Published in final edited form as:

Pancreas. 2018 March ; 47(3): 265–271. doi:10.1097/MPA.0000000000000995.

Leukocyte Telomere Length and Pancreatic Cancer Risk: Updated Epidemiologic Review

Samuel O. Antwi, PhD^{*} and Gloria M. Petersen, PhD[†]

^{*}Division of Epidemiology, Department of Health Sciences Research, Mayo Clinic, Jacksonville, FL

[†]Division of Epidemiology, Department of Health Sciences Research, Mayo Clinic, Rochester, MN

Abstract

Many risk factors have been firmly established for pancreatic cancer (PC), but the molecular processes by which known risk factors influence susceptibility to PC are not clear. There has been a recent upsurge of interest in the role of telomere length (TL), the protective DNA sequence repeats at chromosome ends, in pancreatic carcinogenesis. Given this heightened interest, we performed an in-depth, focused, and up-to-date review of the epidemiological evidence linking leukocyte TL (LTL) with PC risk. We searched MEDLINE, Embase, and the Cochrane Library databases for all published studies on LTL and PC risk, up to May 2017. Five studies were identified for review: four nested case-control studies and one retrospective case-control study. Two studies found opposite associations between LTL and PC risk; one found a dose-response positive association and the other found a dose-response inverse association. Two studies also found a “U-shaped” association, while another reported a weak nonlinear relationship. We offer potential reasons for the conflicting findings including variation in study design, biospecimen characteristics, and differences in inter-laboratory measurements of TL. Future studies should carefully control for risk factors of PC that are associated also with telomere attrition, and investigate the role of genetic variation in TL maintenance.

Keywords

telomere length; telomeres; telomere genes; pancreatic cancer; pancreatic adenocarcinoma

Introduction

Pancreatic cancer (PC) is the ninth most common cancer and fourth leading cause of cancer death in Western countries.¹ In the United States, PC is projected to become the second leading cause of cancer death by 2030.² The firmly established risk factors for PC include tobacco smoking, pre-existing diabetes mellitus, chronic pancreatitis, obesity, and non-O blood type.³⁻⁵ Nonetheless, the molecular processes by which these risk factors influence pancreatic carcinogenesis are not clear. It is postulated that some of these factors (e.g.,

Correspondence: Samuel O. Antwi, PhD, Mayo Clinic, 4500 San Pablo Road S. Vincent Stabile Building, North, 756N, Jacksonville, FL 32224 (antwi.samuel@mayo.edu). Tel: 1-904-953-0310; Fax: 1-904-953-1013.

Disclosure: The authors declare no conflict of interest.

tobacco smoking, diabetes, and obesity) modulate PC risk by accelerating the shortening of telomeres or telomere length (TL),^{6,7} the non-coding DNA sequence repeats at the ends of eukaryotic chromosomes that protect chromosomal composition from degradation and maintain chromosomal stability during cell replication.⁸⁻¹⁰ Findings from studies that have investigated the association between peripheral blood leukocyte telomere length (LTL) and PC risk have been mixed (reviewed in¹¹). However, these studies differ substantially in methods, design, and study population characteristics. Thus, despite inconsistent findings, it remains plausible that LTL may represent a comprehensive biological marker of exposure to risk factors of pancreatic cancer. A better understanding of how TL influences pancreatic carcinogenesis would potentially lead to more effective prevention and intervention strategies to reduce incidence and improve outcomes for this deadly malignancy.

Recent data from two large prospective studies on the association between peripheral blood LTL and PC risk,^{12,13} and from studies relating genetically inferred TL to PC risk^{14,15} necessitate a deeper review of the evidence linking TL with PC risk. We present a focused discussion on the structure and function of TL, its suggested role in tumor suppression, and carcinogenesis hypotheses for critically short TL. We next provide an overview of reported associations between TL and overall cancer risk from an updated review of publications on the association between LTL and PC risk. Finally, we discuss potential reasons for variation in study findings and offer suggestions to guide future telomere research in pancreatic cancer.

Telomere Biology and Implications for Cancer

Telomeres, the protective DNA tandem repeats (TTAGGG) and DNA binding proteins (shelterin) that cap the ends of linear chromosomes, are essential for maintaining genomic stability.⁸ The nucleoprotein telomere complex is catalyzed by the telomerase enzyme⁹ and involves the *TERC* gene that encodes the RNA template for the repetitive DNA sequence repeats, a reverse transcriptase subunit (*TERT*), and the shelterin proteins (e.g., *TRF1*, *TRF2*, *POT1*, *TIN2*, *TPP1*, and *Rap1*), which attach the DNA sequence repeats to chromosome ends and regulate telomere maintenance.^{10,16-18} The main functions of telomeres are to protect chromosomal material from degradation, prevent chromosomal end-to-end fusion, and ensure proper segregation of chromosomes during cell division.⁸ In humans, TLs range between 10–16.4 kilobase pairs (kbp) at birth¹⁸⁻²⁰ but decrease by roughly 50-100 base pairs (bp) with each cell division owing to incomplete replication by DNA polymerases.^{21,22} Thus, TL decreases throughout a person's life course and is thought to be a marker of biological age.^{10,23,24} In addition to aging, conditions that promote chronic inflammation (e.g., diabetes and obesity) and oxidative stress (e.g., tobacco smoking, exposure to ionizing radiation, and habitually low antioxidant intake) contribute further to telomere attrition.²⁵⁻²⁸

Although TLs vary across human tissues, there is far greater variation in TL between individuals than between tissues of the same individual.^{19,29,30} In leukocytes, an estimated minimum TL of 3.81 kbp is necessary for fully functional telomeres,^{10,31} whereas in germ cells, TLs that are less than 4 kbp are thought to compromise chromosomal integrity.³² Cells with critically short or dysfunctional telomeres are highly unstable and prone to

genomic aberration, increasing cancer risk.³³ The recognition of such cells with telomere dysfunction by immune effector cells triggers cellular damage responses, such as the activation of *p53*, *pRB*, *ATM*, *RAD17*, and *p16^{INK4a}* pathways, which may avert proliferation of potential cancer cells through cellular senescence or apoptosis.³²⁻³⁷

Two main hypotheses have emerged on how cells with critically short TL may influence cancer risk. The first postulates that although a majority of cells with extremely short telomeres undergo cellular senescence or apoptosis, on rare occasions, some cells with critically short telomeres evade both mechanisms, and undergo uncontrolled proliferation, a hallmark of tumorigenesis.³²⁻³⁴ The second posits that although senescent cells lack the ability to proliferate, they remain metabolically active for many years and are known to secrete pro-carcinogenic substances, such as inflammatory cytokines (e.g., IL6 and IL8), growth factors, and metalloproteases.^{32,38,39} Therefore, the accumulation of telomere-related senescent cells may promote a microenvironment that favors carcinogenesis.⁴⁰

TL and Cancer Risk

It has been suggested that lifestyle factors, such as cigarette smoking,⁴¹⁻⁴⁴ obesity,^{28,41,45} and physical inactivity⁴⁶⁻⁴⁹ and health states, such as diabetes and insulin resistance,^{50,51} which have been associated with increased cancer risk, may function through a mechanism of shortening of TL.^{10,52} Many studies have investigated the association between LTL and cancer risk, and these have generally indicated that having short LTL may increase risk.^{40,53-58} The earliest, most comprehensive epidemiological report linking TL with cancer risk was by Wu and colleagues in 2003.⁵⁴ They examined the association between peripheral blood lymphocyte TL and cancer risk in a pooled analysis of individual level data from four studies that included 92 head and neck cancers, 135 bladder cancers, 54 lung cancers, and 32 renal cell carcinomas with equal numbers of controls for each cancer type matched on age, sex, and ethnicity.⁵⁴ They found that having short TL was associated with increased risk of head and neck cancer. Compared to the fourth quartile category of TL (representing the longest TL), odds ratios (ORs) and 95% confidence intervals (CIs) for decreasing quartiles of TL in relation to head and neck cancer risk were 0.84 (95% CI, 0.36–1.97), 1.77 (95% CI, 0.72–4.36), and 5.11 (95% CI, 1.90–13.77). In a combined analysis of tobacco-related cancers (lung, bladder, and renal cell carcinomas), they observed an increased risk among individuals with short TL, with ORs (95% CIs) for decreasing quartiles of TL of 1.06 (1.90–8.68), 5.08 (2.40–10.75), and 4.41 (2.10–9.28).⁵⁴ In general, there is strong evidence for an inverse association between TL and risk of gastric,⁵⁵⁻⁵⁸ bladder,^{43,54,59} ovarian,^{57,60} and esophageal^{61,62} cancers, whereas reports from studies on breast, colorectal, lung, and non-Hodgkin's lymphoma have varied considerably, and the majority of studies on melanoma, prostate, and endometrial cancers show a null association.^{40,53}

Three overlapping meta-analyses have examined the association between TL and cancer risk.^{53,63,64} The first meta-analysis included 11,255 cancer cases and 13,101 controls from 21 studies published up to November 2010⁶³ and found increased overall cancer risk among individuals with short telomeres (OR, 1.35; 95% CI, 1.14–1.60; < median *versus* median TL), with evidence of heterogeneity between studies ($P_{\text{heterogeneity}} < 0.001$). In separate analyses by cancer type, they found that short telomeres (< median) were associated with

increased risk of bladder cancer (OR, 1.84; 95% CI, 1.38-2.44; $P_{\text{heterogeneity}}=0.88$), lung cancer (OR, 2.39; 95% CI, 1.18-4.88; $P_{\text{heterogeneity}}=0.88$), and smoking-related cancers (OR, 2.25; 95% CI, 1.83-2.78; $P_{\text{heterogeneity}}=0.009$), but not breast cancer. Short TL was also associated with an increased risk of cancers of the digestive tract, stomach, esophagus and colon (OR, 1.69; 95% CI, 1.53-1.87; $P_{\text{heterogeneity}}=0.88$) and cancers of the urogenital system, bladder, kidney and prostate (OR, 1.73; 95% CI, 1.12-2.67; $P_{\text{heterogeneity}}=0.88$).⁶³ The second meta-analysis included 27 studies published between August 2003 and August 2010.⁵³ The authors reported the OR for overall cancer risk comparing the longest TL quartile with the shortest quartile was 1.96 (95% CI, 1.37-2.81; $P_{\text{heterogeneity}}<0.001$), and there was a stronger association among the 16 retrospective studies (OR, 2.90; 95% CI, 1.75-4.80; $P_{\text{heterogeneity}}<0.0001$), but not among the 11 prospective studies (OR, 1.16, 95% CI, 0.87-1.54; $P_{\text{heterogeneity}}=0.32$).⁵³ The third meta-analysis included 51 studies published between August 2003 and September 2015 and identified a non-significant increased overall cancer risk associated with short TL (OR, 1.10; 95% CI, 0.98–1.23; $P_{\text{heterogeneity}}<0.0001$). However, there was evidence for an association between short TL and gastrointestinal cancers (OR, 1.62; 95% CI, 1.33–1.97; $P_{\text{heterogeneity}}<0.0001$) and head and neck cancers (OR, 1.86; 95% CI, 1.23–2.82; $P_{\text{heterogeneity}}<0.02$).⁶⁴

Association between LTL and PC Risk

A thorough search of MEDLINE, Embase, and the Cochrane Library databases using the search terms, “telomere length” OR “telomeres” OR “telomere” AND “pancreatic cancer” OR “pancreatic adenocarcinoma” OR “pancreatic ductal adenocarcinoma” OR “pancreas” OR “pancreatic” showed that as of May 26, 2017, five epidemiological studies have been published on association between LTL and PC risk.^{6,7,12,13,65} Three reported a nonlinear or U-shaped association,^{6,12,65} one reported a linear positive association,⁶⁵ and another reported a linear inverse association;¹³ details are provided in Table 1. The first study was a 2012 clinic-based case-control study that consisted of 499 rapidly ascertained PC cases and 963 non-cancer control patients frequency-matched on age, sex and state/region of residence.⁶ In that study, Skinner *et al.* used generalized additive logistic regression models treating TL as a continuous variable and observed a U-shaped association, such that individuals with extremely short TL (shortest 1%) and those with extremely long TL (longest 10%) were both found to have an increased risk of PC.⁶ When they used the median TL among controls as the reference category, they found an increased risk among those in the 1st to 40th percentiles of TL, decreased risk among those between the 60th-90th percentiles, and a trend toward increased risk among those in the 99th-100th percentiles of TL based on distribution among controls (Table 1). In 2013, Lynch *et al.* reported on a nested case-control study conducted among heavy Finnish male smokers in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study.⁷ The study, which included 193 incident PC cases and 660 controls matched on date of birth (± 5 years),⁷ showed a dose-response positive association between longer TL and PC risk, with an OR of 1.58 (95% CI, 1.1.02–2.46) in the highest *versus* the lowest quartile and a significant linear trend across quartiles ($P_{\text{trend}}=0.003$). In 2014, Campa *et al.* examined the association between leukocyte TL and PC by pooling individual level data from 10 cohorts in the European Prospective Investigation into Cancer and Nutrition (EPIC) study.⁶⁵ Their study included 331 cases and 331 controls

matched on age, sex, and country of origin, and they did not find an association between TL and PC risk when using quartiles of TL-based distribution among controls (OR, 1.38; 95% CI, 0.80-2.41, highest *versus* lowest quartile). However, when modeling TL as a continuous variable, they found a positive association between longer TL and PC risk (OR, 1.13; 95% CI, 1.01-1.27). Further, analysis by cubic spline regression showed evidence of a nonlinear association ($P_{\text{nonlinearity}}=0.022$),⁶⁵ similar to that observed by Skinner *et al.*⁶ In 2016, Bao and colleagues also examined the association between LTL and PC in a pooled analysis of five protective cohort studies consisting of 386 cases and 896 controls.¹³ These investigators found that short LTL was associated with increased PC risk (OR, 1.72; 95% CI, 1.07–2.78; lowest *versus* highest quintile, $P_{\text{trend}}=0.048$). The results from a cubic spline regression were consistent with a linear inverse association between longer TL and PC risk ($P_{\text{nonlinearity}}>0.05$).¹³ Additionally, in 2016, Zhang and colleagues examined the association between LTL and PC risk in a population-based nested case-control study of 900 cases and 900 controls in Liaoning, China and reported a U-shaped association.¹² Using the third quartile as the reference category, they found an increased risk of PC among individuals in the lowest quartile, representing the shortest TL category (OR, 3.10; 95% CI, 1.84–5.21), and an increased risk among those in the highest quartile, representing the longest TL category (OR, 1.49; 95% CI, 1.11–2.00).¹² The relationship between measured LTL and PC therefore remains inconclusive.

Possible Reasons for Inconsistent Results

These mixed findings on the LTL and PC association can be attributed to many factors, including differences in the studied populations, variation in study design, differences in the time between blood collection and the diagnosis of pancreatic cancer, between-laboratory variability in methods used to measure TL, or confounding by factors known to influence TL. As an example, the nested case-control study by Lynch *et al.*⁷ that found a positive association between TL and PC was conducted among Finnish males who smoked at least five cigarettes per day.⁷ By comparison, the nested case-control study by Bao *et al.*¹³ that reported an inverse association was conducted among Americans of European descent, including males (35%) and females (65%), and less than 15% of the study subjects were current smokers. It is plausible that the differences in smoking prevalence and sex may in part explain the discordant findings between these two prospective studies. Moreover, four of the studies used a nested case-control design and measured TL in pre-diagnostic peripheral blood leukocytes^{7,12,13,65} whereas one was a clinic-based case-control study that measured TL in post-diagnostic blood samples.⁶ Even findings from the four prospective studies that used pre-diagnostic blood samples are contradictory, and only two of these studies^{7,13} provided data on the median time between blood collection and the diagnosis of pancreatic cancer. Bao *et al.* found no difference in the association between LTL and PC when they performed a stratified analysis by the median time between blood collection and the diagnosis of PC (< 6.7 *vs.* ≥ 6.7 years).¹³ Lynch and colleagues did not stratify by the median time between blood collection and diagnosis (6.3 years); however, when stratified by years of follow-up, they found that the association between long TL and increased risk of PC was restricted to cases diagnosed within 5 years of enrollment.⁷ This suggests reverse causation—the idea that cancer may utilize telomere lengthening mechanisms to evade

apoptosis and senescence, leading to uncontrolled proliferation of cancer and the accumulation of gene mutations.³²⁻³⁴

The differential findings could also be due to inter-laboratory variation in the methods used to measure TL.¹¹ Most of the studies used single quantitative real-time polymerase chain reaction (qPCR)⁶⁶ to measure LTL,^{6,12,13,65} only Lynch et al⁷ used monochrome multiplex qPCR (MMqPCR).⁷ Even among studies that used qPCR, differences in the DNA purity of blood samples or in the methods used to identify, quantify, or normalize the telomeric sequence repeats may affect TL measurements between studies.^{67,68} Indeed, Martin-Ruiz et al. observed 40% inter-laboratory variation in TL measurement when qPCR was used.⁶⁹ As described in detail by Cawthon,⁷⁰ multiplexing the qPCR method (i.e., MMqPCR) is a better approach to measure TL because it is not affected by differences in the amount of DNA used to estimate the ratio of telomere (T) signals to single-copy gene (S) signals (i.e., T/S ratio; the standard measure of relative TL). MMqPCR has also been found to reduce measurement error and increase throughput.⁷⁰ Other methods of telomere measurement, such as telomere restriction fragment (TRF) length, single TL analysis (STELA), and quantitative fluorescent in situ hybridization (Q-FISH), have been described^{67,68} but are not often used in large epidemiological studies because they tend to be labor intensive and time consuming.⁶⁷

Notably, the discrepancies in study findings may be due to poor control of confounding factors, particularly tobacco smoking, diabetes mellitus, and obesity. Tobacco smoking is estimated to account for up to 25% of all PCs³ and smoking accelerates TL shortening.^{41,43,44,49} Indeed, Valdes *et al.* reported a dose-response association between pack-years of tobacco smoking and TL shortening, such that every 1 pack-year of smoking history was found to be associated with a 5-bp reduction in LTL.⁴¹ Mirabello et al also observed a significant inverse association between smoking and leukocyte telomere length among both healthy controls and prostate cancer patients.⁴⁴ Diabetes mellitus is also a firmly established risk factor for pancreatic cancer,⁴ and studies have consistently shown that LTLs are shorter among diabetics compared to non-diabetics.⁷¹⁻⁷⁴ Obesity and physical inactivity, known risk factors for pancreatic cancer, have all been associated with TL shortening.^{28,41,45-49} Poor measurement of these confounding factors would residually confound the association between TL and pancreatic cancer. Most existing studies on TL and PC risk adjusted for smoking status (i.e., never, former, current).^{6,12,13,65} However, it is well known that PC risk increases with smoking dose and duration,^{75,76} and the risk among former smokers approaches that of never smokers after about 15 years of smoking cessation.⁷⁵⁻⁷⁷ Thus, adjusting for pack-years of smoking among current smokers and the duration of smoking cessation among former smokers may more adequately control for confounding by smoking. Similarly, adjusting for insulin resistance (a marker of metabolic perturbations that influence TL^{51,78}) as opposed to self-reported history of diabetes, and adjusting for measured usual adult BMI instead of self-reported BMI, may help clarify the association between TL and PC risk.

Role of Genetic Variation in Telomere Maintenance Genes

As high as 80% of individual differences in TL are thought to be attributable to germline variation in telomere-related genes.¹⁰ In view of the conflicting findings from studies

involving measured LTL, Antwi and colleagues examined associations between genetically predicted TL and PC risk as a means of overcoming inter-laboratory variation in TL measurement and concerns of reverse causation.¹⁴ They computed genetic risk scores involving eight polymorphisms that were previously associated with inter-individual differences in LTL in genome-wide association studies (GWAS). The study, which included 1500 incident PC cases and 1499 non-cancer controls, found no association between genetically predicted TL and PC.¹⁴ Indeed, they found that one short telomere-related allele (rs10936599, T) associated with lower PC risk, whereas another short telomere-related allele (rs2736100, A) was associated with an increased PC risk.¹⁴ Haycock *et al.* have also reported a null association between genetic markers that determine TL and PC risk in a pooled analysis of GWAS data that included 5105 PC cases and 8739 controls.¹⁵ However, no study has yet examined whether the association between measured TL and PC risk is modulated by individual variation in telomere maintenance genes (e.g., *TRC*, *TERT*, *TRF1*, *TRF2*, *POT1*, and *Rap1*). Because polymorphic variants generally have modest effects, their impact may become more noticeable in the presence of relevant factors.^{79,80} Delineating the potential interactive effect of variation in telomere-related genes and host TL on the risk of PC may help further clarify telomere dynamics in pancreas carcinogenesis. Moreover, it has been suggested that *SURVIVIN*, a gene known to inhibit apoptosis and enhance telomerase activity by up-regulating *TERT* and found to be overexpressed in PC patients compared to healthy controls,⁸¹⁻⁸³ may modify the association between TL and PC risk. To our knowledge, no study has examined the relationship between *SURVIVIN* expression in blood or tumor tissue relative to TL in the respective media. Because *SURVIVIN* promotes telomere lengthening through the activation of telomerase (the enzyme responsible for the elongation of TL), investigations into how *SURVIVIN* expression or how inherited mutations in *SURVIVIN* may influence TL would help further elucidate the telomere-PC dynamics. Examining gene-gene interactions in the *SURVIVIN* and the *TERT* signaling pathways in relation to TL and PC risk may also help better understand the role of telomeres and their influencers in pancreatic cancer.

Future Directions

Epidemiological studies examining the association between TL and PC risk have universally relied on LTL as a surrogate for the overall telomere status of an individual. Many studies have reported significant variation in TL across tissues of the same individual;⁸⁴⁻⁸⁷ thus, LTL may not reflect TL in pancreatic tissue. Studies must investigate the intra-individual correlation of TL in leukocytes and matching tumors of PC patients and between leukocytes and matching pancreatic tissue of non-cancer individuals to determine the appropriateness of LTL to estimate PC risk. Pancreatic tissue-based studies have reported shorter TL in pancreatic tumors compared to adjacent normal tissue or normal-normal tissue (i.e., pancreatic tissue from non-cancer patients).⁸⁸⁻⁹⁰ Further, Hashimoto *et al.* examined the temporal sequence of TL progression in pancreatic carcinogenesis and found progressive loss of TL during the transition of intraductal papillary mucinous neoplasm (IPMN) from adenoma IPMN to borderline IPMN lesions and to carcinoma in situ IPMN,⁸⁸ indicating that telomere shortening precedes the malignant transformation of precancerous somatic cells. A similar finding was reported by Matsuda *et al.*⁹⁰ It is plausible that the temporal changes in

TL of pancreatic tissue could have also occurred in the leukocytes of patients who later developed pancreatic cancer. Although the timing of telomere dysfunction in the leukocytes of PC patients is unknown, it could be determined in longitudinal studies with multiple measurements of TL at stages in life that are most relevant to carcinogenesis. Determining the critical point of change in LTL among individuals who later developed PC would facilitate the development of a telomere-based early detection model for PC.

Four^{6,12,13,65} of the five studies relating LTL to PC risk measured TL using the qPCR assay.⁶⁶ The precision of the qPCR assay has been questioned^{67,68} given that it is affected by variation in the amount of DNA used to amplify the telomere signal (T) versus the single gene signal (S) wells. An improved version of the qPCR assay (i.e., MMqPCR) that addresses issues relating to differences in DNA quantity in each well has been introduced and could help improve the accuracy of telomere measurement across studies.⁷⁰ Other methods, such as the Southern blot analysis of TRFs, Q-FISH and SETLLA, have also been used in telomere research, but these are not generally well suited for large epidemiological studies because they are not high throughput.⁶⁷ Notably, TL tends to differ in subpopulations of leukocytes; B-cell lymphocytes have longer TLs than T-cell or natural killer cell lymphocytes.⁹¹⁻⁹³ Studies are necessary to determine the yield of each leukocyte subtype needed for optimal TL measurement in leukocytes. However, sampling leukocytes with >80% B-cell lymphocytes or isolating B-cell lymphocytes only for telomere measurement may be the most informative method. DNA extraction methods also affect comparability of TL measurement between studies.^{94,95} Standardizing DNA extraction for telomere measurement between studies and adopting the high-throughput MMqPCR method instead of the qPCR method widely used in epidemiological research may help minimize variability in the measurement of TL between studies.⁶⁷

Because TL is determined partly by inter-individual germline variation, age (a measure of cellular replicative history), environmental exposures (e.g., smoking) and chronic conditions (e.g., diabetes) are associated with PC risk; thus, it is important that studies examining the association between TL and PC adequately control for potential confounding effects of these factors. To reduce confounding by tobacco smoking history, studies must consider adjusting for pack-years of smoking among current smokers and the duration of smoking cessation among former smokers. Moreover, because PC is often associated with significant weight loss and patients often have experienced substantial weight loss before clinical diagnosis of the disease,⁹⁶ it would be of interest for studies to use measured usual adult weight and height to calculate and adjust for BMI, rather than the frequently used self-reported BMI or measured BMI at diagnosis. Adjusting for a clinically verified diagnosis of diabetes or insulin resistance would also help to minimize confounding by metabolic disturbances that influence TL.^{51,71} Although studies that have examined the association between genetically predicted TL and PC have reported null findings,^{14,15} an advancement in this area could be the investigation of interactive effects between inherited variation in telomere maintenance and tobacco smoking or personal history of diabetes. Evaluation of the joint effects of known risk factors (i.e., diabetes, smoking or obesity) and TL in relation to the risk of PC also could inform tailored interventions based on genomic profiles to maximize the impact of PC prevention efforts.

In summary, we have presented a focused overview on the association between TL and pancreatic cancer. The data presented here show that the association between LTL and PC is not completely clear. However, the most comprehensive study involving pooled data from five prospective cohorts, points to a dose-response increase in PC risk among individuals with short LTL.¹³ Possible reasons for the differences in study findings have been discussed. Suggestions ranging from choice of study design, choice of technology for the measurement of LTL in large epidemiological studies, methods for controlling for confounding by extraneous factors. We propose approaches to address gaps in knowledge to guide future research regarding the association of PC and telomere length.

Acknowledgments

Funding: This research was supported by an NCI Specialized Program of Research Excellence (SPOR) in Pancreatic Cancer grant (P50 CA102701) and another NCI grant (R25 CA92049)

References

1. American Cancer Society. Global Cancer Facts & Figures. 3. Atlanta: American Cancer Society; 2015.
2. Rahib L, Smith BD, Aizenberg R, et al. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res.* 2014; 74:2913–2921. [PubMed: 24840647]
3. Lowenfels AB, Maisonneuve P. Epidemiology and risk factors for pancreatic cancer. *Best Pract Res Clin Gastroenterol.* 2006; 20:197–209. [PubMed: 16549324]
4. Antwi SO, Oberg AL, Shivappa N, et al. Pancreatic cancer: associations of inflammatory potential of diet, cigarette smoking and long-standing diabetes. *Carcinogenesis.* 2016; 37:481–490. [PubMed: 26905587]
5. Wolpin BM, Chan AT, Hartge P, et al. ABO blood group and the risk of pancreatic cancer. *J Natl Cancer Inst.* 2009; 101:424–431. [PubMed: 19276450]
6. Skinner HG, Gangnon RE, Litzelman K, et al. Telomere length and pancreatic cancer: a case-control study. *Cancer Epidemiol Biomarkers Prev.* 2012; 21:2095–2100. [PubMed: 23093543]
7. Lynch SM, Major JM, Cawthon R, 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; 133:2672–2680. [PubMed: 23674344]
8. Blackburn EH. Telomere states and cell fates. *Nature.* 2000; 408:53–56. [PubMed: 11081503]
9. Bryan TM, Cech TR. Telomerase and the maintenance of chromosome ends. *Curr Opin Cell Biol.* 1999; 11:318–324. [PubMed: 10395557]
10. Blackburn EH, Epel ES, Lin J. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. *Science.* 2015; 350:1193–1198. [PubMed: 26785477]
11. Duell EJ. Telomere length and pancreatic cancer risk: breaking down the evidence. *Gut.* 2017; 66:986–987. [PubMed: 27974551]
12. Zhang R, Zhao J, Xu J, et al. Association of peripheral leukocyte telomere length and its variation with pancreatic cancer and colorectal cancer risk in Chinese population. *Oncotarget.* 2016; 7:38579–38585. [PubMed: 27509261]
13. Bao Y, Prescott J, Yuan C, et al. Leucocyte telomere length, genetic variants at the TERT gene region and risk of pancreatic cancer. *Gut.* 2017; 66:1116–1122. [PubMed: 27797938]
14. Antwi SO, Bamlet WR, Broderick BT, et al. Genetically predicted telomere length is not associated with pancreatic cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2017; 26:971–974. [PubMed: 28264873]
15. Haycock PC, Burgess S, Nounu A, et al. Association Between Telomere Length and Risk of Cancer and Non-Neoplastic Diseases: A Mendelian Randomization Study. *JAMA Oncol.* 2017; 3:636–651. [PubMed: 28241208]

16. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev.* 2005; 19:2100–2110. [PubMed: 16166375]
17. Sfeir A, de Lange T. Removal of shelterin reveals the telomere end-protection problem. *Science.* 2012; 336:593–597. [PubMed: 22556254]
18. Palm W, de Lange T. How shelterin protects mammalian telomeres. *Annu Rev Genet.* 2008; 42:301–334. [PubMed: 18680434]
19. Frenck RW Jr, Blackburn EH, Shannon KM. The rate of telomere sequence loss in human leukocytes varies with age. *Proc Natl Acad Sci U S A.* 1998; 95:5607–5610. [PubMed: 9576930]
20. Drury SS, Esteves K, Hatch V, et al. Setting the Trajectory: Racial Disparities in Newborn Telomere Length. *J Pediatr.* 2015; 166:1181–1186. [PubMed: 25681203]
21. Proctor CJ, Kirkwood TB. Modelling telomere shortening and the role of oxidative stress. *Mech Ageing Dev.* 2002; 123:351–363. [PubMed: 11744046]
22. Allsopp RC, Vaziri H, Patterson C, et al. Telomere length predicts replicative capacity of human fibroblasts. *Proc Natl Acad Sci U S A.* 1992; 89:10114–10118. [PubMed: 1438199]
23. Mather KA, Jorm AF, Parslow RA, et al. Is telomere length a biomarker of aging? A review. *J Gerontol A Biol Sci Med Sci.* 2011; 66:202–213. [PubMed: 21030466]
24. von Zglinicki T, Martin-Ruiz CM. Telomeres as biomarkers for ageing and age-related diseases. *Curr Mol Med.* 2005; 5:197–203. [PubMed: 15974873]
25. von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci.* 2002; 27:339–344. [PubMed: 12114022]
26. Jurk D, Wilson C, Passos JF, et al. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. *Nat Commun.* 2014; 2:4172. [PubMed: 24960204]
27. Demissie S, Levy D, Benjamin EJ, et al. Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. *Aging cell.* 2006; 5:325–330. [PubMed: 16913878]
28. Rode L, Nordestgaard BG, Weischer M, et al. Increased body mass index, elevated C-reactive protein, and short telomere length. *J Clin Endocrinol Metab.* 2014; 99:E1671–E1675. [PubMed: 24762112]
29. Youngren K, Jeanclos E, Aviv H, et al. Synchrony in telomere length of the human fetus. *Hum Genet.* 1998; 102:640–643. [PubMed: 9703424]
30. Okuda K, Bardeguez A, Gardner JP, et al. Telomere Length in the Newborn. *Pediatr Res.* 2002; 52:377–381. [PubMed: 12193671]
31. Lin TT, Norris K, Heppel NH, et al. Telomere dysfunction accurately predicts clinical outcome in chronic lymphocytic leukaemia, even in patients with early stage disease. *Br J Haematol.* 2014; 167:214–223. [PubMed: 24990087]
32. Campisi J. Cellular senescence as a tumor-suppressor mechanism. *Trends Cell Biol.* 2001; 11:S27–S31. [PubMed: 11684439]
33. Hoeijmakers JHJ. Genome maintenance mechanisms for preventing cancer. *Nature.* 2001; 411:366–374. [PubMed: 11357144]
34. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, et al. A DNA damage checkpoint response in telomere-initiated senescence. *Nature.* 2003; 426:194–198. [PubMed: 14608368]
35. Jacobs JJ, de Lange T. p16INK4a as a second effector of the telomere damage pathway. *Cell Cycle.* 2005; 4:1364–1368. [PubMed: 16177573]
36. Saretzki G, Sitte N, Merkel U, et al. Telomere shortening triggers a p53-dependent cell cycle arrest via accumulation of G-rich single stranded DNA fragments. *Oncogene.* 1999; 18:5148–5158. [PubMed: 10498864]
37. Ohtani N, Yamakoshi K, Takahashi A, et al. The p16INK4a-RB pathway: molecular link between cellular senescence and tumor suppression. *J Med Invest.* 2004; 51:146–153. [PubMed: 15460900]
38. Coppé JP, Desprez PY, Krtolica A, et al. The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. *Annu Rev Pathol.* 2010; 5:99–118. [PubMed: 20078217]
39. Campisi J. Aging, Cellular Senescence, and Cancer. *Ann Rev Physiol.* 2013; 75:685–705. [PubMed: 23140366]

40. Prescott J, Wentzensen IM, Savage SA, et al. Epidemiologic evidence for a role of telomere dysfunction in cancer etiology. *Mutat Res.* 2012; 730:75–84. [PubMed: 21756922]
41. Valdes AM, Andrew T, Gardner JP, et al. Obesity, cigarette smoking, and telomere length in women. *Lancet.* 2005; 366:662–664. [PubMed: 16112303]
42. Morla M, Busquets X, Pons J, et al. Telomere shortening in smokers with and without COPD. *Eur Respir J.* 2006; 27:525–528. [PubMed: 16507852]
43. McGrath M, Wong JY, Michaud D, et al. Telomere length, cigarette smoking, and bladder cancer risk in men and women. *Cancer Epidemiol Biomarkers Prev.* 2007; 16:815–819. [PubMed: 17416776]
44. Mirabello L, Huang WY, Wong JY, et al. The association between leukocyte telomere length and cigarette smoking, dietary and physical variables, and risk of prostate cancer. *Aging Cell.* 2009; 8:405–413. [PubMed: 19493248]
45. Kim S, Parks CG, DeRoo LA, et al. Obesity and Weight Gain in Adulthood and Telomere Length. *Cancer Epidemiol Biomarkers Prev.* 2009; 18:816. [PubMed: 19273484]
46. Du M, Prescott J, Kraft P, et al. Physical activity, sedentary behavior and leukocyte telomere length in women. *Am J Epidemiol.* 2012; 175:414–422. [PubMed: 22302075]
47. Garland SN, Johnson B, Palmer C, et al. Physical activity and telomere length in early stage breast cancer survivors. *Breast Cancer Res.* 2014; 16:1.
48. Kim JH, Ko JH, Lee DC, et al. Habitual physical exercise has beneficial effects on telomere length in postmenopausal women. *Menopause.* 2012; 19:1109–1115. [PubMed: 22668817]
49. Latifovic L, Peacock SD, Massey TE, et al. The Influence of Alcohol Consumption, Cigarette Smoking, and Physical Activity on Leukocyte Telomere Length. *Cancer Epidemiol Biomarkers Prev.* 2016; 25:374–380. [PubMed: 26656293]
50. Verhulst S, Dalgård C, Labat C, et al. A short leukocyte telomere length is associated with development of insulin resistance. *Diabetologia.* 2016; 59:1258–1265. [PubMed: 27020448]
51. Demissie S, Levy D, Benjamin EJ, et al. Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. *Aging Cell.* 2006; 5:325–330. [PubMed: 16913878]
52. Ornish D, Lin J, Chan JM, et al. Effect of comprehensive lifestyle changes on telomerase activity and telomere length in men with biopsy-proven low-risk prostate cancer: 5-year follow-up of a descriptive pilot study. *Lancet Oncol.* 2013; 14:1112–1120. [PubMed: 24051140]
53. Wentzensen IM, Mirabello L, Pfeiffer RM, et al. The association of telomere length and cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev.* 2011; 20:1238–1250. [PubMed: 21467229]
54. Wu X, Amos CI, Zhu Y, et al. Telomere dysfunction: a potential cancer predisposition factor. *J Natl Cancer Inst.* 2003; 95:1211–1218. [PubMed: 12928346]
55. Hou L, Savage SA, Blaser MJ, et al. Telomere length in peripheral leukocyte DNA and gastric cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2009; 18:3103–3109. [PubMed: 19861514]
56. Liu X, Bao G, Huo T, et al. Constitutive telomere length and gastric cancer risk: case-control analysis in Chinese Han population. *Cancer Sci.* 2009; 100:1300–1305. [PubMed: 19432888]
57. Martinez-Delgado B, Yanowsky K, Inglada-Perez L, et al. Shorter telomere length is associated with increased ovarian cancer risk in both familial and sporadic cases. *J Med Genet.* 2012; 49:341–344. [PubMed: 22493152]
58. Tahara T, Shibata T, Kawamura T, et al. Telomere length shortening in gastric mucosa is a field effect associated with increased risk of gastric cancer. *Virchows Archiv.* 2016; 469:19–24. [PubMed: 27173780]
59. Broberg K, Bjork J, Paulsson K, et al. Constitutional short telomeres are strong genetic susceptibility markers for bladder cancer. *Carcinogenesis.* 2005; 26:1263–1271. [PubMed: 15746160]
60. Mirabello L, Garcia-Closas M, Cawthon R, et al. Leukocyte telomere length in a population-based case-control study of ovarian cancer: a pilot study. *Cancer Causes Control.* 2010; 21:77–82. [PubMed: 19784860]
61. Xing J, Ajani JA, Chen M, et al. Constitutive short telomere length of chromosome 17p and 12q but not 11q and 2p is associated with an increased risk for esophageal cancer. *Cancer Prev Res (Phila).* 2009; 2:459–465. [PubMed: 19401529]

62. Risques RA, Vaughan TL, Li X, et al. Leukocyte telomere length predicts cancer risk in Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev.* 2007; 16:2649–2655. [PubMed: 18086770]
63. Ma H, Zhou Z, Wei S, et al. Shortened telomere length is associated with increased risk of cancer: a meta-analysis. *PloS One.* 2011; 6:e20466. [PubMed: 21695195]
64. Zhu X, Han W, Xue W, et al. The association between telomere length and cancer risk in population studies. *Sci Rep.* 2016; 6:22243. [PubMed: 26915412]
65. Campa D, Mergarten B, De Vivo I, et al. Leukocyte telomere length in relation to pancreatic cancer risk: a prospective study. *Cancer Epidemiol Biomarkers Prev.* 2014; 23:2447–2454. [PubMed: 25103821]
66. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res.* 2002; 30:e47. [PubMed: 12000852]
67. Aubert G, Hills M, Lansdorp PM. Telomere length measurement—Caveats and a critical assessment of the available technologies and tools. *Mutat Res.* 2012; 730:59–67. [PubMed: 21663926]
68. Montpetit AJ, Alhareeri AA, Montpetit M, et al. Telomere length: a review of methods for measurement. *Nurs Res.* 2014; 63:289–299. [PubMed: 24977726]
69. Martin-Ruiz CM, Baird D, Roger L, et al. Reproducibility of telomere length assessment: an international collaborative study. *Int J Epidemiol.* 2015; 44:1673–1683. [PubMed: 25239152]
70. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res.* 2009; 37:e21. [PubMed: 19129229]
71. Monickaraj F, Aravind S, Gokulakrishnan K, et al. Accelerated aging as evidenced by increased telomere shortening and mitochondrial DNA depletion in patients with type 2 diabetes. *Mol Cell Biochem.* 2012; 365:343–350. [PubMed: 22411737]
72. Adaikalakoteswari A, Balasubramanyam M, Mohan V. Telomere shortening occurs in Asian Indian Type 2 diabetic patients. *Diabet Med.* 2005; 22:1151–1156. [PubMed: 16108841]
73. Zee RY, Castonguay AJ, Barton NS, et al. Mean leukocyte telomere length shortening and type 2 diabetes mellitus: a case-control study. *Transl Res.* 2010; 155:166–169. [PubMed: 20303464]
74. Salpea KD, Talmud PJ, Cooper JA, et al. Association of telomere length with type 2 diabetes, oxidative stress and UCP2 gene variation. *Atherosclerosis.* 2010; 209:42–50. [PubMed: 19889414]
75. Lynch SM, Vrieling A, Lubin JH, et al. Cigarette smoking and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium. *Am J Epidemiol.* 2009; 170:403–413. [PubMed: 19561064]
76. Bosetti C, Lucenteforte E, Silverman DT, et al. Cigarette smoking and pancreatic cancer: an analysis from the International Pancreatic Cancer Case-Control Consortium (Panc4). *Ann Oncol.* 2012; 23:1880–1888. [PubMed: 22104574]
77. Fuchs CS, Colditz GA, Stampfer MJ, et al. A prospective study of cigarette smoking and the risk of pancreatic cancer. *Arch Intern Med.* 1996; 156:2255–2260. [PubMed: 8885826]
78. Strazhesko I, Tkacheva O, Boytsov S, et al. Association of Insulin Resistance, Arterial Stiffness and Telomere Length in Adults Free of Cardiovascular Diseases. *PloS one.* 2015; 10:e0136676. [PubMed: 26308091]
79. Wang WY, Barratt BJ, Clayton DG, et al. Genome-wide association studies: theoretical and practical concerns. *Nat Rev Genet.* 2005; 6:109–118. [PubMed: 15716907]
80. Hunter DJ. Gene-environment interactions in human diseases. *Nat Rev Genet.* 2005; 6:287–298. [PubMed: 15803198]
81. Satoh K, Kaneko K, Hirota M, et al. Expression of survivin is correlated with cancer cell apoptosis and is involved in the development of human pancreatic duct cell tumors. *Cancer.* 2001; 92:271–278. [PubMed: 11466679]
82. Endoh T, Tsuji N, Asanuma K, et al. Survivin enhances telomerase activity via up-regulation of specificity protein 1- and c-Myc-mediated human telomerase reverse transcriptase gene transcription. *Exp Cell Res.* 2005; 305:300–311. [PubMed: 15817155]
83. Dong H, Qian D, Wang Y, et al. Survivin expression and serum levels in pancreatic cancer. *World J Surg Oncol.* 2015; 13:189. [PubMed: 26016480]

84. Dlouha D, Maluskova J, Kralova Lesna I, et al. Comparison of the relative telomere length measured in leukocytes and eleven different human tissues. *Physiol Res*. 2014; 63(Suppl 3):S343–S350. [PubMed: 25428739]
85. Wu KD, Orme LM, Shaughnessy J, et al. Telomerase and telomere length in multiple myeloma: correlations with disease heterogeneity, cytogenetic status, and overall survival. *Blood*. 2003; 101:4982–4989. [PubMed: 12609839]
86. Demerath EW, Cameron N, Gillman MW, et al. Telomeres and telomerase in the fetal origins of cardiovascular disease: a review. *Hum Biol*. 2004; 76:127–146. [PubMed: 15222684]
87. Daniali L, Benetos A, Susser E, et al. Telomeres shorten at equivalent rates in somatic tissues of adults. *Nat Commun*. 2013; 4:1597. [PubMed: 23511462]
88. Hashimoto Y, Murakami Y, Uemura K, et al. Telomere shortening and telomerase expression during multistage carcinogenesis of intraductal papillary mucinous neoplasms of the pancreas. *J Gastrointest Surg*. 2008; 12:17–28. [PubMed: 17960465]
89. Hong SM, Heaphy CM, Shi C, 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; 24:256–266. [PubMed: 20871595]
90. Matsuda Y, Ishiwata T, Izumiyama-Shimomura N, 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:e0117575, 1–13. [PubMed: 25658358]
91. Lin J, Epel E, Cheon J, et al. Analyses and comparisons of telomerase activity and telomere length in human T and B cells: insights for epidemiology of telomere maintenance. *J Immunol Methods*. 2010; 352:71–80. [PubMed: 19837074]
92. Lin Y, Damjanovic A, Metter EJ, et al. Age-associated telomere attrition of lymphocytes in vivo is co-ordinated with changes in telomerase activity, composition of lymphocyte subsets and health conditions. *Clin Sci (Lond)*. 2015; 128:367–377.
93. Lin J, Cheon J, Brown R, et al. Systematic and Cell Type-Specific Telomere Length Changes in Subsets of Lymphocytes. *J Immunol Res*. 2016; 2016:5371050. [PubMed: 26977417]
94. Denham J, Marques FZ, Charchar FJ. Leukocyte telomere length variation due to DNA extraction method. *BMC Res Notes*. 2014; 7:877. [PubMed: 25475541]
95. Raschenberger J, Lamina C, Haun M, et al. Influence of DNA extraction methods on relative telomere length measurements and its impact on epidemiological studies. *Sci Rep*. 2016; 6:25398. [PubMed: 27138987]
96. Fearon KC, Baracos VE. Cachexia in pancreatic cancer: new treatment options and measures of success. *HPB (Oxford)*. 2010; 12:323–324. [PubMed: 20590907]

Abbreviations

CI	confidence interval
IPMN	intraductal papillary mucinous neoplasm
kbp	kilobase pairs
LTL	leukocyte telomere length
MMqPCR	monochrome multiplex quantitative real-time polymerase chain reaction
OR	odds ratio
PC	pancreatic cancer
qPCR	quantitative real-time polymerase chain reaction
TL	telomere length

Table 1
Summary of Studies on Peripheral Blood Leukocyte Telomere Length and Pancreatic Cancer Risk

Study	Setting/Participants	Design	Sample Size	Time of Blood Collection in Cases	Assay Method	Covariates	OR (95% CI)
Skinner et al, 2012 ⁶	Clinic-based study: Pancreatic cancer patients and non-cancer control patients	Retrospective, No follow-up	Cases: 499 Controls: 963	~ 80% of samples collected within one month of diagnosis	qPCR	Adjustment for age, sex, race, BMI, diabetes, and fasting blood glucose levels	1 st %: 1.47 (1.11–1.95) 10 th %: 1.33 20 th %: 1.21 (1.06–1.38) 30 th %: 1.11 (1.03–1.20) 40 th %: 1.05 (1.01–1.09) 50 th %: ref 60 th %: 0.95 (0.91–0.99) 70 th %: 0.87 (0.78–0.96) 80 th %: 0.78 (0.65–0.95) 90 th %: 0.72 (0.53–0.98) 95 th %: 0.77 (0.50–1.20) 99 th %: 1.26 (0.61–2.59) 100 th %: 2.87 (0.55–14.91)
Lynch et al, 2013 ⁷	Randomized trial of Finnish male smokers	Prospective	Cases: 193 Controls: 660	Median of 6.3 years before cancer diagnosis	MMqPCR	Adjustment for age, pack-years of smoking, and diabetes	Q1: ref Q2: 0.87 (0.53–1.43) Q3: 1.29 (0.82–2.03) Q4: 1.58 (1.02–2.46) Cont.: 1.26 (1.08–1.46); $P_{trend} = 0.003$
Campa et al, 2014 ⁶⁵	Population-based Pooled data from 10 countries European countries	Prospective	Cases: 331 Controls: 331	Not reported	qPCR	Matching factors: age, sex, country of origin, and adjusted for C-peptides, HbA1c levels.	Q1: ref Q2: 0.86 (0.51–1.76) Q3: 0.92 (0.55–1.56) Q4: 1.38 (0.80–2.41) Cont.: 1.13 (1.01–1.27); $P_{trend} = 0.31$
Bao et al, 2016 ¹³	Population-based pooled data from five US cohort studies	Prospective	Cases: 331 Controls: 331	Median of 6.7 years before cancer diagnosis	qPCR	Conditional analysis on matching factors: year of birth, cohort (which matched on sex), smoking status, fasting status at blood collection, and month/year of blood collection.	Q1: 1.72 (1.07–2.78) Q2: 1.27 (0.79–2.02) Q3: 1.23 (0.79–1.93) Q4: 1.35 (0.90–2.04) Q5: ref $P_{trend} = 0.048$
Zhang et al, 2016 ¹²	Population-based study in Liaoning Province, Chinese	Prospective	Cases: 900 Controls: 900	Baseline	qPCR	Adjustment for age, sex, smoking status, drinking status, hypertension, BMI, and diabetes	Q1: 3.10 (1.84–5.21) Q2: 1.29 (0.98–1.71) Q3: ref Q4: 1.49 (1.13–1.97)

Cont. indicates continuous; qPCR, quantitative polymerase chain reaction; MMqPCR, monochrome multiplex quantitative polymerase chain reaction; OR, odds ratio; CI, confidence intervals.