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

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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 (PBLs) have been associated with elevated cancer risk. Further, 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 Study, a population-based study of ovarian cancer. In addition, we assessed germline genetic variation in five telomere maintenance genes among 2112 cases and 2456 controls from the New England Case Control Study and the Nurses' Health Study, a prospective cohort study. Odds ratios and 95% confidence intervals were estimated using logistic regression.

Results—Overall, we observed no differences in telomere length between cases and controls. Compared to 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, 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 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.

Keywords

telomeres; ovarian cancer; polymorphisms; *TERT*

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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 undergo apoptosis. A rare cell may reactivate telomerase expression allowing the cell to escape apoptosis, become immortalized (2). Shorter telomeres in peripheral blood leukocytes have been associated with a variety of cancers, including ovarian cancer (3) (4, 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 90-97% 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 measurements in peripheral blood leukocytes of 911 ovarian cancer cases and 948 controls and germline genetic analyses in five telomere maintenance genes among 2112 ovarian cancer cases and 2456 controls.

METHODS

Study populations

New England Case-Control Study (NECC)—Data and specimens from the New England-based case-control study of ovarian cancer come from three enrollment phases (1992-1997, 1998-2003, 2003-2008). Briefly, 3957 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 three 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 non-ovarian primary tumor after review. Of the 3083 eligible cases, 2203 (71% of the eligible cases, 2076 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, 4366 potential controls were identified, 2940 were eligible, 1362 declined to participate by phone or by mail via an "opt-out" postcard, and 1578 (54%) were enrolled. Potential controls were required to meet the same eligibility criteria as cases. In addition, controls were required to have at least one 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 (NHS)—The NHS cohort was established in 1976 among 121,700 U.S. female registered nurses, ages 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 four years. In 1989-90, 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 non-melanoma skin cancer. We randomly selected three controls per case from the study participants with DNA available, no prior bilateral oophorectomy, and no history of cancer, other than non-melanoma 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 1021 controls in the NECC study were standardized and concentrations were measured using PicoGreen quantification by 96-well spectrophotometer (Molecular Devices, Sunnyvale, CA). 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 peripheral blood leukocytes. Briefly, 5ng of buffy-coat derived genomic DNA was dried down in a 384-well plate and resuspended in 10μL of either the Telomere or 36B4 PCR reaction mixture for 2 hours at 4C. The telomere reaction mixture consisted of 1x Qiagen Quantitect Sybr Green Master Mix, 2.5mM of DTT, 270nM of Tel-1b primer (GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT), and 900nM of Tel-2b primer (TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA). The reaction proceeded for 1 cycle at 95C for 5 min, followed by 30 cycles of at 95C for 15 sec, and 54C for 2 min. The 36B4 reaction consisted of 1x Qiagen Quantitect Sybr Green Master Mix, 300nM of 36B4U primer (CAGCAAGTGGGAAGGTGTAATCC), and 500nM of 36B4D primer (CCCATTCTATCATCAACGGGTACAA). The 36B4 reaction proceeded for 1 cycle at 95C for 5 min, followed by 30 cycles at 95C for 15 sec, and 58C for 1 min 10 sec. Each 384-well plate contained a 6-point standard curve from 0.6ng to 20ng 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 Ct value from the average telomere Ct value. The relative T/S ratio (-ddCt) was determined by subtracting the T/S ratio value of the 5ng standard curve point from the T/S ratio of each unknown sample. Assays were performed in triplicate. 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 eight quality control samples, generated by aliquotting four replicates from two designated samples. The intraplate CV for T/S ratio of QC samples was 10%. Our assay was validated using cancer cell lines (SAOS, CYS-SV, ATS-V1), which showed a strong correlation between T/S ratio and telomere length determined by Southern blot with correlations ranging from 0.82-0.99. Cell lines were kindly provided by the laboratory of Dr. Nikhil Munshi of the Dana Farber Cancer Institute. The cell lines were obtained greater than

6 months prior to the assay from ATCC and were authenticated by the repository using standard cytogenetic, morphological, antigen expression, and DNA profiling criteria.

SNP selection and genotyping

We identified 53 tagging SNPs in seven 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 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 1173 cases, 1200 controls from the first two 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) (11).

DNA was extracted from buffy coat samples using QIAmp (Qiagen, Chatsworth, CA); samples were amplified using Genomiphi (GE Healthcare, Piscataway, NJ). All genotyping was performed at the DF/HCC High Throughput Polymorphism Core. First, we genotyped 39 SNPs on the first two phases of the NECC study using 5′ nuclease assays (Taqman®) on the Applied Biosystems Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, California). 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 performed 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

Relative telomere length (RTL) analysis—RTL values were adjusted for 384-well plate using batch adjustment methods described previously (22). Outliers were identified using 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 relative telomere length 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 odds ratios (OR) and 95% confidence intervals (95% CI) for the association between tertiles of RTL and ovarian cancer risk. Multivariate models were additionally adjusted for oral contraceptive use (<3 months, 3 months- 2 years, 2-5 years, >5 years), parity (0, 1, 2, 3, 4+), tubal ligation, BMI (<21.9, 22-24.6, 25.7-28.3, >28.3), smoking (never, past, current), family history of breast or ovarian cancer, and race (white, non-white).

Histology specific analyses were performed comparing RTL in a particular histologic group to all controls. We evaluated the association between RTL and ovarian cancer risk within subgroups of ovulatory cycles, genital talc use, smoking, and BMI. We performed 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 performed 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 polytomous 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 dataset. SNP associations were evaluated using a log-additive model where the odds ratio 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&2, NECC phase 3, and NHS were combined by meta-analysis using fixed effects models to calculate summary odds ratios and 95% confidence intervals. We used P_{ACT} 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 approach described previously by Gauderman and colleagues that accounts for linkage disequilibrium between SNPs (26). Briefly, we estimated the combinations of SNPs, grouped as principal components (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 principal components to determine the association between the gene of interest and ovarian cancer risk. All analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC) or Intercooled Stata 9.2 (StataCorp, College Station, TX).

RESULTS

Population characteristics for the first two 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 two 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, standard deviation (std) = 0.40) versus 948 controls (mean = 0.77, std = 0.51). Compared to 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) or serous invasive cancer (OR= 1.11, 95% CI: 0.82, 1.51) (Table 2). Associations did not significantly differ between high-grade (OR=1.09, 95% CI: 0.80, 1.50) 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-heterogeneity=0.01). Among overweight women (BMI > 28.3), women with the shortest telomeres had a borderline decreased risk compared to 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 to those with blood drawn longer after diagnosis (OR=1.21, 95% CI: 0.90, 1.62, $p_{heterogeneity} = 0.13$). Although non-significant, the association between RTL and ovarian cancer risk tended to increase as cases who had recently had chemotherapy were excluded from the analyses. For example, odds ratios for ovarian cancer, comparing shortest to longest tertiles of relative telomere length, were 1.20 (95% CI: 0.66-2.19) when restricting to cases with blood drawn more than 3 months after chemotherapy (n=82) and 1.51 (95% 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 five telomere maintenance genes (*TERT, TRF2, TRF1, TNKS,* and *POT1*) in the NECC and NHS studies. Minor allele frequencies were similar across all three datasets. Overall, we observed seven significant associations for *TERT* SNPs, but no significant associations for other SNPs. *TERT* SNPs rs4246742, rs4975605, rs10069690, rs2736100, rs2853676 and rs7726159 were associated with 11-19% increase in ovarian cancer risk with each additional variant allele, while rs2736122 was associated with an 11% decrease in risk with each allele (Table 3), but none of these associations remained significant after permutation testing. On a gene level, estimated principal components accounting for 88% of the genetic variation in *TERT* were significantly associated with ovarian cancer (p=0.00008), but principal components of other genes were not associated with risk.

Interestingly, histology-specific associations revealed that *TERT* SNPs rs4246742 (pooled OR=1.43, 95% CI: 1.12, 1.83), rs4975605 (pooled OR=1.26, 95%CI: 1.05, 1.51), and rs10069690 (pooled OR=1.28, 95% CI: 1.04, 1.57) were most strongly associated with endometrioid tumors, while rs2853676 was more strongly associated with serous invasive ovarian cancer (OR=1.14, 95%CI: 1.00, 1.30). RS2736100 was associated with a significant increase in borderline tumors (pooled OR=1.24, 95% CI: 1.05, 1.46). In stratified analyses, we evaluated the association between telomere 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 peripheral blood leukocytes. We observed significant age-adjusted differences in telomere length by genotype for *POT1* SNPs rs929365 (p=0.02) and rs4360236 (p=0.04), but these SNPs were not associated with ovarian cancer risk.

DISCUSSION

Our data shows an association between genetic variation in the *TERT* gene and ovarian cancer risk in three datasets with significant associations between six 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 peripheral blood leukocytes with increasing age, former smoking, and higher BMI as previously reported in the literature (28, 29) but no significant association between RTL in peripheral blood leukocytes 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 genotyped and ovarian cancer risk was small and of borderline significance, and a combined analysis of four ovarian cancer GWAS studies showed no significant associations between these individual *TERT* SNPs and overall ovarian cancer risk (unpublished data). However, when we considered the *TERT* variants together in a principal components 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 to ovarian cancer susceptibility. In a study of 1,309 SNPs in 173 genes involved in stromal epithelial cross-talk, Johnatty and colleagues observed that *TERT* rs7726159 was significantly associated with serous invasive ovarian cancer ($OR_{per\text{-}allele}$ = 1.14, 95% CI=1.04-1.24) and an inverse association of borderline significance between rs2736100 and serous ovarian cancer (OR=0.88, 95% CI=0.77-1.01) (11). Furthermore, rs2736100 has emerged from recent GWAS studies as a potential susceptibility allele for glioma (OR=1.27, $p=2 \times 10^{-17}$) (15), lung cancer (OR=1.14, $p=4\times10^{-6}$) (12), and testicular cancer (OR=1.33, $p=8\times10^{-15}$) (17). Based on this accumulating evidence, the area around the *TERT* gene has been hypothesized to be cancer polymorphism “hot-spot” that may influence many types of cancer (11, 13, 14).

Given the emerging evidence that p53 mutations are likely precursors to most serous ovarian cancers (37) and p53 mutations are also observed in endometrioid ovarian tumors (38), the telomere maintenance pathway may have particular relevance to these tumors. Interestingly, Kuhn and colleagues found that telomeres measured in tissue from serous tubal intraepithelial carcinoma (STIC) were significantly shorter than those found in concurrent high grade serous ovarian carcinoma and suggested that the shortened telomeres STICs represents an early carcinogenic event while longer telomeres in coexisting high grade serous tumors are a result of subsequent activation of telomerase and immortalization (39).

Although none of the *TERT* SNPs we found to be associated with ovarian cancer was associated with telomere length in peripheral blood leukocytes of controls, the influence of these SNPs on other telomere attributes cannot be ruled out. Telomere attrition can occur due to a range of insults that increase cellular turnover, accelerate telomeric DNA damage, or result in defective telomere repair. Therefore, the influence of a single SNP on telomere length may be difficult to detect. Furthermore, these SNPs may influence carcinogenesis through a pathway that does not involve telomere length. For instance, Artandi and colleagues have shown that *TERT* interacts directly with WNT transcription factors and cooperate in activating WNT responsive promoters while Mancke and colleagues have recently shown telomerase interacts with β-catenin (reviewed in (40)). Interestingly, WNT and β-catenin have been implicated in ovarian carcinogenesis (41).

Our telomere length and ovarian cancer risk results contrast those of two small case-control studies that previously reported significantly increased ovarian cancer risk with shorter telomeres (4, 5). Idei and colleagues observed shorter mean telomere lengths in DNA from plasma from 32 cases (≥ stage 3) versus controls in a Japanese population. In addition, long-term (≥10 years) ovarian cancer survivors had significantly longer telomeres than other cases, suggesting a survival benefit with increased telomere length (4). Similarly, Mirabello and colleagues reported shorter leukocyte telomere length in 99 serous ovarian cancer cases compared to 100 controls in a Polish population, with a 3-fold increase in risk for women with telomere length in the shortest tertile (OR=3.39, 95% CI: 1.54-7.46) (5). Differences in study populations, study design, sample size, timing of chemotherapy, and type of specimen or assay may explain inconsistencies between our own results and previous studies. Telomere length has been shown to vary by ethnicity (30), which vary between these studies. Furthermore, telomere length in DNA obtained from plasma (4) may differ from that derived from buffy coat, and cases in the Polish study were recruited sooner after diagnosis (median 2.4 months) than in our study (median 9.3 months), such that time since chemotherapy likely differed between the studies.

Several studies have suggested that chemotherapy may influence measureable telomere length in peripheral blood leukocytes, but conclusions are mixed showing both increases and decreases in length as well as changes in telomerase activity after chemotherapy (4, 31-34). In prospectively collected serial samples, one study reported that telomere length post-chemotherapy was approximately 550 base pairs shorter than pre-chemotherapy (34) while 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 telomeric 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: 0.89-0.99) and after chemotherapy (telomere length=0.91, 95% CI: 0.82-0.99). Regardless, telomere length measurements in post-diagnostic samples can be difficult to interpret because samples collected 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 relative telomere length 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 to tissue. However, telomere length in a variety of tissues are highly synchronous (42), and matched blood and tissue samples correlated (4, 43). Although this is a large study with over 2112 cases and 2456 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.

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Table 1

Association between relative telomere length in peripheral blood leukocytes and selected characteristics among women without ovarian cancer, New England Case Control Study (1992-2003)

Characteristic	Number of controls	Batch adjusted telomere length mean (std)	$p_{regression}$ *	Correlation	$p_{correlation}$
All controls	947	0.77 (0.51)			
Age					
≤ 40 years	230	0.84 (0.53)	ref	−0.21	<0.0001
41 - 50 years	230	0.83 (0.56)	0.62		
51 - 60 years	234	0.75 (0.43)	0.01		
61 - 70 years	210	0.68 (0.48)	<0.0001		
> 70 years	43	0.79 (0.63)	0.19		
Smoking					
Never	439	0.81 (0.54)	ref		0.08§
Former	366	0.73 (0.42)	0.04		
Current	142	0.79 (0.61)	0.16		
Body mass index†					
Quartile 1 (≤ 21.9)	225	0.82 (0.55)	ref	−0.12	0.0002
Quartile 2 (22 - 24.6)	251	0.83 (0.62)	0.69	−0.08‡	
Quartile 3 (24.7 - 28.3)	230	0.73 (0.34)	0.31		
Quartile 4 (≥ 28.4)	241	0.71 (0.47)	0.06		

*Based on age-adjusted linear regression comparing batch-adjusted relative telomere length of each category to reference group

†BMI missing for 3 controls

§Based on the Kruskal Wallis test

‡Correlation between BMI and relative telomere length adjusted for age.

std = standard deviation

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2013 March 01.

Table 2

Association between relative telomere length (RTL) in peripheral blood leukocytes and ovarian cancer risk, New England Case Control Study Phases 1 & 2 (1992-2003)

			Tertile of relative telomere length		
	n	**RTL mean (std)**	**T1 (shortest) OR (95% CI)***	**T2 OR (95% CI)***	**T3 (longest)**
Controls n (%)	947	0.77 (0.51)	314 (33)	305 (32)	328 (35)
Cases n (%)	911	0.75 (0.40)	319 (35)	275 (30)	317 (35)
Overall			1.01 (0.80, 1.28)	0.95 (0.75, 1.20)	1.00
Serous	482	0.73 (0.37)	1.05 (0.79, 1.39)	0.94 (0.71, 1.25)	1.00
Mucinous	120	0.79 (0.39)	0.77 (0.46, 1.32)	1.41 (0.89, 2.24)	1.00
Endometrioid	132	0.77 (0.46)	0.88 (0.55, 1.42)	0.89 (0.55, 1.43)	1.00
Clear Cell	121	0.72 (0.41)	1.07 (0.66, 1.74)	0.84 (0.51, 1.39)	1.00

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

Table 3

Association between SNPs in telomere-related genes and risk of ovarian cancer in NECC (1992-2008) and NHS (1976-2006)*

Gene	rs number	OR†	Per allele 95% CI	P_{gene}‖
TERT	RS2736122	0.89	(0.81, 0.99)	0.00008
TERT	RS2075786	1.06	(0.97, 1.17)	
TERT	RS4246742	1.19	(1.05, 1.35)	
TERT	RS6882077‡	1.31	(0.27, 6.29)	
TERT	RS4975605	1.11	(1.02, 1.22)	
TERT	RS10069690	1.11	(1.00, 1.23)	
TERT	RS2242652‡	1.06	(0.91, 1.24)	
TERT	RS2736100	1.12	(1.02, 1.23)	
TERT	RS2853676	1.13	(1.02, 1.25)	
TERT	RS2736098	0.99	(0.89, 1.09)	
TERT	RS7726159‡	1.17	(1.02, 1.34)	
TRF2	RS251796	0.94	(0.85, 1.04)	0.59
TRF2	RS153045	0.94	(0.86, 1.04)	
TRF2	RS3785074	1.06	(0.96, 1.18)	
TRF2	RS166134‡	0.79	(0.48, 1.28)	
TRF2	RS8061382‡	1.07	(0.74, 1.55)	
TRF1	RS2975842	1.01	(0.93, 1.11)	0.99
TRF1	RS2975852	1.03	(0.94, 1.14)	
TRF1	RS6989159‡	0.95	(0.40, 2.25)	
TRF1	RS6989493‡	1.19	(0.44, 3.26)	
TRF1	RS12334686	1.02	(0.93, 1.12)	
TRF1	RS6982126	0.97	(0.87, 1.08)	
TRF1	RS2981096‡	0.94	(0.69, 1.26)	
TRF1	RS10107605	1.00	(0.87, 1.15)	
TRF1	RS1545827	1.01	(0.93, 1.10)	
TNKS	RS1539041	0.98	(0.89, 1.08)	0.34
TNKS	RS3802650	1.02	(0.94, 1.12)	
TNKS	RS10509637	1.01	(0.89, 1.15)	
TNKS	RS1772180	0.93	(0.85, 1.02)	
TNKS	RS10509639	1.11	(0.94, 1.30)	
TNKS	RS1772186	0.98	(0.86, 1.11)	
TNKS	RS10881982‡	1.03	(0.72, 1.49)	
TNKS	RS12412538	1.07	(0.97, 1.18)	
TNKS	RS7087365	1.06	(0.96, 1.17)	
POT1	RS929365	0.96	(0.79, 1.18)	0.79
POT1	RS7801661	1.02	(0.92, 1.13)	

Gene	rs number	OR†	Per allele 95% CI	p_{gene}‖
POT1	RS11972248	0.96	(0.86, 1.07)	
POT1	RS12532038	1.01	(0.91, 1.11)	
POT1	RS4360236	1.14	(0.99, 1.32)	
POT1	RS2896361	0.98	(0.90, 1.08)	

*Restricted to white women.

†Adjusted for age (continuous), center, oral contraceptive use (never, 1-24, 25-72, >72 months), parity (0, 1, 2+), family history of breast or ovarian cancer, and tubal ligation. The combined analysis is additionally adjusted for genotyping batch.

§Meta-analysis of NECC phases 1&2, NECC phase 3, and NHS using fixed effects model. We observed significant heterogeneity between datasets for *TRF1* SNP RS12334686 ($p_{heterogeneity}$ = 0.04) and *TNKS* SNP RS1772180 ($p_{heterogeneity}$ = 0.02).

‡RS6882077, RS2242652, RS166134, RS8061382, RS6989159, RS6989493, RS2981096, RS10881982 were not genotyped in NECC phase 3 and NHS datasets and RS7726159 was not genotyped in NECC phases 1 and 2.

‖Principal components analyses were used to determine gene-level associations with ovarian cancer risk accounting for linkage disequilibrium between SNPs