

# Telomere Length in Peripheral Leukocyte DNA and Gastric Cancer Risk

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## Abstract

Telomere length reflects lifetime cumulative oxidative stress from environmental exposures, such as cigarette smoking and chronic inflammation. Shortened telomere length is thought to cause genomic instability and has been associated with several cancers. We examined the association of telomere length in peripheral leukocyte DNA with gastric cancer risk as well as potential confounding factors and risk modifiers for telomere length–related risk. In a population-based study of gastric cancer conducted in a high-risk population in Warsaw, Poland, between 1994 and 1996, we measured relative telomere length in 300 cases and 416 age- and gender-matched controls using quantitative real-time PCR. Among controls, telomeres were significantly shorter in association with aging ( $P < 0.001$ ), increasing pack-years of cigarette smoking ( $P = 0.02$ ), decreasing fruit intake ( $P = 0.04$ ), and *Helicobacter pylori* positivity ( $P = 0.03$ ). Gastric cancer cases had

significantly shorter telomere length (mean  $\pm$  SD relative telomere length,  $1.25 \pm 0.34$ ) than controls ( $1.34 \pm 0.35$ ;  $P = 0.0008$ ). Gastric cancer risk doubled [odds ratio (OR), 2.04; 95% confidence interval (95% CI), 1.33–3.13] among subjects in the shortest compared with the highest quartile of telomere length ( $P_{\text{trend}} < 0.001$ ). Telomere length–associated risks were higher among individuals with the lowest risk profile, those *H. pylori*–negative (OR, 5.45; 95% CI, 2.10–14.1), non-smokers (OR, 3.07; 95% CI, 1.71–5.51), and individuals with high intake of fruits (OR, 2.43; 95% CI, 1.46–4.05) or vegetables (OR, 2.39; 95% CI, 1.51–3.81). Our results suggest that telomere length in peripheral leukocyte DNA was associated with *H. pylori* positivity, cigarette smoking, and dietary fruit intake. Shortened telomeres increased gastric cancer risk in this high-risk Polish population. (Cancer Epidemiol Biomarkers Prev 2009;18(11):3103–9)

## Introduction

Telomeres consist of repetitive nucleotide sequences and an associated terminal protein complex that help prevent loss of chromosomal integrity (1). Telomere shortening in genomic DNA appears to reflect lifetime cumulative oxidative stress from environmental exposures, such as smoking, poor nutrition, and chronic inflammation (2–6). Short telomeres are associated with cellular senescence and decreased tissue renewal capacity (7, 8). Telomerase-knockout mouse models, in which animals possess critically short telomeres, exhibit increased cancer rates (9–11). Shortened telomere length were found in human epithelial cancers due to the formation of complex nonreciprocal translocations and increased chromosome instability (12–15). Telomere length measured in either blood leukocyte

or buccal cell genomic DNA has been associated with increased risks for bladder, lung, head and neck, and renal cancers (5, 16–20).

*Helicobacter pylori* infection is a known risk factor for gastric cancer and chronic inflammation is closely linked with telomere length shortening (21, 22). Furthermore, shorter telomere length was identified in *H. pylori*–positive gastric epithelial tissue (23, 24) and tumor tissues (25, 26) compared with normal gastric tissues. We therefore hypothesized that telomere length in blood leukocyte DNA is associated with *H. pylori* infection and gastric cancer risk. In the present study, we examined the association between telomere length and gastric cancer risk, the associations between the risk factors and telomere length, and their potential effect modification of these factors on telomere length–related gastric cancer risk.

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## Materials and Methods

**Study Population and Design.** The study design has been described elsewhere (27). Briefly, a population-based case-control study was conducted in Warsaw, Poland. Residents ages 21 to 79 years, who were newly diagnosed with gastric adenocarcinoma (*International Classification*

of Diseases for Oncology code 151 or *International Classification of Diseases for Oncology, Second Edition* code C16) between March 1, 1994, and April 30, 1996, were identified by collaborating physicians in all the 22 hospitals of Warsaw. A total of 72 clinics and endoscopic departments within these hospitals and 8 private endoscopic units were covered. In addition, the population-based Cancer Registry files were reviewed regularly to ensure completeness of case ascertainment. Diagnostic information was abstracted in a standardized manner from hospital records of endoscopy and surgical and pathology reports by a collaborating physician or by the study physician, who visited the hospitals once a month. All pathologic slides were reviewed for confirmation of the diagnosis and standardized reclassification using the Lauren (1965) and WHO criteria (28) by two pathologists, one from Poland and the other from the United States. The final decision on classification of borderline cases was made by a senior U.S. pathologist specializing in gastro-

intestinal tumor pathology. We only included cases with invasive adenocarcinoma.

Controls were randomly selected among Warsaw residents from a computerized registry of all residents in Poland, the Polish Electronic System of Residence Evidency, and frequency-matched to cases by sex and age in 5-year groups. The system is updated monthly, and completeness of registration is estimated to be ~100%. After written consent was obtained, controls and cases or next of kin of deceased cases were interviewed by trained interviewers to elicit information on demographic background, usual diet before 1990, childhood living conditions, family history of cancer, history of selected medical conditions and medication use, lifetime occupational history, and consumption of cigarettes, alcohol, and other beverages.

An ever-smoker was defined as a smoker of at least 1 cigarette smoked per day for  $\geq 6$  months. An ever-drinker was defined as a person drinking at least 1 serving of beer

**Table 1. Characteristics of study subjects**

Demographic and risk factors	Cases <i>n</i> (%), <i>n</i> = 300	Controls <i>n</i> (%), <i>n</i> = 416	<i>P</i> *
Age (tertile), y			
$\leq 61$	111 (37.0)	150 (36.0)	
62-69	99 (33.0)	138 (33.2)	
$\geq 70$	90 (30.0)	128 (30.8)	0.96
Sex			
Male	199 (66.3)	269 (64.7)	
Female	101 (33.7)	147 (35.3)	0.64
Education levels			
Low	141 (47.0)	154 (37.0)	
Medium	102 (34.0)	144 (34.6)	
High	57 (19.0)	118 (28.4)	0.005
Family history of cancer			
No family history	175 (58.3)	275 (66.1)	
Stomach cancer	36 (12.0)	17 (4.1)	
Other cancers	77 (25.7)	117 (28.1)	
Unknown	12 (4.0)	7 (1.7)	<0.001
<i>H. pylori</i> status <sup>†</sup>			
Negative	50 (16.7)	63 (15.2)	
Positive	250 (83.3)	352 (84.8)	0.59
Smoking status			
Nonsmoker	85 (28.5)	166 (39.9)	
Former smoker	90 (30.2)	133 (32.0)	
Current smoker	123 (41.3)	117 (28.1)	<0.001
Pack-years of smoking <sup>‡</sup>			
0	85 (28.6)	166 (40.0)	
0.1-30	100 (33.7)	137 (33.0)	
>30	112 (37.7)	112 (27.0)	0.002
Alcohol drinking status			
Nondrinker	105 (35.8)	136 (32.7)	
Former drinker	95 (32.5)	62 (14.9)	
Current drinker	93 (31.7)	218 (52.4)	<0.001
Total years of drinking alcohol			
Nondrinker	105 (35.7)	136 (32.9)	
<10	74 (25.2)	137 (33.1)	
10-29	51 (17.3)	85 (20.5)	
>29	64 (21.8)	56 (13.5)	0.008
Fruit intake			
Daily/weekly	131 (46.3)	202 (48.7)	
Several times/month	101 (35.7)	137 (33.0)	
Rarely/never	51 (18.0)	76 (18.3)	0.75
Vegetables intake			
Daily/weekly	141 (49.7)	250 (60.7)	
Several times/month	108 (38.0)	122 (29.6)	
Rarely/never	35 (12.3)	40 (9.7)	0.02
Mean (SD) RTL	1.25 (0.34)	1.34 (0.35)	0.0008 <sup>§</sup>

\**P* value obtained from a Fisher's exact test comparing cases and controls.

<sup>†</sup>Negative, tested negative for IgG antibodies both to *H. pylori* and to *cagA*; positive, tested positive for either IgG antibodies to *H. pylori* or *cagA* antibody or both.

<sup>‡</sup>Division into nonsmokers and low and high numbers of pack-years used the median of pack-years among smokers.

<sup>§</sup>*P* value obtained from a Student's *t* test comparing telomere length in cases and controls.

**Table 2. Relation of gastric cancer risk factors to telomere length among controls**

Demographic and risk factors	n	RTL, mean (95% CI)*	P
Age (tertile), y <sup>†</sup>			
≤61	150	1.43 (1.37-1.48)	
62-69	138	1.30 (1.24-1.35)	
≥70	128	1.28 (1.22-1.34)	<0.001
Sex <sup>‡</sup>			
Male	269	1.32 (1.28-1.36)	
Female	147	1.38 (1.32-1.43)	0.09
Body mass index (tertile), kg/m <sup>2</sup>			
≤24	138	1.36 (1.30-1.42)	
24-26.8	140	1.33 (1.27-1.38)	
≥26.8	142	1.33 (1.27-1.38)	0.40
Education level			
Low	154	1.30 (1.25-1.36)	
Medium	144	1.34 (1.28-1.39)	
High	118	1.39 (1.33-1.45)	0.05
Family history of cancer			
No family history	275	1.32 (1.28-1.36)	
Stomach cancer	17	1.25 (1.09-1.41)	
Other cancers	117	1.39 (1.33-1.45)	
Unknown	7	1.52 (1.27-1.77)	0.13
<i>H. pylori</i> status <sup>§</sup>			
Negative	63	1.42 (1.34-1.51)	
Positive	352	1.32 (1.29-1.36)	0.03
Smoking status <sup>  </sup>			
Never	166	1.37 (1.32-1.43)	
Former	133	1.33 (1.27-1.39)	
Current	117	1.31 (1.25-1.37)	0.14
Pack-years of smoking <sup>  </sup>			
0	166	1.38 (1.32-1.43)	
0.1-30	137	1.36 (1.31-1.42)	
>30	112	1.26 (1.20-1.33)	0.02
Total years of drinking alcohol			
Nondrinker	136	1.36 (1.30-1.43)	
<10	137	1.37 (1.31-1.43)	
10-29	85	1.30 (1.23-1.38)	
>29	56	1.26 (1.16-1.35)	0.06
Fruit			
Daily/weekly	202	1.37 (1.32-1.41)	
Several times/month	137	1.34 (1.29-1.40)	
Rarely/never	76	1.26 (1.19-1.34)	0.04
Vegetables			
Daily/weekly	250	1.35 (1.31-1.39)	
Several times/month	122	1.33 (1.27-1.39)	
Rarely/never	40	1.29 (1.18-1.39)	0.30

\*RTL means estimated from multivariable models adjusting for age, gender, smoking status, and pack-years of smoking using the post-estimation command adjust in Stata 10.0.

<sup>†</sup>Only adjusted for sex, smoking status, and pack-years of smoking.

<sup>‡</sup>Only adjusted for age, smoking status, and pack-years of smoking.

<sup>§</sup>Negative, tested negative for both IgG antibodies to *H. pylori* and *cagA*; positive, tested positive for either IgG antibodies to *H. pylori* or *cagA* antibody or both.

<sup>||</sup>Adjusted for each other.

(12 oz.), wine (4 oz.), or liquor (1.5 oz.) per month for ≥6 months. Among smokers and drinkers, information was collected on the age when exposure to each product started and stopped and total years and frequency of use. Pack-years of smoking were calculated as the product of packs of cigarettes smoked per day and total years of smoking. Total drink-years were calculated as the product of yearly frequency and total years of alcohol use. The information on dietary intakes was collected as described previously (29). Briefly, usual frequency of intake of 118 food and beverage items was elicited. Nutrient intake was estimated from the weekly consumption of food items, the average portion size, and the nutrient composition of each food item. Total intake of each nutrient then was summed across all food items. The information on cancer treatment was collected by asking if the patients

had obtained radiation or chemotherapy before the blood draw.

Of the 515 eligible gastric adenocarcinoma cases identified, 34 (6.6%) refused and 17 (3.3%) were untraceable or unavailable for other reasons. Interviews were obtained in person for 324 (62.9%) cases and with next-of-kin of 140 (27.2%) cases who died or were too ill to participate. Of the 586 potential controls identified, 37 (6.3%) had moved. Of the 549 remaining subjects, 480 (87.4%) agreed to be interviewed. The predominant reason for noninterview was subject refusal (10.9%). Among the 464 gastric cancer cases and 480 controls included in the study, 345 cases and 442 controls agreed to donate a 30-mL blood sample (30-32). Peripheral leukocyte DNA was obtained from 305 cases and 427 controls (33-35). In the present study, telomere length measurement was successfully conducted in 300 cases and 416 controls.

The study was approved by the institutional review boards of the U.S. National Cancer Institute and The M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology. Written informed consent was obtained from all participants.

**Measurement of Serum Levels of IgG Antibodies to *H. pylori* and *cagA*.** Serum levels of IgG antibodies to *H. pylori* and to the *cagA* protein were determined by antigen-specific ELISA as described previously (36, 37). We defined individuals who tested negative for both serum IgG antibodies to *H. pylori* and *cagA* antibody as *H. pylori*-negative and those who tested positive for either or both markers as *H. pylori*-positive subjects.

**Telomere Length Measurement by Quantitative PCR.** Telomere length was measured in blood leukocyte DNA using quantitative real-time PCR as described by Cawthon (38) and modified by McGrath et al. (19). This method measures relative telomere length (RTL) in genomic DNA by determining the ratio of telomere repeat copy number (T) to single copy gene (S) copy number (T/S ratio) in individual samples relative to a reference pooled DNA. The reference pooled DNA was created using DNA from 60 subjects who were randomly selected from the 416 control subjects of the current study (400 ng from each sample) and used to generate a fresh standard curve ranging from 0.25 to 8 ng/μL in every T and S PCR run. The T (telomere) PCR mix was iQ SYBR Green Supermix (Bio-Rad) 1×, tel 1b 100 nmol/L, tel 2b 900 nmol/L, DMSO 1%, and EDTA 1×. The S (human β-globin) PCR mix was iQ SYBR Green Supermix (Bio-Rad) 1×, hbg1 300 nmol/L, hbg2 700 nmol/L, DMSO 1%, DTT 2.5 mmol/L, and EDTA 1×. After addition of *Escherichia coli* DNA (Sigma-Aldrich), DNA samples were heated at 96°C for 10 min and then cooled to room temperature. All PCRs were done on a DNA Engine thermal cycler Chromo4 (Bio-Rad). DNA (15 ng) was used in each PCR (final volume 20 μL). The thermal cycling profile for both amplicons began with 95°C incubation for 3 min. The T PCR included 25 cycles of 95°C for 15 s and annealing/extension at 54°C for 49 s. The S PCR included 35 cycles of 95°C for 15 s, annealing at 58°C for 1 s, and extension at 72°C for 15 s. At the end of each reaction, a melting curve was used for both T and S PCRs. All samples were run in duplicate and the mean of two measurements was used in the statistical analyses. The interbatch variability (coefficient of variation) in the present study was 8.1%.

**Statistical Analyses.** Graphical inspection of RTL distribution separately in cases and controls showed no departures from the normal distribution. RTL data normality was confirmed using the Shapiro-Wilk test. Linear regression models were used to evaluate differences in RTL (continuous-dependent variable in the models) among controls in relation to age at blood draw, gender, *H. pylori* infection status, family history of cancer, and other oxidative stress-related factors, including smoking, alcohol drinking, and fruit and vegetable consumption. Mean RTL for each of the variables evaluated was estimated using the post-estimation command `adjust` in Stata 10.0 from models including age, gender, smoking status, and pack-years as independent variables. Unconditional logistic regression was used to estimate odds ratios (OR) for gastric cancer and corresponding 95% confidence intervals (95% CI). Quartile cut-points were based on distributions among controls. All models were adjusted for age, gender, smoking status (nonsmokers, former smokers, and current smokers), and pack-years of smoking (nonsmokers, 0.1-30, and >30). Further adjustment by other potential confounding variables, including education, body mass index, alcohol drinking, caloric intake, intake of fruits, vegetables, sausages, red meats, or preserved vegetables, family history of gastric cancer or other cancers, and *H. pylori* status, did not alter the risk estimates. Therefore, these variables were not included in the final model. All tests were two-sided and  $\alpha < 0.05$  was considered significant.

## Results

Among 345 cases and 442 controls who donated a blood sample, RTL results were available in 300 cases (87.0%) and 416 controls (94.1%). Cases and controls had similar age and sex distributions (Table 1), as they were frequency-matched. When compared with the controls, gastric cancer cases tended to have lower levels of education ( $P = 0.004$ ) and reported having more first-degree relatives diagnosed with gastric cancer ( $P < 0.001$ ). Cases also smoked more ( $P = 0.002$ ), drank more alcohol ( $P = 0.008$ ), and consumed fewer vegetables ( $P = 0.02$ ).

As expected, telomere length shortened significantly in controls with increasing age at blood collection ( $P_{\text{trend}} < 0.001$ ). RTL decreased from 1.43 among controls ages  $\leq 61$  to 1.28 years among those ages  $\geq 70$  years (Table 2). *H. pylori*-positive controls had significantly shorter telomere length (RTL, 1.32; 95% CI, 1.29-1.36) the *H. pylori*-negative controls (RTL, 1.42; 95% CI, 1.34-1.51;

$P = 0.03$ ). Telomere length tended to decrease with increasing pack-years of cigarette smoking ( $P = 0.02$ ) and decreasing frequencies of fruit intake ( $P = 0.04$ ). RTL was marginally associated with alcohol consumption ( $P = 0.06$ ) and level of education ( $P = 0.05$ ) but not with body mass index, family history of cancer, smoking status, and vegetable intake.

Gastric cancer cases had significantly shorter telomeres (mean  $\pm$  SD RTL,  $1.25 \pm 0.34$ ) than controls ( $1.34 \pm 0.35$ ;  $P = 0.0008$ ; Table 1). Analyses of RTL in quartiles, based on the distribution in the controls, showed that the risk of gastric cancer was doubled (OR, 2.04; 95% CI, 1.33-3.13) among cases in the lowest quartile of RTL (RTL  $\leq 1.13$ ) when compared with those in the highest quartile (RTL  $> 1.53$ ; Table 3). The risks among those in the second (RTL, 1.14-1.30) and third (RTL, 1.31-1.53) quartiles of RTL were comparable with those in the highest quartile. These three groups therefore were combined into a group with "long" telomere length for the stratified analyses.

When stratified by known or potential risk factors, the association between short telomere length and gastric cancer risk tended to be stronger among men and older persons, although the interaction did not reach statistical significance (Table 4). The magnitude of association with short telomere length tended to be stronger among persons with the lowest risk profile, *H. pylori*-negative individuals (OR, 5.45; 95% CI, 2.10-14.1), nonsmokers (OR, 3.07; 95% CI, 1.71-5.51), and persons with high intake of fruits (OR, 2.43; 95% CI, 1.46-4.05) or vegetables (OR, 2.39; 95% CI, 1.51-3.81). In contrast, the short telomere length-associated gastric cancer risks were 1.78 (95% CI, 1.25-2.54) for *H. pylori*-positive subjects, 1.40 (95% CI, 0.79-2.45) among current smokers, and 1.73 (95% CI, 0.80-3.76) and 2.00 (95% CI, 0.73-5.50) for those who rarely consumed fruits and vegetables, respectively.

Telomere length did not vary significantly by Lauren pathologic classification (67.7% intestinal type, 16.3% diffuse, and 16.0% indeterminate or unknown;  $P = 0.86$ ) or tumor subsite of origin (11.7% cardia, 72.7% distal, and 15.7% indeterminate or unknown;  $P = 0.35$ ; data not shown). In addition, excluding patients who received chemotherapy before the blood draw ( $n = 42$ ) produced no meaningful difference in results, with no changes in statistical significance.

## Discussion

In the present population-based investigation, we showed that short telomeres are related to an increased risk of

**Table 3. Gastric cancer risk and telomere length**

	RTL	Cases, n (%)	Controls, n (%)	OR (95% CI)*
Quartile				
Fourth	>1.53	60 (20.0)	104 (25.0)	1.00 (reference)
Third	1.31-1.53	56 (18.7)	104 (25.0)	0.91 (0.58-1.45)
Second	1.14-1.30	63 (21.0)	104 (25.0)	1.06 (0.67-1.67)
First	$\leq 1.13$	121 (40.3)	104 (25.0)	2.04 (1.33-3.13)
				$P_{\text{trend}} < 0.001$
Length category				
Second, third, and fourth	Long	179 (59.7)	312 (75.0)	1.00 (reference)
First	Short	121 (40.3)	104 (25.0)	2.06 (1.48-2.86)
				$P < 0.001$

\*Adjusted for age, gender, smoking status, and pack-years of smoking.

**Table 4. Gastric cancer risk and telomere length by known or potential risk factors**

Demographic and risk factors	RTL*	Cases, n (%)	Controls, n (%)	OR (95% CI) <sup>†</sup>
Age (tertile), y				
≤61	Long	84 (75.7)	127 (84.7)	1.00 (reference)
	Short	27 (24.3)	23 (15.3)	1.61 (0.85-3.04)
62-69	Long	55 (55.6)	97 (70.3)	1.00 (reference)
	Short	44 (44.4)	41 (29.7)	1.83 (1.06-3.15)
≥70	Long	40 (44.4)	88 (68.7)	1.00 (reference)
	Short	50 (55.6)	40 (31.3)	2.78 (1.57-4.90)
Sex				
Male	Long	113 (56.8)	199 (74.0)	1.00 (reference)
	Short	86 (43.2)	70 (26.0)	2.25 (1.50-3.38)
Female	Long	66 (65.3)	113 (76.9)	1.00 (reference)
	Short	35 (34.7)	34 (23.1)	1.77 (1.00-3.12)
<i>H. pylori</i> status <sup>‡</sup>				
Negative	Long	27 (54.0)	55 (87.3)	1.00 (reference)
	Short	23 (46.0)	8 (12.7)	5.45 (2.10-14.1)
Positive	Long	152 (60.8)	256 (72.7)	1.00 (reference)
	Short	98 (39.2)	96 (27.3)	1.78 (1.25-2.54)
Smoking status <sup>§</sup>				
Nonsmokers	Long	48 (56.5)	133 (80.1)	1.00 (reference)
	Short	37 (43.5)	33 (19.9)	3.07 (1.71-5.51)
Former	Long	49 (54.4)	94 (70.7)	1.00 (reference)
	Short	41 (45.6)	39 (29.3)	2.02 (1.13-3.63)
Current	Long	81 (65.8)	85 (72.6)	1.00 (reference)
	Short	42 (34.2)	32 (27.4)	1.40 (0.79-2.45)
Pack-years of smoking <sup>§</sup>				
0	Long	48 (56.5)	133 (80.1)	1.00 (reference)
	Short	37 (43.5)	33 (19.9)	3.07 (1.71-5.51)
0.1-30	Long	64 (64.0)	107 (78.1)	1.00 (reference)
	Short	36 (36.0)	30 (28.9)	2.11 (1.17-3.80)
>30	Long	65 (58.0)	72 (64.3)	1.00 (reference)
	Short	47 (42.0)	40 (35.7)	1.39 (0.80-2.42)
Total years of drinking alcohol				
Nondrinker	Long	60 (57.1)	99 (72.8)	1.00 (reference)
	Short	45 (42.9)	37 (27.2)	1.97 (1.13-3.44)
<10	Long	45 (60.8)	106 (77.4)	1.00 (reference)
	Short	29 (39.2)	31 (22.6)	2.38 (1.26-4.49)
10-29	Long	37 (72.5)	70 (82.3)	1.00 (reference)
	Short	14 (27.5)	15 (17.7)	2.28 (0.93-5.60)
>29	Long	34 (53.1)	36 (64.3)	1.00 (reference)
	Short	30 (46.9)	20 (35.7)	1.67 (0.78-3.61)
Fruit intake				
Daily/weekly	Long	82 (62.6)	162 (80.2)	1.00 (reference)
	Short	49 (37.4)	40 (19.8)	2.43 (1.46-4.05)
Several times/month	Long	60 (59.4)	100 (73.0)	1.00 (reference)
	Short	41 (40.6)	37 (27.0)	1.90 (1.08-3.34)
Rarely/never	Long	28 (54.9)	49 (64.5)	1.00 (reference)
	Short	23 (45.1)	27 (35.5)	1.73 (0.80-3.76)
Vegetable intake				
Daily/weekly	Long	85 (60.3)	195 (78.0)	1.00 (reference)
	Short	56 (39.7)	55 (22.0)	2.39 (1.51-3.81)
Several times/month	Long	66 (61.1)	85 (69.7)	1.00 (reference)
	Short	42 (38.9)	37 (30.3)	1.63 (0.92-2.88)
Rarely/never	Long	19 (54.3)	29 (72.5)	1.00 (reference)
	Short	16 (45.7)	11 (27.5)	2.00 (0.73-5.50)

\*Long: second, third, and fourth quartiles of telomere length; short: first quartile.

<sup>†</sup>Adjusted for age, gender, smoking status, and pack-years of smoking.

<sup>‡</sup>Negative, tested negative for IgG antibodies both to *H. pylori* and to cagA; positive, tested positive for either IgG antibodies to *H. pylori* or cagA antibody or both.

<sup>§</sup>Adjusted for each other.

gastric cancer. Among our control population, shortened telomeres were related to several recognized gastric cancer risk factors, including older age, smoking, low fruit intake, and *H. pylori* positivity, suggesting possible etiologic pathways.

Our observation that telomere length in peripheral leukocyte DNA declined with increasing age is consistent with previous reports (39–43). However, the extent of telomere shortening may vary considerably among individuals within age groups, suggesting that environmental and lifestyle factors could play critical roles in the rate of telomere attrition. Because of high guanine content in spe-

cific telomere sequences, telomeres are remarkably sensitive to damage by oxidative stress (44) and telomeric DNA is deficient in the repair of single-strand breaks induced by oxidative DNA damage (3, 45, 46). *H. pylori*-positive gastric mucosa has been shown to have shorter telomere length than *H. pylori*-negative mucosa (23, 24). We found that telomere length in leukocyte DNA was also significantly shortened in *H. pylori*-positive persons compared with those without the organism. This observation provides evidence that carriage of gastric *H. pylori*, like other persistent microbes (5, 6, 47, 48), can contribute to the progressive shortening of telomeres in blood leukocyte

genomic DNA. Infection-induced chronic inflammation increases the turnover of peripheral leukocytes, leading to a greater loss of leukocyte telomere repeats over time. *H. pylori* infection may also facilitate telomere shortening process by increasing cumulative oxidative stress (3, 4) that has been proposed as a potential mechanism for *H. pylori* infection-related gastric cancer (49).

Cigarette smoke contains a mixture of compounds that generate reactive oxygen species in hosts (50). In our control population, we found that persons with >30 pack-years of smoking had shorter telomere length than nonsmokers or subjects who smoked less. This finding supports previous observations that telomere length is related to cumulative lifetime cigarette smoke exposure in a dose-related manner (51). Others have shown that age-adjusted telomere length was 5 bp shorter per pack-year of smoking (4).

Treatment with *N*-acetylcysteine, an antioxidant, has been shown to decrease the telomere attrition rate (52). Fruits and vegetables are important natural antioxidant sources (53), and their high intake has been associated with reduced risk of gastric cancer (29, 50). Our observation of shortened telomere length with decreasing fruit intake among controls suggests that diets rich in antioxidants may decelerate telomere shortening, thus modifying cancer risk (3). Gastric cancer cases had shorter telomeres than the controls in our study. This observation is in agreement with findings from most studies of cancer, including cancers of the bladder, lung, kidney, and head and neck (5, 17, 19, 20), but not with a recent study reporting longer blood telomeres in breast cancer patients than controls (54). Our results lend further support to the hypothesis that telomere shortening in blood leukocyte DNA is a marker of cancer risk (5, 9, 12, 17, 19). Telomere shortening may increase cancer risk through impairment of cellular functions resulting from chromosome instability (13-15, 55) and cell senescence (9, 56).

In our stratified analyses, we observed a higher risk related to telomere length shortening among individuals without some of the risk factors for gastric cancer (nonsmokers, *H. pylori* infection negative, frequent fruit consumption, or frequent vegetable consumption) compared with individuals with these risk factors. This phenomenon may be, in part, due to competing risks among high-risk individuals, who might have been exposed to multiple risk factors that involve carcinogenic mechanisms other than telomere length shortening. The plausibility of this phenomenon is supported by the similarity of the RTL among our controls with these risk factors (mean RTL, 1.26, 1.32, 1.26, and 1.29 for controls who were heavy smokers, *H. pylori* infection positive, rarely consumed fruit, and rarely consumed vegetables, respectively) to that seen in cancer cases (mean RTL, 1.25).

Because this is the first study evaluating blood leukocyte genomic DNA telomere shortening in relation to gastric cancer risk, our findings need to be confirmed in future studies, preferably with prospectively collected genomic DNA samples. Despite the efforts to recruit cases immediately after diagnosis, 30% of eligible cases had died before they could be contacted (27). To the extent that telomere shortening might be related to survival, our results might not be generalizable to patients with advanced gastric cancer. However, we observed no consistent pattern when we stratified the results by tumor grade or tumor stage at diagnosis (data not shown), sug-

gesting that survival bias in our study was likely minimal. The study sample size is limited for stratified analyses. Therefore, caution needs to be exercised in interpreting these results. Another potential limitation was the relatively low rate of blood donation among cases (65.7%). However, we found no significant difference in selected demographic or lifestyle characteristics, including age, gender, education, alcohol drinking, smoking status, fresh vegetables and fruits intake, body mass index and family history of gastric cancer, between cases with and without blood donation data (32, 34). In addition, telomere length in peripheral leukocyte DNA may be different from telomere length in gastric tumor tissue in the same patient because of differences in telomerase activity. Although tumor samples were collected from most patients, the quality and quantity of their DNA were insufficient for any informative genetic analyses (57). In the present study, *H. pylori* infection is not positively associated with gastric cancer risk. The disappearance of *H. pylori* after cancer diagnosis may partially explain the observed association, especially in populations with relatively high baseline prevalence (58, 59). The *H. pylori* prevalence is 84.8% among controls in the present study.

In summary, telomere shortening in blood leukocyte genomic DNA in this high-risk Polish population is associated with gastric *H. pylori* colonization, cigarette smoking, and fruit intake. Shortened telomeres increased gastric cancer risk.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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