarily be extrapolated to other races and ethnicities. Population characteristics are comparable with those of other Western communities, but transferability of our findings to geographical regions with a prominently distinct genetic background remains to be established. Although the study was adequately powered to reliably assess an association between telomere length and both cancer incidence and mortality (see eFigure 3 at <http://www.jama.com>), the overall number of cases is limited to 92

and 44, respectively, and the sample size is too small to precisely assess associations between telomere length and each specific type of cancer. Heterogeneity beyond cancer fatality is likely to exist and remains to be addressed in future research.<sup>6</sup> Moreover, telomere length was measured in circulating blood leukocytes, which is an easily accessible source of DNA, rather than in a variety of distinct tissues.

Finally, tumors were classified as fatal or nonfatal using data collected during the last follow-up examination in 2005 and this classification will be subject to further refinement based on data collected in the subsequent years.

In conclusion, our study shows that short telomeres are associated with an enhanced risk of cancer and fatal cancer in particular.

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tegrity of the data and the accuracy of the data analysis. *Study concept and design:* P. Willeit, J. Willeit, Mayr, Weger, Oberhollenzer, Kiechl.

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