Telomere Length and Genetic Variation in Telomere Maintenance Genes in Relation to Ovarian Cancer Risk

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Abstract

Background: Telomeres protect chromosomal ends, shorten with cellular division, and signal cellular senescence, but unchecked telomere attrition can lead to telomere dysfunction, upregulation of telomerase, and carcinogenesis. Shorter telomeres in peripheral blood leukocytes (PBL) have been associated with elevated cancer risk. Furthermore, genetic variants in and around the TERT gene have been implicated in carcinogenesis.

Methods: We measured relative telomere length (RTL) in PBLs of 911 cases and 948 controls from the New England case–control (NECC) study, a population-based study of ovarian cancer. In addition, we assessed germline genetic variation in five telomere maintenance genes among 2,112 cases and 2,456 controls from the NECC study and the Nurses’ Health Study, a prospective cohort study. ORs and 95% CIs were estimated by logistic regression.

Results: Overall, we observed no differences in telomere length between cases and controls. Compared with women with RTL in the longest tertile, women with RTL in the shortest tertile had no increase in risk (OR = 1.01, 95% CI: 0.80–1.28). However, several SNPs in the TERT gene, including rs2736122, rs4246742, rs4975605, rs10069690, rs2736100, rs2853676, and rs7726159, were significantly associated with ovarian cancer risk. We observed a significant gene-level association between TERT and ovarian cancer risk (P = 0.00008).

Conclusion: Our observations suggest that genetic variation in the TERT gene may influence ovarian cancer risk, but the association between average telomere length in PBLs and ovarian cancer remains unclear.

Impact: The role of telomeres in ovarian carcinogenesis remains unsettled and warrants further investigation. Cancer Epidemiol Biomarkers Prev; 21(3); 504–12. ©2012 AACR.

Background

Telomeres are repeated DNA sequences that protect chromosomal ends, shorten with each cellular division, and signal cellular senescence. However, inactivation of tumor suppressors such as p53 allow cells to circumvent senescence and telomeres erode until a crisis point is reached (1). At this stage, cells are characterized by chromosomal aberrations and the majority undergoes apoptosis. A rare cell may reactivate telomerase expression allowing the cell to escape apoptosis and become immortalized (2). Shorter telomeres in peripheral blood leukocytes (PBL) have been associated with a variety of cancers, including ovarian cancer (3–5).

Telomerase activity has been identified in some normal ovarian and fallopian tube tissue and is thought to contribute in a controlled manner to oogenesis and fertility (6, 7). Conversely, telomerase is not detectable in premalignant lesions but is upregulated in 90% to 97% of ovarian cancers, suggesting deregulation of telomerase is a step in ovarian carcinogenesis (6). Twin studies suggest that the majority (40%–80% heritability) of interindividual variation in telomere length is genetic (8, 9). Telomere length and stability is maintained by several proteins, including telomerase (encoded in part by the TERT gene), TRF1, TRF2, and POT1 that act directly on telomeres as well as TRF2IP, TINF2, and TNKS that interact directly with components of the telomere complex (10). In addition, the importance of genetic variation in TERT to cancer susceptibility has emerged from recent genome-wide association studies (GWAS) and candidate gene studies (11–17). Here, we report the results of relative telomere length (RTL) measurements in PBLs of 911 ovarian cancer cases and 948 controls and germline genetic analyses in 5 telomere maintenance genes among 2,112 ovarian cancer cases and 2,456 controls.

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Methods

Study populations

New England case–control study. Data and specimens from the New England case–control (NECC) study of ovarian cancer come from 3 enrollment phases (1992–1997, 1998–2003, and 2003–2008). Briefly, 3,957 women residing in eastern Massachusetts or New Hampshire with a diagnosis of incident ovarian cancer were identified through hospital tumor boards and statewide cancer registries over 3 study phases. Women were not eligible to be a case in the study if they had died, moved, lived outside the study area, had no phone, did not speak English, or had a nonovarian primary tumor after review. Of the 3,083 eligible cases, 2,203 (71% of the eligible cases, 2,076 epithelial cases) agreed to participate. All pathology reports were reviewed by a study pathologist for consistency. Controls were identified through a combination of random digit dialing, drivers’ license lists, and town resident lists. In the first phase, 420 (72%) of the eligible women identified through random digit dialing agreed to participate, and 102 (51%) of the eligible women identified through town resident lists agreed to participate. In the second and third phases, 4,366 potential controls were identified, 2,940 were eligible, 1,362 declined to participate by phone or by mail via an “opt-out” postcard, and 1,578 (54%) were enrolled. Potential controls were required to meet the same eligibility criteria as cases. In addition, controls were required to have at least 1 ovary. Controls were frequency matched to cases on age and state of residence. All study participants were interviewed at the time of enrollment about known and suspected ovarian cancer risk factors and asked to complete a food frequency questionnaire. Over 97% of the participants provided a blood specimen. In 558 cases diagnosed at Brigham and Women’s Hospital or Massachusetts General Hospital, we abstracted chemotherapy data from medical records. Of the 434 cases with RTL and abstracted clinical data, 302 received chemotherapy. Of these, 51 had blood drawn before her chemotherapy. For the remainder, the mean time between chemotherapy and blood draw was 35 days with an interquartile range of 20 to 165 days.

Nurses’ Health Study. The Nurses’ Health Study (NHS) cohort was established in 1976 among 121,700 U. S. female registered nurses, aged 30 to 55 years. Women completed an initial questionnaire, were followed biennially by questionnaire to update exposure status and disease diagnoses, and completed a food frequency questionnaire every 4 years. In 1989 to 1990, 32,826 participants submitted a blood sample (18). Follow-up of the NHS blood study cohort was 96% in 2006. Self-reported diagnoses and deaths reported by family members or identified through the U.S. National Death Index or U.S. Postal Service were followed up for confirmation. A gynecologic pathologist confirmed the diagnosis of all cases through a record review (19). For this analysis, we included all epithelial cases who submitted a blood sample prior to diagnosis or within 4 years after diagnosis (n = 162). All cases were diagnosed before June 1, 2006 and had no history of a prior cancer other than nonmelanoma skin cancer. We randomly selected 3 controls per case from the study participants with DNA available, no prior bilateral oophorectomy, and no history of cancer, other than nonmelanoma skin cancer, at the questionnaire cycle coinciding with diagnosis of the case, matching on age, menopausal status, postmenopausal hormone use, and timing of blood draw.

Telomere length measurements

Genomic DNA from 990 cases and 1,021 controls in the NECC study were standardized and concentrations were measured with PicoGreen quantification by 96-well spectrophotometer (Molecular Devices). We used quantitative PCR as described previously (20, 21) to estimate the ratio (T/S) of telomeric DNA to that of a single gene (36B4) copy number derived from PBLs. Briefly, 5 ng ofuffy coat–derived genomic DNA was dried down in a 384-well plate and resuspended in 10 μL of either the telomere or 36B4 PCR reaction mixture for 2 hours at 4°C. The telomere reaction mixture consisted of 1× QIAGEN QuantiTect SYBR Green Master Mix, 2.5 mmol/L of DT, 270 nmol/L of Tel-1b primer (GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGGT), and 900 nmol/L of Tel-2b primer (TCCCGACTAATCCCATCCTATCCTATATCCTATCCCTA). The reaction proceeded for 1 cycle at 95°C for 5 minutes, followed by 30 cycles of at 95°C for 15 seconds, and 54°C for 2 minutes. The 36B4 reaction consisted of 1× QIAGEN QuantiTect SYBR Green Master Mix, 300 nmol/L of 36B4U primer (CAGCAAGTGGAAGGTTAATCC), and 500 nmol/L of 36B4D primer (CCCCCCCTATCCTATCCTATCTATATCCTATCCCTA). The 36B4 reaction proceeded for 1 cycle at 95°C for 5 minutes, followed by 30 cycles at 95°C for 15 seconds, and 58°C for 1 minute 10 seconds. Each 384-well plate contained a 6-point standard curve from 0.6 to 20 ng using pooled buffy coat–derived genomic DNA. By obtaining slopes within –3.33 ± 0.15 for each standard curve, we were able to achieve 94% PCR efficiency. The T/S ratio (–dCt) for each sample was calculated by subtracting the average 36B4 C_t value from the average telomere C_t value. The relative T/S ratio (–ddCt) was determined by subtracting the T/S ratio value of the 5 ng standard curve point from the T/S ratio of each unknown sample. Assays were conducted in triplicate. A total of 926 cases and 961 controls were successfully assayed. Telomere and single gene coefficients of variation (CV) for triplicates were 1.7 and 1.6, respectively. Cases and controls were evenly distributed across plates. Each plate contained its own unique set of 8 quality control samples, generated by aliquotting 4 replicates from 2 designated samples. The intraplate CV for T/S ratio of QC samples was 10%. Our assay was validated with cancer cell lines (SAOS, CYS-SV, and ATS-V1), which showed a strong correlation between T/S ratio and telomere length determined by Southern blot with correlations ranging from 0.82 to 0.99. Cell lines were kindly provided by the laboratory of Dr. Nikhil Munshi of the...
Dana Farber Cancer Institute. The cell lines were obtained more than 6 months prior to the assay from American Type Culture Collection and were authenticated by the repository with standard cytogenetic, morphologic, antigen expression, and DNA profiling criteria.

**SNP selection and genotyping**

We identified 53 tagging SNPs in 7 genes involved in telomere maintenance (TERT, POT1, TNKS, TRF1, TINF2, TRF2, and TRF2IP) using publicly available data from the HapMap phase II. SNPs in TINF2 and TRF2IP were excluded because they were monomorphic in whites and POT1 SNP rs6946757 was excluded because we were unable to design an assay for this SNP, resulting in 39 SNPs genotyped on 1,173 cases, 1,200 controls from the first 2 phases of the NECC study. In phase 3 NECC and NHS, we used an alternative platform that only allows multiples of 16 SNPs, therefore, we limited our SNP set to the top 31 SNPs and added a promising candidate SNP from the TERT gene (rs7726159; ref. 11).

DNA was extracted from buffy coat samples with QI Amp (QIAGEN); samples were amplified with Genomi phi (GE Healthcare). All genotyping was carried out at the DF/HCC High Throughput Polymorphism Core. First, we genotyped 39 SNPs on the first 2 phases of the NECC study using 3' nuclease assays (Taqman) on the Applied Biosystems Pr isotm 7900HT Sequence Detection System (Applied Biosystems). Then, we genotyped 32 SNPs on phase 3 NECC samples and the NHS samples using the Applied Biosystems Taqman OpenArray genotyping platform. Replicate samples (approximately 10%) included for quality control had 100% concordance. Genotyping was carried out by laboratory personnel blinded to case–control status and quality control replicates. Over 95% of the samples were successfully genotyped for each polymorphism.

**Statistical analysis**

**RTL analysis.** RTL values were adjusted for 384-well plate using batch adjustment methods described previously (22). Outliers were identified by the extreme studentized deviate many-outlier procedure (23) and excluded from further analysis (n = 29). Tertiles were determined based on the distribution in controls. Among controls, we tested for differences in the distribution of RTL by categories of epidemiologic characteristics using the Wilcoxon rank-sum and Kruskal–Wallis tests as well as linear regression for multivariate adjusted analyses. Unconditional logistic regression adjusted for the matching factors and study phase were used to calculate ORs and 95% CI for the association between RTL and ovarian cancer risk. Multivariate models were additionally adjusted for oral contraceptive use, parity, tubal ligation, and family history of breast or ovarian cancer. Estimates from NECC phases 1 and 2, NECC phase 3, and NHS were combined by meta-analysis using fixed effects models to calculate summary ORs and 95% CIs. We used P value to account for multiple testing in correlated tests (25). Variables evaluated for effect modification included those associated with ovarian cancer and/or thought to influence telomere length, including estimated lifetime number of ovulatory cycles, talc use, BMI, exercise, dietary folate, and total folate (which includes vitamins and supplements).

To evaluate gene level associations, we employed a principal components (PC) approach described previously by Gauderman and colleagues that accounts for linkage disequilibrium between SNPs (26). Briefly, we estimated the combinations of SNPs, grouped as PCs that represent the genetic variation across the gene. Then, we included the fewest number of PCs that together describe at least 80% of the variation in a logistic regression with ovarian cancer as the outcome. Using a likelihood ratio test, we compared models with and without selected PCs to determine the association between the gene of interest and ovarian cancer risk. All analyses were conducted with SAS (version 9.2; SAS Institute) or Intercooled Stata 9.2 (StataCorp).

Histology-specific analyses were conducted comparing RTL in a particular histologic group with all controls. We evaluated the association between RTL and ovarian cancer risk within subgroups of ovulatory cycles, genital talc use, smoking, and BMI. We conducted tests for interaction using a likelihood ratio test to compare models with and without the interaction terms.

Among the subset of cases with telomere length measurements and date of last chemotherapy before blood draw (n = 256), we conducted a sensitivity analysis to assess the influence of recent chemotherapy on the association between RTL in PBLs and ovarian cancer by excluding cases with blood drawn within 3 or 6 months of chemotherapy. Further, we used polychotomous logistic regression (24) to assess whether the association between RTL and risk of serous invasive ovarian cancer differed by time between diagnosis and blood draw, as this may also reflect chemotherapy status. We calculated estimates for the association between RTL and ovarian cancer risk for cases with blood draw within 283 days of diagnosis (population median) versus more than 283 after diagnosis.

**SNP analysis.** There were no SNPs out of Hardy–Weinberg Equilibrium at P < 0.001 in any data set. SNP associations were evaluated by a log-additive model in which the OR represents the incremental increase or decrease in risk with each additional allele. Unconditional logistic regression adjusted for the matching factors (age and study center) was used for the NECC analyses and conditional logistic regression was used in the NHS analysis. We restricted to whites because we observed that several SNP frequencies varied by race. Multivariate models were additionally adjusted for oral contraceptive use, parity, tubal ligation, and family history of breast or ovarian cancer. Estimates from NECC phases 1 and 2, NECC phase 3, and NHS were combined by meta-analysis using fixed effects models to calculate summary ORs and 95% CIs. We used P value to account for multiple testing in correlated tests (25). Variables evaluated for effect modification included those associated with ovarian cancer and/or thought to influence telomere length, including estimated lifetime number of ovulatory cycles, talc use, BMI, exercise, dietary folate, and total folate (which includes vitamins and supplements).

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Results

Population characteristics for the first 2 phases of the NECC study and the NHS-nested case–control study have been reported previously (27). Participants in NECC phase 3 were similar to those in the previous 2 phases. However, phase 3 participants were slightly older, more likely to have taken oral contraceptives, and cases were more likely to be invasive. Among controls with telomere length measurements (Table 1), RTL was inversely associated with age \( (r = -0.21, P < 0.0001) \) and BMI \( (r = -0.12, P = 0.0002) \). BMI remained independently, although modestly, associated with RTL after adjustment for age \( (r = -0.08, P = 0.02) \). Former smokers had significantly shorter telomeres on average than women who never smoked \( (age-adjusted P = 0.04) \), but there were no differences between current and never smokers. Oral contraceptive use, parity, tubal ligation, family history of breast or ovarian cancer, estimated lifetime number of ovulatory cycles, and Jewish ancestry were not associated with telomere length.

Overall, we observed no significant difference in mean RTL between 911 women with epithelial ovarian cancer \( (mean = 0.75, SD = 0.40) \) versus 948 controls \( (mean = 0.77, SD = 0.51) \). Compared with women with telomeres in the longest tertile of length, those with telomeres in the shortest tertile of length had no increased risk of ovarian cancer overall \( (OR = 1.01, 95\% CI: 0.80–1.28; Table 2) \) or serous invasive cancer \( (OR = 1.11, 95\% CI: 0.82–1.51) \). Associations did not significantly differ between high-grade \( (OR = 1.09, 95\% CI: 0.80–1.50) \) serous invasive ovarian cancer and low-grade \( (OR = 0.88, 95\% CI: 0.30–2.64) \) serous invasive ovarian cancer. Results were similar for other histologic subtypes and when considering quartile or quintile cutpoints. Evaluating the RTL-ovarian cancer association within subgroups did not reveal any interactions with ovulatory cycles, genital talc use, endometriosis, or smoking. However, the association between RTL and ovarian cancer differed significantly by quartiles of BMI \( (P_{\text{heterogeneity}} = 0.01) \). Among overweight women \( (BMI > 28.3) \), women with the shortest telomeres had a borderline decreased risk compared with women with the longest telomeres \( (OR = 0.62, 95\% CI: 0.38–1.01) \), but among women with a BMI less than 21.9 \( (OR = 1.29, 95\% CI: 0.79–2.10) \) and women with a BMI between 22 and 24.6 \( (OR = 1.06, 95\% CI: 0.67–1.66) \) shorter telomeres were not associated with ovarian cancer risk.

The association between RTL and risk of serous invasive ovarian cancer did not differ significantly between cases with blood drawn less than or equal to median time between diagnosis and blood draw \( (OR = 0.89, 95\% CI: 0.67–1.18) \) compared with those with blood drawn longer after diagnosis \( (OR = 1.21, 95\% CI: 0.90–1.62, P_{\text{heterogeneity}} = 0.13) \). Although nonsignificant, the association between RTL and ovarian cancer risk tended to increase as cases that had recently had chemotherapy were excluded from the analyses. For example, ORs for ovarian cancer, comparing shortest with longest tertiles of RTL, were 1.20 \( (95\% CI: 0.66–2.19) \) when restricting to cases with blood

### Table 1. Association between RTL in PBLs and selected characteristics among women without ovarian cancer, NECC study (1992–2003)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of controls</th>
<th>Batch adjusted telomere length</th>
<th>( P_{\text{regression}}^a )</th>
<th>Correlation</th>
<th>( P_{\text{correlation}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>All controls</td>
<td>947</td>
<td>0.77 (0.51)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>230</td>
<td>0.84 (0.53)</td>
<td>ref.</td>
<td>-0.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>41–50</td>
<td>230</td>
<td>0.83 (0.56)</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51–60</td>
<td>234</td>
<td>0.75 (0.43)</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61–70</td>
<td>210</td>
<td>0.68 (0.48)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;70</td>
<td>43</td>
<td>0.79 (0.63)</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>439</td>
<td>0.81 (0.54)</td>
<td>ref.</td>
<td>0.08c</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>366</td>
<td>0.73 (0.42)</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>142</td>
<td>0.79 (0.61)</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index(^b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 ((\leq 21.9))</td>
<td>225</td>
<td>0.82 (0.55)</td>
<td>ref.</td>
<td>-0.12</td>
<td>0.0002</td>
</tr>
<tr>
<td>Quartile 2 (22–24.6)</td>
<td>251</td>
<td>0.83 (0.62)</td>
<td>0.69</td>
<td>-0.08d</td>
<td></td>
</tr>
<tr>
<td>Quartile 3 (24.7–28.3)</td>
<td>230</td>
<td>0.73 (0.34)</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 4 ((&gt;28.4))</td>
<td>241</td>
<td>0.71 (0.47)</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Based on age-adjusted linear regression comparing batch-adjusted RTL of each category to reference group.

\(^b\)BMI missing for 3 controls.

\(^c\)Based on the Kruskal–Wallis test.

\(^d\)Correlation between BMI and RTL adjusted for age.
drawn more than 3 months after chemotherapy \((n = 82)\)
and \(1.51 (95\% \text{ CI: } 0.67–3.40)\) when restricting to cases
with blood drawn more than 6 months after chemotherapy \((n = 53)\).

We genotyped 40 tagging SNPs in 5 telomere maintenance
genes \((TERT, TRF2, TRF1, TNKS,\) and \(POT1)\) in the
NECC and NHS studies. Minor allele frequencies were
similar across all 3 data sets. Overall, we observed 7
significant associations for \(TERT\) SNPs, but no significant
associations for other SNPs. \(TERT\) SNPs \(r_s4246742,\)
\(r_s975605, rs10069690, rs2736100, rs2853676,\) and
\(rs7226159\) were associated with 11% to 19% increase
in ovarian cancer risk with each additional variant allele,
whereas \(rs2736122\) was associated with an 11% decrease
in risk with each allele \((Table 3)\), but none of these
associations remained significant after permutation test-
ing. On a gene level, estimated PCs accounting for 88% of
the genetic variation in \(TERT\) were significantly associat-
ed with ovarian cancer \((P = 0.00008)\), but PCs of other
genes were not associated with risk.

Interestingly, histology-specific associations revealed
that \(TERT\) SNPs \(rs4246742\) \((pooled \text{ OR} = 1.43, 95\% \text{ CI:}
1.12–1.83), rs975605 \((pooled \text{ OR} = 1.26, 95\% \text{ CI: } 1.05–
1.51),\) and \(rs10069690 \((pooled \text{ OR} = 1.28, 95\% \text{ CI: } 1.04–
1.57)\) were most strongly associated with endometrioid
tumors, whereas \(rs2853676\) was more strongly associated
with serous invasive ovarian cancer \((OR = 1.14, 95\% \text{ CI:}
1.00–1.30)\). \(rs2736100\) was associated with a significant
increase in borderline tumors \((pooled \text{ OR} = 1.24, 95\% \text{ CI:}
1.05–1.46)\). In stratified analyses, we evaluated the association
teleomere maintenance SNPs and ovarian cancer risk by estimated lifetime number of ovulatory
cycles, BMI, exercise, genital talc use, folate intake, and
smoking; however, we did not observe any significant
interactions after adjustment for multiple testing.

Among 918 white controls from the NECC study, we
evaluated the association between SNPs in telomere
maintenance genes and RTL in PBLs. We observed significant

<table>
<thead>
<tr>
<th>Tertile of RTL</th>
<th>n</th>
<th>RTL mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (shortest)</td>
<td>947</td>
<td>0.77 (0.51)</td>
</tr>
<tr>
<td>T2</td>
<td>911</td>
<td>0.75 (0.40)</td>
</tr>
<tr>
<td>T3 (longest)</td>
<td>Overall</td>
<td>1.01 (0.80–1.28)</td>
</tr>
<tr>
<td>Serous</td>
<td>482</td>
<td>0.73 (0.37)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>120</td>
<td>0.79 (0.39)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>132</td>
<td>0.77 (0.46)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>121</td>
<td>0.72 (0.41)</td>
</tr>
</tbody>
</table>

*Odds ratios and 95% CIs are age adjusted, study center, study, oral contraceptive use (<3 mo, 3 mo–2 y, 2–5 y, and >5 y), parity (0, 1, 2, 3, and 4+), tubal ligation, BMI (<21.9, 22–24.6, 25.7–28.3, and >28.3), smoking (never, past, and current), family history of breast or ovarian cancer, and race.

Discussion

Our data show an association between genetic variation
in the \(TERT\) gene and ovarian cancer risk in 3 data sets with
significant associations between 6 individual \(TERT\) SNPs
and ovarian cancer risk and a highly significant gene level
association with risk. In our telomere length analysis, we
observed shorter telomeres in PBLs with increasing age,
former smoking, and higher BMI as previously reported in
the literature \((28, 29)\) but no significant association
between RTL in PBLs and ovarian cancer risk in the largest
study of telomere length and ovarian cancer risk to date.

When considered independently, the magnitude of the association
between any individual \(TERT\) SNP we geno-
typed and ovarian cancer risk was small and of borderline
significance, and a combined analysis of 4 ovarian cancer
GWAS studies showed no significant associations
between these individual \(TERT\) SNPs and overall ovarian
cancer risk \((unpublished\ data)\). However, when we con-
sidered the \(TERT\) variants together in a PC analysis that
accounts for linkage disequilibrium between the SNPs, we
observed a highly significant association \((P = 0.00008)\),
suggesting that these individual SNPs do not capture the
importance of genetic variation in this region. In the
context of other recent reports of significant associations
between \(TERT\) SNPs and risk of various cancers,
including ovarian cancer \((11–17)\), our data suggest that
genetic variation in the \(TERT\) gene may be important
towards ovarian cancer susceptibility. In a study of 1,309 SNPs in
173 genes involved in stromal epithelial crosstalk, Joh-
natty and colleagues observed that \(TERT\) \(rs7726159\) was
particularly associated with serous invasive ovarian cancer
\((\text{OR}_{\text{per allele}} = 1.14, 95\% \text{ CI: } 1.04–1.24)\) and an inverse
<table>
<thead>
<tr>
<th>Gene</th>
<th>rs number</th>
<th>Per allele</th>
<th>$P_{\text{gene}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TERT</td>
<td>rs2736122</td>
<td>0.89 (0.81–0.99)</td>
<td>0.00008</td>
</tr>
<tr>
<td>TERT</td>
<td>rs2075786</td>
<td>1.06 (0.97–1.17)</td>
<td></td>
</tr>
<tr>
<td>TERT</td>
<td>rs4246742</td>
<td>1.19 (1.05–1.35)</td>
<td></td>
</tr>
<tr>
<td>TERT</td>
<td>rs6882077</td>
<td>1.31 (0.27–6.29)</td>
<td></td>
</tr>
<tr>
<td>TERT</td>
<td>rs4975605</td>
<td>1.11 (1.02–1.22)</td>
<td></td>
</tr>
<tr>
<td>TERT</td>
<td>rs10069690</td>
<td>1.11 (1.00–1.23)</td>
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<td>TERT</td>
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*Restricted to white women.

*Adjusted for age (continuous), center, oral contraceptive use (never, 1–24, 25–72, and >72 months), parity (0, 1, and 2+), family history of breast or ovarian cancer, tubal ligation, and genotyping batch. Meta-analysis of NECC phases 1 and 2, NECC phase 3, and NHS using fixed effects model. We observed significant heterogeneity between data sets for **TRF1** SNP rs12334686 ($P_{\text{heterogeneity}} = 0.04$) and **TNKS** SNP rs1772180 ($P_{\text{heterogeneity}} = 0.02$).

*rs6882077, rs2242652, rs166134, rs8061382, rs6989159, rs6989493, rs2981096, and rs10881982 were not genotyped in NECC phase 3 and NHS data sets and rs7726159 was not genotyped in NECC phases 1 and 2.

*PC analyses were used to determine gene-level associations with ovarian cancer risk accounting for linkage disequilibrium between SNPs.
Interestingly, WNT and 
beta-catenin have been implicated 
to cooperate in activating WNT responsive promoters, 
may influence many types of cancer (11, 13, 14).

Hypothesized to be cancer polymorphism “hot spot” that 
shorter mean telomere lengths in DNA from plasma from 
previously reported significantly increased ovarian cancer risk 
contrast those of 2 small case–control studies that previ-
ously collected serial samples, one study reported that telo-
mer length postchemotherapy was approximately 550 base pairs shorter than prechemotherapy (34), whereas another reported significant telomere shortening in peripheral blood mononuclear cells after multiple cycles of chemotherapy (33). Cisplatin, a standard therapy for ovarian cancer, forms DNA adducts with telo-
meric sequences and results in telomere loss in cell lines (31). In our study, telomere lengths were shorter on average as time since chemotherapy increased, suggesting 
an influence of chemotherapy. Thus, the association between RTL and ovarian cancer risk may be obscured in samples collected after treatment. Yet, Mirabello and 
colleagues observed no difference in telomere length between samples collected before (telomere length = 0.89, 95% CI: 0.89–0.90) and after chemotherapy (tele-
mere length = 0.91, 95% CI: 0.82–0.99). Regardless, 
telomere length measurements in postdiagnostic sam-
ple can be difficult to interpret because samples col-
clected soon after diagnosis are likely to be influenced by 
chemotherapy or active disease and those collected later 
are likely influenced by survival (35).

Given the well documented inverse correlation 
between BMI and RTL noted in our study and by others 
(28, 29), our subgroup analyses showing a reduced risk of 
ovarian cancer for women in the highest quartile of weight is counterintuitive as one would expect the 
highest cancer risk for obese women with short telomeres. 
These results are likely a chance finding due to a limited 
distribution of telomere length in this subgroup.

A limitation of our study is the use of telomere length in 
PBLs as a surrogate for telomere length in the ovaries or 
fallopian tubes. A range of exposures including chronic infection or other immune insults could accelerate cellular 
turnover in white blood cells leading to shorter telomeres 
compared with tissue. However, telomere length in a 
variety of tissues are highly synchronous (41), and 
matched blood and tissue samples correlated (4, 42).

Although this is a large study with over 2,112 cases and 
2,456 controls in the SNP analysis and 911 cases in 948 
controls in the telomere length analysis, power is limited 
to detect significant associations within subgroups of 
histologic types, particularly rarer subtypes.

Our findings regarding genetic variation in the TERT 
gene and ovarian cancer risk add to the growing body of
literature suggesting the importance of this genetic region to cancer development. Further research is needed in this area to understand how changes in telomere length over time may influence ovarian carcinogenesis in a prospective setting and interaction with a p53 pathway of development.

Disclosure of Potential Conflicts of Interest
No conflicts of interest were disclosed.

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References


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Kathryn L. Terry, Shelley S. Tworoger, Allison F. Vitonis, et al.


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