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Leucocyte telomere length, genetic variants at the TERT gene region and risk of pancreatic cancer

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ABSTRACT

Objective Telomere shortening occurs as an early event in pancreatic tumorigenesis, and genetic variants at the telomerase reverse transcriptase (TERT) gene region have been associated with pancreatic cancer risk. However, it is unknown whether prediagnostic leucocyte telomere length is associated with subsequent risk of pancreatic cancer.

Design We measured prediagnostic leucocyte telomere length in 386 pancreatic cancer cases and 896 matched controls from five prospective US cohorts. ORs and 95% CIs were calculated using conditional logistic regression. Matching factors included year of birth, cohort (which also matches on sex), smoking status, fasting status and month/year of blood collection. We additionally examined single-nucleotide polymorphisms (SNPs) at the TERT region in relation to pancreatic cancer risk and leucocyte telomere length using logistic and linear regression, respectively.

Results Shorter prediagnostic leucocyte telomere length was associated with higher risk of pancreatic cancer (comparing extreme quintiles of telomere length, OR 1.72; 95% CI 1.07 to 2.78; p(trend)=0.048). Results remained unchanged after adjustment for diabetes, body mass index and physical activity. Three SNPs at TERT (linkage disequilibrium r²<0.25) were associated with pancreatic cancer risk, including rs401681, rs2736100 and rs2736098.

pancreatic cancer risk, including rs401681, per minor allele OR 1.33; 95% CI 1.12 to 1.59; p=0.002), rs2736100 (per minor allele OR 1.36; 95% CI 1.13 to 1.63; p=0.001) and rs2736098 (per minor allele OR 1.63; 95% CI 0.63 to 0.90; p=0.002). The minor allele for rs401681 was associated with shorter telomere length (p=0.023).

Conclusions Prediagnostic leucocyte telomere length and genetic variants at the TERT gene region were associated with risk of pancreatic cancer.

INTRODUCTION

Pancreatic cancer is the third leading cause of cancer death in the USA.1 The large number of deaths from pancreatic cancer is due in part to late diagnosis, with >80% of patients presenting with advanced, incurable disease.2 To facilitate early detection, it is critical to identify those individuals at increased risk.3 Nevertheless, known predisposing factors for pancreatic cancer, including obesity, diabetes, smoking and chronic pancreatitis, are inadequate to meaningfully risk stratify the general population.4

Telomeres are DNA repeats at the ends of chromosomes with a critical role in maintaining
The nested case–control set consisted of 472 pancreatic cancer cases and 1071 controls. Because telomere dynamics may differ across races/ethnicities and SNPs at the TERT gene region were identified in individuals of European descent, Caucasian subjects were excluded, resulting in 456 cases and 1035 matched controls. To minimise the influence of subclinical malignancy on leucocyte telomere length, we excluded case-control sets in which the pancreatic cancer case was diagnosed within two years of blood collection, resulting in 386 pancreatic cancer cases and 896 matched controls for analysis.

**Prediagnostic leucocyte telomere length measurement**

Genomic DNA was extracted from peripheral blood leucocytes using QIAamp (Qiagen, Chatsworth, California, USA) 96-spin blood protocol. PicoGreen quantitation of genomic DNA was performed using Molecular Devices 96-well spectrophotometer. The ratio of telomere repeat copy number to a single-gene copy number (T/S) was determined by modified, high-throughput quantitative real-time PCR telomere assay run Applied Biosystems 7900HT Sequence Detection System (Foster City, California, USA). Triplicate reactions were performed. Leucocyte relative telomere length is reported as exponentiated sample T/S ratio corrected for a reference sample. Telomere and single-gene assay coefficients of variation (CVs) for triplicates were 0.6% and 0.5%, respectively. CVs for the exponentiated T/S ratio were 12.9%.

**SNP selection and genotyping**

We genotyped four SNPs at the TERT gene region previously associated with cancer risk, including rs401681 (pancreatic cancer), rs2736100 (glioma and lung cancer), rs402770 (lung cancer) and rs2853676 (glioma). DNA was extracted centrally from archived buffy coat samples with QIAGEN QIAmp and whole genome amplified with GE Healthcare Genomiphi. All genotyping was performed at Partners HealthCare Center for Personalized Genetic Medicine using a custom-designed Illumina Golden Gate genotyping assay. Replicate samples included for quality control (N=44 sample groups) had mean genotype concordance of 97.2% across the four SNPs. No SNPs deviated from Hardy–Weinberg equilibrium at p<0.01.

In addition, 644 pancreatic cancer cases and 1171 controls from the five cohorts were included in recent GWAS of pancreatic cancer (PanScan studies). From these studies, we obtained SNP genotypes for our cohort participants to mark the six independent risk loci at TERT identified in a recent, large, multicancer analysis. Genotyping and imputation methods in the PanScan studies have been described previously. Among these subjects, 290 cases and 232 controls were included in the nested case–control study of telomere length. Online supplementary table 1 summarises the numbers of cases and controls with leucocyte telomere length, locally genotyped SNPs and SNPs genotyped in PanScan.

**Statistical analysis**

To standardise telomere length values, we calculated z-scores (SDs from the mean) of log-transformed telomere length within each cohort. Participants were categorised into quintiles based on the z-score distributions among all controls. To compute ORs and 95% CIs, we used conditional logistic regression. In multivariable models, we adjusted for history of diabetes (yes, no), body mass index (BMI, <18.5, 18.5–24.9, 25–29.9, ≥30 kg/m²) and physical activity (quartiles). p Values for trend were calculated by the Wald test of a score variable that
RESULTS
The median time from collection of DNA to cancer diagnosis was 6.7 years in cases. As expected, individuals with shorter telomere length were older and more likely to be current smokers (table 1, characteristics among controls). The Spearman correlation coefficient between leukocyte telomere length and age was −0.14 (p<0.0001) among controls. Online supplementary table 2 shows the characteristics of pancreatic cancer cases and matched controls in each cohort.

We observed a statistically significant inverse association between prediagnostic leukocyte telomere length and risk of pancreatic cancer (table 2). Compared with the highest quintile of telomere length z-score, individuals in quintiles four to one had multivariable ORs (95% CIs) of 1.35 (0.90 to 2.04), 1.23 (0.79 to 1.93), 1.27 (0.79 to 2.02) and 1.72 (1.07 to 2.78), respectively (p=0.048). Adjustment for history of diabetes, BMI and physical activity did not materially alter the results (table 2).

Table 1  Characteristics by quintile of leucocyte telomere length among controls in five prospective cohorts

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Q5 (longest)</th>
<th>Q4</th>
<th>Q3</th>
<th>Q2</th>
<th>Q1 (shortest)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of controls</td>
<td>179</td>
<td>180</td>
<td>179</td>
<td>178</td>
<td>180</td>
</tr>
<tr>
<td>Age, years, mean (SD)</td>
<td>61.1 (8.3)</td>
<td>61.1 (8.5)</td>
<td>61.9 (9.1)</td>
<td>63.8 (9.2)</td>
<td>64.1 (8.6)</td>
</tr>
<tr>
<td>Men, %</td>
<td>35.8</td>
<td>35.2</td>
<td>35.7</td>
<td>35.2</td>
<td>34.6</td>
</tr>
<tr>
<td>Height, inches, mean (SD)</td>
<td>66.5 (3.5)</td>
<td>66.2 (3.8)</td>
<td>66.7 (3.9)</td>
<td>66.5 (3.7)</td>
<td>66.7 (3.8)</td>
</tr>
<tr>
<td>Body mass index, kg/m², mean (SD)</td>
<td>25.7 (4.8)</td>
<td>26.1 (4.6)</td>
<td>25.6 (4.2)</td>
<td>25.1 (3.7)</td>
<td>25.6 (4.0)</td>
</tr>
<tr>
<td>Physical activity, MET-hour/week, mean (SD)</td>
<td>20.5 (21.3)</td>
<td>21.5 (23.9)</td>
<td>20.2 (26.3)</td>
<td>20.5 (29.4)</td>
<td>18.0 (22.6)</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>43</td>
<td>49.6</td>
<td>42.1</td>
<td>40.3</td>
<td>42.2</td>
</tr>
<tr>
<td>Past</td>
<td>43.6</td>
<td>37.1</td>
<td>44.7</td>
<td>47.2</td>
<td>39</td>
</tr>
<tr>
<td>Current</td>
<td>13.4</td>
<td>13.3</td>
<td>13.2</td>
<td>12.5</td>
<td>18.8</td>
</tr>
<tr>
<td>History of diabetes mellitus, %</td>
<td>4.4</td>
<td>4.2</td>
<td>2.2</td>
<td>1.1</td>
<td>5.4</td>
</tr>
<tr>
<td>Regular multivitamin use, %</td>
<td>43</td>
<td>41.8</td>
<td>45.2</td>
<td>32.2</td>
<td>38.4</td>
</tr>
<tr>
<td>Alcohol (≥1 drink/day), %</td>
<td>22.4</td>
<td>22.8</td>
<td>23.4</td>
<td>19.3</td>
<td>23.6</td>
</tr>
</tbody>
</table>

All variables were age-standardised and study-standardised except age.
predisposing factors. Three SNPs at the TERT gene region were also associated with pancreatic cancer risk, including two SNPs identified in the PanScan GWAS.\textsuperscript{10–11} In our five cohorts, the minor allele of rs401681 was associated with both shorter leucocyte telomere length and higher risk of pancreatic cancer. In aggregate, these data implicate telomere maintenance in the development of pancreatic cancer. Furthermore, they support further investigation of non-invasive testing of telomere biology as a component of pancreatic cancer risk stratification strategies.

Large studies and meta-analyses have assessed the association of peripheral leucocyte telomere length and overall cancer risk, with inconsistent results.\textsuperscript{29–30 31 32} Initial studies suggested that shorter telomere length was associated with increased cancer risk and mortality, while others have suggested only an association of shorter telomere length with worse cancer survival.\textsuperscript{32 33} These inconsistencies may result from differences in study design and assay performance and the potential impact of confounding factors.\textsuperscript{34} The kinetics of telomere loss appear to vary by timing of measurement before cancer diagnosis\textsuperscript{35} and treatment after diagnosis,\textsuperscript{36} impairing the ability to combine data from studies with different designs. In the current study, all blood samples were collected prospectively >2 years prior to diagnosis and before any cancer-directed treatments, limiting the impact of these factors on our results. Pooling data across studies and laboratories can be complex due to methodological differences in measuring telomere length.\textsuperscript{37} In the current study, all samples were analysed together in the same laboratory using the same analytic protocol. Confounding by exposures such as smoking and obesity may affect associations. We included multiple potential confounding factors in our multivariable-adjusted models, noting little change in our results. Notably, recent studies have constructed genetic scores based on the number of inherited telomere-shortening alleles and noted associations with cancer risk, suggesting an independent effect of telomere length on cancer risk.\textsuperscript{38–40 41}

Previous smaller studies focused on leucocyte telomere length and pancreatic cancer risk have yielded inconsistent results. In a hospital-based case–control study, shorter telomere length was associated with higher risk for pancreatic cancer across most of the distribution of length.\textsuperscript{24} However, the temporality of this relationship could not be established due to the retrospective study design, with blood samples collected at the time of cancer diagnosis. A prospective study of 193 pancreatic cancer cases in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention study found that longer telomere length was associated with higher pancreatic cancer risk among individuals diagnosed within the first five years of blood collection.\textsuperscript{13} However, this study was conducted among male smokers in Finland, limiting generalisability of the results. A prospective study in the European Prospective Investigation into Cancer and Nutrition suggested no overall association of leucocyte telomere length and pancreatic cancer risk.\textsuperscript{14} Secondary analyses suggested modest increases in risk for individuals with both short and long telomere length.

Telomere shortening is known to be present at the early stages of pancreatic tumorigenesis.\textsuperscript{3 42} When telomeres shorten to a critical length, the DNA damage response triggers senescence or apoptosis.\textsuperscript{5} In preneoplastic cells, if protective mechanisms such as the p53 tumour suppressor are inactive, the cell continues to proliferate and telomeres become extremely short.

### Table 2

Association between prediagnostic leucocyte telomere length and future risk of pancreatic cancer

<table>
<thead>
<tr>
<th>Quintiles of leucocyte telomere length, z score</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>OR (95% CI)\textsuperscript{*}</th>
<th>p Value, trend\textsuperscript{*}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q5 (longest)</td>
<td>64</td>
<td>179</td>
<td>1.0 (0.90–2.04)</td>
<td>0.904</td>
</tr>
<tr>
<td>Q4</td>
<td>84</td>
<td>180</td>
<td>1.27 (0.79–2.02)</td>
<td>0.078</td>
</tr>
<tr>
<td>Q3</td>
<td>72</td>
<td>179</td>
<td>1.0 (0.80–2.04)</td>
<td>0.100</td>
</tr>
<tr>
<td>Q2</td>
<td>171</td>
<td>178</td>
<td>1.27 (0.79–2.02)</td>
<td>0.078</td>
</tr>
<tr>
<td>Q1 (shortest)</td>
<td>95</td>
<td>180</td>
<td>1.27 (0.79–2.02)</td>
<td>0.078</td>
</tr>
</tbody>
</table>

\*p Value for trend calculated by the Wald test of a variable containing median values of quintiles.

### Table 3

Association of genotyped single-nucleotide polymorphisms (SNPs) at the TERT gene region with pancreatic cancer risk in five prospective cohorts

<table>
<thead>
<tr>
<th>SNP</th>
<th>LD\textsuperscript{†}</th>
<th>r\textsuperscript{‡}</th>
<th>Major allele</th>
<th>Minor allele</th>
<th>Participants</th>
<th>MAF</th>
<th>Per minor allele OR (95% CI)\textsuperscript{*}</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs401681</td>
<td></td>
<td></td>
<td>G</td>
<td>A</td>
<td>362</td>
<td>846</td>
<td>1.33 (1.12–1.59)</td>
<td>0.002</td>
</tr>
<tr>
<td>rs2736100</td>
<td>0.01</td>
<td>0.84</td>
<td>C</td>
<td>A</td>
<td>363</td>
<td>845</td>
<td>1.36 (1.13–1.63)</td>
<td>0.001</td>
</tr>
<tr>
<td>rs402710</td>
<td>0.59</td>
<td></td>
<td>G</td>
<td>A</td>
<td>360</td>
<td>847</td>
<td>1.20 (1.00–1.44)</td>
<td>0.047</td>
</tr>
<tr>
<td>rs2853676</td>
<td>0.007</td>
<td></td>
<td>G</td>
<td>A</td>
<td>363</td>
<td>841</td>
<td>0.94 (0.76–1.15)</td>
<td>0.540</td>
</tr>
</tbody>
</table>

\*p Value for trend calculated by the Wald test of a variable containing median values of quintiles.

\textsuperscript{†}LD of SNPs with rs401681 based on 1000G CEU data.

\textsuperscript{‡}LD, linkage disequilibrium; MAF, minor allele frequency; TERT, telomerase reverse transcriptase.
ultimately causing chromosomal instability and invasive malignancy. Thus, telomere length in tissues may be important in determining whether preneoplastic lesions progress to invasive disease. Whether telomere length measured in white blood cells reflects telomere length in the pancreas is unknown. However, prior studies have indicated that telomere shortening proceeds at similar rates across human somatic tissues and may be influenced by both genetic and environmental factors.

The TERT gene encodes the catalytic subunit of telomerase reverse transcriptase, a component of the protein and RNA complex that maintains telomere ends. SNPs at chromosome 5p15.33, adjacent to or within the TERT gene, are associated with the development of multiple malignancies. Previously, the PanScan studies observed altered pancreatic cancer risk in association with two independent loci at the TERT gene region marked by rs401681 and rs2736098. In the present study, we observed similar associations of pancreatic cancer risk with these two SNPs and also noted an association of a third SNP (rs2736100) in low linkage disequilibrium with the others. Of these SNPs, rs401681 was also associated with leucocyte telomere length, such that the minor allele at rs401681 was associated with higher pancreatic cancer risk and shorter telomere length. Similarly, multiple independent variants have been identified at the TERT gene region related to breast and ovarian cancer risk, with a subset of these variants also associated with leucocyte telomere length.

Similar to our results related to pancreatic cancer, the minor allele at rs2736098 was associated with longer telomeres and lower risk of oestrogen receptor-positive breast cancer. Furthermore, the A allele at rs2736100 was associated with shorter telomere length in a prior GWAS and with increased pancreatic cancer risk in the current study. In aggregate, these data suggest that SNPs at the TERT gene region might be associated with shorter telomere length and higher risk of pancreatic cancer.

Strengths of the present study include the prospective design, large sample size for studies of pancreatic cancer and long, nearly complete follow-up. The exclusion of cases diagnosed within two years of blood draw further minimised potential bias due to reverse causation. The multidimensional data from the five cohorts provided a unique opportunity to conduct integrative analyses of leucocyte telomere length, SNPs at the TERT gene region and pancreatic cancer risk. The extensive covariate data from these cohorts also allowed for rigorous control of potential confounding and evaluation of effect modification.

A limitation of our study was an inability to assess risk related to telomere attrition rate as we were only able to measure telomere length at one point in time. However, most prospective studies have evaluated outcomes related to a one-time telomere length measurement, and whether longitudinal measurement will better predict cancer risk is unknown. We cannot be certain that leucocyte telomere length reflects telomere length in pancreatic tissue. However, telomere length is highly synchronised in fetal tissues and among white blood cells, umbilical artery endothelial cells and skin cells at birth, and interindividual variation in telomere length far exceeds variation between different tissues from the same individual. A recent study also showed that the rate of telomere shortening was similar across leucocytes, skin, skeletal muscle and subcutaneous fat, suggesting that blood serves as an adequate proxy for peripheral tissues. Although the blood samples were collected from different cohorts, it is unlikely that differences in blood collection affected the quality of the genomic DNA because genomic DNA is coiled around histones and very stable when housed in intact nuclei of cells, even when frozen. Furthermore, the effect of potential differences in the handling of biological materials between studies would likely be mitigated because the T/S ratios from each study were normalised using z-scores, which ranks each participant in each study by telomere length based on their respective distributions. Finally, our study population consisted only of white participants, so we were not able to examine other ethnic groups.

In conclusion, in this large study of five prospective cohorts, we found that shorter leucocyte telomere length and genetic variants at the TERT gene locus were associated with higher subsequent risk of pancreatic cancer. Overall, these data support...
a role for telomere maintenance in the development of pancreatic cancer. Furthermore, they suggest that further investigation of non-invasive testing related to telomere biology may have potential as a component of pancreatic cancer risk stratification strategies.

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Contributors YB had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Statistical analysis

YB. BW. The manuscript for important intellectual content: JP, CY, MZ, PK, AB, VM-O, ZRQ, JEB, BBC, JMG, ELG, JAEM, KN, SO, TER, HDS, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data.

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