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A Prospective Analysis of Telomere Length and Pancreatic Cancer in the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Study

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Abstract

Smoking and diabetes, consistent risk factors for pancreatic cancer, are also factors that influence telomere length maintenance. To test whether telomere length is associated with pancreatic cancer risk, we conducted a nested case-control study in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study cohort of male smokers, aged 50–69 years at baseline. Between 1992 and 2004, 193 incident cases of pancreatic adenocarcinoma occurred (mean follow-up from blood draw: 6.3 years) among participants with whole blood samples available for telomere length assays. For these cases and 660 controls, we calculated odds ratios (OR) and 95% confidence intervals using unconditional logistic regression, adjusting for age, number of years smoked regularly, and history of diabetes mellitus. Telomere length was categorized into quartiles (shortest to longest) and analyzed as both a categorical and a continuous normal variable (reported per 0.2 unit increase in telomere length). All statistical tests were two-sided. Longer telomere length was significantly associated with increased pancreatic cancer risk (continuous OR=1.26 95% CI=1.09–1.46; highest quartile compared to lowest, OR=1.57, 95% CI=1.01–2.43, p-trend=0.007). This association remained for subjects diagnosed within the first five years of blood draw (continuous OR=1.46, 95% CI=1.19–1.79 highest quartile OR=2.92, 95%CI=1.47–5.77, p-trend=0.002), but not those diagnosed greater than five years after blood draw (continuous OR=1.03, 95%CI=0.85–1.22; highest quartile OR=1.04, 95%CI=0.60–1.79). This is the first prospective study to suggest an association between longer blood leukocyte telomere length and increased pancreatic cancer risk.

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Introduction

Pancreatic adenocarcinoma is the fifth leading cause of worldwide cancer death [1] and the fourth leading cause of cancer mortality in the U.S [2]. The lifetime risk of developing pancreatic cancer is the same in men and women (about 1 in 71) [2]. An estimated 43,920 Americans were diagnosed with pancreatic cancer in 2012, and over 37,390 were estimated to die from the disease[2], making pancreatic cancer the most deadly cancer in the U.S. Few biomarkers have been identified to detect pancreatic cancer, and it is likely that a lack of effective screening methods to detect early disease contributes to its low 5-year survival rate of less than 6% [2].

Telomeres are complex nucleoprotein structures that are essential for the maintenance of chromosomal integrity. Telomeres consist of stretches of (TTAGGG) repeat DNA at the end of chromosomes that bind to proteins and form a “capping” structure designed to protect chromosomal DNA from end-to-end fusions, misrepair, and degradation [3] during cell division. Telomeres naturally shorten with age in all replicating somatic cells [4]. This telomeric erosion continues through the life of the cell unless telomeres are elongated by the activity of telomerase[5, 6] or other processes [5]. Telomerase acts to maintain telomere length, but in normal cells, is found in low levels [7]. In the absence of elongation, telomeres are continually shortened eventually resulting in their inability to bind to their proteins and maintain their structure, thus leading to a condition known as telomere crisis. In normal cells, telomere crisis or short telomeres result in apoptosis or senescence [8, 9]. Cells with critically short telomeres that escape apoptosis or senescence [10] and continue to replicate and undergo mitosis are believed to mark the critical step to malignant transformation for most cancers. This is why short telomeres have been related to both pro-(when they escape apoptosis or senescence) and anti-tumor (under normal conditions when they undergo apoptosis or senescence) effects in mouse models and tumor studies [4, 11].

Studying the relationship between telomere length and pancreatic cancer is particularly relevant based on published literature. Previous tumor-based studies have reported a significant relationship between shorter telomere length and pancreatic intraepithelial neoplasia (PIN) and metaplasia lesions resulting from PIN [12, 13] and suggest that telomere length can change during different stages of pancreatic cancer progression [11, 14]. Genome-wide association studies suggest that the TERT or telomerase gene [15] is associated with pancreatic cancer. In addition, higher levels of smoking [16–18], increased body mass index [17, 19–22] and the presence of diabetes [23, 24], consistent risk factors for pancreatic cancer, are also factors that influence the rate at which telomeres can shorten. While two recent meta-analyses suggest an association between shorter telomere length measured in blood leukocytes and overall incident cancer [25, 26], most tumor-specific epidemiological studies report inconsistencies in the association between telomere length and cancer risk [25, 26], and no prospective, epidemiologic studies have investigated the relationship between telomere length and pancreatic cancer, specifically. Therefore, we tested the hypothesis that altered telomere length measured in blood leukocytes might be associated with a risk of developing pancreatic cancer in a prospective cohort, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study [27].

Methods

Study Population

The ATBC Study was a double-blind, placebo-controlled, 2 X 2 factorial-primary prevention trial. The goal of the trial was to test whether alpha-tocopherol or beta-carotene supplementation reduced cancer incidence in Finnish male smokers [27]. The study design, data collection and methods have been described previously [27]. Briefly, 29,133 eligible men in Southwestern Finland between the ages of 50–69 who smoked at least 5 cigarettes per day were randomized between 1985 and 1988 to receive active supplements or placebo through the course of the study. All study participants provided written informed consent, and the study protocol was approved by the institutional review boards of both the National Public Health Institute in Finland and the National Cancer Institute in the United States.

Case Ascertainment and Control Selection

Pancreatic cancer cases within the ATBC cohort were identified and confirmed through central review of all relevant hospital records for cases diagnosed from baseline through April 1999, whereas cases diagnosed after April 1999 were identified solely using Finnish Cancer Registry data. The Finnish Cancer Registry provides nearly 100% case ascertainment in Finland and accurately reports 89% of pancreatic cancer that are primary cases [27, 28]. Cases were defined as incident primary malignant neoplasm of the exocrine pancreas (ICD9-157) only. We excluded endocrine tumors (ICD9-157.4) for this analysis and only included subjects with whole blood samples for DNA extraction. Whole blood samples were collected at one time point towards the end of the study between 1992 and 1993. With a follow-up of 12 years (from 1992–2004), the median time from whole blood draw to pancreatic cancer diagnosis was 6.3 years.

Controls were selected from ATBC Study participants who had whole blood samples for DNA extraction, and were alive and free of cancer (except nonmelanoma skin cancer) at the time the case was diagnosed. Two controls (n=386) were matched to each pancreatic cancer case by date of birth (±5 years). We also included additional controls from other ATBC nested case-control studies (n=274) with telomere data that were measured concurrently with our study measurements [29, 30]. There were no significant differences between the two control groups for the characteristics listed in Tables 1 and 2 (data not shown). For instance, in the matched control set, the median telomere length was 1.08 with an interquartile range of 0.95–1.22, and in the additional control set, the median was 1.07 with an interquartile range of 0.92–1.20. Therefore, to increase study power, we used the pooled control set for this analysis (n=660).

Telomere Length

DNA was extracted from whole blood using the phenol–chloroform method, and a monochrome quantitative multiplex PCR assay was used to measure the relative telomere length [31]. In brief, the reagents in the 25 _l PCR were 10 mM Tris–HCl pH 8.3, 50 mM KCl, 3 mM MgCl2, 0.2 mM each dNTP, 1 mM DTT, 1 M betaine, 0.75× SYBR Green I, and AmpliTaq Gold DNA polymerase, 0.625 U. Four primers were used (5′to 3′): telg (at 900 nM), telc (at 900 nM), hbgu (at 500 nM), and hbgd (at 500 nM), and added to each reaction

well with five to 70ng of DNA. Three-fold serial dilutions of a reference genomic DNA sample were used to generate two standard curves for each PCR plate (five concentrations with a high of 150ng per reaction, and a low of 1.85ng per reaction). The MyiQ software (Bio-Rad iQ5 2.0 Standard Edition Optical System Software) was used to determine the T (telomere) and S (single copy gene) values for each experimental sample by the standard curve method. T/S in this study is a relative and dimensionless value which is proportional to the average telomere length per cell. Samples with a T/S of >1.0 have an average telomere length greater than that of the standard DNA (reference 1.0); samples with a T/S of <1.0 have an average telomere length shorter than that of the standard DNA. The multiplex quantitative PCR method described here eliminates the pipetting variation between wells present using other quantitative PCR methods [31] and the correlation between T/S ratios determined from multiplex quantitative PCR and Terminal Restriction Fragment (TRF) lengths measured by the Southern Blot, the more tedious and costly standard approach, is high ($R^2 = 0.84$) [31].

Cases and their matched controls were blindly assayed consecutively within each batch. Quality control duplicate samples (5%) were interspersed in each batch to determine assay reproducibility. Samples were analyzed in duplicate. If there was discordance between the samples, telemere length was measured again and the two most similar measurements averaged. Across all assays (including pancreas, lung, and lymphoma control groups), the intra-class correlation coefficient of the assay was 80% and the overall coefficient of variation was 7%.

Statistical Analysis

We compared distributions of selected case and control characteristics using Wilcoxon rank-sum tests for median values and the chi-square tests for proportions (Table 1). Among control participants, median values for continuous variables and proportions (percentages) for the categorical variables were calculated to describe selected characteristics across telomere length quartiles (Table 2). We compared distributions of these selected characteristics using the Kruskal Wallis test for continuous variables and the chi-square test for proportions (Table 2). We also evaluated the correlation between continuous variables using Spearman correlations, and results were similar to those reported in Table 2 (data not shown). Variables examined in the analyses and as potential confounders in the risk models were based on previous findings from literature [32–39] (see Table 1). Nutrients and foods highly correlated with energy intake were adjusted for energy using the residual method described by Willett and Stampfer [40].

Matching between cases and controls was not retained for the analyses because we used a pooled control set. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CI) for pancreatic cancer, with men in the lowest telomere length quartile serving as the reference category. Telomere length (T/S ratio) was analyzed as both categorical and continuous variables. Telomere length was entered in as a normal continuous variable based on the T/S ratio for statistical models and results can be interpreted as a 0.2 unit increase in telomere length. As a categorical variable, telomere length was categorized into quartiles based on the distribution of controls for the main

analysis. We also report results with telomere length characterized into dichotomous groups to better assess patterns. Tests for trend were calculated by treating the level of telomere length as a continuous variable.

Multivariable models were developed using forward and backward regression by individually entering or removing potentially confounding variables into the model. Variables remained in the model if they were associated with both the disease and telomere length, had a P value of 0.10 or less in the full model, or changed the risk estimate by more than 10%. No factors met this definition of a confounder in our analysis; therefore, the final multivariable models included age at randomization (continuous), smoking history (pack-years of cigarette smoking) and history of diabetes mellitus (yes/no) only, since they are putative risk factors for pancreatic cancer. Based on literature and findings from Tables 1 and 2, interactions between telomere length and factors including age, trial intervention, education, living in a city, alcohol intake (grams), history of diabetes, body mass index, smoking (number of cigarettes smoked per day and years smoked) and baseline serum retinol, alpha-tocopherol and beta-carotene, were evaluated using stratified analysis and multiplicative interactions tested with cross-product terms of the variables of interest. No statistically significant interactions were observed. We also stratified our analysis by follow-up time from whole blood collection to case diagnosis to assess the potential for reverse causation. Specifically, we conducted lag analyses stratifying by follow-up time at 4, 5, 6, 7, 8, 9, and 10 years in order to determine if risk changed during follow-up. We present data using 5 years as the cutpoint in Table 3 since the risk estimate patterns across quartiles remained the same regardless of which follow-up time cutpoint was used. All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, North Carolina). The P values for all statistical tests were 2-sided, and an alpha level of 0.05 was used to determine statistical significance.

Results

The median time from whole blood collection to pancreatic cancer diagnosis was 6.3 years. Telomere length was significantly different between cases and controls (p=0.007), with cases having a higher median telomere length (1.13; interquartile range 0.97–1.27 vs 1.07; range 0.92–1.21) (Table 1). Cases and controls did not significantly differ with respect to most characteristics; controls were less likely to report a history of diabetes or pancreatitis (Table 1). The mean telomere length (T/S ratio) was 1.13 for cases (standard deviation, 0.21; range, 0.64–1.7) and 1.08 for controls (standard deviation, 0.22; range, 0.30–2.01).

Few significant trends existed between selected baseline characteristics and telomere length among controls (Table 2). Men in the lowest quartile of telomere length had lower serum retinol (p=0.0004), while men in the highest quartile of telomere length smoked cigarettes the least number of years (p=0.03), had the lowest number of pack-years (p=0.08), and drank the least amount of alcohol (p=0.02) and were less likely to live in a city (p=0.02).

Telomere length was significantly associated with pancreatic cancer risk in multivariate-adjusted models (continuous OR=1.26, 95%CI=1.09–1.46; OR=1.57, 95%CI=1.01–2.43, p-trend=0.007 for highest vs. lowest telomere length quartile) (Table 3). The association

between longer telomere length and pancreatic cancer was also observed when examining telomere length as a dichotomous variable using median telomere length as the cutpoint (multivariate-adjusted OR=1.61, 95% CI=1.15–2.23). The association between telomere length and pancreatic cancer was similar when we restricted our analysis to the smaller matched case-control set (n=193 cases; 386 controls) and used conditional logistic regression to estimate risk (highest compared to lowest quartile, multivariate adjusted OR=1.50, 95% CI=0.90–2.57).

The association between telomere length and pancreatic cancer remained for cases diagnosed within the first 5 years of blood draw (n=77) (continuous OR=1.46, 95% CI=1.19–1.79); however, our findings from the lag analysis showed no association between telomere length and pancreatic cancer risk after excluding cases diagnosed within the first five years after blood draw (continuous OR=1.03, 95% CI=0.85–1.22). Study intervention group did not significantly modify the association between telomere length and pancreatic cancer risk. The association between telomere length and pancreatic cancer was not modified by factors listed in Table 1 (all P-values >0.05) when they were analyzed as both confounding and interacting variables.

Discussion

We found a statistically significant positive association between longer telomere length and pancreatic cancer risk. The association between longer telomeres and an increased risk of pancreatic cancer remained for cases diagnosed closer to the time of blood draw, starting at 5 years from blood draw. Smoking[16–18] and diabetes [23, 24], well-known risk factors for pancreatic cancer that have been shown to affect telomere length, did not appear to alter the association we observe between telomere length and pancreatic cancer in this analytic cohort of all male smokers.

Our observation linking longer telomeres to an elevated risk of pancreatic cancer might be attributed to immune system activation that occurs primarily during latent pancreatic cancer, prior to cancer diagnosis [41]. Studies suggest the immune system is activated along the course of carcinogenesis, from initiation to progression[42]. Therefore, it is plausible that in response to carcinogenesis, more white blood cells might be produced, thus increasing our general measure of blood leukocyte telomere length. More specifically, telomere lengthening mechanisms could be activated in circulating leukocytes and/or the mobilization of younger immune response cells, like T cells, with longer telomeres may be released[43–45]. Additionally, white blood cell proliferation is favored over cellular senescence [41, 46]. This preference may favor delayed cell senescence for mutated cells with activated telomere lengthening mechanisms and allow for more opportunities for these cells to develop chromosomal instability and to continue to replicate, which can lead to advancing carcinogenesis[41,29]. Because studies suggest that the immune system plays dual roles in both promoting and inhibiting cancer formation[47], it is difficult to ascertain at this time both the timing and degree of immune system activation along the path to carcinogenesis in general, let alone for pancreatic cancer. However, the development of pancreatic cancer from initiation to diagnosis is thought to take 20 years with cases living with latent disease up to 10 years prior to diagnosis[48]. Therefore, the association between telomere length and

pancreatic cancer in participants with blood samples taken closest to the date of diagnosis suggests that the longer telomere length we observe might be due, in part, to the presence of underlying disease at blood draw. An alternative explanation could be that blood leukocyte telomere length is particularly sensitive to risk factors, like smoking or stress, and subsequently shorten in response to these environmental factors. This shortening of telomeres due to environmental factors can subsequently trigger cellular mechanisms that then work to increase telomere length, like telomerase activity or activation of the telomerase independent or alternative lengthening of telomeres (ALT) pathway[5]. However, in our analysis, very few risk factors appeared to be related to telomere length.

Our results contradict published meta-analyses of mostly case-control studies that have reported an overall association between cancer risk and shorter telomere length measured in surrogate tissues such as lymphocytes [25, 26]. Although these studies did not include pancreatic cancer, in stratified analysis by tumor type, associations remained significant in subgroups of bladder, esophageal, gastric, head and neck, lung, ovarian, and renal cancers[25, 26]. Smoking-related (OR = 2.25, 95% CI = 1.83–2.78) and digestive system cancers (OR = 1.69, 95% CI = 1.53–1.87) were also associated with shorter telomeres compared to longer telomeres, and an association between relative telomere length and overall cancer risk was reported in studies using retrospective designs, hospital-based controls and smaller sample sizes [25]. Most of the studies included in the meta-analysis measured telomere length differently, with few using the validated monochrome multiplex quantitative PCR method employed in this study[29]. A recent clinic-based case-control study, found a U-shaped association between telomere length and pancreatic cancer, such that short telomeres and extremely long telomeres were associated with increased risk.[49] A limitation of most retrospective pancreatic cancer studies is that blood samples are collected from cases with late stage disease. In the case-control study that investigated telomere length and pancreatic cancer, most blood samples were taken within 1 month of diagnosis[49], and temporal associations could not be established. Therefore, further investigation into the role of telomere length as a potential disease marker, particularly in prospective studies, is warranted.

The strength of our study is its prospective nature, with blood samples and other risk factor data collected prior to cancer diagnosis. Moreover, recent, prospective nested case-controls studies similarly report that longer telomeres are associated with increased risk for lymphoma, lung, and breast cancers [29, 30, 41]; two of these studies (lymphoma and lung) were also conducted with ATBC participants[29, 30]. The cases and controls from our study were both obtained from the larger ATBC cohort study; therefore, our study has internal validity, and no survival or selection bias that can result from case-control studies of pancreatic cancer. Residual confounding by cigarette smoking is possible, however, all subjects were current smokers at baseline and only 7% of the present analytic cohort stopped smoking during the course of the study (defined as a self-report of quitting smoking for 10 or more study visits in a row). Adjusting for smoking cessation did not change risk estimates and the smoking exposures were not effect modifiers of the associations (data not shown). While our findings in male smokers may not be generalizable to populations that include women and nonsmokers, they may provide novel insight into the role of telomeres in pancreatic cancer pathogenesis.

Our study also has limitations. Studies of pancreatic tissue show that telomere length shortens with age in normal tissue[50], and tumor-based studies on the progression of pancreatic cancer suggest that shorter telomeres occur early in disease development[5111, 14], whereas longer telomeres are observed in more advanced or metastasized pancreatic cancer [11, 14]. However, because the timing of blood draw and pancreatic cancer diagnosis were not concurrent, i.e. not collected at the same time, we cannot examine the association between telomere length and stage of cancer diagnosis. Therefore, we can draw few conclusions about whether blood leukocyte telomere length can serve as a surrogate marker for pancreatic tumors in our analysis. Blood leukocyte telomere length is a broad measure of telomere length that can be influenced by genetic variation (like the TERT gene)[52], chromosomal location [53], and blood composition (granulocytes, lymphocytes, monocytes, etc) [54, 55], particularly type of blood leukoctye (e.g., CD41 and CD81 T cells have varying telomere length)[56]. Our telomere assay is based on DNA extracted from a mixture of various types of white blood cells that may be affected by the presence of tumor, but they can also be affected by other chronic or acute infections that can cause inflammation. However, because our cases and controls had similar smoking status, were comparable in age, and blood samples were collected prior to diagnosis, inflammation in and composition of blood is unlikely to be systematically different between cases and controls. We did not have repeated measures of telomere length, which may help clarify the temporal relationship between telomere length and pancreatic cancer progression. In addition, the number of cases in our study is relatively small, particularly to evaluate subgroups and interactions.

In conclusion, we observed a significant association between longer telomere length and increased pancreatic cancer risk; however, our sensitivity analyses suggest that reverse causation may explain these findings. Further research is needed to confirm our findings in prospective studies of other populations, which include nonsmokers and women, and in studies with longer follow-up.

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Novelty and Impact Statement

This is the first prospective study to examine the association between blood leukocyte telomere length and pancreatic cancer. In tumor, longer telomeres have been observed in advanced pancreatic cancer and risk factors for pancreatic cancer(cigarette smoke/diabetes) affect telomere length. We found that longer telomeres were significantly associated with increased pancreatic cancer risk. Our results add insight into the role of telomeres in pancreatic cancer, a disease that can benefit from the identification of early detection markers.

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Table 1

Baseline Characteristics of Case & Control Subjects, Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study.

	Cases	(n=193)	Controls	(n=660)	
Characteristics	**Median**	**IQR**	**Median**	**IQR**	**p-value** [b]
Telomere length, median[a]	1.13	0.97–1.27	1.07	0.92–1.21	0.002
Age, years	58	54–61	58	54–62	0.87
Body mass index (kg/m^2)	26.2	23.9–28.3	25.7	23.7–28.5	0.42
Smoking History (%)					
Total cigarettes smoked/day	20	15–25	20	15–24	0.16
Years of smoking	37	31–42	37	31–42	0.98
Pack-years of smoking	36	25–46	34	23–44	0.12
Medical History (%)					
Diabetes mellitus	7.5		4.3		0.07
Pancreatitis	2.0		0.8		0.13
Gallstones	4.0		5.5		0.40
Peptic Ulcers	18.1		17.1		0.73
Primary school education or less(%)	73.4		78.3		0.15
Living in city (%)	43.7		47.2		0.42
Daily dietary intake[cd]					
Energy, kcal	2660.0	2154.4–3135.0	2587.5	2153.8–3104.9	0.58
Total fat, g[c]	123.4	114.6–134.9	122.8	113.1–132.5	0.25
Saturated fat, g[c]	53.6	44.9–64.0	52.6	43.7–60.5	0.09
Carbohydrate, g[c]	264	236.2–289.4	269	246.3–289.3	0.13
Protein, g[c]	94.2	86.6–107.7	94.2	86.7–101.3	0.13
Folate, ug[c]	333.2	275.3–402.4	328.3	269.7–394.9	0.57
Alcohol, g	8.9	2.4–24.5	10.7	2.5–24.2	0.96
Physical activity (%)					
Occupational activity					0.73
Sedentary	16.1		13.2		
Moderate	32.7		33.2		

Characteristics	Cases (n=193) Median	IQR	Controls (n=660) Median	IQR	p-value [b]
Heavy	7.5		8.8		
Not working	43.7		44.8		
Leisure activity					0.49
Sedentary	39.9		36.3		
Light or moderate	53.5		55.1		
Exercising to keep fit	6.6		8.7		
Baseline serum concentration					
Alpha-Tocopherol, mg/L	12	9.9–13.8	11.8	10.0–13.6	0.77
Beta-Carotene, ug/L	162	110.0–272	189	121.5–283.0	0.22
Retinol, ug/L	574	486–669	574	496.0–668	0.66
Randomization Group(%)					
Alpha-Tocopherol (AT)	28.6		25.5		0.67
Beta-Carotene (BC)	24.6		25.1		
AT and BC	20.6		24.2		
Placebo	26.2		25.2		

[a] Units of measurement=T/S ratio

[b] P values for categorical variables were based on the chi-squared test or Fisher's exact test; P values for continuous variables were based on Wilcoxon's rank-sum test.

[c] Adjusted by total energy intake.

[d] Cases=192; Controls=622

Table 2

Select Characteristics of Control subjects by Telomere Length (Quartiles)[a]

	Q1	Q2	Q3	Q4
Characteristic	**≤0.92**	**>0.92-≤1.07**	**>1.07-≤1.21**	**>1.21**
Telomere Length	0.85	1.02	1.14	1.32
Age, years	58	58.2	57.9	57.6
BMI	25.7	25.4	25.9	26.1
Smoking history				
Total cigarettes smoked	20.0	20.0	20.0	20.0
Years of smoking	39.0	39.0	37.0	36.0
Pack-years of smoking	35.0	34.0	35.0	30.0
Medical History				
Diabetes mellitus	5.2	3.1	2.3	6.8
Pancreatitis	1.2	0.6	1.2	0.0
Gallstones	6.9	5.6	6.4	3.1
Peptic Ulcers	16.7	12.9	20.5	17.8
Primary education or less, %	78.6	82.7	71.9	80.4
Living in city, %	53.8	46.9	49.1	38.7
Daily dietary intake[bc]				
Energy, kcal	2602.9	2590.4	2632.1	2528.2
Total fat, g	123.0	121.2	122.2	124.6
Saturated fat, g	53.5	51.4	52.2	53.1
Carbohydrate, g	268.0	271.4	265.8	272.3
Protein, g	94.2	94.2	93.6	94.7
Folate, ug	330.1	327.4	333.2	323.7
Alcohol, g	11.9	10.7	13.6	8.3
Physical Activity				
Occupational activity				
Sedentary	11.5	14.5	13.1	15.5
Moderate	31.7	30.5	31.4	39.8
Heavy	7.8	7.7	11	6.5

	Q1	Q2	Q3	Q4
Characteristic	**≤0.92**	**>0.92-≤1.07**	**>1.07-≤1.21**	**>1.21**
Not working	48.9	47.3	44.5	38.2
Leisure activity				
Sedentary	33.1	32.0	35.9	44.7
Light or moderate	58.3	58	54.7	48.8
Exercising to keep fit	8.6	9.9	9.5	6.5
Baseline Serum Concentration[b]				
Alpha-tocopherol, ug/L	11.3	12.0	12.0	11.7
Beta-Carotene, ug/L	169.0	190.0	193.5	195.0
Retinol, ug/L	553.0	575.0	602.0	566.0
Randomization Group				
Alpha-Tocopherol (AT)	26.6	24.4	29.9	23.6
Beta-Carotene (BC)	22.3	27.5	23.4	27.6
ATBC	28.1	21.4	24.1	25.2
Placebo	23	26.7	22.6	23.6

[a] Based on Total number of controls n=650, where quartile 1(Q1)=170, Q2=159, Q3=168; Q4=163 controls; median values are presented for continuous variables and percentages are reported for categorical variables.

[b] Number of controls=622

[c] Adjusted for Energy Intake

Table 3

Crude and Adjusted Odds Ratios and 95% Confidence Intervals for Telomere Length and Pancreatic Cancer Risk, Quartiles

	Telomere Length					
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**		
	≤0.92	**>0.92-≤1.07**	**>1.07-≤1.21**	**>1.21**	**p-trend[a]**	**Continuous OR**
n (cases/controls)	38/164	35/162	55/171	65/163		193/660
Crude	1.00	0.85 (0.52–1.39)	1.27(0.81–1.98)	1.57 (1.01–2.43)	.007	1.26 (1.09–1.46)
Adjusted Models:						
Age-adjusted	1.00	0.85 (0.52–1.39)	1.27(0.81–1.98)	1.57 (1.01–2.44)	.002	1.26 (1.09–1.46)
Multivariate-adjusted	1.00	0.87 (0.53–1.43)	1.29 (0.82–2.03)	1.58 (1.02–2.46)	.003	1.26 (1.08–1.46)
LAG Analysis:						
Cases diagnosed ≤5 years						
N (cases/controls)	8/164	10/162	24/171	35/163		77/660
Crude	1.00	0.82 (0.35–1.93)	1.87 (0.92–3.79)	2.86 (1.46–5.59)	0.002	1.46 (1.20–1.79)
Multivariate-adjusted	1.00	0.87 (0.37–2.05)	1.99 (0.98–4.07)	2.92 (1.47–5.77)	0.002	1.46 (1.19–1.79)
Cases diagnosed >5 years						
N (cases/controls)	30/164	25/162	31/171	30/163		116/660
Crude	1.00	0.86 (0.49–1.52)	1.01(0.59–1.74)	1.03 (0.60–1.77)	0.77	1.02 (0.86–1.23)
Multivariate-adjusted	1.00	0.87 (0.49–1.54)	1.01(0.59–1.74)	1.04 (0.60–1.79)	0.84	1.03 (0.85–1.22)

[a]Tests for trend calculated using telomere length as continuous variable.

OR=Odds ratio; Multivariate model adjusted for age, pack-years of cigarette smoke and diabetes.