

Telomere Length and Pancreatic Cancer: A Case–Control Study

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Abstract

Background: Telomeres, the ends of chromosomes, are critical for maintaining genomic stability and grow shorter with age. Shortened telomeres in pancreatic tissue play a key role in the pathogenesis of pancreatic cancer, and shorter telomeres in peripheral blood leukocytes (PBL) have been associated with increased risk for several cancer types. We hypothesized that shorter blood telomeres are associated with higher risk for pancreatic cancer.

Methods: Telomere length was measured in PBLs using quantitative real-time PCR in 499 cases with pancreatic cancer and 963 cancer-free controls from the Mayo Clinic. ORs and confidence intervals (CI) were computed using logistic generalized additive models (GAM) adjusting for multiple variables.

Results: In multivariable adjusted models, we observed a significant nonlinear association between telomere length in peripheral blood samples and the risk for pancreatic cancer. Risk was lower among those with longer telomeres compared with shorter telomeres across a range from the 1st percentile to 90th percentile of telomere length. There was also some evidence for higher risk among those with telomeres in the longest extreme.

Conclusions: Short telomeres in peripheral blood are associated with an increased risk for pancreatic cancer across most of the distribution of length, but extremely long telomeres may also be associated with higher risk.

Impact: Although the temporality of this relationship is unknown, telomere length may be useful as either a marker of pancreatic cancer risk or of the presence of undetected pancreatic cancer. If telomere shortening precedes cancer incidence, interventions to preserve telomere length may be an effective strategy to prevent pancreatic cancer. *Cancer Epidemiol Biomarkers Prev*; 21(11); 2095–100. ©2012 AACR.

Introduction

Telomere length and telomere erosion are strongly implicated in the molecular biology of pancreatic cancer (1). Telomeres are the end caps of chromosomes and are composed of telomeric proteins and hexamer repeats of DNA (TTAGGG; ref. 2) that serve to protect the integrity of the DNA sequence during cell division. Across the lifespan of an organism, telomeres become shorter with each cell division and eventually become too short to function, termed telomere crisis. Although normal cells undergo apoptosis or senescence at telomere crisis, abnormal cells, for example, those with p53 or retinoblastoma

dysfunction, may proceed through cell division with inadequate telomeres. This can lead to gross chromosomal abnormality and a malignant phenotype in the daughter cell (3). Abnormal cells that survive division with inadequate telomeres do so by activating telomerase, or extending their telomeres through recombination (alternative lengthening of telomeres, ALT).

Shorter telomeres in peripheral blood leukocytes (PBL) have been associated with higher risk for several cancers including bladder (4–6), lung (7), esophageal (8, 9), gastric (10, 11), head and neck (6), ovarian (12), and renal (13), as well as cancer overall (14, 15). In addition, telomere shortening in tissue has been reported for pancreatic ductal adenocarcinoma (16) and premalignant pancreatic intraepithelial neoplasia (PanIN) as compared with normal tissue and has been implicated in the pathogenesis of a variety of other malignancies (16–20). Moreover, telomere shortening has been associated with cigarette smoking (21) and diabetes (22–25), as well as factors associated with both diabetes and pancreatic cancer, including obesity/body mass index (BMI; refs. 21, 26) and insulin resistance (26, 27). We investigated the association between pancreatic cancer and telomere length in PBLs in a large hospital-based case–control study.

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Materials and Methods

This study draws upon data collected through the patient registry of Mayo Clinic's Pancreatic Cancer Specialized Program of Research Excellence (SPORE) between 2000 and February 2009. A total of 1,500 participants were included in this study, 500 patients with pancreatic cancer (cases) and 1,000 noncancer controls (500 controls with diabetes and 500 controls without diabetes). Detailed ascertainment and recruitment information has been published elsewhere (28). Briefly, cases were ascertained from patients attending Mayo Clinic who had histologically proven or clinically diagnosed pancreatic cancer and were recruited using an ultrarapid approach. More than 80% of the case subjects were recruited within a month of their diagnosis. Controls were recruited through a Mayo Clinic-based sample of primary care patients having routine check-up visits (general medical examination). Controls were frequency-matched to pancreatic cancer cases on age (5-year groups), sex, and state/region of residence; controls with diabetes were oversampled to provide statistical efficiency when evaluating contrasts by diabetes status. Cases and controls were limited to non-Hispanic Caucasians. Those with previous diagnosis of cancer (except nonmelanoma skin cancer) at the time of enrollment were excluded from the study.

Once informed consent was obtained, participants completed detailed questionnaires about family history, lifestyle, and risk factors and had blood samples drawn for research purposes. Individuals were classified as current smokers if they reported smoking more than 100 cigarettes in their lifetime and had smoked in the past year, former smokers if they reported smoking greater than 100 cigarettes in their lifetime but had not smoked for the past year, and never smokers if they reported 100 or fewer cigarettes smoked in their lifetime. Because the onset of pancreatic cancer is often associated with profound weight loss, BMI was computed using self-reported "usual adult weight" for cases and controls. All other covariates (age, sex, diabetes status, and fasting blood glucose concentration) were ascertained from the electronic medical record concurrent with recruitment. One case and 37 controls were dropped from the analyses due to missing data, resulting in a final sample size of 499 cases and 963 controls.

Because of the ultrarapid nature of case ascertainment, all blood samples were collected before administration of chemotherapy. Telomere length in PBLs was measured using the PCR method described by Cawthon (29). This PCR-based assay uses a set of primers to the telomeric hexamer repeats to amplify telomeric DNA. The average telomere length for each sample was measured by comparing the intensity of the sample's telomere signal (T) with the signal from a single-copy gene (S) to compute the T/S ratio. The T and S values were taken from the median of 3 repeats for each sample.

Two master mixes of PCR reagents were prepared, one with the T primer pair, the other with the S primer pair. Fifteen microliters of the T master mix were added to each sample well, control well, and standard curve well of the

first plate and 15 μ L of the S master mix were added to each sample well, control well, and standard curve well of the second plate. For each sample assayed, 3 identical 5 μ L aliquots of the DNA sample (15 ng/aliquot) were added to plate 1 and another 3 aliquots were added to the same well position in plate 2. For each standard curve, one reference DNA sample was serially diluted in Tris EDTA buffer (TE) by 1:2-fold per dilution to produce 6 concentrations of DNA ranging from 0.78 to 25 ng/ μ L. Five microliters of each concentration was distributed to the standard curve wells on each plate. The plates were then sealed with a transparent adhesive cover, centrifuged briefly at $800 \times g$ and transported on ice to the ABI 7900HT instrument for analysis.

The T and S PCRs were prepared identically with the exception of the oligonucleotide primers. The final concentrations of the reagents in the PCR were 20 mmol/L Tris-HCl, 0.2 mmol/L each dNTPs, 2.0 mmol/L $MgCl_2$, 1% dimethyl sulfoxide, 150 nmol/L ROX dye, 0.2 \times SYBR Green I (Molecular Probes), 5 mmol/L DL-Dithiothreitol (DTT), 1.25 U AmpliTaq Gold DNA polymerase (Applied Biosystems). The final telomere primer concentrations was tel 1b, 600 nmol/L; tel 2b 900 nm.

The single-copy (S) control gene (*B2-globin* on chromosome 11) concentrations was B2-globin forward primer (hbg1) 300 nm; B2-globin reverse primer (hbg2) 700 nm. The primer sequences (written 5'-3') were:

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tel 1b CGGTTTGGTGGGTTGGGTTGGGTTGGG-
TTTGGGTT;
tel 2b GGCTTGCCCTACCCTACCCTACCCTACC-
TTACCCT;
hbg1 GCTTCTGACACAAGTGTGTTCACTAGC;
hbg2 CACCAACTTCATCCACGTTACC.
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All PCRs were conducted on the ABI Fast Real-Time 7900HT (Applied Biosystems). The thermal cycle conditions for both primers pairs began with a 95°C incubation for 10 minutes to activate the AmpliTaq Gold DNA polymerase. For telomere PCR, this was followed by 40 cycles of 95°C for 15 seconds, 54°C for 2 minutes. For the hbg PCR, this was followed by 40 cycles of 95°C for 15 seconds, 58°C for 60 seconds, and 72°C for 30 seconds. The data were then analyzed with the ABI SDS software to generate the standard curve for each plate.

Relative telomere length was determined by substituting the ratio of the median of 3 telomere PCR measurements (T) and the median of the 3 single copy gene PCR values (S) into the equation: telomere length in base pairs = $(T/S) \times 1,910 + 4,157$, in which 1,910 represents the slope of the line comparing the relative T/S ratio with the measurement of telomere length in base pairs by the mean telomere restriction fragment (TRF) and 4,157 is the y -intercept (29).

The relative telomere length (T/S) was transformed to the natural log, as log-transformed T/S ratios were approximately normally distributed and generally better behaved than the skewed distribution of the

untransformed *T/S* ratios. The relationships between continuous variables and pancreatic cancer were examined using generalized additive models (GAM) with binary regression and a logit link. Continuous variables (telomere length, age, BMI, fasting blood glucose, etc.) were included in the models as thin-plate regression splines with the degree of smoothness selected by generalized cross-validation. Results were quantified in terms of ORs with the corresponding 95% confidence intervals (CI). Effect modification by cigarette smoking, BMI, age, diabetes status, or fasting blood glucose was examined by including interaction terms into the GAM modeled as tensor product smooths.

Results

Table 1 depicts selected characteristics of the cases and controls. Among cases, 74.5% reported current diabetes, or a fasting blood glucose greater than 100 mg/dL. Cases were more likely to report being current smokers than controls (10.8% vs. 3.2%).

In GAMs adjusted for age, smoking status, sex, BMI, diabetes status, and fasting blood glucose concentration, we observed a significant inverse association between PBL telomere length and pancreatic cancer risk for those with telomere lengths across the 1st to 90th percentile (4,184 to 6,520 bp; Fig. 1). Among those with telomeres in the top 1% (lengths greater than 11,740 bp), we observed some evidence for an increase in pancreatic cancer risk although the estimates are imprecise. The OR for pancreatic cancer among those with shortest telomeres was 1.48 (95% CI, 1.12%–1.97%), as compared with a reference telomere length of 4,911 bp (the median telomere length in controls; Table 2). The lowest point estimate of pancreatic cancer risk was observed for those with telomere length at the 90th percentile (6,520 bp) with an OR of 0.72 (95% CI, 0.53–0.98%) as compared with the reference of 4,911 bp. Although there was a strong inverse trend in risk for longer telomere lengths, there was also evidence that cases were more likely to have extremely long telomeres than controls; the multivariable-adjusted OR for pancreatic cancer among those with the longest telomeres (27,723 bp) was 2.84 (0.55–14.76). We did not detect evidence for effect modification by cigarette smoking, BMI, age, diabetes status, or fasting blood glucose concentration.

Conclusions

We found that telomere length in PBLs was associated with risk for pancreatic cancer. There was a continuous linear trend of higher pancreatic cancer risk with shorter telomeres across a range of telomere lengths representing the shortest 1% to the longest 10%. This trend translates to a 2-fold increase in risk for pancreatic cancer across the extremes of the range (1st vs. 90th percentile of telomere length). We also observed some evidence for a greater prevalence of extremely long telomeres in pancreatic cancer cases than controls. The long telomeres in these cases (the top 1% of telomere length) may represent an

Table 1. Selected characteristics of pancreatic cancer cases and controls

Characteristic	Case	Control
N	499	963
Age, y (SD)	66.0 (10.2)	66.7 (9.8)
Sex (% male)	53.1%	54.0%
Fasting blood glucose, mg/dL (SD)	138.1 (54.9)	103.2 (23.5)
Diabetic	74.5%	48.3%
Cigarette smoking		
Current	10.8%	3.2%
Former	48.7%	44.1%
Never	40.5%	52.7%
BMI, kg/m ² (SD)	28.3 (5.5)	27.3 (4.7)

abnormal activation of telomerase in leukocytes, or the effects of ALT (ref. 30) in pancreatic cancer cases. This aspect of the association requires confirmation and further investigation. The overall result is a skewed "U-shaped" relationship between the constitutional telomere length and the prevalence of pancreatic cancer. To our knowledge, this is the first report of an association between pancreatic cancer risk and PBL telomere length.

These results are consistent with numerous other studies reporting similar associations for other types of cancer. In a meta-analysis of 11 retrospective and 16 prospective studies of telomere length in relation to cancer, the pooled OR was 1.96 (95% CI, 1.37%–2.81%) for cancer overall comparing people in the shortest quartile of telomere

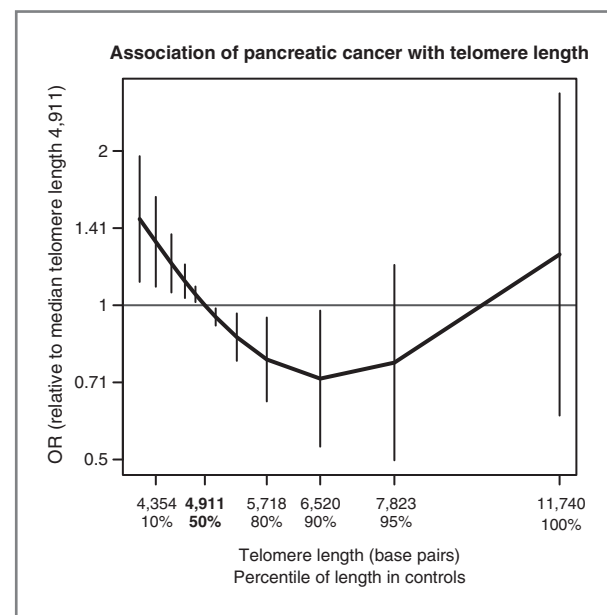


Figure 1. Multivariable adjusted ORs and CIs for pancreatic cancer risk by telomere length in peripheral blood cells compared with the reference category of 4,911 bp, the median telomere length in nondiabetic controls.

Table 2. Associations between telomere lengths and pancreatic cancer in cases and controls from the Mayo Clinic SPORE patient registry

Telomere length	Percentile	Age adjusted OR (95% CI)	Multivariable adjusted OR (95% CI)
4,184	1	1.46 (1.16–1.84)	1.47 (1.11–1.95)
4,354	10	1.33 (1.12–1.56)	1.33 (1.09–1.63)
4,524	20	1.21 (1.08–1.34)	1.21 (1.06–1.38)
4,676	30	1.11 (1.05–1.19)	1.11 (1.03–1.20)
4,799	40	1.05 (1.02–1.08)	1.05 (1.01–1.09)
4,911	50	1.00 (Reference)	1.00 (Reference)
5,043	60	0.95 (0.92–0.98)	0.95 (0.91–0.99)
5,312	70	0.86 (0.79–0.94)	0.87 (0.78–0.96)
5,718	80	0.78 (0.67–0.91)	0.78 (0.65–0.95)
6,520	90	0.72 (0.56–0.92)	0.72 (0.53–0.98)
7,823	95	0.77 (0.54–1.08)	0.77 (0.50–1.20)
11,740	99	1.18 (0.66–2.12)	1.26 (0.61–2.59)
27,723	100	4.09 (1.06–15.72)	2.87 (0.55–14.91)

NOTE: Multivariable ORs adjusted for age, sex, race, BMI, diabetes status, and fasting blood glucose. The reference category (in bold) is 4,911 base pairs, the median telomere length in the nondiabetic control group.

length with those in the longest quartile (31). A second meta-analysis reported a pooled OR of 1.69 (95% CI 1.53%–1.87%) for cancers of the digestive system, and found that results of studies for cancers overall were consistent between Caucasian and Asian populations, with no evidence of publication bias (32).

Telomere shortening plays a critical role in the molecular pathology of pancreatic cancer and is a component of the standard model of progression from normal pancreas to adenocarcinoma (1). For example, telomeres are found to be shorter or absent in all pancreatic adenocarcinoma tissues as compared with adjacent normal tissue (18), a finding that extends to the earliest precursors of malignancy, pancreatic intraepithelial neoplasia 1a (PanIN 1a; ref. 18). Likewise, telomere shortening has been observed in acinar to ductal metaplasia associated with PanINs but not in metaplasia not associated with PanINs (18). In the context of the role of telomere shortening in the development of pancreatic cancers, we reason that the association we observe between shorter telomeres in peripheral blood cells and pancreatic cancer risk may reflect an underlying relationship between short telomeres in pancreatic tissue and cancer susceptibility.

Among the strengths of this study, cases were ascertained in an ultrarapid fashion, minimizing the chance for survival bias and ensuring that telomere length was not influenced by treatment. Although survival from pancreatic cancer is remarkably poor, with 50% of patients dying within 6 months, 80% of our cases were recruited within 1 month of diagnosis. Second, although recall bias is a concern in case–control studies, our primary independent variable, telomere length, is not subject to biased recall. Likewise, all of our covariates except cigarette smoking status and usual adult weight were derived from the

electronic medical record and not self-reported (e.g., fasting blood glucose concentration, age, etc.). Therefore, recall bias is unlikely to have influenced our results. Finally, the use of GAMs revealed an important nonlinear component to the association, whereas we would have concluded that no association existed had we relied on a comparison of mean telomere lengths between cases and controls, or used logistic regression models that enforced a linear relationship between the odds of pancreatic cancer and telomere length.

Our results should be interpreted in consideration of some potential limitations. A primary concern in a case–control study is the potential for selection bias. As a large referral center, pancreatic cancer cases are attracted to Mayo Clinic for treatment from a broader population than the controls sampled from the general internal medicine clinics, which represent a more local population. Selection bias could arise if the populations underlying the case and control samples have inherently different constitutional telomere lengths. In particular, as a hospital-based study, we must consider the potential for Berkson's bias. For example, if shorter constitutional telomere length is related to the likelihood that pancreatic cancer cases attended Mayo Clinic, or conversely if longer telomeres somehow predicted use of Mayo Clinic's general internal medicine clinics, bias could result. However, we are unaware of any biologic basis to suppose that telomere length is related to the likelihood of selection as a case or control. Finally, because our sample was limited to non-Hispanic Whites, our results may not be generalizable to populations with different demographic characteristics. Therefore, our hypothesis should be examined in other populations to determine if the associations we observed hold in different race/ethnic groups.

A limitation of our study is that leukocyte telomere length is assumed to reflect telomere length in the pancreas. Although no study has directly measured the correlation of telomere lengths in blood and pancreatic tissue within individuals, an autopsy study reported that telomere length in pancreatic tissue was progressively shorter in people of older ages ranging from 13.9 kb in neonates to 8.4 kb in centenarians (33). Moreover, several studies have examined how telomeres length in blood relates to telomere length in other tissues. For example, telomere length in blood correlates well with telomere length in skin ($r = 0.79$; ref. 34). Likewise, the length of telomeres in skin is highly correlated with telomere length in lingual epithelium ($r = 0.84$; ref. 35). Future work aims to directly describe the relationship between tissue and PBL telomere lengths in pancreatic cancer cases.

An etiologic hypothesis for telomere shortening in cancer posits that shorter telomeres in peripheral blood are an indicator of an older cellular or biologic age, and that peripheral blood telomere length reflects shorter telomeres across other tissues (e.g., the pancreas; ref. 6). With globally shorter telomeres, more cells would be near telomere crisis and with a greater potential for malignant transformation. Our results are consistent with this hypothesis, and indicate that people with shorter peripheral blood telomeres may be at higher risk for pancreatic cancer than those with longer telomeres across most of the range of telomere length. However, because of the retrospective nature of the study, we were unable to determine the timing of the association between telomere length measurement and pancreatic cancer. We must therefore consider an alternative hypothesis that some factor(s) associated with the presence of pancreatic cancer may have lead to shorter telomeres in peripheral blood cells (i.e., "reverse causation"). One candidate factor for this phenomenon could be an increase in fasting blood glucose. For example, one study has reported that an increase in BMI and increasing insulin resistance (HOMA-IR) over time were associated with more rapid shortening of telomeres (26). Thus, changes in glucose metabolism concurrent with the development of pancreatic cancer could explain shorter telomeres in pancreatic cancer cases than controls. However, our analyses controlled for both diabetes status and fasting blood glucose concentration. Moreover, examination of the relationship between telomere length and diabetes status and fasting blood glucose in controls revealed little if any evidence for an association ($P = 0.89$ and 0.16 , respectively). Likewise, we did not

observe heterogeneity of effect measures when stratifying by diabetes status.

In conclusion, we observed a significant inverse association between constitutional telomere length and risk for pancreatic cancer in a large hospital-based case-control study. If confirmed, our results indicate that telomere length may be a novel indicator of pancreatic cancer risk and that preventive strategies targeting the preservation of telomere length could be developed against pancreatic cancer. Conversely, depending on the timing of the relationship, telomere length may be a useful indicator of the presence of an undetected pancreatic cancer. Additional work is needed to determine if constitutional telomere shortening is cause or consequence of pancreatic cancer, and whether telomere length has any clinical use in risk prediction or diagnosis of pancreatic cancer. In other words, telomere length may be either a biomarker of risk, or of disease. Future studies of constitutional telomere length in relation to pancreatic cancer should allow for a nonlinear association between telomere length and cancer, should include information on fasting blood glucose concentration, and should examine telomere length as a time-dependent variable to resolve the remaining questions of the temporal relationship.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: H.G. Skinner, G.M. Petersen, L.A. Boardman
Development of methodology: H.G. Skinner, R.A. Johnson, L.A. Boardman

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.G. Skinner, G.M. Petersen, L.A. Boardman
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H.G. Skinner, R.E. Gangnon, G.M. Petersen
Writing, review, and/or revision of the manuscript: H.G. Skinner, R.E. Gangnon, K.R. Litzelman, G.M. Petersen, L.A. Boardman
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.G. Skinner, K.R. Litzelman, S.T. Chari

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