Null Results in Brief

No Association between TERT-CLPTM1L Single Nucleotide Polymorphism rs401681 and Mean Telomere Length or Cancer Risk

Karen A. Pooley1, Jonathan Tyrer2, Mitul Shah2, Kristy E. Driver2, Jean Leyland1, Judith Brown1, Tina Audley1, Lesley McGuffog3, Bruce A.J. Ponder3, Paul D.P. Pharoah2, Douglas F. Easton1, and Alison M. Dunning2

Abstract

Background: A recent study reported genetic variants in the TERT-CLPTM1L locus that were associated with mean telomere length, and with risk of multiple cancers.

Methods: We evaluated the association between single nucleotide polymorphism (SNP) rs401681 (C > T) and mean telomere length, using quantitative real-time PCR, in blood-extracted DNA collected from 11,314 cancer-free participants from the Sisters in Breast Screening study, the Melanoma and Pigmented Lesions Evaluative Study melanoma family study, and the SEARCH Breast, Colorectal, Melanoma studies. We also examined the relationship between rs401618 genotype and susceptibility to breast cancer (6,800 cases and 6,608 controls), colorectal cancer (2,259 cases and 2,181 controls), and melanoma (787 cases and 999 controls).

Results: The "per T allele" change in mean telomere length (ΔCt), adjusted for age, study plate, gender, and family was 0.001 [95% confidence intervals (CI), 0.01-0.02; P trend = 0.61]. The "per T allele" odds ratio for each cancer was 1.01 for breast cancer (95% CI, 0.96-1.06; P trend = 0.64), 1.02 for colorectal cancer (95% CI, 0.94-1.11; P trend = 0.66), and 0.99 for melanoma (95% CI, 0.84-1.15; P trend = 0.87).

Conclusions: We found no evidence that this SNP was associated with mean telomere length, or with risk of breast cancer, colorectal cancer, or melanoma.

Impact: Our results indicate that the observed associations between rs401681 and several cancer types might be weaker than previously described. The lack of an association in our study between this SNP and mean telomere length suggests that any association with cancer risk at this locus is not mediated through TERT.

Cancer Epidemiol Biomarkers Prev; 19(7); 1862–5. ©2010 AACR.

Introduction

Telomeres are repetitive (TTAGGG),n sequences, present on the ends of chromosomes, which protect against coding sequence erosion and consequent DNA damage repair, resulting in genome instability, chromosomal fusions, and rearrangements (1-3). The relationship between telomere length and cancer risk has been investigated in several publications, but the results have been inconclusive, and few truly prospective studies have thus far been carried out (4-6). Many common sequence variants associated with susceptibility to cancer have been discovered by genome-wide association studies (7-11).

One such study has found sequence variants on chromosome 5p15, in the TERT-CLPTM1L genomic region associated with the risk of basal cell carcinoma (12, 13). Telomerase reverse transcriptase (TERT) encodes the protein subunit of telomerase, is responsible for telomere synthesis and, hence, the maintenance of telomere length. Subsequent analysis showed that the major (C) allele of rs401681 was associated with an increased risk of various cancers, including lung, bladder, prostate, and cervix, but with a decreased risk of colorectal cancer and melanoma. The C allele was also associated with shorter mean telomere length in lymphocytes. No association between rs401681 and breast cancer risk was observed.

To confirm and extend these observations, we have investigated the association between rs401681 and risk of three types of cancer, and mean telomere length, as measured by quantitative real-time PCR, in almost 10,000 individuals with cancer and >11,000 disease-free controls.

Materials and Methods

Study summaries

SEARCH. The SEARCH Study is an ongoing population-based study in Eastern England. Cases were
ascertained through the Eastern Cancer Registry and Information Centre (14), and were aged between 18 and 70 years at diagnosis. Controls were drawn from SEARCH and EPIC-Norfolk. The details of these studies have been previously published (8, 15). In total, 6,800 breast cancer cases and 6,608 controls, 2,259 colorectal cancer cases and 2,248 controls, and 378 melanoma cases and 380 controls were genotyped for the polymorphism studied here.

MAPLES. Additional melanoma association study subjects (404 cases and 619 controls) were recruited via the Melanoma and Pigmented Lesions Evaluative Study (MAPLES), the aim of which was the identification of genetic mutations responsible for moliness and, consequently, to identify individuals at high risk of melanoma. Cases and controls were ascertained through pigmented lesion clinics and general practices in the Cambridge area.

SIBS. The Sisters in Breast Screening (SIBS) study is an ongoing investigation of intermediate phenotypes related to breast cancer (16). Its aim is the mapping of genes underlying these quantitative traits, specifically mammographic density and sex steroid hormone levels. One thousand seven hundred and forty cancer-free subjects were recruited via SIBS.

Additional melanoma association study samples (3.2%), SIBS samples (4.2%), and the failing genotypes were not repeated. A proportion of the subjects (404 cases and 619 controls) were recruited via the Melanoma and Pigmented Lesions Evaluative Study (MAPLES), the aim of which was the identification of genetic mutations responsible for moliness and, consequently, to identify individuals at high risk of melanoma. Cases and controls were ascertained through pigmented lesion clinics and general practices in the Cambridge area.

TaqMan genotyping
Genotyping was done by TaqMan assay as previously described (15). The call rate was >98% for all studies; failed genotypes were not repeated. A proportion of the SEARCH samples (3.2%), SIBS samples (4.2%), and the combined melanoma study (2.9%) were duplicated for assessment of quality control. The concordance between duplicate calls was 100%.

Real-time PCR
Relative mean telomere length was ascertained by high-throughput SYBR Green real-time PCR, the method for which has been previously described (4, 17, 18). The disease-free samples from each study were assayed: SIBS ($n = 1,655$), MAPLES ($n = 619$), and SEARCH ($n = 9,050$). Twenty-one percent of the combined melanoma study, 22% of the SIBS study, and 12% of the SEARCH breast and colorectal studies were duplicated for quality control. Failed PCR reactions were not repeated.

Statistical methods
Analyses were done using Intercooled Stata 10.1 statistical package (Stata). Methods are detailed in the legends of Tables 1 and 2.

Results
We found no association between rs401681 genotype and mean telomere length in a combined sample set of 11,314 cancer history–free study participants ages 18 to 81 years (mean, 57 years; Table 1). Similarly, none of the individual studies showed any significant effect when analyzed separately. As a validation of the assay, we examined the association of mean telomere length with age. There was a significant decrease in mean telomere length with age: “per annum” increase in ΔCt, adjusted for study, gender, and 384-well plate = 0.014 [95% confidence intervals (95% CI), 0.012-0.016; $P$ trend = 1.5 × 10$^{-39}$].

There was no significant association between rs401681 genotype and risk of any of the three cancers assessed (Table 2). The “per T allele” odds ratio (OR) was 1.01 (95% CI, 0.92-1.06; $P$ trend = 0.64) for breast cancer; OR, 1.02 (95% CI, 0.94-1.11; $P$ trend = 0.66) for colorectal cancer; and OR, 0.99 (95% CI, 0.84-1.15; $P$ trend = 0.87) for melanoma. There was no heterogeneity in the per allele OR between the two melanoma studies.

Table 1. TERT SNP genotype and mean telomere length

<table>
<thead>
<tr>
<th>TERT rs401681</th>
<th>Breast cancer (6,434 controls)</th>
<th>Melanoma (979 controls)</th>
<th>SIBS (1,655 controls)</th>
<th>Colorectal cancer (2,246 controls)</th>
<th>Combined (11,314 controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5ΔCt (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.00 reference</td>
<td>0.00 reference</td>
<td>0.00 reference</td>
<td>0.00 reference</td>
<td>0.00 reference</td>
</tr>
<tr>
<td>CT</td>
<td>−0.002 (-0.02 to 0.02)</td>
<td>0.06 (0.003-0.1)</td>
<td>−0.002 (-0.04 to 0.04)</td>
<td>0.01 (-0.02 to 0.05)</td>
<td>0.02 (-0.005 to 0.04)</td>
</tr>
<tr>
<td>TT</td>
<td>−0.02 (-0.03 to 0.02)</td>
<td>0.03 (-0.05 to 0.1)</td>
<td>0.03 (-0.02 to 0.08)</td>
<td>0.004 (-0.04 to 0.05)</td>
<td>0.04 (-0.03 to 0.04)</td>
</tr>
<tr>
<td>Per T allele</td>
<td>$P$ trend = 0.86</td>
<td>$P$ trend = 0.38</td>
<td>$P$ trend = 0.29</td>
<td>$P$ trend = 0.78</td>
<td>$P$ trend = 0.61</td>
</tr>
</tbody>
</table>

NOTE: Genotype frequencies and mean telomere length, as represented by the continuous ΔCt variable, were analyzed in control samples using linear regression for the “per T allele” change in mean telomere length (ΔΔCt), with associated 95% CI. Analyses were adjusted for 384-well plate and age in all the studies, and for gender and family where applicable.
Table 2. TERT SNP genotype and cancer risk

<table>
<thead>
<tr>
<th>TERT rs401681</th>
<th>Breast cancer OR (95% CI), P-het</th>
<th>Colorectal cancer OR (95% CI), P-het</th>
<th>Melanoma OR (95% CI), P-het</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(6,800 cases, 6,608 controls)</td>
<td>(2,259 cases, 2,246 controls)</td>
<td>(782 cases, 999 controls)</td>
</tr>
<tr>
<td>CC</td>
<td>1.00 reference</td>
<td>1.00 reference</td>
<td>1.00 reference</td>
</tr>
<tr>
<td>CT</td>
<td>1.02 (0.94-1.10), 0.49</td>
<td>1.09 (0.96-1.25), 0.19</td>
<td>1.01 (0.79-1.29), 0.95</td>
</tr>
<tr>
<td>TT</td>
<td>1.01 (0.92-1.12), 0.70</td>
<td>1.02 (0.86-1.21), 0.80</td>
<td>0.98 (0.70-1.37), 0.90</td>
</tr>
<tr>
<td>Per T allele</td>
<td>1.01 (0.96-1.06), P trend = 0.64</td>
<td>1.02 (0.94-1.11), P trend = 0.66</td>
<td>0.99 (0.84-1.17), P trend = 0.91</td>
</tr>
</tbody>
</table>

NOTE: Genotype frequencies in cases and controls were compared using a 2 df $\chi^2$ test for heterogeneity (P-het) and a 1 df Cochran-Armitage $\chi^2$ test for trend in risk by T allele dose (P trend). Genotype-specific risks were estimated as ORs with associated 95% CI using unconditional logistic regression. For each study, the deviation of genotype distribution in controls from Hardy-Weinberg equilibrium was assessed by a $\chi^2$ test with 1 df (data not shown).

Discussion

We found no association between mean telomere length and rs401681 genotype in ~11,000 cancer-free individuals using an assay that successfully detects the known reduction of mean telomere length with increasing age in the same subjects. Rafnar et al. (12) reported an association between genotype and age- and platelet-corrected mean telomere length in 276 healthy controls, aged between 85 and 95 years ($P = 0.017$). These findings were not significant in a group of 260 younger women in the same study (60-70 years; $P = 0.081$).

Furthermore, we found no association between rs401681 genotype and risk of three different cancers. Rafnar et al. (12) also reported no association between this single nucleotide polymorphism (SNP) and breast cancer risk. However, the major C allele was associated with protection against both colorectal cancer and cutaneous melanoma in the same study. The cutaneous melanoma risk association was subsequently replicated in a larger study by the same group (13). Our colorectal cancer analysis was sufficiently large to exclude any substantial risk (95% CI, 0.94-1.11). Our melanoma association analysis was based on a smaller sample size and our estimated “per T allele” OR was 0.99 (95% CI, 0.84-1.15; $P = 0.91$). In comparison with the published “per C allele” estimates (0.82-0.95 and 0.81-0.91; refs. 12, 13), our “per C allele” data shows a significant overlap (OR, 1.01; 95% CI, 0.86-1.19; $P$ trend = 0.91). Thus, although we did not observe an association, our results are consistent with the published data.

The previous publications examined a second variant in the region, rs2736098, and found a significant association with cancer risk and with mean telomere length independent of rs401681 genotype. We were unable to manufacture a TaqMan assay to interrogate the second SNP, which is correlated with rs401681 ($r^2 = 0.39$ and $D^2 = 0.94$ in Europeans; ref. 19). We would have less power to detect an association at this locus; however, assuming that rs2736098 is more strongly correlated with a putative causal variant, our results would still exclude any substantial association with breast or colorectal cancer.

Our results indicate that the observed associations between rs401681 and several cancer types do not extend to breast or colorectal cancer, and that the melanoma association might be weaker than previously described. The lack of an association in our study between this SNP and mean telomere length in lymphocytes in middle-aged adults suggests either that any association with cancer risk at this locus is not mediated through TERT, or that the modification of TERT expression materially affects telomere length in lymphocytes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received 03/18/2010; accepted 04/15/2010; published OnlineFirst 06/22/2010.

References

6. Zee RY, Castonguay AJ, Barton NS, Buring JE. Mean telomere lengths...


No Association between TERT-CLPTM1L Single Nucleotide Polymorphism rs401681 and Mean Telomere Length or Cancer Risk


Updated version
Access the most recent version of this article at:
doi:10.1158/1055-9965.EPI-10-0281

Cited articles
This article cites 16 articles, 9 of which you can access for free at:
http://cebp.aacrjournals.org/content/19/7/1862.full.html#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
/content/19/7/1862.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.