

# Prediagnosis Leukocyte Telomere Length and Risk of Ovarian Cancer

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## Abstract

**Background:** The associations between telomere length and cancer risk are equivocal, and none have examined the association between prediagnosis leukocyte telomere length (LTL) and the risk of developing ovarian cancer.

**Methods:** We prospectively measured LTL collected from 442 ovarian cancer cases and 727 controls in the Nurses' Health Studies and the Northern Sweden Health and Disease Study. Cases were matched to one or two controls on age, menopausal status, and date of blood collection. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using conditional logistic regression.

**Results:** LTL was measured a median of 9.5 years before ovarian cancer diagnosis among cases. We observed a decreased risk of ovarian cancer with longer LTL. In multivariable models, women in the top quartile of LTL had an OR for

ovarian cancer of 0.67 (95% CI, 0.46–0.97) compared with those in the bottom quartile. Inverse associations were stronger for nonserous cases (OR<sub>quartile 4 vs. quartile 1 of LTL</sub> = 0.55, 95% CI, 0.33–0.94) and rapidly fatal cases (i.e., cases who died within 3 years of diagnosis; OR<sub>quartile 4 vs. quartile 1 of LTL</sub> = 0.55, 95% CI, 0.32–0.95).

**Conclusions:** Our prospective findings suggest that longer circulating LTL may be associated with a lower ovarian cancer risk, especially for nonserous and rapidly fatal cases. The evaluation of LTL in relation to ovarian cancer risk by tumor subtypes is warranted in larger prospective studies.

**Impact:** Prediagnosis LTL may reflect an early event in the ovarian cancer development and could serve as a biomarker to predict future risk. *Cancer Epidemiol Biomarkers Prev*; 26(3); 1–7. ©2017 AACR.

## Introduction

Risk of ovarian cancer, the fifth leading cause of cancer-related death among U.S. women (1), is hypothesized to increase with greater number of lifetime ovulations. Ovulation-induced trauma to the ovarian surface epithelium generates reactive oxidants (2), a local inflammatory response, and stimulates the epithelium proliferation, leading to an accumulation of genetic errors that may augment ovarian cancer risk (3). Telomeres, which protect the physical integrity of linear chromosomes (4), are shortened with each cell division (5), a process that may be accelerated by damage incurred by oxidative stress (6). Tissue-based studies

reveal patterns of telomere shortening, genomic instability, and upregulated telomerase expression for many tumor types, including ovarian cancer, as cells progress from noninvasive precursor lesions to cancer, implicating telomere shortening as a common event early in malignant transformation (7–10).

Three retrospective epidemiologic studies have explored the associations between telomere length and ovarian cancer risk and reported mixed results. In a small pilot study, Polish women in the shortest versus longest tertile of relative leukocyte telomere length (LTL) had a 3-fold increased risk of serous ovarian carcinoma compared to cancer-free controls [odds ratio (OR), 3.4; 95% confidence interval (CI), 1.5–7.5], with the strongest association observed among women diagnosed with poorly differentiated tumors (11). A subsequent study observed a weaker, but significant association between shorter LTL and higher ovarian cancer risk (12). In contrast, the largest study to date conducted within the New England Case–Control Study, did not find evidence of an association between LTL and ovarian cancer risk overall or by histologic subtype; nevertheless, an association in the expected direction (inverse) emerged but was not statistically significant when cases who had recently been treated with chemotherapy were excluded (13). As blood samples were collected after ovarian cancer diagnosis in these retrospective studies, telomere shortening may have occurred as a result of the physiological changes stemming from the disease itself, cancer treatment, and/or the psychological impact of a cancer diagnosis. It remains inconclusive as to whether telomere shortening precedes development of ovarian cancer. Therefore, we prospectively investigated whether LTL from prediagnosis blood samples was associated with ovarian cancer risk using data from the Nurses' Health Study

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**Note:** Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

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(NHS), NHSII, and Northern Sweden Health and Disease Study (NSHDS).

## Materials and Methods

### Study population

**NHS/NHSII.** The NHS cohort was established in 1976 among 121,700 U.S. female registered nurses ages 30 to 55 years, and the NHSII began in 1989 among 116,430 female registered nurses ages 25 to 42 years. All women completed an initial questionnaire and have been followed biennially by questionnaires to update their demographics, lifestyle, and medical history. In 1989 to 1990, 32,826 NHS participants (ages 43–69 years) provided blood samples and completed a short questionnaire (14); follow-up was 93% in 2010. Between 1996–1999, 29,611 NHSII participants (ages 32–54 years) provided blood samples (18,521 eligible premenopausal women provided a sample timed in the luteal phase of the menstrual cycle) and completed a short questionnaire (15); follow-up was 95% in 2011. Plasma, buffy coat, and red blood cell aliquots have been stored in liquid nitrogen freezers since collection. These studies were approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Ovarian cancers were identified via self-report on questionnaires or from death certificates, and then confirmed by medical record review or linkage to the relevant tumor registry. Cases had no previous history of cancer, except nonmelanoma skin cancer, before blood collection and were diagnosed with primary invasive epithelial ovarian or peritoneal cancer after blood draw and before June 1, 2012 (NHS) or June 1, 2011 (NHSII). Cases were matched to one or two controls, who were alive and had at least one intact ovary at the time of the case diagnosis, on menopausal status at baseline and diagnosis (premenopausal, postmenopausal, unknown), age ( $\pm 1$  year), month of blood collection ( $\pm 1$  month), time of day of blood draw ( $\pm 2$  hours), fasting status ( $> 8$  hours and  $\leq 8$  hours), and postmenopausal hormone use at blood draw (yes, no). For NHSII cases with timed samples, we additionally matched on day of the luteal blood draw (date of next menstrual cycle minus date of blood draw,  $\pm 1$  day).

**NSHDS.** The NSHDS cohort consists of three sub-cohorts [the Västerbotten Intervention Program (VIP) cohort, the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) cohort, and the mammary (mammography) screening cohort], and was established in 1985. At recruitment, participants provided venous blood samples that were stored at  $-80^{\circ}\text{C}$  (16). Information on demographic factors, lifestyle factors (including exogenous hormone use and smoking), reproductive factors, and medical history were collected at the time of recruitment and/or through follow-up questionnaires.

Cases were identified through the cancer registry as women with primary invasive epithelial ovarian cancer diagnosed after blood donation, who had no preceding invasive cancer diagnosis (except nonmelanoma skin cancer), did not use exogenous hormones at the time of blood donation and who were diagnosed before December 2013. A pathology report review was carried out by a gynecologic pathologist. One control who was alive and free of cancer, who did not report a bilateral oophorectomy, and did not use exogenous hormones at blood donation at case diagnosis was matched to each case on sub-cohort, menopausal status

(premenopausal, postmenopausal, unknown), age ( $\pm 6$  months), and date of blood donation ( $\pm 3$  months).

### Telomere assay

Genomic DNA was extracted from the buffy coat fraction of peripheral blood using QIAmp DNA blood kits (QIAGEN). We used Real-Time Quantitative PCR (qPCR) to determine average relative LTL (17, 18). The assay determined the copy-number ratio between telomere repeats and a single-copy (36B4) reference gene (T/S Ratio,  $-\Delta C_t$ ). Relative LTL was reported as the exponentiated T/S ratio corrected for a reference sample. A modified version of the qPCR telomere assay was performed in a 384-well format with a 7900HT PCR System (Life Technologies). Briefly, 5 ng of buffy-coat derived genomic DNA was dried down in a 384-well plate and suspended in 10  $\mu\text{L}$  of either the telomere or 36B4 reaction mixture for 2 hours at  $4^{\circ}\text{C}$ . The telomere reaction mixture consisted of 1x Quantitect SYBR Green Master Mix (Qiagen), 2.5 mmol/L of DTT, 270 nmol/L of Tel-1 primer-(GGTTTTGAG-GGTGAGGGTGAGGGTGAGGGTGAGGGT), and 900 nmol/L of Tel-2 primer-(TCCCAGACTATCCCTATCCCTATCCCTATCCCTATCCCTA). The 36B4 reaction consisted of 1x Quantitect SYBR Green Master Mix, 300 nmol/L of 36B4U primer (CAGCAAG-TGGGAA GGTGTAATCC), and 500 nmol/L of 36B4D primer-(CCCATTCTATCATCAACG GGTACAA). All samples for both the telomere and 36B4 reactions were performed in triplicate on different plates. Each 384-well plate contained a 6-point standard curve from 0.625 ng to 20 ng to assess PCR efficiency. A slope of  $-3.33 \pm 0.3$  ( $> 90\%$  pcr efficiency) for the standard curve of both the telomere and 36B4 reactions was considered acceptable. Quality control samples was interspersed throughout the plates to assess inter-plate and intra-plate variability of  $C_t$  values. Mean coefficients of variation (CV) for the exponential T/S ratio of blinded QC samples ranged from 7.8% to 17.6% across batches. The correlation between T/S ratios and absolute telomere lengths determined by southern blot was 0.82 ( $P < 0.001$ ; ref. 18). Samples with failed qPCR data ( $n = 26$ ) and those with a within-triplicate CVs greater than 20% ( $n = 52$ ) were removed for final analyses.

### Statistical analysis

Relative LTL values were z-transformed to improve normality within batches. To control for variation across laboratory batches, we used the batch correction method proposed by Rosner and colleagues (19) adjusting for age, BMI, postmenopausal hormone use, smoking status and seasons of blood draw to obtain a batch-adjusted LTL in each cohort, with values in NHS as the reference batch. Cohort-specific quartiles of the batch-adjusted z-scores were determined on the basis of the LTL distributions in the controls in each cohort. Demographics, reproductive factors, and clinical characteristics at blood draw were estimated across cohorts for cases and matched controls.

Conditional logistic regression was used to calculate ORs and 95% CIs across cohort-specific quartiles of LTL, with a higher quartile indicating longer LTL. In each cohort, LTL was inversely associated with ovarian cancer risk but the associations did not reach statistical significance (Supplementary Table S1). Heterogeneity across cohorts was assessed using random effects meta-analysis techniques (20). There was little evidence for heterogeneity across cohorts ( $P_{\text{heterogeneity}} = 0.78$ ). Hence, we pooled NHS, NHSII and NSHDS data, and re-determined batch-corrected LTL quartiles using the control distribution in the pooled study.

**Table 1.** Characteristics of ovarian cancer cases and controls in NHS, NHSII, and NSHDS at the time of blood collection<sup>a</sup>

	NHS		NHSII		NSHDS	
	Case (n = 246)	Control (n = 493)	Case (n = 51)	Control (n = 92)	Case (n = 145)	Control (n = 142)
Age at blood draw, y, mean (SD)	56.9 (6.5)	56.9 (6.5)	44.8 (4.6)	44.7 (4.8)	52.4 (9.2)	53.0 (9.3)
Age at diagnosis, y, mean (SD)	68.4 (8.3)	—	50.9 (5.7)	—	60.1 (9.3)	—
Time between blood draw and diagnosis, y, mean (SD)	11.6 (6.2)	—	6.2 (4.3)	—	7.7 (5.5)	—
BMI at blood draw, kg/m <sup>2</sup> , mean (SD)	28.1 (22.2)	28.7 (23.3)	32.9 (25.7)	28.4 (16.7)	25.9 (4.6)	24.7 (3.5)
Fasting status, %	63	67	63	72	63	57
Menopausal status at blood draw, %						
Premenopausal	22	21	81	78	27	22
Postmenopausal	65	65	11	10	59	49
Missing	13	15	8	12	14	29
Use of postmenopausal hormones at blood draw (%)	43	41	9	12	7	10
Oral contraceptive use (%)	45	45	88	88	41	40
Number of children (%)						
Nulliparous	7	4	29	18	27	20
1 child	4	4	18	10	14	12
2 children	32	28	38	43	37	39
3 children	32	28	9	19	15	19
4+ children	26	37	6	10	7	10
Tubal ligation (%)	15	17	16	30	5	3
Family history of ovarian or breast cancer (%)	16	12	12	14	10	13
Smoking status at blood draw (%)						
Never	46	48	64	75	33	33
Former	42	39	31	15	20	21
Current	12	14	5	10	21	14
Missing	0	0	0	0	26	31
Histology subtypes, N <sup>c</sup>						
Serous/nonserous	158/72	—	25/24	—	79/53	—
Rapidly fatal/nonfatal	86/103	—	7/32	—	38/93	—
Relative LTL, median (10 <sup>th</sup> –90 <sup>th</sup> percentile) <sup>b</sup>						
Values on original scale	0.47 (0.32–0.66)	0.48 (0.33–0.67)	0.55 (0.32–0.81)	0.57 (0.37–0.89)	0.46 (0.31–0.66)	0.46 (0.33–0.69)
Values after batch correction	0.47 (0.32–0.66)	0.48 (0.33–0.67)	0.49 (0.27–0.73)	0.50 (0.29–0.81)	0.48 (0.33–0.68)	0.48 (0.36–0.72)

<sup>a</sup>Values are means (SD) or percentages and are standardized to the age distribution of the study population.

<sup>b</sup>Batch correction method proposed by Rosner and colleagues (19) was used to control for variation across laboratory batches, adjusting for age, BMI, postmenopausal hormone use, smoking status and seasons at blood draw, with values in NHS as reference.

<sup>c</sup>Cases with undetermined histology subtypes were deleted for the subtype analyses in each cohort.

Spearman correlation coefficients were performed between age at blood draw and LTL among controls. As expected, LTL was inversely correlated with age at blood draw ( $r_{\text{Spearman}} = -0.12$ ,  $P = 0.008$ ) among control participants (Supplementary Fig. S1). We used conditional logistic regression to estimate the ORs and 95% CIs across cohort-common quartiles of LTL conditioning on matching factors. In multivariable models, we adjusted for oral contraceptive use (ever vs. never), tubal ligation (yes vs. no), family history of ovarian or breast cancer (yes vs. no), parity (nulliparous, 1 child, 2 children, 3 children, 4+ children), smoking status (never smoker, former smoker, current smoker, missing), and BMI at blood draw (kg/m<sup>2</sup>, continuous). We also modeled LTL as a continuous measure (per one SD).

In secondary analyses, we evaluated whether associations were stronger for certain tumor subtypes, that is, serous versus non-serous cases, and tumors that were rapidly fatal (i.e., case died within 3 years of diagnosis) versus less aggressive, using polytomous logistic regression. We assessed whether associations were modified by menopausal status (premenopausal vs. postmenopausal), age at blood draw (<55, 55–65, >65 years), smoking status (never smoking vs. ever smoking) and time interval between blood draw and diagnosis (<9.5 years vs. ≥9.5 years). Interaction terms were created by multiplying the variables above with indicators of quartiles of LTL. The statistical significance of

the interaction was assessed using likelihood ratio tests. In addition, we also repeated all the analyses by applying the age-adjusted LTL using residual methods (21) given the inverse correlation between LTL and age at blood draw. All  $P$  values were two sided and analyses were conducted using SAS release 9.4 (SAS Institute).

## Results

The final samples size in the pooled analysis consisted of 442 cases and 727 controls. Demographic and reproductive factors were similar among cases and controls across three cohorts (Table 1). Among cases, the mean age at diagnosis was 68.4 years in NHS, 50.9 years in NHSII and 60.1 years in NSHDS. The mean time between blood collection and cancer diagnosis among cases was 11.6 years in NHS, 6.2 years in NHSII, and 7.7 years in NSHDS.

We observed an inverse association between LTL and ovarian cancer risk after pooling (Table 2). Women in the top quartile of LTL had an OR for ovarian cancer of 0.67 compared to those in the bottom quartile (95% CI, 0.46–0.97) with a borderline significant trend across quartiles ( $P_{\text{trend}} = 0.07$ ). When modeling LTL continuously, a one SD increase in LTL was significantly associated with 11% decreased risk of developing ovarian cancer (OR, 0.89; 95% CI, 0.78–1.01;  $P = 0.04$ ). Further adjusting for potential confounders did not considerably change the results.

**Table 2.** OR and 95% CI of ovarian cancer in three pooled nested case-control studies (NHSI, NHSII, and NSHDS) according to common quartiles of circulating relative LTL<sup>a</sup>

Relative LTL	Case	Control	Model 1 <sup>c</sup> OR (95% CI)	Model 2 <sup>d</sup> OR (95% CI)
Quartile (median level) <sup>b</sup>				
Q1 (0.34)	122	179	1.00	1.00
Q2 (0.44)	111	184	0.83 (0.57-1.22)	0.81 (0.55-1.20)
Q3 (0.53)	114	183	0.83 (0.58-1.18)	0.84 (0.58-1.21)
Q4 (0.67)	95	181	0.68 (0.47-0.97)	0.67 (0.46-0.97)
$P_{\text{trend}}^e$			0.06	0.07
1 SD increase (0.14) <sup>f</sup>	442	727	0.88 (0.77-0.99)	0.89 (0.78-1.01)
$P$ value			0.04	0.07

<sup>a</sup>Common quartiles of circulating relative LTL were obtained by using the control distribution in the pooled three cohorts. There was no heterogeneity across three studies so we pooled all data.

<sup>b</sup>Relative LTL was z-scored and batch-corrected.

<sup>c</sup>Conditional logistic regression model conditioned on matching factors. Cases were matched to one or two controls on age, menopausal status, and date of blood collection.

<sup>d</sup>Conditional logistic model conditioned on matching factors and adjusted for oral contraceptive use (yes vs. no), tubal ligation (yes vs. no), family history of ovarian or breast cancer (yes vs. no), parity (nulliparous, 1 child, 2 children, 3 children, 4+ children), smoking status (never smoke, former smoker, current smoker, missing), and BMI at blood draw (kg/m<sup>2</sup>, continuous).

<sup>e</sup> $P_{\text{trend}}$  was calculated by modeling the median of each category as a continuous term. All statistical tests were two-sided.

<sup>f</sup>We modeled relative LTL as a continuous variable (per one SD increase). One SD of relative LTL = 0.14.

When we further evaluated potential differences by ovarian cancer subtype, longer LTL was significantly related to lower risk of nonserous ovarian cancer and rapidly fatal cases that died within three years of diagnosis (Table 3). However, there was no significant heterogeneity by subtype. The ORs comparing extreme quartiles of LTL were 0.55 (95% CI, 0.33-0.94) for nonserous cases ( $P_{\text{trend}} = 0.02$ ) and 0.81 (95% CI, 0.53-1.24) for serous cases ( $P_{\text{trend}} = 0.50$ ;  $P_{\text{heterogeneity}} = 0.17$ ). In addition, the ORs were 0.55 (95% CI, 0.32-0.95) among rapidly fatal cases ( $P_{\text{trend}} = 0.02$ ) and 0.77 (95% CI, 0.49-1.23) among less aggressive ovarian cancer ( $P_{\text{trend}} = 0.37$ ;  $P_{\text{heterogeneity}} = 0.18$ ), comparing highest vs. lowest quartile of LTL. Furthermore, the inverse association between LTL and ovarian cancer was not significantly modified by menopausal status ( $P_{\text{interaction}} = 0.27$ ), age at diagnosis ( $P_{\text{interaction}} = 0.20$ ), smoking status ( $P_{\text{interaction}} = 0.73$ ), or time interval between blood draw and diagnosis ( $P_{\text{interaction}} = 0.97$ ). In addition, to more carefully account for the association of age with LTL, we obtained an age-adjusted LTL by residual method to model the association and observed similar results. The OR comparing extreme quartiles of LTL for ovarian cancer was 0.62 (95% CI, 0.43-0.90), similar to conventionally adjusted analyses.

## Discussion

In this first prospective nested case-control study of ovarian cancer, longer LTL was associated with a lower risk of developing ovarian cancer. This association was not modified by menopausal status, age at diagnosis, smoking status or time interval between blood draw and diagnosis. Moreover, stronger inverse associations were observed for risk of nonserous and rapidly fatal ovarian cancer.

Inconsistent associations have been reported between telomere length and various cancers. For some studies, longer circulating telomere length was associated with lower risk of several cancers, including lung cancer (22-24), colorectal cancer (25), and breast cancer (26, 27), with stronger associations reported in retrospective studies with LTL measured after cancer onset. Other studies

**Table 3.** OR and 95% CI of ovarian cancer risk according to the common quartiles of circulating relative LTL in three pooled nested case-control studies by histologic subtype and rapidly fatal versus less aggressive disease<sup>a,b</sup>

Relative LTL	Serous/poorly differentiated (n = 262)	Nonserous (n = 149)	$P_{\text{heterogeneity}}^c$
	OR (95% CI)	OR (95% CI)	
Quartile			
Q1	1.00	1.00	
Q2	0.90 (0.60-1.36)	0.74 (0.45-1.20)	0.52
Q3	1.14 (0.77-1.69)	0.64 (0.38-1.07)	0.08
Q4	0.81 (0.53-1.24)	0.55 (0.33-0.94)	0.27
$P_{\text{trend}}^d$	0.50	0.02	0.17
1 SD increase <sup>e</sup>	0.98 (0.85-1.13)	0.79 (0.65-0.95)	
$P$ value	0.77	0.01	0.07
	Rapidly fatal <sup>f</sup> (n = 131)	Less aggressive (n = 228)	
	OR (95% CI)	OR (95% CI)	
Quartile (median)			
Q1 (0.34)	1.00	1.00	
Q2 (0.44)	0.68 (0.41-1.12)	1.01 (0.65-1.56)	0.24
Q3 (0.53)	0.49 (0.28-0.85)	1.22 (0.79-1.86)	0.01
Q4 (0.68)	0.55 (0.32-0.95)	0.77 (0.49-1.23)	0.34
$P_{\text{trend}}^d$	0.02	0.37	0.18
1 SD increase <sup>e</sup>	0.79 (0.64-0.97)	0.93 (0.80-1.09)	
$P$ value	0.02	0.37	0.20

<sup>a</sup>Common quartiles of circulating relative LTL were obtained by using the control distribution in the pooled three cohorts. There was no heterogeneity across three studies so we pooled all data. Relative LTL was z-scored and batch-corrected.

<sup>b</sup>Polytomous logistic regression model conditioned on matching factors (age, menopausal status, and date of blood collection) and adjusted for oral contraceptive use (yes vs. no), tubal ligation (yes vs. no), family history of ovarian or breast cancer (yes vs. no), parity (nulliparous, 1 child, 2 children, 3 children, 4+ children), smoking status (never smoke, former smoker, current smoker, missing), and BMI at blood draw (kg/m<sup>2</sup>, continuous).

<sup>c</sup> $P_{\text{heterogeneity}}$  was obtained by likelihood ratio test.

<sup>d</sup> $P_{\text{trend}}$  was calculated by modeling the median of each category as a continuous term. All statistical tests were two-sided.

<sup>e</sup>We modeled relative LTL as a continuous variable (per one SD increase). One SD of relative LTL = 0.14.

<sup>f</sup>Rapidly fatal cases are defined as those that died within 3 years of diagnosis.



have documented an increased risk with longer telomere length for lung cancer (28, 29), melanoma (30), pancreatic cancer (31), breast cancer (32, 33), and prostate cancer (34), primarily among prospective studies. Null results have also been reported (35, 36). Greater cancer risk with shorter telomere length is biologically plausible, given evidence that shortened telomeres can play a causal role in carcinogenesis by instigating chromosomal instability, promoting genetic lesions, inactivating tumor suppressor checkpoints, and ultimately inducing cancer (10, 37, 38). However, there are also reasonable hypotheses for the alternate scenario, as cells with longer telomeres might be at higher risk of acquiring genetic abnormality because having longer telomeres may delay cellular senescence (39, 40). Furthermore, recent genome wide association studies have revealed bi-directional associations between genetic determinants of telomere length and different cancers (41, 42). Overall, the association between telomere length and cancer risk may vary by cancer site and may depend on other characteristics of the tumors at those sites (e.g., amount of genomic instability).

For ovarian cancer specifically, three retrospective case-control studies have examined circulating telomere length in relation to ovarian cancer risk, with two reporting an inverse association (11, 12) and one with null findings (13). However, the latter study did observe a suggestive association between genetic variation in the *TERT* gene and the risk (13). Because of their retrospective design, the estimates in these studies might be biased, because telomere shortening may occur after diagnosis, potentially due to treatment or disease processes. In contrast, our study associated risk of developing ovarian cancer with prediagnosis telomere length in blood collected years before diagnosis (mean 6.2–11.6 years across the studies), and observed an inverse association between LTL and overall ovarian cancer risk. These findings, with the advantage of prospective design, though generally consistent with previous retrospective investigations, may support the hypothesis that circulating telomere length can predict ovarian cancer risk. Tissue studies indicated that telomere shortening may be a critical early event in ovarian cancer development. Compared with normal tubal epithelium, progressively shorter telomeres have been observed in tubo-ovarian dysplasia (TOD) and serous tubal intraepithelial carcinoma (STIC), the putative precursor to high-grade serous carcinomas (HGSC; refs. 43, 44). Furthermore, the number and size of chromosomal aberrations increased from TOD to STIC to HGSC, suggesting that genetic instability may be an early alteration in ovarian carcinogenesis (43, 44). Ovarian cancers, particularly HGSCs, are characterized by *p53* mutations, a deregulated pRb pathway, and a high degree of genomic instability (45), features consistent with the telomere dysfunction hypothesis of carcinogenesis (46). Nevertheless, despite tissue evidence of shorter telomere length in serous tumors, we did not find a clear association between LTL and serous ovarian cancer risk; the association was stronger for nonserous tumors, although the number of cases with these tumors were limited. We also further conducted cross-classification with histology subtypes and found that (after excluding undetermined subtypes) among rapidly fatal cases there were 90 serous cases and 34 nonserous cases; while the numbers were 129 and 100 respectively among less aggressive cases. Moreover, we explored associations for high-grade versus low-grade serous cases but did not find any associations. Although these findings were limited by modest sample sizes of the various histologic types, telomerase reactivation, and immortalization has been identified in high-grade serous ovarian

tumor cells, in which longer and shorter telomeres coexisted in the same tumors (43). In addition, telomere length measured in leukocytes might not be the optimal surrogate for prediagnosis telomere length in ovarian and tubal tissue; nevertheless, telomere length does appear to be highly correlated across a variety of tissues within the same individual (47). Furthermore, although it is unclear whether our observed association among nonserous cases represents true biologic differences by tumor subtype, we recently reported that key risk factors exhibited significant heterogeneity by histology (48). Notably, most established ovarian cancer risk factors were more strongly associated with nonserous versus serous subtypes. Our observations that the associations of telomere length with ovarian cancer risk differ by histologic type, along with our prior publication on differences by traditional risk factors, add to the growing evidence that ovarian cancer is a highly heterogeneous disease and that evaluations by subtype are necessary for identifying novel risk factors. This is particularly important for developing risk prediction models, as, to date, such models have not taken differences by histologic type into account. As a result, future evaluations of telomere length by tumor subtypes in larger studies are warranted.

Interestingly, genetic research has been mixed with respect to variants in the *TERT* gene as well as variants associated with telomere length in relation to ovarian cancer risk. Several SNPs in *TERT* and its promoter have been associated with ovarian cancer risk, particularly with the serous subtype (13, 49–51), although one study noted that the SNPs associated with ovarian cancer risk were not associated with telomere length (51). Conversely, more recent studies using Mendelian randomization provided no evidence of a relationship between telomere-associated SNPs and overall ovarian cancer as well as three histologic subtypes (41, 42). Therefore, we cannot rule out the possibility that these associations in our study are due to chance or may reflect another exposure that can alter telomere length. For example, depression, which has been associated with reduced telomere length (52, 53), was recently associated with an increased risk of ovarian cancer (54). Strengths and limitations of this study are worth careful consideration. On one hand, the study used a prospective design, multiple independent cohorts, rigorous case-control matching, and rich covariate information to adjust for potential confounders. Nevertheless, as discussed above, peripheral blood LTL might not be an adequate surrogate for telomere length in ovaries or fallopian tubes given the dynamic immune system. We had a limited number of specific histologic subtypes, which reduced the precision of those relative risk estimates. The lack of racial/ethnic backgrounds might be another limitation.

In conclusion, our prospective findings indicate that longer LTL may be associated with a lower ovarian cancer risk, particularly for rapidly fatal disease and nonserous histology. These findings suggest that prediagnosis LTL may reflect an early event in the ovarian cancer development and could serve as a biomarker to predict future risk. Given the significant findings in this first prospective study, additional research to replicate these results is warranted, particularly to examine associations by tumor subtype. Confirmation of telomere length as a risk biomarker for ovarian cancer could have implications for improving identification of women at high risk of ovarian cancer.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Disclaimer

The authors assume full responsibility for analyses and interpretation of these data.

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## References

- Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013;63:11–30.
- Murdoch WJ. Carcinogenic potential of ovulatory genotoxicity. *Biol Reprod* 2005;73:586–90.
- Fleming JS, Beaugie CR, Haviv I, Chenevix-Trench G, Tan OL. Incessant ovulation, inflammation and epithelial ovarian carcinogenesis: revisiting old hypotheses. *Mol Cell Endocrinol* 2006;247:4–21.
- Abbott A. Chromosome protection scoops Nobel. *Nature* 2009;461:706–7.
- Olovnikov AM. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol* 1973;41:181–90.
- von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci* 2002;27:339–44.
- van Heek NT, Meeker AK, Kern SE, Yeo CJ, Lillemoed KD, Cameron JL, et al. Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. *Am J Pathol* 2002;161:1541–7.
- Meeker AK, Hicks JL, Platz EA, March GE, Bennett CJ, Delannoy MJ, et al. Telomere shortening is an early somatic DNA alteration in human prostate tumorigenesis. *Cancer Res* 2002;62:6405–9.
- Kawai T, Hiroi S, Nakanishi K, Meeker AK. Telomere length and telomerase expression in atypical adenomatous hyperplasia and small bronchioloalveolar carcinoma of the lung. *Am J Clin Pathol* 2007;127:254–62.
- Meeker AK, Hicks JL, Iacobuzio-Donahue CA, Montgomery EA, Westra WH, Chan TY, et al. Telomere length abnormalities occur early in the initiation of epithelial carcinogenesis. *Clin Cancer Res* 2004;10:3317–26.
- Mirabello L, Garcia-Closas M, Cawthon R, Lissowska J, Brinton LA, Peplonska B, et al. Leukocyte telomere length in a population-based case-control study of ovarian cancer: a pilot study. *Cancer Causes Control* 2010;21:77–82.
- Martinez-Delgado B, Yanowsky K, Inglada-Perez L, de la Hoya M, Caldes T, Vega A, et al. Shorter telomere length is associated with increased ovarian cancer risk in both familial and sporadic cases. *J Med Genet* 2012;49:341–4.
- Terry KL, Tworoger SS, Vitonis AF, Wong J, Titus-Ernstoff L, De Vivo J, et al. Telomere length and genetic variation in telomere maintenance genes in relation to ovarian cancer risk. *Cancer Epidemiol Biomarkers Prev* 2012;21:504–12.
- Hankinson SE, Willett WC, Manson JE, Colditz GA, Hunter DJ, Spiegelman D, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 1998;90:1292–9.
- Tworoger SS, Sluss P, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. *Cancer Res* 2006;66:2476–82.
- Hallmans G, Agren A, Johansson G, Johansson A, Stegmayr B, Jansson JH, et al. Cardiovascular disease and diabetes in the Northern Sweden Health and Disease Study Cohort—evaluation of risk factors and their interactions. *Scand J Public Health Suppl* 2003;61:18–24.
- Wang H, Chen H, Gao X, McGrath M, Deer D, De Vivo I, et al. Telomere length and risk of Parkinson's disease. *Mov Disord* 2008;23:302–5.
- Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res* 2002;30:e47.
- Rosner B, Cook N, Portman R, Daniels S, Falkner B. Determination of blood pressure percentiles in normal-weight children: some methodological issues. *Am J Epidemiol* 2008;167:653–66.
- DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177–88.
- Willett WC. *Nutritional epidemiology*. Oxford, United Kingdom: Oxford University Press; 2012.
- Jang JS, Choi YY, Lee WK, Choi JE, Cha SI, Kim YJ, et al. Telomere length and the risk of lung cancer. *Cancer Sci* 2008;99:1385–9.
- Wu X, Amos CI, Zhu Y, Zhao H, Grossman BH, Shay JW, et al. Telomere dysfunction: a potential cancer predisposition factor. *J Natl Cancer Inst* 2003;95:1211–8.
- Hosgood HD III, Cawthon R, He X, Chanock S, Lan Q. Genetic variation in telomere maintenance genes, telomere length, and lung cancer susceptibility. *Lung Cancer* 2009;66:157–61.
- Pooley KA, Sandhu MS, Tyrer J, Shah M, Driver KE, Luben RN, et al. Telomere length in prospective and retrospective cancer case-control studies. *Cancer Res* 2010;70:3170–6.
- Shen J, Gammon MD, Terry MB, Wang Q, Bradshaw P, Teitelbaum SL, et al. Telomere length, oxidative damage, antioxidants and breast cancer risk. *Int J Cancer* 2009;124:1637–43.
- Shen J, Terry MB, Gurvich I, Liao Y, Senie RT, Santella RM. Short telomere length and breast cancer risk: a study in sister sets. *Cancer Res* 2007;67:5538–44.
- Shen M, Cawthon R, Rothman N, Weinstein SJ, Virtamo J, Hosgood HD III, et al. A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of lung cancer. *Lung Cancer* 2011;73:133–7.
- Lan Q, Cawthon R, Gao Y, Hu W, Hosgood HD III, Barone-Adesi F, et al. Longer telomere length in peripheral white blood cells is associated with risk of lung cancer and the rs2736100 (CLPTM1L-TERT) polymorphism in a prospective cohort study among women in China. *PLoS ONE* 2013;8:e59230.
- Han J, Qureshi AA, Prescott J, Guo Q, Ye L, Hunter DJ, et al. A prospective study of telomere length and the risk of skin cancer. *J Invest Dermatol* 2009;129:415–21.

31. Lynch SM, Major JM, Cawthon R, Weinstein SJ, Virtamo J, Lan Q, et al. A prospective analysis of telomere length and pancreatic cancer in the alpha-tocopherol beta-carotene cancer (ATBC) prevention study. *Int J Cancer* 2013;133:2672–80.
32. Svenson U, Nordfjall K, Stegmayr B, Manjer J, Nilsson P, Tavelin B, et al. Breast cancer survival is associated with telomere length in peripheral blood cells. *Cancer Res* 2008;68:3618–23.
33. Gramatges MM, Telli ML, Balise R, Ford JM. Longer relative telomere length in blood from women with sporadic and familial breast cancer compared with healthy controls. *Cancer Epidemiol Biomarkers Prev* 2010;19:605–13.
34. Julin B, Shui I, Heaphy CM, Joshi CE, Meeker AK, Giovannucci E, et al. Circulating leukocyte telomere length and risk of overall and aggressive prostate cancer. *Br J Cancer* 2015;112:769–76.
35. De Vivo I, Prescott J, Wong JY, Kraft P, Hankinson SE, Hunter DJ. A prospective study of relative telomere length and postmenopausal breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2009;18:1152–6.
36. Weischer M, Nordestgaard BG, Cawthon RM, Freiberg JJ, Tybjaerg-Hansen A, Bojesen SE. Short telomere length, cancer survival, and cancer risk in 47102 individuals. *J Natl Cancer Inst* 2013;105:459–68.
37. Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, et al. Telomere dysfunction promotes nonreciprocal translocations and epithelial cancers in mice. *Nature* 2000;406:641–5.
38. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet* 2005;6:611–22.
39. Mooi WJ, Peeper DS. Oncogene-induced cell senescence—halting on the road to cancer. *N Engl J Med* 2006;355:1037–46.
40. Jones AM, Beggs AD, Carvajal-Carmona L, Farrington S, Tenesa A, Walker M, et al. TERC polymorphisms are associated both with susceptibility to colorectal cancer and with longer telomeres. *Gut* 2012;61:248–54.
41. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, et al. Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet* 2013;45:422–7.
42. Zhang C, Doherty JA, Burgess S, Hung RJ, Lindstrom S, Kraft P, et al. Genetic determinants of telomere length and risk of common cancers: a Mendelian randomization study. *Hum Mol Gen* 2015;24:5356–66.
43. Kuhn E, Meeker A, Wang TL, Sehdev AS, Kurman RJ, Shih Ie M. Shortened telomeres in serous tubal intraepithelial carcinoma: an early event in ovarian high-grade serous carcinogenesis. *Am J Surg Pathol* 2010;34:829–36.
44. Chene G, Tchirkov A, Pierre-Eymard E, Dauplat J, Raoelfils I, Cayre A, et al. Early telomere shortening and genomic instability in tubo-ovarian pre-neoplastic lesions. *Clin Cancer Res* 2013;19:2873–82.
45. The Cancer Genome Research Atlas Network. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011;474:609–15.
46. Campisi J, Kim SH, Lim CS, Rubio M. Cellular senescence, cancer and aging: the telomere connection. *Exp Gerontol* 2001;36:1619–37.
47. Youngren K, Jeanclous E, Aviv H, Kimura M, Stock J, Hanna M, et al. Synchrony in telomere length of the human fetus. *Human Genet* 1998;102:640–3.
48. Wentzensen N, Poole EM, Trabert B, White E, Arslan AA, Patel AV, et al. Ovarian cancer risk factors by histologic subtype: an analysis from the ovarian cancer cohort consortium. *J Clin Oncol* 2016;34:2888–98.
49. Johnatty SE, Beesley J, Chen X, Macgregor S, Duffy DL, Spurdle AB, et al. Evaluation of candidate stromal epithelial cross-talk genes identifies association between risk of serous ovarian cancer and TERT, a cancer susceptibility "hot-spot." *PLoS Genet* 2010;6:e1001016.
50. Beesley J, Pickett HA, Johnatty SE, Dunning AM, Chen X, Li J, et al. Functional polymorphisms in the TERT promoter are associated with risk of serous epithelial ovarian and breast cancers. *PLoS ONE* 2011;6:e24987.
51. Bojesen SE, Pooley KA, Johnatty SE, Beesley J, Michailidou K, Tyrer JP, et al. Multiple independent variants at the TERT locus are associated with telomere length and risks of breast and ovarian cancer. *Nat Genet* 2013;45:371–84.
52. Lynch SM, Peek MK, Mitra N, Ravichandran K, Branas C, Spangler E, et al. Race, ethnicity, psychosocial factors, and telomere length in a multicenter setting. *PLoS ONE* 2016;11:e0146723.
53. Ridout KK, Ridout SJ, Price LH, Sen S, Tyrka AR. Depression and telomere length: a meta-analysis. *J Affect Disord* 2016;191:237–47.
54. Huang T, Poole EM, Okereke OI, Kubzansky LD, Eliassen AH, Sood AK, et al. Depression and risk of epithelial ovarian cancer: results from two large prospective cohort studies. *Gynecol Oncol* 2015;139:481–6.

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