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Leukocyte telomere length and its interaction with germline variation in telomere-related genes in relation to pancreatic adenocarcinoma risk

Samuel O. Antwi¹, William R. Bamlet², Kari G. Rabe², Richard M. Cawthon³, Isoken Umudi¹, Brooke R. Druliner⁴, Hugues Sicotte², Ann L. Oberg², Aminah Jatoi⁴, Lisa A. Boardman⁴, Gloria M. Petersen⁵

¹Division of Epidemiology, Mayo Clinic, Jacksonville, FL

²Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN

³Department of Human Genetics, University of Utah, Salt Lake City, Utah

⁴Department of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN

⁵Division of Epidemiology, Mayo Clinic, Rochester, MN

Abstract

Background—Leukocyte telomere length (LTL) has been associated with risk of multiple cancers, but its association with pancreatic ductal adenocarcinoma (PDAC) is unclear. We investigated the association between peripheral blood LTL and PDAC risk, and examined effect modification by candidate single-nucleotide polymorphisms (SNPs) previously reported to be associated with variation in LTL.

Methods—A case-control study of 1460 PDAC cases and 1459 frequency-matched controls was performed using biospecimens and data from the Mayo Clinic Biospecimens Resource for Pancreas Research. Quantitative PCR was used to measure LTL and then categorized into tertiles based on sex-specific control distribution. Eleven telomere-related SNPs were also genotyped. Logistic regression was used to calculate multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs).

Results—Shorter peripheral blood LTL was associated with a higher risk of PDAC ($OR_{T1vsT3}=1.26$, 95%CI=1.03–1.54, $P_{trend}=0.02$, $OR_{continuous}=1.14$, 95%CI=1.02–1.28), but the association was restricted to cases with treatment-naïve blood samples ($OR_{T1vsT3}=1.51$, 95%CI=1.16–1.96, $P_{trend}=0.002$; $OR_{continuous}=1.25$, 95%CI=1.08–1.45) and not cases whose blood samples were collected after initiation of cancer therapy ($OR_{T1vsT3}=1.10$, 95%CI=0.87–

Corresponding author: Samuel O. Antwi, PhD, Mayo Clinic, 4500 San Pablo Road South, Vincent Stabile Building 756N, Jacksonville, FL, 32224, USA Tel: 1-904-953-0310; Fax: 1-904-953-1447; Antwi.samuel@mayo.edu.

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Disclosures: Dr. Richard M. Cawthon (or RMC) and the University of Utah hold a patent for the technology used herein to measure average telomere length by quantitative PCR, and have licensed the commercial use of this technology to Telomere Diagnostics, Inc. of Menlo Park, California. However, Dr. Cawthon was blind regarding all clinical data (sex, age, clinical outcomes, etc.) for the subjects studied here, at the time he performed and reported the results of the telomere length assays.

Data availability statement: The data may be made available to researchers upon request to Dr. Gloria Petersen (Petersen.gloria@mayo.edu). Ethical and legal restrictions apply to these data.

1.39, $P_{\text{trend}}=0.42$; $OR_{\text{continuous}}=1.08$, 95% CI=0.94–1.23). Three SNPs (*TERC*-rs10936599, *ACYP2*-rs11125529, and *TERC*-rs1317082) were each associated with inter-individual variation in LTL among controls, but there was no evidence of effect modification by these SNPs.

Conclusions—Treatment-naïve short LTL is associated with higher risk of PDAC, and the association does not differ by germline variation in the candidate telomere-related SNPs examined.

Impact—Peripheral blood LTL might serve as a molecular marker for risk modeling to identify persons at high-risk of PDAC.

Keywords

Telomere length; telomere genes; pancreatic cancer; pancreatic ductal adenocarcinoma; PDAC

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an often fatal malignancy with 5-year survival rate of 9% (1,2). The poor prognosis of PDAC is due largely to incomplete understanding of the etiology, the generally aggressive disease course, and the often late stage of diagnosis (1). A thorough understanding of the causes of PDAC and the pathways through which various risk factors influence susceptibility to PDAC will enhance risk stratification toward early detection. The firmly established risk factors of PDAC include chronic pancreatitis, cigarette smoking, pre-existing diabetes, certain inherited genetic disorders or variants, and obesity (1,3–5). However, the molecular mechanisms by which these risk factors influence PDAC development is not completely clear. A proposed mechanism for some of these risk factors (e.g., smoking, obesity, and diabetes) involves the acceleration of telomere shortening (6–9).

Telomeres, the tandemly repeated non-coding DNA sequences (TTAGGG) that together with shelterin proteins form capping structures at chromosome ends and ensures segregational fidelity of chromosomes during mitosis (6,10), have been implicated in risk of cancer development (9,11–13). Normally, host telomere length (TL) is determined by the activities of the telomerase enzyme and involves several genes (e.g., *TERC*, *TERT*, and *OBFC1*) and the shelterin proteins (e.g., *TRF1*, *RAP1*, and *POT1*) that bind the DNA sequences at chromosome ends (6,10,14). Hence, TL is influenced in part by germline variation in telomere-related genes (15–18). Moreover, because of incomplete replication of the 3' ends of chromosomal DNA at each cell division, TL shortens progressively with successive cell division and thus correlates inversely with age (6,9,19). Several other factors have been associated with TL attrition, including cigarette smoking (20,21), obesity (20,22), physical inactivity (23), and insulin resistance (24)—all of which are known cancer risk factors and are thought to influence cancer risk by inducing telomere-shortening processes, such as chronic inflammation and oxidative stress (6,8).

Epidemiological studies have reported associations between short LTL and increased risk of multiple cancers, including gastric, bladder, ovarian, and head and neck cancers (25–28). Five prospective studies (including nested case-control studies)(29–33) and one retrospective study (34) have investigated the association between LTL and PDAC risk and have reported

mixed findings. Of the prospective studies, one reported a linear inverse association between LTL and higher PDAC risk (32), and in marked contrast, two studies reported a linear positive association between LTL and higher PDAC risk (29,33). One other prospective study reported a modest non-linear association between shorter LTL and higher PDAC risk (30), while another reported a “U-shaped” association wherein both shorter and longer LTLs were associated with higher PDAC risk (31). The lone retrospective study also reported a “U-shaped” association (34)(summarized in supplementary Figure 1). Thus, the nature of any association between LTL and PDAC risk remains unclear. Additionally, no study has investigated potential interactions between germline variation in telomere-related genes and measured LTL in relation to PDAC risk. In this study, we tested the hypothesis that short peripheral blood LTL is associated with higher risk of PDAC in a clinic-based, case-control study using the largest number of PDAC cases of any study conducted to date. We further tested the hypothesis that LTL interacts with germline variation in telomere-related genes or known cancer risk factors to influence susceptibility to PDAC.

Materials and Methods

Study Population

Following approval by the Mayo Clinic Institutional Review Board, data and biospecimens were obtained from the Mayo Clinic Biospecimen Resource for Pancreas Research, a patient registry supported by the Mayo Clinic Specialized Program of Research Excellence (SPORE) in Pancreatic Cancer (3,5). The registry utilizes an ultra-rapid case ascertainment process, which ensures that ~86% of PDAC participants identified at Mayo Clinic are recruited within 30 days of diagnosis, with an average of two weeks between first contact and recruitment. The cases included in this study had pathologically (~95%) or clinically confirmed diagnosis of PDAC, recruited between October 2000 and June 2016. The methods used to identify controls have been described (35). In brief, controls were healthy patients recruited from primary care clinics at Mayo Clinic during regular wellness check visits. The controls did not have a prior history of cancer (except non-melanoma skin cancer) and were recruited between May 2004 and March 2012. The controls used in this study were selected from a larger pool of controls through frequency matching to cases based on age (± 5 years), sex, and race (White, other). In the patient registry, participation rates are approximately 67% for cases and 70% for controls (3,5,35). In our experience, non-participation by cases is due mainly to the debilitating nature of PDAC and the often rapid fatality associated with PDAC. In all, 1500 controls were frequency-matched to 1500 cases for the study. All participants provided written informed consent.

Data Collection

The participants' completed standardized risk factor questionnaires that sought information on demographics (age, sex, and race/ethnicity), highest level of education, self-reported personal history of diabetes, cigarette smoking history, and usual adult weight and height. Smoking history was categorized as current, former and never, and self-reported usual adult weight and height were used to calculate body mass index (BMI) in kg/m^2 .

Telomere Length Measurement

Genomic DNA was isolated from peripheral blood buffy coat leukocytes stored at -80°C until DNA extraction. DNA extraction was performed using the Maxwell® RSC Instrument with appropriate kits (Promega) and quantitated using a Qubit Fluorometer. Details of the methods used for LTL measurement have been described (36). Briefly, quantitative polymerase chain reaction (qPCR) was used to measure LTL, expressed as relative telomere to single-copy gene (T/S) ratio and was based on the average of three measurements per sample. The qPCR-based assay uses one pair of primers to amplify the telomeric hexanucleotide repeats and another pair to amplify a single-copy gene. The primer sequences, written 5'–3', were:

telg: AACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT

telc: TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA

HBBugc: CGGCGGCGGGCGGCGGGCTGGGCGGCTTCATCCACGTTACCTTG

HBBdgc: GCGCGGCGGGCGGCGGGCTGGGCGGCTTCATCCACGTTACCTTG

The DNA samples were diluted in Dilution Buffer, consisting of 10mM Tris-HCl 0.1 mM EDTA, pH 8.0, “TE buffer”, with BSA added to 100 ng of DNA per microliter. Dilution Buffer without DNA also was assayed in quadruplicate on each 384-well plate as the No Template Control. A concentrated stock (175 ng of DNA/microliter of TE buffer) of pooled samples from several healthy Utah subjects aged 65 years (“Standard Pooled DNA”) was serially diluted into the Dilution Buffer to produce five DNA concentrations: 27, 9, 3, 1, and 0.333 ng of DNA per five microliters, in sufficient quantities for 4 replicate qPCR reactions per DNA concentration for each 384-well qPCR plate. The Standard Curve serial dilution, always based on the same Standard Pooled DNA, was freshly prepared for each 384-well qPCR run. Three reference DNAs with known low, middle, and high telomere lengths (T/S values) relative to that of the standard pool DNA (whose T/S value is, by definition, 1.00) were assayed in quadruplicate on each 384-well qPCR plate. Input DNA amount was approximately 3 ng per reaction. DNA samples from the participants were diluted into the Dilution Buffer in a 96-well source plate, with one subject’s DNA sample per well, at a concentration of approximately 3 ng per 5 microliter, in sufficient quantities for 3 replicate qPCR reactions per subject per 384-well qPCR plate. All qPCR reactions were run on a Bio-Rad CFX384 Real-Time PCR Detection System. Five microliters of 2x Master Mix (2x KlenTaq1 Reaction Buffer, pH 7.9, DNA Polymerase Technology; 2M betaine solution, Sigma-Aldrich; 0.4mM of each of the 4 dNTPs, ThermoFisher; 2x SYBR Green I, ThermoFisher; 100 ng/microliter of BSA (bovine serum albumin), New England Biolabs; 2x Titanium Taq DNA Polymerase, Takara Bio USA, Inc.; 400 nM of each of the four primers) was added to each well that would subsequently be receiving DNA (or Dilution Buffer without DNA), after which 5 microliters of DNA (Standard Curve, Reference, or Subjects) or Dilution Buffer alone were added. Plates were then sealed with adhesive transparent film, shaken to mix master mix and sample, and centrifuged at $700 \times g$ for a few seconds. Thermal cycling was performed as follows: (a) $94^{\circ}\text{C} \times 3'$, (b) $98^{\circ}\text{C} \times 2''$, $49^{\circ}\text{C} \times 15''$; 2 cycles, (c) $98^{\circ}\text{C} \times 2''$, $65^{\circ}\text{C} \times 10''$, $74^{\circ}\text{C} \times 15''$ with signal acquisition, $84^{\circ}\text{C} \times 10''$, $86^{\circ}\text{C} \times 15''$ with

signal acquisition; 31 cycles, and (d) melt curve from 72C to 96C, 0.5C steps, each for 5" with signal acquisition. Analyses were performed using the Bio-Rad CFX Manager 3.1 software with relative quantification by the Standard Curve method. Following this protocol, LTLs (T/S values) were determined for the 1500 cases and 1500 controls; however, 40 case samples and 41 control samples failed assay design or quality control, leaving a final sample of 1460 cases and 1459 controls. The intra- and inter-assay coefficient of variation (CV) based on the assay standards were < 3%. The median intra-assay CV for the input DNA was 5.5%. Additional details about intra- and inter-assay reproducibility of T/S ratio measurements by qPCR has been provided by Cawthon (36).

SNP Selection and Genotyping

We selected 11 single nucleotide polymorphisms (SNPs) that have been associated with inter-individual variation in measured LTL in genome-wide scans (15–17) or candidate gene studies (18). The SNPs are located in or near the following genes: *TERC* (rs10936599, rs1317082), *TERT* (rs2736100, rs7726159), *NAFI* (rs7675998), *OBFC1* (rs9420907, rs2487999), *ZNF208* (rs8105767), *ACYP2* (rs11125529), *PXK* (rs6772228), and *CTCI* (rs3027234) (15–18). Genotyping of leukocyte DNA was performed by the Mayo Clinic Genome Analysis Core using the Sequenom multiplex assay (37). The genotyping call rates and concordance with blinded duplicates were each 100%. All allele frequencies were in Hardy-Weinberg equilibrium.

Statistical Analyses

Distribution of participant characteristics and selected telomere-related SNPs were compared between the cases and controls using means and proportions. The measured LTLs were categorized into tertiles based on sex-specific distribution among controls, and to maximize sample size and avoid small cell sizes in subgroup analyses. For age stratified analyses, both age- and sex-specific distributions among controls were used to categorize the LTLs into tertiles. Before performing the main association analyses, we examined the distribution of participant characteristics across LTL tertiles among control subjects using analysis of variance for continuous variables and chi-square tests for categorical variables. We also verified reported relationships between the candidate SNPs and LTL using linear regression, with adjustment for age (continuous), sex, BMI (continuous), smoking status (never, former, current), and diabetes (yes, no; both pre-existing diabetes and possibly PDAC-induced diabetes (1)). For these analyses, each SNP was modeled separately as predictor and LTL as the outcome and per-allele β -estimates were calculated for alleles reported to be associated with short LTL, using the reported long telomere allele as reference (15–17).

The association between LTL and PDAC risk was examined using unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs). The association was examined in multivariable models, adjusting for age, sex, race, diabetes, BMI and smoking history, and using the longest LTL tertile as reference. Linear trend across tertiles was assessed based on the ordinal method. We also modeled LTL as a continuous predictor variable, adjusting for covariates. Some of the PDAC cases included in the study had already started cancer-directed therapy before blood collection. Because cancer

treatment can affect LTL (38), we performed subset analyses among cases with treatment-naïve blood samples (n=653) and cases who had started cancer treatment before blood collection (n=773), with each group compared with the same controls. Data on treatment status were not available for 34 participants who were excluded from the subset analyses. Stratified analyses were performed by age (< 65, ≥65 years), sex, diabetes (yes, no), BMI (< 25, 25–29.9, ≥30 kg/m²), and smoking history (never, former, current). To determine whether the association between LTL and PDAC differed by germline variation in telomere-related SNPs, we performed stratified analyses by SNPs found to be significantly associated with variation in LTL among controls based on additive allele modeling. We also performed stratified analyses by the total number of alleles associated with shorter LTL carried by each participant across all 11 SNPs (9, 10–11, 12 short allele counts) and number of short telomere alleles based on three SNPs (rs1317082, rs2487999 and rs7726159; 0–2, 3–4, 5–6 short allele counts) as has been done by others (18). All stratified analyses included assessment of multiplicative interaction. Exploratory analyses were performed with restricted cubic spline functions with LTL as predictor variable in the unconditional logistic regression model to assess the possibility of a nonlinear association between LTL and PDAC risk. Analyses were performed with SAS v9.4 (SAS Institute, Cary, NC), and a two-sided p-value < 0.05 was considered statistically significant.

Results

Characteristics of the study participants and distribution of selected telomere-related SNPs are presented in Table 1 for the 1460 PDAC cases and the 1459 controls. The cases and controls did not differ by age, sex, or race. However, the cases were more likely than controls to be obese (BMI ≥30 kg/m²). Furthermore, the cases included greater proportions of current and former smokers and individuals with self-reported personal history of diabetes and had slightly shorter LTLs than controls. Among cases only, the LTLs were longer in cases who had already started cancer treatment before blood collection (n=773) than cases with treatment-naïve samples (n=653). No differences were noted in the distribution of the selected SNPs.

The distribution of participant characteristics across tertiles of LTL among controls is presented in Supplementary Table 1. Current smokers were more likely to belong in the shortest LTL tertile (T1) than in the longest tertile (T3), while never smokers were more likely to belong in the longest LTL tertile than in the shortest tertile. There were no other significant differences noted.

Relationships between alleles previously reported to be associated with variation in LTL and the measured LTL were examined among control subjects using multivariable-adjusted linear regression (Table 2). Alleles of three SNPs, *TERC*-rs1317082, *TERC*-rs10936599 and *ACYP2*-rs11125529, were found to be associated with variation in LTL. Each A allele of rs1317082 was associated with a 0.01628 reduction in T/S ratio, after adjusting for age, sex, BMI, smoking history, and diabetes. Per C allele of rs10936599 also was associated with a 0.01599 reduction in T/S ratio, and per A allele of rs11125529 was associated with a 0.01840 reduction in T/S ratio. An overall genetic score computed based on total number of short telomere alleles carried by each participant across all 11 SNPs showed a 0.00573

reduction in T/S ratio for every additional short telomere allele carried by a participant. The three SNPs in *TERC* and *ACYP2* and the overall genetic score were evaluated further for potential interaction with LTL in regards to PDAC risk.

ORs and 95% CIs for the association between LTL and PDAC risk are shown in Figure 1. After adjusting for multiple risk factors of PDAC, shorter LTL was associated with higher PDAC risk ($OR_{T1vsT3}=1.26$, 95% CI=1.03–1.54, $P_{trend}=0.02$, $OR_{continuous}=1.14$, 95% CI=1.02–1.28). Subgroup analyses based on treatment status showed that the association between shorter LTL and higher PDAC risk is restricted to treatment-naïve cases ($OR_{T1vsT3}=1.51$, 95% CI=1.16–1.96, $P_{trend}=0.002$; $OR_{continuous}=1.25$, 95% CI=1.08–1.45), and not cases who had samples collected after initiation of cancer therapy ($OR_{T1vsT3}=1.10$, 95% CI=0.87–1.39, $P_{trend}=0.42$; $OR_{continuous}=1.08$, 95% CI=0.94–1.23) when compared with controls. Additionally, no evidence was found in exploratory analyses to support a nonlinear association between treatment-naïve short LTL and increased PDAC risk. Based on the difference in association between the treatment-naïve samples and treated sample, and the relevance of treatment-naïve samples to cancer risk stratification, the remaining analyses were performed among the treatment-naïve cases and controls.

In stratified analyses by risk factors of PDAC, we found a statistical interaction by BMI for the association between shorter treatment-naïve LTL and higher PDAC risk ($P_{interaction}=0.001$) (Table 3). The association was statistically significant among participants with BMI ≥ 30 kg/m² ($OR_{T1vsT3}=2.59$, 95% CI=1.57–4.27, $P_{trend}=0.0002$; $OR_{continuous}=1.78$, 95% CI=1.31–2.43), but not those with BMI between 25–29.9 kg/m² ($OR_{T1vsT3}=1.13$, 95% CI=0.77–1.67, $P_{trend}=0.52$; $OR_{continuous}=1.01$, 95% CI=0.81–1.26) or <25 kg/m² ($OR_{T1vsT3}=1.47$, 95% CI=0.88–2.45, $P_{trend}=0.14$; $OR_{continuous}=1.32$, 95% CI=1.00–1.74). No other statistical interaction was observed. However, some associations were observed in certain subgroups. For instance, treatment-naïve shorter LTL was associated with higher PDAC risk among female participants ($OR_{T1vsT3}=1.91$, 95% CI: 1.27–2.86, $P_{trend}=0.002$, $OR_{continuous}=1.39$, 95% CI=1.11–1.75) but not males, and among never smokers ($OR_{T1vsT3}=1.63$, 95% CI: 1.10–2.42, $P_{trend}=0.01$; $OR_{continuous}=1.32$, 95% CI=1.06–1.66) but not former or current smokers. There also was a suggestion of association among participants with and without a self-reported history of diabetes.

Stratified analyses by the SNPs found to be associated with variation in LTL among controls and by the number of short telomere alleles carried by the participants did not show any interaction (Table 4). However, associations were observed among carriers of the CC genotype of *ACYP2*-rs11125529 ($OR_{T1vsT3}=1.46$, 95% CI: 1.08–1.98, $P_{trend}=0.01$; $OR_{continuous}=1.20$, 95% CI=1.01–1.43), and carriers of ≥ 9 short telomere alleles across all 11 SNPs ($OR_{T1vsT3}=1.77$; 95% CI: 1.07–2.95, $P_{trend}=0.03$; $OR_{continuous}=1.37$, 95% CI=1.03–1.81). No other discernible pattern of association was observed.

Discussion

This clinic-based case-control study shows that shorter treatment-naïve LTL is associated with a higher risk of PDAC. Germline variation in three telomere-related SNPs, *TERC*-rs1317082, *TERC*-rs10936599 and *ACYP2*-rs11125529, was associated with individual

differences in LTL among controls; however, no interaction or effect modification was observed by these SNPs. In stratified analyses, interaction was observed by BMI, such that a significant association was observed between shorter treatment-naïve LTL and higher PDAC risk among participants with BMI ≥ 30 kg/m², but not those with BMI between 25–29.9 kg/m² or < 25 kg/m². Overall, the findings lend support for the hypothesis that telomere shortening is associated with increased susceptibility to PDAC and is compatible with known telomere kinetics in pancreatic tumorigenesis (39,40).

TL plays a critical role in the maintenance of genomic stability by ensuring fidelity of chromosomal segregation to daughter cells, preventing the ends of linear chromosomes from fusing with each other, and maintaining the integrity of chromosomal composition (8,9,14). When TL becomes critically short, it generally leads to induction of senescence (proliferation arrest) or apoptosis (programed cell death) (14,41). However, in some instances, cells with critically short TL evade both processes leading to the proliferation of daughter cells with gross chromosomal abnormalities, a phenomenon that tends to favor cancer development (41). In this context, short LTL has been associated with increased overall cancer risk and increased risk of individual cancers, including bladder, renal, gastric, ovarian, head and neck, and lung cancers (11–13). For PDAC, tissue-based studies have shown that PDAC tumors have shorter TL than benign pancreatic tissues (39,40,42). Studies have also shown that TL shortening occurs early in pancreatic tumorigenesis (39,40), with progressive loss of TL through the progression of the PDAC precursor lesion, intraductal papillary mucinous neoplasia (IPMN), from adenoma IPMN to borderline IPMN to carcinoma in situ IPMN (39). Thus, telomere shortening appears to be a fundamental event in pancreatic tumorigenesis.

Our finding is compatible with results from a case-control study nested within five US-based prospective cohorts (32). In that study, Bao *et al.* measured pre-diagnosis LTL among 386 PDAC cases and 896 controls, and found that shorter LTL is associated with higher PDAC risk (OR_{T3vs.T1}=1.72, 95% CI: 1.07–2.78)(32). Campa *et al.* also examined the association between pre-diagnosis LTL and PDAC risk in a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort that included 331 PDAC cases and 331 controls, but the results were null (30). However, in secondary analysis based on continuous LTL variable, they found a modest association between short LTL and higher PDAC risk (OR=1.13, 95% CI: 1.01–1.27)(30). By contrast, a US-based retrospective case-control study (499 cases, 963 controls)(34) and a China-based nested case-control study (900 cases, 900 controls)(31) both reported a “U-shaped” association between LTL and PDAC, wherein individuals with extremely short or extremely long LTL were found to have higher PDAC risk (31). Two other studies, one nested case-control study within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Trial (193 cases, 660 controls)(29) and a Chinese-based prospective cohort study that included only 116 PDAC cases (33), reported that long (as opposed to short) LTL is associated with increased PDAC risk. The reasons for these conflicting reports, could range from differences in host biology to characteristics of the study populations to variation in assay performance across laboratories to small sample sizes in some studies, as discussed elsewhere (7).

It is worth noting that LTL may be affected by the timing of blood collection relative to cancer diagnosis (43) or cancer treatment (38), but not all prior studies provided information on the length of time between blood collection and cancer diagnosis (30,31). In our study, the majority (>80%) of blood samples were collected within one month of diagnosis, by which time some case subjects had already started cancer treatment. When cases with treatment-naïve blood samples were combined with cases who had samples collected after treatment initiation, we found a modest association between short LTL and PDAC risk. The association became clearer and more robust when we restricted the case samples to treatment-naïve cases only. We also found interaction by BMI for the association treatment-naïve shorter LTL and higher PDAC risk, in that, the association tended to be restricted to participants with BMI ≥ 30 kg/m², but not those with BMI between 25–29.925 kg/m² or < 25 kg/m². Associations were observed also among female participants and never smokers, but not their respective counterparts, and the reasons for these observations are not completely clear but may be explained by small samples sizes in some subgroups or could be due to chance.

To circumvent limitations associated with measured LTL, some studies have used telomere-related SNPs to compute genetic risk scores (GRS) intended to predict LTL then determine the association between the genetically predicted LTL and PDAC risk, but here too, findings have varied, possibly owing to differences in methods and variants used for computing the GRS (37,44,45). We previously examined associations between individual telomere-related SNPs and telomere-related GRS in regards to PDAC risk (37). In age- and sex-adjusted models, we found that two of eight SNPs examined had opposite associations with PDAC, such that the short telomere allele of *TERT*-rs2736100 (A) was associated with higher risk, while the short allele of *TERC*-rs10936599 (T) was associated lower risk, but no association was found for the GRS (37). Another study that used a method similar to ours also did not find an association between telomere-related GRS and PDAC (45). However, Campa and colleagues used a different computational method and reported an association between genetically determined short LTL and higher PDAC risk (44). In the present study, we investigated whether an association between LTL and PDAC risk differs by variation in candidate SNPs previously reported to be associated with differences in LTL (15–18). While three short telomere alleles were confirmed to be associated with lower LTL, there was no evidence of effect modification by the telomere-related SNPs.

Our study has several strengths and limitations. Strengths of the study include its large sample size and assessment of interaction by germline variation in telomere-related genes. The use of an ultra-rapid case ascertainment process for recruitment of PDAC cases adds to the study strengths. Limitations include the predominantly White population and the use of LTLs as a surrogate marker of overall TL, which may not reflect TL in pancreatic tissue. Although no study has yet examined the relationship between LTL and TL in pancreatic tissue, it has been shown that LTL correlates well with TL across multiple tissues, including TL of skeletal muscle ($r = 0.84$), skin cells ($r = 0.83$), and subcutaneous fat cells ($r = 0.83$) (46). Because of the retrospective design, we were unable to determine whether short LTL is a cause or consequence of PDAC. However, our findings are consistent with that of a prospective study that measured LTL in blood samples collected a median of 6.7 years before cancer diagnosis (32). In that study, no difference was noted in the association

between shorter LTL and higher PDAC risk in stratified analyses among samples collected before or after the median time between blood collection relative to cancer diagnosis (32). Our study also underscores the importance of utilizing treatment-naïve biospecimens in case-control studies of LTL. Some of our study variables (e.g., diabetes, smoking, and BMI) were based on self-report; thus, we cannot rule out the possibility of residual confounding or potential confounding by unmeasured factors.

In conclusion, this study shows that treatment-naïve shorter LTL is associated with higher risk of PDAC. The association did not differ by germline variation in the telomere-related SNPs examined, but there was suggestion of effect modification by BMI. Peripheral blood LTL might serve as a marker for risk modeling to identify persons at high-risk of PDAC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BMI	Body mass index
CI	Confidence intervals
GRS	Genetic risk scores
IPMN	Intraductal papillary mucinous neoplasia
LTL	Leukocyte telomere length
OR	Odds ratio
PDAC	Pancreatic ductal adenocarcinoma
SNPs	Single nucleotide polymorphisms
TL	Telomere length

References

1. Antwi SO, Jansen RJ, Petersen GM. Cancer of the pancreas In: Thun MJ, Linet MS, Cerhan JR, Haiman C, Schottenfeld D, editors. Schottenfeld and Fraumeni Cancer Epidemiology and Prevention, Fourth Edition: Oxford University Press; 2017 p 611–34.
2. American Cancer Society. Cancer Facts & Figures 2019. Atlanta: American Cancer Society; 2019.
3. Antwi SO, Oberg AL, Shivappa N, Bamlet WR, Chaffee KG, Steck SE, et al. Pancreatic cancer: associations of inflammatory potential of diet, cigarette smoking and long-standing diabetes. *Carcinogenesis* 2016;37(5):481–90 doi 10.1093/carcin/bgw022. [PubMed: 26905587]
4. Hu C, Hart SN, Polley EC, Gnanaolivu R, Shimelis H, Lee KY, et al. Association Between Inherited Germline Mutations in Cancer Predisposition Genes and Risk of Pancreatic Cancer. *JAMA* 2018;319(23):2401–9 doi 10.1001/jama.2018.6228. [PubMed: 29922827]

5. Antwi SO, Fagan SE, Chaffee KG, Bamlet WR, Hu C, Polley EC, et al. Risk of Different Cancers Among First-degree Relatives of Pancreatic Cancer Patients: Influence of Proband's Susceptibility Gene Mutation Status. *J Natl Cancer Inst* 2019;111(3):264–71 doi 10.1093/jnci/djx272. [PubMed: 29982661]
6. Blackburn EH, Epel ES, Lin J. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. *Science* 2015;350(6265):1193–8 doi 10.1126/science.aab3389. [PubMed: 26785477]
7. Antwi SO, Petersen GM. Leukocyte Telomere Length and Pancreatic Cancer Risk: Updated Epidemiologic Review. *Pancreas* 2018;47(3):265–71 doi 10.1097/mpa.0000000000000995. [PubMed: 29424808]
8. Prescott J, Wentzensen IM, Savage SA, De Vivo I. Epidemiologic evidence for a role of telomere dysfunction in cancer etiology. *Mutat Res* 2012;730(1–2):75–84 doi 10.1016/j.mrfmmm.2011.06.009. [PubMed: 21756922]
9. Artandi SE, DePinho RA. Telomeres and telomerase in cancer. *Carcinogenesis* 2010;31(1):9–18 doi 10.1093/carcin/bgp268. [PubMed: 19887512]
10. de Lange T Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev* 2005;19(18):2100–10 doi 10.1101/gad.1346005. [PubMed: 16166375]
11. 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):1238–50 doi 10.1158/1055-9965.Epi-11-0005. [PubMed: 21467229]
12. Ma H, Zhou Z, Wei S, Liu Z, Pooley KA, Dunning AM, et al. Shortened telomere length is associated with increased risk of cancer: a meta-analysis. *PLoS One* 2011;6(6):e20466 doi 10.1371/journal.pone.0020466. [PubMed: 21695195]
13. Zhu X, Han W, Xue W, Zou Y, Xie C, Du J, et al. The association between telomere length and cancer risk in population studies. *Sci Rep* 2016;6:22243 doi 10.1038/srep22243. [PubMed: 26915412]
14. Blackburn EH. Switching and signaling at the telomere. *Cell* 2001;106(6):661–73 doi 10.1016/s0092-8674(01)00492-5. [PubMed: 11572773]
15. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, et al. Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet* 2013;45(4):422–7, 7e1–2 doi 10.1038/ng.2528. [PubMed: 23535734]
16. Pooley KA, Bojesen SE, Weischer M, Nielsen SF, Thompson D, Amin Al Olama A, et al. A genome-wide association scan (GWAS) for mean telomere length within the COGS project: identified loci show little association with hormone-related cancer risk. *Hum Mol Genet* 2013;22(24):5056–64 doi 10.1093/hmg/ddt355. [PubMed: 23900074]
17. Mangino M, Hwang SJ, Spector TD, Hunt SC, Kimura M, Fitzpatrick AL, et al. Genome-wide meta-analysis points to CTC1 and ZNF676 as genes regulating telomere homeostasis in humans. *Hum Mol Genet* 2012;21(24):5385–94 doi 10.1093/hmg/dds382. [PubMed: 23001564]
18. Rode L, Nordestgaard BG, Bojesen SE. Peripheral blood leukocyte telomere length and mortality among 64 637 individuals from the general population. *J Natl Cancer Inst* 2015;107(6):djv074. [PubMed: 25862531]
19. von Zglinicki T, Martin-Ruiz CM. Telomeres as biomarkers for ageing and age-related diseases. *Curr Mol Med* 2005;5(2):197–203 doi 10.2174/1566524053586545. [PubMed: 15974873]
20. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, et al. Obesity, cigarette smoking, and telomere length in women. *Lancet* 2005;366(9486):662–4 doi 10.1016/s0140-6736(05)66630-5. [PubMed: 16112303]
21. Mirabello L, Huang WY, Wong JY, Chatterjee N, Reding D, Crawford ED, et al. The association between leukocyte telomere length and cigarette smoking, dietary and physical variables, and risk of prostate cancer. *Aging Cell* 2009;8(4):405–13 doi 10.1111/j.1474-9726.2009.00485.x. [PubMed: 19493248]
22. Kim S, Parks CG, DeRoo LA, Chen H, Taylor JA, Cawthon RM, et al. Obesity and weight gain in adulthood and telomere length. *Cancer Epidemiol Biomarkers Prev* 2009;18(3):816–20 doi 10.1158/1055-9965.Epi-08-0935. [PubMed: 19273484]

23. Du M, Prescott J, Kraft P, Han J, Giovannucci E, Hankinson SE, et al. Physical activity, sedentary behavior, and leukocyte telomere length in women. *Am J Epidemiol* 2012;175(5):414–22 doi 10.1093/aje/kwr330. [PubMed: 22302075]
24. Demissie S, Levy D, Benjamin EJ, Cupples LA, Gardner JP, Herbert A, et al. Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. *Aging Cell* 2006;5(4):325–30 doi 10.1111/j.1474-9726.2006.00224.x. [PubMed: 16913878]
25. Wu X, Amos CI, Zhu Y, Zhao H, Grossman BH, Shay JW, et al. Telomere dysfunction: a potential cancer predisposition factor. *J Natl Cancer Inst* 2003;95(16):1211–8 doi 10.1093/jnci/djg011. [PubMed: 12928346]
26. Hou L, Savage SA, Blaser MJ, Perez-Perez G, Hoxha M, Dioni L, et al. Telomere length in peripheral leukocyte DNA and gastric cancer risk. *Cancer Epidemiol Biomarkers Prev* 2009;18(11):3103–9 doi 10.1158/1055-9965.Epi-09-0347. [PubMed: 19861514]
27. Martinez-Delgado B, Yanowsky K, Inglada-Perez L, de la Hoya M, Caldes T, Vega A, et al. Shorter telomere length is associated with increased ovarian cancer risk in both familial and sporadic cases. *J Med Genet* 2012;49(5):341–4 doi 10.1136/jmedgenet-2012-100807. [PubMed: 22493152]
28. Broberg K, Bjork J, Paulsson K, Hoglund M, Albin M. Constitutional short telomeres are strong genetic susceptibility markers for bladder cancer. *Carcinogenesis* 2005;26(7):1263–71 doi 10.1093/carcin/bgi063. [PubMed: 15746160]
29. Lynch SM, Major JM, Cawthon R, Weinstein SJ, Virtamo J, Lan Q, et al. A prospective analysis of telomere length and pancreatic cancer in the alpha-tocopherol beta-carotene cancer (ATBC) prevention study. *Int J Cancer* 2013;133(11):2672–80 doi 10.1002/ijc.28272. [PubMed: 23674344]
30. Campa D, Mergarten B, De Vivo I, Boutron-Ruault MC, Racine A, Severi G, et al. Leukocyte telomere length in relation to pancreatic cancer risk: a prospective study. *Cancer Epidemiol Biomarkers Prev* 2014;23(11):2447–54 doi 10.1158/1055-9965.Epi-14-0247. [PubMed: 25103821]
31. Zhang R, Zhao J, Xu J, Liu F. Association of peripheral leukocyte telomere length and its variation with pancreatic cancer and colorectal cancer risk in Chinese population. *Oncotarget* 2016;7(25):38579–85 doi 10.18632/oncotarget.9536. [PubMed: 27509261]
32. Bao Y, Prescott J, Yuan C, Zhang M, Kraft P, Babic A, et al. Leucocyte telomere length, genetic variants at the TERT gene region and risk of pancreatic cancer. *Gut* 2017;66(6):1116–22 doi 10.1136/gutjnl-2016-312510. [PubMed: 27797938]
33. Luu HN, Huang JY, Wang R, Adams-Haduch J, Jin A, Koh WP, et al. Association between leukocyte telomere length and the risk of pancreatic cancer: Findings from a prospective study. *PLoS One* 2019;14(8):e0221697 doi 10.1371/journal.pone.0221697. [PubMed: 31465482]
34. Skinner HG, Gangnon RE, Litzelman K, Johnson RA, Chari ST, Petersen GM, et al. Telomere length and pancreatic cancer: a case-control study. *Cancer Epidemiol Biomarkers Prev* 2012;21(11):2095–100 doi 10.1158/1055-9965.Epi-12-0671. [PubMed: 23093543]
35. Wang L, Bamlet WR, de Andrade M, Boardman LA, Cunningham JM, Thibodeau SN, et al. Mitochondrial genetic polymorphisms and pancreatic cancer risk. *Cancer Epidemiol Biomarkers Prev* 2007;16(7):1455–9 doi 10.1158/1055-9965.Epi-07-0119. [PubMed: 17627010]
36. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res* 2002;30(10):e47 doi 10.1093/nar/30.10.e47. [PubMed: 12000852]
37. Antwi SO, Bamlet WR, Broderick BT, Chaffee KG, Oberg A, Jatoi A, et al. Genetically Predicted Telomere Length is not Associated with Pancreatic Cancer Risk. *Cancer Epidemiol Biomarkers Prev* 2017;26(6):971–4 doi 10.1158/1055-9965.Epi-17-0100. [PubMed: 28264873]
38. Benitez-Buelga C, Sanchez-Barroso L, Gallardo M, Apellaniz-Ruiz M, Inglada-Perez L, Yanowski K, et al. Impact of chemotherapy on telomere length in sporadic and familial breast cancer patients. *Breast Cancer Res Treat* 2015;149(2):385–94 doi 10.1007/s10549-014-3246-6. [PubMed: 25528024]
39. Hashimoto Y, Murakami Y, Uemura K, Hayashidani Y, Sudo T, Ohge H, et al. Telomere shortening and telomerase expression during multistage carcinogenesis of intraductal papillary mucinous neoplasms of the pancreas. *J Gastrointest Surg* 2008;12(1):17–28; discussion –9 doi 10.1007/s11605-007-0383-9. [PubMed: 17960465]

40. Matsuda Y, Ishiwata T, Izumiyama-Shimomura N, Hamayasu H, Fujiwara M, Tomita K, et al. Gradual telomere shortening and increasing chromosomal instability among PanIN grades and normal ductal epithelia with and without cancer in the pancreas. *PLoS One* 2015;10(2):e0117575 doi 10.1371/journal.pone.0117575. [PubMed: 25658358]
41. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144(5):646–74 doi 10.1016/j.cell.2011.02.013. [PubMed: 21376230]
42. Hong SM, Heaphy CM, Shi C, Eo SH, Cho H, Meeker AK, et al. Telomeres are shortened in acinar-to-ductal metaplasia lesions associated with pancreatic intraepithelial neoplasia but not in isolated acinar-to-ductal metaplasias. *Mod Pathol* 2011;24(2):256–66 doi 10.1038/modpathol.2010.181. [PubMed: 20871595]
43. Hou L, Joyce BT, Gao T, Liu L, Zheng Y, Penedo FJ, et al. Blood Telomere Length Attrition and Cancer Development in the Normative Aging Study Cohort. *EBioMedicine* 2015;2(6):591–6 doi 10.1016/j.ebiom.2015.04.008. [PubMed: 26288820]
44. Campa D, Matarazzi M, Greenhalf W, Bijlsma M, Saum KU, Pasquali C, et al. Genetic determinants of telomere length and risk of pancreatic cancer: A PANDoRA study. *Int J Cancer* 2019;144(6):1275–83 doi 10.1002/ijc.31928. [PubMed: 30325019]
45. Haycock PC, Burgess S, Nounu A, Zheng J, Okoli GN, Bowden J, et al. Association Between Telomere Length and Risk of Cancer and Non-Neoplastic Diseases: A Mendelian Randomization Study. *JAMA Oncol* 2017;3(5):636–51 doi 10.1001/jamaoncol.2016.5945. [PubMed: 28241208]
46. Daniali L, Benetos A, Susser E, Kark JD, Labat C, Kimura M, et al. Telomeres shorten at equivalent rates in somatic tissues of adults. 2013;4:1597 doi 10.1038/ncomms2602.

Figure 1:

Association between peripheral blood leukocyte telomere length and pancreatic ductal adenocarcinoma risk, showing tertiles of telomere lengths, sample sizes, and plots of odds ratios. The tertile for longest telomere length (T3) was used as reference group. Results are from multivariable models with adjustment for age (continuous), sex, body mass index (continuous), smoking status (never, former, current), and personal history of diabetes (yes, no). Abbreviations: T1–3, tertile 1 to tertile 3; LTL, leukocyte telomere length; OR, odds ratio; CI, confidence interval.

Table 1.

Distribution of participant characteristics and selected telomere-related genotype frequencies among the PDAC cases and cancer-free controls, $N=2919$.

	Cases $N=1460$	Controls $N=1459$	p-value
Age, years^a			0.4667
Mean (SD)	65.4 (10.2)	65.0 (10.3)	
Sex			0.6663
Female	646 (44.2%)	634 (43.5%)	
Male	814 (55.8%)	825 (56.5%)	
Race			0.4636
White	1434 (98.2%)	1438 (98.6%)	
Other	26 (1.8%)	21 (1.4%)	
BMI, kg/m²			<0.0001
< 25	413 (28.3%)	456 (31.3%)	
25–29.9	590 (40.4%)	667 (45.7%)	
30	457 (31.3%)	336 (23.0%)	
Smoking Status			<0.0001
Never	607 (41.6%)	804 (55.1%)	
Former	629 (43.1%)	607 (41.6%)	
Current	224 (15.3%)	48 (3.3%)	
Diabetes			<0.0001
No	1042 (71.4%)	1306 (89.5%)	
Yes	418 (28.6%)	153 (10.5%)	
<i>TERC</i>-rs10936599^b			0.1700
CC	841 (57.9%)	807 (55.5%)	
CT	525 (36.1%)	538 (37.0%)	
TT	87 (6.0%)	110 (7.6%)	
<i>ACYP2</i>-rs11125529^b			0.9914
CC	1080 (74.3%)	1083 (74.4%)	
AC	338 (23.3%)	338 (23.2%)	
AA	35 (2.4%)	34 (2.3%)	
<i>TERC</i>-rs1317082^b			0.1609
AA	844 (58.0%)	808 (55.5%)	
AG	523 (36.0%)	537 (36.9%)	
GG	87 (6.0%)	110 (7.6%)	
LTL (T/S ratio), Mean (SD)			
Overall sample	0.77 (0.17)	0.79 (0.17)	0.0002
Treated cases (n=773)	0.79 (0.17)	n/a	0.0001 ^c
Treatment naïve cases (n=653)	0.76 (0.16)	n/a	

Abbreviations: BMI, body mass index; PDAC, pancreatic ductal adenocarcinoma; LTL, leukocyte telomere length; T/S ratio, relative telomere to single copy gene (T/S) ratio

^a Age at diagnosis for cases and at recruitment for controls.

^b Some of categories may not sum to 100% due to missing genotype data (n=11).

^c P-value comparing treatment naïve versus treated cases using Wilcoxon rank-sum test. Treatment information is missing for 34 cases.

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Table 2.

Association between measured leukocyte telomere length and telomere-related SNP alleles among cancer-free controls in the present study.

SNP	Position (GRCh38.p12)	Nearby Gene	Short Allele ^a	Long Allele	N	β -value ^b	SE	p-value	SNP Reference
rs1317082	chr3: 169779797	<i>TERC</i>	A	G	1455	-0.01628	0.00644	0.0116	Rode <i>et al.</i> (18)
rs10936599	chr3: 169774313	<i>TERC</i>	C	T	1455	-0.01599	0.00644	0.0132	Codd <i>et al.</i> (15)
rs11125529	chr2: 54248729	<i>ACYP2</i>	A	C	1455	-0.01840	0.00814	0.0239	Codd <i>et al.</i> (15)
rs2487999	chr10: 103900068	<i>OBFC1</i>	T	C	1455	-0.01528	0.00947	0.1069	Rode <i>et al.</i> (18)
rs7675998	chr4: 163086668	<i>NAF1</i>	G	A	1453	-0.00805	0.00711	0.2577	Codd <i>et al.</i> (15)
rs9420907	chr10: 103916707	<i>OBFC1</i>	C	A	1455	-0.00857	0.00810	0.2901	Codd <i>et al.</i> (15)
rs7726159	chr5: 1282204	<i>TERT</i>	A	C	1454	-0.00576	0.00594	0.3326	Rode <i>et al.</i> (18)
rs3027234	chr17: 8232774	<i>CTCI</i>	C	T	1454	-0.00644	0.00675	0.3403	Mangino <i>et al.</i> (17)
rs6772228	chr3: 58390292	<i>PXK</i>	T	A	1455	-0.00630	0.01384	0.6490	Pooley <i>et al.</i> (16)
rs8105767	chr19: 22032639	<i>ZNF208</i>	G	A	1454	-0.00179	0.00614	0.7706	Codd <i>et al.</i> (15)
rs2736100	chr5: 1286401	<i>TERT</i>	C	A	1441	-0.00155	0.00573	0.7870	Codd <i>et al.</i> (15)
All 11 SNPs ^c	-	-	-	-	1437	-0.00573	0.00169	0.0007	-

Abbreviation: SNP, single nucleotide polymorphism

^a Short allele is the allele associated with short leukocyte telomere length in previously published studies.

^b β -estimates represent relative telomere to single copy gene (T/S) ratio per short allele. The estimates were derived from linear regression models fitted for each SNP and the genetic score with adjustment for age (continuous), sex, body mass index (continuous), smoking status (never, former, current), and diabetes (yes, no).

^c The genetic score was computed based on the total number of short telomere allele carried by each individual across all 11 SNPs, ranging from 3 short telomere alleles to 19 short alleles, with mean of 10.97, median of 11, and inter-quartile range of 9 to 13.

Table 3.

Stratified analyses of the association between peripheral blood leukocyte telomere length and PDAC risk by known risk factors of PDAC among the treatment-naïve cases and cancer-free controls; $N=2,112$.

	Teriles ^a of leukocyte telomere length				<i>P</i> trend	Continuous	<i>P</i> interaction ^d
	T3 (longest LTL)	T2	T1 (shortest LTL)				
Age, years^b							0.5935, 0.7815
65							
<i>N</i> , case : control	288 : 249	222 : 244	200 : 245				
Multivariable-adjusted ^c	1.00 (ref)	1.09 (0.73–1.61)	1.43 (0.99–2.06)	0.0567	1.29 (0.94–1.59)		
>65							
<i>N</i> , case : control	266 : 243	243 : 238	241 : 240				
Multivariable-adjusted ^c	1.00 (ref)	1.13 (0.81–1.57)	1.21 (0.88–1.68)	0.2441	1.25 (0.89–1.53)		
Sex							0.0492, 0.2371
Female							
<i>N</i> , case : control	257 : 212	227 : 211	162 : 211				
Multivariable-adjusted ^c	1.00 (ref)	1.73 (1.16–2.58)	1.91 (1.27–2.86)	0.0019	1.39 (1.11–1.75)		
Male							
<i>N</i> , case : control	316 : 276	257 : 275	241 : 274				
Multivariable-adjusted ^c	1.00 (ref)	0.90 (0.63–1.27)	1.29 (0.92–1.82)	0.1385	1.15 (0.94–1.40)		
Diabetes							0.4897, 0.9630
No							
<i>N</i> , case : control	405 : 440	343 : 428	294 : 438				
Multivariable-adjusted ^c	1.00 (ref)	1.23 (0.92–1.65)	1.42 (1.06–1.91)	0.0176	1.23 (1.04–1.45)		
Yes							
<i>N</i> , case : control	168 : 48	141 : 58	109 : 47				
Multivariable-adjusted ^c	1.00 (ref)	1.10 (0.62–1.95)	1.93 (1.07–3.47)	0.0278	1.39 (0.99–1.94)		
BMI							0.0012, 0.0084
< 25 kg/m ²							

	Tertiles ^a of leukocyte telomere length				P trend	Continuous	P interaction ^d
	T3 (longest LTL)	T2	T1 (shortest LTL)				
N, case : control	167 : 161	141 : 130	105 : 165				
Multivariable-adjusted ^c	1.00 (ref)	1.91 (1.15–3.17)	1.47 (0.88–2.45)	0.1383	1.32 (1.00–1.74)		
25–29.9 kg/m ²							
N, case : control	233 : 243	183 : 217	174 : 207				
Multivariable-adjusted ^c	1.00 (ref)	1.01 (0.69–1.50)	1.13 (0.77–1.67)	0.5222	1.01 (0.81–1.26)		
30 kg/m ²							
N, case : control	173 : 84	160 : 139	124 : 113				
Multivariable-adjusted ^c	1.00 (ref)	1.01 (0.62–1.64)	2.59 (1.57–4.27)	0.0002	1.78 (1.31–2.43)		
Smoking Status							
Never							0.4601, 0.2342
N, case : control	218 : 236	195 : 267	194 : 301				
Multivariable-adjusted ^c	1.00 (ref)	1.34 (0.91–1.98)	1.63 (1.10–2.42)	0.0149	1.32 (1.06–1.66)		
Former							
N, case : control	259 : 232	215 : 207	155 : 168				
Multivariable-adjusted ^c	1.00 (ref)	0.97 (0.66–1.42)	1.35 (0.93–1.97)	0.1190	1.16 (0.94–1.45)		
Current							
N, case : control	96 : 20	74 : 12	54 : 16				
Multivariable-adjusted ^c	1.00 (ref)	1.94 (0.75–4.98)	1.84 (0.75–4.50)	0.1804	1.47 (0.86–2.52)		

Abbreviations: BMI, body mass index; LTL, leukocyte telomere length; PDAC, pancreatic ductal adenocarcinoma; T1–T3, tertile 1 to tertile 3.

^a Telomere length was categorized into tertiles based relative telomere to single copy gene (T/S) ratio using sex-specific cut-points. For male participants, the T/S ratio was categorized among controls from tertile 1 to 3 as: 0.341–0.701, 0.702–0.842, and 0.843–1.410. For female participants, it was categorized among controls from tertile 1 to 3 as: 0.381–0.729, 0.730–0.892, and 0.893–1.470.

^b For the age stratified analyses, age- and sex-specific cut-points were used to categorize T/S ratio into tertiles among controls and grouped from tertile 1 to 3 for males who were 65 years old as: 0.423–0.750, 0.751–0.884, and 0.885–1.410; and for males who were >65 years old as: 0.341–0.654, 0.655–0.774, and 0.775–1.400. For females who were 65 years old the T/S ratio was categorized among controls as: 0.448–0.779, 0.781–0.941, and 0.943–1.470; and for female who were >65 years old as: 0.381–0.683, 0.684–0.825, and 0.826–1.370.

^c Adjusted for age (continuous), sex, body mass index (continuous), smoking status (never, former, current), and diabetes (yes, no), with exclusion of the stratified variable in the respective stratified analysis.

^d The first interaction p-values was calculated using a tertile telomere variable. The second was calculated using a continuous telomere variable.

Table 4.

Stratified analyses of the association between leukocyte telomere length and pancreatic cancer risk among the treatment naïve cases and cancer-free controls by SNPs previously reported to be associated with variation in leukocyte telomere length; $N=2,112$.

	Tertiles ^a of leukocyte telomere length				P trend	Continuous	P interaction ^c
	T3 (longest LTL)	T2	T1 (shortest LTL)				
TERC-rs10936599							0.1189, 0.9582
CC							
N, case : control	301 : 266	305 : 265	235 : 276				
Multivariable-adjusted ^b	1.00 (ref)	1.42 (1.01–2.00)	1.37 (0.97–1.95)		0.0759	1.21 (0.99–1.48)	
CT							
N, case : control	232 : 179	149 : 178	144 : 181				
Multivariable-adjusted ^b	1.00 (ref)	0.96 (0.61–1.51)	1.70 (1.12–2.60)		0.0137	1.24 (0.96–1.59)	
TT							
N, case : control	40 : 41	28 : 42	19 : 27				
Multivariable-adjusted ^b	1.00 (ref)	0.61 (0.21–1.75)	1.57 (0.55–4.48)		0.4006	1.75 (0.93–3.29)	
ACYP2-rs11125529							0.4964, 0.5289
CC							
N, case : control	431 : 380	364 : 349	285 : 354				
Multivariable-adjusted ^b	1.00 (ref)	1.28 (0.95–1.74)	1.46 (1.08–1.98)		0.0141	1.20 (1.01–1.43)	
AC							
N, case : control	129 : 96	108 : 128	101 : 114				
Multivariable-adjusted ^b	1.00 (ref)	0.93 (0.54–1.60)	1.72 (1.01–2.94)		0.0444	1.38 (1.00–1.89)	
AA							
N, case : control	12 : 10	11 : 8	12 : 16				
Multivariable-adjusted ^b	1.00 (ref)	1.51 (0.24–9.49)	1.24 (0.22–6.90)		0.8066	1.57 (0.65–3.78)	
TERC-rs1317082							0.1177, 0.9605
AA							
N, case : control	303 : 266	306 : 265	235 : 277				

	Tertiles ^a of leukocyte telomere length					<i>P</i> interaction ^c
	T3 (longest LTL)	T2	T1 (shortest LTL)	<i>P</i> trend	Continuous	
Multivariable-adjusted ^b	1.00 (ref)	1.43 (1.02–2.01)	1.38 (0.97–1.96)	0.0711	1.22 (1.00–1.48)	
AG						
<i>N</i> case : control	230 : 179	149 : 178	144 : 180			
Multivariable-adjusted ^b	1.00 (ref)	0.96 (0.61–1.50)	1.69 (1.11–2.59)	0.0152	1.23 (0.96–1.58)	
GG						
<i>N</i> case : control	40 : 41	28 : 42	19 : 27			
Multivariable-adjusted ^b	1.00 (ref)	0.61 (0.21–1.75)	1.57 (0.55–4.48)	0.4006	1.75 (0.93–3.29)	0.7603, 0.8423
No. of short LTL allele counts for all 11 SNPs^d						
9						
<i>N</i> case : control	127 : 118	132 : 121	118 : 140			
Multivariable-adjusted ^b	1.00 (ref)	1.51 (0.90–2.52)	1.77 (1.07–2.95)	0.0273	1.37 (1.03–1.81)	
10–11						
<i>N</i> case : control	166 : 140	151 : 159	133 : 160			
Multivariable-adjusted ^b	1.00 (ref)	1.21 (0.77–1.91)	1.43 (0.90–2.27)	0.1325	1.25 (0.96–1.64)	
12						
<i>N</i> case : control	275 : 219	191 : 203	144 : 177			
Multivariable-adjusted ^b	1.00 (ref)	0.90 (0.59–1.38)	1.38 (0.92–2.08)	0.1181	1.18 (0.92–1.50)	0.4520, 0.8238
No. of short LTL allele counts from 3 SNPs^e						
0–2						
<i>N</i> case : control	37 : 109	39 : 124	81 : 118			
Multivariable-adjusted ^b	1.00 (ref)	1.28 (0.62–2.62)	1.01 (0.47–2.15)	0.9850	1.17 (0.76–1.81)	
3–4						
<i>N</i> case : control	71 : 216	93 : 217	126 : 224			
Multivariable-adjusted ^b	1.00 (ref)	1.21 (0.88–1.65)	1.47 (1.07–2.01)	0.0159	1.22 (1.02–1.46)	
5–6						
<i>N</i> case : control	47 : 159	74 : 144	84 : 144			

Tertiles ^a of leukocyte telomere length				
T3 (longest LTL)	T2	T1 (shortest LTL)	P trend	Continuous
<hr/>				
1.00 (ref)	1.05 (0.56–1.97)	1.96 (1.07–3.60)	0.0296	1.39 (0.96–2.00)
<hr/>				
Multivariable-adjusted ^b			P interaction ^c	

Abbreviations: BMI, body mass index; SNP, single nucleotide polymorphism; LTL, leukocyte telomere length; T1-T3, tertile 1 to tertile 3.

^aTelomere length was categorized into tertiles based relative telomere to single copy gene (T/S) ratio using sex-specific cut-points. For male participants, the T/S ratio was categorized among controls from tertile 1 to 3 as: 0.341–0.701, 0.702–0.842, and 0.843–1.410. For female participants, it was categorized among controls from tertile 1 to 3 as: 0.381–0.729, 0.730–0.892, and 0.893–1.470.

^bAdjusted for age (continuous), sex, body mass index (continuous), smoking status (never, former, current), and diabetes (yes, no).

^cThe first interaction p-values was calculated using a tertile telomere variable. The second was calculated using a continuous telomere variable.

^dThe number of short allele counts was based on all 11 SNPs (rs1317082, rs10936599, rs11125529, rs2487999, rs7675998, rs9420907, rs7726159, rs3027234, rs6772228, rs8105767, rs2736100). Genotype data were missing for 23 cases and 21 controls.

^eThe computation was based on the following 3 SNPs as has been done previously by Rode *et al.* (18): rs1317082, rs2487999, and rs7726159. For this analysis, 10 cases and 5 controls had missing genotype data.