Int J Mol Epidemiol Genet 2012;3(3):252-261
www.ijmeg.org /ISSN1948-1756/IJMEG1207006

Original Article

Genetic variation in telomere maintenance genes in relation to ovarian cancer survival

Holly R Harris[1,2], Immaculata De Vivo[3,4], Linda J Titus[5], Allison F Vitonis[1], Jason Y Y Wong[3,4], Daniel W Cramer[1,4], Kathryn L Terry[1,4]

[1]Obstetrics and Gynecology Epidemiology Center, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, 02115, United States; [2]Division of Nutritional Epidemiology, National Institute for Environmental Medicine, Karolinska Institutet, Stockholm, 171 77, Sweden; [3]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, 02115, United States; [4]Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts, 02115, United States; [5]Department of Community & Family Medicine, Dartmouth Medical School, Lebanon, New Hampshire, 03755, United States

Received July 29, 2012; accepted August 17, 2012; Epub August 31, 2012; Published September 15, 2012

Abstract: Telomeres are repetitive non-coding DNA sequences at the ends of chromosomes that provide protection against chromosomal instability. Telomere length and stability are influenced by proteins, including telomerase which is partially encoded by the *TERT* gene. Genetic variation in the *TERT* gene is associated with ovarian cancer risk, and predicts survival in lung cancer and glioma. We investigated whether genetic variation in five telomere maintenance genes was associated with survival among 1480 cases of invasive epithelial ovarian cancer in the population-based New England Case-Control Study. Cox proportional hazard models were used to calculate hazard ratios and 95% confidence intervals. Overall we observed no significant associations between SNPs in telomere maintenance genes and mortality using a significance threshold of p=0.001. However, we observed some suggestive associations in subgroup analyses. Future studies with larger populations may further our understanding of what role telomeres play in ovarian cancer survival.

Keywords: Ovarian cancer, survival, telomere length, SNPs, telomeres

Introduction

Telomeres are repetitive non-coding DNA sequences at the ends of chromosomes that provide protection against chromosomal instability. Telomeres shorten with cell division and at a critical length eventually signal cellular senescence. Telomerase, an enzyme which maintains telomere length, is typically inactive in somatic cells but is expressed in 90% of human tumors, resulting in cellular immortalization [1, 2]. Telomere length and stability are likely influenced by variation in telomere maintenance genes including *TERT* (which partially encodes telomerase), *TRF1*, *TRF2*, *TNKS*, and *POT1* [3].

Previous studies have shown that telomere length is predictive of overall mortality [4] as well as cancer mortality [5-7]. The majority of inter-individual variation in telomere length appears to be genetically determined [8, 9] and genome wide association studies (GWAS) and candidate gene studies have highlighted the importance of genetic variation in the *TERT* locus in relation to ovarian cancer risk as well as other cancers [10-18]. Furthermore, this locus has recently emerged as a predictor of survival and prognosis in lung cancer [19] and glioma [20], but has not been examined in relation to ovarian cancer survival. The aim of this study was to investigate whether SNPs in five telomere maintenance genes were associated with survival among women diagnosed with invasive epithelial ovarian cancer in the population-based New England Case-Control Study. We also examined whether the associations between these exposures and survival differed by histologic subtype, age, smoking, body mass index (BMI), estimated lifetime number of ovulatory cycles, and among those receiving chemo-

therapy. Finally we examined whether these exposures were associated with time to relapse and chemo-refractory disease.

Materials and methods

Study population

This study includes participants from the population-based New England Case-Control Study (NECC) of ovarian cancer diagnosed with invasive epithelial ovarian cancer from 1992-2008. Data for these analyses come from three enrollment phases (1992-1997, 1998-2002, 2003-2008) corresponding to three funding periods. Details regarding enrollment are described elsewhere [21, 22]. 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. Of these 3083 were eligible and 2203 (71%) agreed to participate. Controls (n=2100) were not included in this analysis. All study participants were interviewed at the time of enrollment about known and suspected ovarian cancer risk factors. To avoid the possible impact of pre-clinical disease on exposure status, cases were asked about exposures that occurred at least one-year before diagnosis. Over 95% of the participants provided a blood specimen. In a subset of the cases (n=793) who were diagnosed at Brigham and Women's Hospital or Massachusetts General Hospital, we abstracted data on chemotherapy and residual disease from medical records. Date of death was identified through the Social Security Death Index. This study was approved by the Institutional Review Boards of Brigham and Women's Hospital and Dartmouth Medical School; each participant provided a signed informed consent.

SNP selection and genotyping

We genotyped 40 tagging SNPs in five genes involved in telomere maintenance (*TERT, POT1, TNKS, TRF1,* and *TRF2*) identified through publicly available data from the HapMap Phase II (www.hapmap.org) as described previously[18]. Duplicate samples were in 100% concordance. DNA was extracted from buffy coat samples using QIAmp (Qiagen, Chatsworth, CA); due to limited availability of genomic DNA, samples were amplified using Genomiphi (GE Healthcare, Piscataway, NJ). All genotyping was performed at the Dana-Farber/Harvard Cancer Center (DF/HCC) High Throughput Polymorphism Core, an affiliate of the Partners HealthCare Center for Personalized Genetic Medicine. First, we genotyped 39 SNPs on samples collected between 1992-2002 (n=881) 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 samples collected between 2003-2008 (n=663) using the Applied Biosystems Taqman OpenArray genotyping platform. Taqman® primers, probes, and conditions for genotyping assays are available upon request. Replicate samples (approximately 10%) were included for quality control and had 100% concordance. Genotyping was performed by laboratory personnel blinded to quality control replicates. Over 95% of the samples were successfully genotyped for each polymorphism.

Statistical analysis

Cases were excluded if their primary tumor was of a borderline histology (n=426) or was non-epithelial (n=127), if they did not have genotype data (n=105), had an implausible date of death (n=1), or were non-white (n=63) because we observed that several SNP frequencies varied by race. Cox proportional hazard models with time since diagnosis in months as the time scale were used to calculate hazard ratios (HRs) and 95% confidence intervals (95% CIs) for the association between each SNP and death. Participants contributed person-time from the date of ovarian cancer diagnosis until death, or the date survival status was last updated (most recent update May 2012). Among 793 invasive cases with abstracted clinical data, 488 had information on dates of chemotherapy, surgery, and relapse. For these cases, we evaluated the association between each SNP and time to relapse after first-line chemotherapy using Cox proportional hazard models. Participants in these analyses contributed person-time from the date of end of first-line chemotherapy until relapse, or date of last follow-up. Logistic regression was used to evaluate the association between each SNP and chemo-refractory disease. Chemo-refractory disease was defined in two ways: relapse within six months of completing first-line chemotherapy or relapse within six months after primary debulking surgery. Multivariate models were adjusted for age

(continuous), enrollment phase (1992-1997, 1998-2002, 2003-2008), study center (Massachusetts, New Hampshire), tubal ligation (yes, no), smoking (never smoker, former/ current smoker where former/current smokers are individuals who have smoked 100 or more cigarettes during their lifetime), oral contraceptive use (never, <2 years, 2-5 years, 5+ years), hormone replacement therapy (ever use, never), and menopausal status (premenopausal/ dubious, postmenopausal). Additional multivariable models were adjusted for the following clinical characteristics: histologic subtype (serous, mucinous, endometrioid, clear cell, other/undifferentiated) and grade (1/2, 3, missing/unknown/ indeterminable).

There were no SNPs out of Hardy-Weinberg Equilibrium at $p<0.001$. SNP associations were evaluated using a log-additive model where the hazard ratio represents the incremental increase or decrease in risk of death or risk of relapse with each additional allele. Variables evaluated for effect modification included those potentially associated with ovarian cancer survival and/or thought to influence telomere length: histologic subtype, age, smoking, BMI, and estimated lifetime number of ovulatory cycles. Histologic subtypes were categorized into serous, mucinous, endometrioid, clear cell, and other/undifferentiated. Age was dichotomized into <50 years and ≥ 50 years; smoking was classified into never smoker and past/current smoker; BMI was categorized into normal weight (<25 kg/m^2) and overweight/obese (≥25 kg/m^2); and ovulatory cycles was divided into two categories based on the median value. All tests for interaction were performed using a likelihood ratio test to compare models with and without the interaction terms.

We conducted sensitivity analyses stratifying by time between blood draw and diagnosis; specifically, we calculated estimates for the association between each SNP and death for cases with blood draw within 154 days of diagnosis (lowest quartile) versus more than 154 days after diagnosis. In addition, we conducted a sensitivity analyses restricting to those with grade 3 cancer and serous cases, the largest histologic subgroup. Among the subset of cases with more detailed medical record data we conducted a sensitivity analyses among cases who received chemotherapy as well as adjusting for debulking status (optimally debulked, not optimally debulked).

To evaluate gene level associations, we employed a principal components approach described previously by Gauderman and colleagues that accounts for linkage disequilibrium between SNPs[23]. 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 model with mortality 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. To account for multiple testing we used a threshold of $p<0.001$ for significance for all analyses.

Results

Overall our study included 1480 cases of invasive epithelial ovarian cancer with 710 deaths from 1992-2012. Participant characteristics are described in **Table 1**. The mean (±SD) age at study entry was 55.8 years (±11.0) and the mean follow-up time was 6.3 years (±5.0). Seventy three percent of deaths were among serous (n=515), 10.6% clear cell (n=75), 7.9% endometrioid (n=56), 2.3% were among mucinous (n=16), and 6.8% other/undifferentiated (n=48).

We genotyped 40 tagging SNPs in five telomere maintenance genes (*TERT, TRF2, TRF1, TNKS,* and *POT1*). Though we observed some suggestive associations, none met our significance threshold of 0.001. *TERT* SNP rs6882077 had a covariate-adjusted HR (95% CI) of death of 3.49 (1.11-11.02) for each additional allele variant. However, variants for this SNP are uncommon (minor allele frequency = 0.2%) and the association between this SNP and mortality did not remain significant when terms for clinical characteristics were added to the covariate-adjusted model (**Table 2**). No significant gene level associations were observed (all $p_{gene}>0.51$). Adjustment for debulking status and restricting to grade 3 ovarian cancer did not influence the results (data not shown).

When histology-specific associations were examined no significant associations between any of the telomere maintenance SNPs and death

Table 1. Characteristics of 1480 invasive epithelial ovarian cancer cases, New England Case Control Study, 1992-2008.

Characteristics	
Follow-up time (years), mean (sd)	7.3 (5.0)
Age at study entry, mean (sd)	55.8 (11.0)
OC use, n (%)	732 (49.5)
OC duration (months), mean (SD)[a]	54.9 (55.6)
Parous, n (%)	1036 (70.0)
Parity, mean (SD)[b]	2.5 (1.3)
Tubal ligation, n (%)	192 (13.0)
BMI (kg/m2), mean (SD)	26.6 (6.3)
Genital talc use, n (%)	495 (33.5)
Family history of breast or ovarian cancer, n (%)[c]	193 (13.0)
Postmenopausal, n (%)	889 (60.1)
Hormone replacement therapy use (>3 months), n (%)[d]	301 (33.9)
Ever smoker, n (%)	809 (54.7)
White, n (%)	1480 (100.0)
Histologic type, n (%)	
Serous	784 (53.0)
Endometrioid	285 (19.3)
Clear cell	224 (15.1)
Mucinous	84 (5.7)
Other	68 (4.6)
Undifferentiated	35 (2.4)
Grade, n (%)	
G1	200 (13.6)
G2	290 (19.7)
G3	861 (58.6)
Indeterminable/Unknown	108 (7.4)
Missing	11 (0.8)
Optimal debulking, n (%)[e]	
Optimally debulked	460 (53.7)
Not optimally debulked	66 (7.7)
Unknown	331 (38.6)

[a]Among oral contraceptive users. [b]Among parous women. [c]Family history is defined as first degree relative with ovarian cancer or breast cancer. [d]Among postmenopausal women. [e]Only available for cases diagnosed at Brigham and Women's Hospital or Massachusetts General Hospital.

were observed in those with serous tumors, the largest histologic subgroup (data not shown). In addition, we observed no substantive differences in the association between any of the SNPs and survival for women with serous tumors when we excluded 47 cases with low-grade tumors (data not shown). A suggestion of heterogeneity between the subtypes was observed for *TNKS* SNPs rs6982126 ($p_{heterogeneity}$=0.04) and rs1545827 ($p_{heterogeneity}$=0.03). Among those with mucinous tumors, SNP rs6982126 was inversely associated with death (HR=0.12; 95% CI 0.02-0.88) while SNP rs1545827 was associated with death (HR=3.18; 95% CI 1.21-8.32); however, these results were based on small numbers (76 and 78 cases, respectively) and no gene level associations were observed. There was no heterogeneity between subtypes for any other SNPs. No effect modification was observed by age, smoking, BMI, or estimated lifetime number of ovulatory cycles (data not shown).

Among 694 cases who received chemotherapy, *TERT* SNPs rs2736100 and rs2853676 were associated with mortality in the covariate-adjusted analyses (HR=1.18; 95% CI 1.01-1.37 and HR=1.20; 95% CI 1.03-1.40, respectively) but these associations did not meet our significance threshold and were attenuated after ad-

Table 2. Hazard ratios and 95% confidence intervals for the association between SNPs in telomere-related genes and death among invasive epithelial ovarian cancer cases, NECC 1992-2008.

					Covariate-adjusted[a]		Covariate and clinical characteristics-adjusted[b]		
					Per allele		Per allele		
Gene	rs number	n	% successfully genotyped	p HWE controls	HR	95% CI	HR	95% CI	p_{gene}[c]
TERT	RS2736122	1396	94.3%	0.63	0.95	(0.84-1.08)	0.98	(0.87-1.12)	0.51
TERT	RS2075786	1414	95.5%	0.04	0.97	(0.87-1.09)	0.99	(0.88-1.10)	
TERT	RS4246742	1410	95.3%	0.01	0.90	(0.77-1.05)	0.95	(0.81-1.11)	
TERT	RS6882077[d]	766	90.2%	0.90	3.49	(1.11-11.00)	2.06	(0.65-6.53)	
TERT	RS4975605	1386	93.6%	0.21	1.10	(0.99-1.22)	1.08	(0.97-1.20)	
TERT	RS10069690	1392	94.1%	0.18	1.10	(0.98-1.23)	1.10	(0.98-1.23)	
TERT	RS2242652[d]	786	92.6%	0.95	1.08	(0.92-1.27)	1.07	(0.91-1.26)	
TERT	RS2736100	1388	93.8%	0.95	1.02	(0.92-1.14)	1.02	(0.91-1.13)	
TERT	RS2853676	1415	95.6%	0.60	1.12	(1.00-1.26)	1.12	(1.00-1.26)	
TERT	RS2736098	1397	94.4%	0.59	1.01	(0.90-1.14)	1.03	(0.92-1.16)	
TERT	RS7726159[d]	611	96.8%	0.23	1.00	(0.83-1.20)	1.06	(0.89-1.27)	
TRF2	RS251796	1419	95.9%	0.01	1.12	(1.00-1.26)	1.11	(0.99-1.25)	0.61
TRF2	RS153045	1415	95.6%	0.01	1.04	(0.93-1.16)	1.03	(0.92-1.15)	
TRF2	RS3785074	1417	95.7%	0.55	0.96	(0.85-1.09)	0.96	(0.85-1.09)	
TRF2	RS166134[d]	790	93.1%	0.63	1.25	(0.74-2.09)	0.96	(0.57-1.62)	
TRF2	RS8061382[d]	782	92.1%	0.35	0.73	(0.48-1.11)	0.88	(0.58-1.36)	
TRF1	RS2975842	1412	95.4%	0.96	1.00	(0.90-1.12)	0.99	(0.89-1.11)	0.73
TRF1	RS2975852	1425	96.3%	0.66	0.96	(0.85-1.07)	0.97	(0.86-1.09)	
TRF1	RS6989159[d]	795	93.6%	0.83	0.18	(0.03-1.29)	0.32	(0.05-2.33)	
TRF1	RS6989493[d]	799	94.1%	0.93	0.56	(0.18-1.76)	0.85	(0.27-2.70)	
TRF1	RS12334686	1411	95.3%	0.18	0.96	(0.86-1.07)	0.98	(0.88-1.10)	
TRF1	RS6982126	1391	94.0%	0.44	1.04	(0.92-1.18)	1.03	(0.92-1.17)	
TRF1	RS2981096[d]	795	93.6%	0.11	1.01	(0.73-1.40)	0.98	(0.70-1.36)	
TRF1	RS10107605	1391	94.0%	0.29	1.11	(0.94-1.31)	1.05	(0.89-1.10)	
TRF1	RS1545827	1421	96.0%	0.60	0.99	(0.89-1.10)	0.99	(0.89-1.10)	
TNKS	RS1539041	1386	93.6%	0.20	0.91	(0.81-1.03)	0.93	(0.83-1.06)	0.60
TNKS	RS3802650	1413	95.5%	0.80	1.03	(0.92-1.14)	1.04	(0.94-1.16)	
TNKS	RS10509637	1397	94.4%	0.32	1.13	(0.97-1.31)	1.11	(0.95-1.29)	
TNKS	RS1772180	1420	95.9%	0.34	0.98	(0.88-1.09)	0.98	(0.88-1.09)	
TNKS	RS10509639	1425	96.3%	0.11	1.00	(0.83-1.20)	0.99	(0.82-1.19)	
TNKS	RS1772186	1383	93.4%	0.68	1.09	(0.94-1.27)	1.05	(0.90-1.23)	
TNKS	RS10881982[d]	787	92.7%	0.88	0.76	(0.50-1.17)	0.84	(0.55-1.30)	
TNKS	RS12412538	1411	95.3%	0.65	1.02	(0.90-1.14)	1.03	(0.91-1.15)	
TNKS	RS7087365	1428	96.5%	0.84	0.96	(0.86-1.08)	0.98	(0.87-1.10)	
POT1	RS929365	1371	92.6%	0.92	1.00	(0.79-1.27)	1.02	(0.80-1.29)	0.63
POT1	RS7801661	1374	92.8%	0.41	0.98	(0.87-1.10)	1.03	(0.91-1.17)	
POT1	RS11972248	1402	94.7%	0.52	1.01	(0.88-1.16)	1.03	(0.90-1.18)	
POT1	RS12532038	1412	95.4%	0.35	0.98	(0.87-1.10)	0.96	(0.86-1.08)	
POT1	RS4360236	1403	94.8%	0.17	0.94	(0.78-1.12)	0.89	(0.75-1.06)	
POT1	RS2896361	1382	93.4%	0.12	1.01	(0.91-1.13)	0.97	(0.87-1.09)	

[a]Adjusted for age (continuous), enrollment phase (1992-1997, 1998-2002, 2003-2008), study center (Massachusetts, New Hampshire), tubal ligation (yes, no), smoking (never, former/current), OC use (never, <2 years, 2-5 years, 5+ years), hormone replacement therapy (ever use, never), menopausal status (premenopausal/dubious, postmenopausal). All analyses are restricted to white women. [b]Adjusted for covariates above plus histology (serous invasive, mucinous, endometrioid, clear cell, other/undifferentiated) and grade (1/2, 3, missing/unknown/indeterminable). [c]Principal components analyses were used to determine gene-level associations with survival accounting for linkage disequilibrium between SNPs. [d]RS6882077, RS2242652, RS166134, RS8061382, RS6989159, RS6989493, RS2981096, RS10881982 were not genotyped in phase 3 and RS7726159 was not genotyped in phases 1 and 2.

justment for clinical characteristics (HR=1.13; 95% CI 0.97-1.31 and HR=1.14; 95% CI 0.98-1.34). Similar associations with *TERT* SNPs rs2736100 and rs2853676 were observed among those receiving carboplatin/cisplatin (rs2736100 covariate and clinical characteristics-adjusted HR=1.18; 95% CI 1.01-1.38 and rs2853676 covariate and clinical characteristics-adjusted HR=1.16; 95% CI 0.99-1.36). Additional analyses suggested *TERT* SNPs rs2736100 and rs2075786 and *POT1* SNP rs7801661, may be relevant to survival among women receiving taxol/paclitaxel (**Table 3**).

Among women who received and had data available on timing of first-line chemotherapy, those with the rs2853676 (covariate-adjusted HR=1.30, 95% CI=1.10-1.54) were at risk of earlier relapse than women without this polymorphism. Furthermore, rs2853676 was associated with a 38% increase in risk of relapse within 6 months of completing first-line chemotherapy (95% CI 1.01-1.88). No association was observed between any SNPs and chemorefractory disease defined as relapse within six months after primary debulking surgery.

Discussion

We observed no significant associations between SNPs in telomere maintenance genes and mortality among this cohort of invasive epithelial ovarian cancer cases. To our knowledge we are the first study to examine SNPs on telomere maintenance genes in relation to ovarian cancer survival. Previous studies have suggested that *TERT* SNPs may be associated with risk of ovarian cancer and other cancers [10-14, 16, 18, 24]. However, we did not observe any significant associations in our analyses with *TERT* SNPs or SNPs in other telomere maintenance genes (*TRF2*, *TRF1*, *TNKS*, or *POT1*) after accounting for multiple comparisons. In addition, we observed suggestive associations among women who received carboplatin/cisplatin and taxol/paclitaxel; however, power was limited in these subgroups.

Epidemiologic studies suggest risk factors vary by histologic type, particularly mucinous vs. non-mucinous [25-27]. Some genetic studies suggest that genetic susceptibility to ovarian cancer may also vary by histologic subtype [28]. Our histology specific analyses suggested histologic differences in genetic polymorphisms as SNPs on the *TNKS* gene were related to survival only in women with mucinous tumors which most closely resemble tumors of the gastrointestinal tract [29], and in some cases may be metastatic tumors from the gastrointestinal tract [30]. The enzyme Tankyrase-1, coded by the *TNKS* gene, regulates telomere length and is down-regulated in colon tumors [31]. Among colon cancer cases, lower Tankyrase-1 mRNA expression has been associated with reduced colon cancer survival [32].

Limitations of our study need to be considered. The most aggressive cases of ovarian cancer will likely be underrepresented in our study as some of these cases will die before enrollment while women who survive longer will be more likely to enroll. Therefore, if a particular SNP predisposes to the most aggressive cases we may miss this association. In addition, while our results were largely null, important associations could have been missed due to limited power in subgroup analyses and the omission of SNPs in the *TERC* gene, which have been identified in recent studies in relation to telomere length [33-35] and colorectal cancer incidence [34].

Our study has several strengths. To our knowledge, this is the first study to examine the relation between SNPs on telomere maintenance genes and ovarian cancer survival and relapse. We also have genotype data on 1,480 cases and detailed information on important clinical characteristics, predictors of telomere length, and ovarian cancer risk factors. Furthermore, we are unlikely to have confounding by ethnicity since the study population was limited to Caucasians.

In conclusion, our analyses showed no associations between polymorphisms on five telomere maintenance genes (*TERT, TRF2, TRF1, TNKS,* and *POT1*) and ovarian cancer survival. Future studies with larger populations as well as those that examine the *TERC* gene may further our understanding of what role telomeres play in ovarian cancer survival.

Acknowledgements

This work was supported by the National Cancer Institute [grants R01 CA054419 and P50 CA105009]; the Department of Defense Ovarian Cancer Academy [grant W18XWH-10-1-0208]; and the Ovarian Cancer Research Fund

Table 3. Hazard ratios and 95% confidence intervals for the association between SNPs in the TERT gene and death among invasive epithelial ovarian cancer cases who received chemotherapy, NECC 1992-2008.

		Any chemotherapy (n=694 cases)				Carboplatin or cisplatin (n=633 cases)				Taxol/paclitaxel (n=551 cases)			
		Covariate-adjusted[a]		Covariate and clinical characteristics-adjusted[b]		Covariate-adjusted[a]		Covariate and clinical characteristics-adjusted[b]		Covariate-adjusted[a]		Covariate and clinical characteristics-adjusted[b]	
		Per allele		Per allele		Per allele		Per allele		Per allele		Per allele	
Gene	rs number	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
TERT	RS2736122	0.93	(0.78-1.12)	0.95	(0.79-1.14)	0.97	(0.81-1.17)	1.00	(0.83-1.20)	0.91	(0.74-1.10)	0.91	(0.75-1.12)
TERT	RS2075786	1.09	(0.93-1.27)	1.16	(0.99-1.36)	1.07	(0.91-1.26)	1.13	(0.96-1.33)	1.15	(0.97-1.36)	1.25	(1.05-1.48)
TERT	RS4246742	0.94	(0.76-1.16)	1.07	(0.86-1.32)	0.97	(0.78-1.20)	1.11	(0.89-1.38)	0.94	(0.75-1.19)	1.05	(0.82-1.33)
TERT	RS6882077[c]	1.90	(0.46-7.82)	1.28	(0.31-5.31)	1.71	(0.23-12.67)	1.21	(0.16-9.03)	1.47	(0.19-1.12)	1.17	(0.15-8.86)
TERT	RS4975605	1.12	(0.96-1.29)	1.08	(0.94-1.25)	1.12	(0.96-1.30)	1.11	(0.95-1.29)	1.06	(0.90-1.25)	1.09	(0.93-1.28)
TERT	RS10069690	1.15	(0.97-1.35)	1.11	(0.94-1.31)	1.12	(0.94-1.33)	1.08	(0.91-1.28)	1.06	(0.88-1.27)	1.02	(0.85-1.23)
TERT	RS2242652[c]	1.17	(0.93-1.47)	1.17	(0.93-1.47)	1.14	(0.89-1.45)	1.14	(0.89-1.45)	0.98	(0.73-1.32)	0.98	(0.72-1.33)
TERT	RS2736100	1.18	(1.01-1.37)	1.13	(0.97-1.31)	1.22	(1.04-1.43)	1.18	(1.01-1.38)	1.20	(1.01-1.43)	1.16	(0.97-1.37)
TERT	RS2853676	1.20	(1.03-1.40)	1.14	(0.98-1.34)	1.21	(1.03-1.42)	1.16	(0.99-1.36)	1.15	(0.97-1.37)	1.08	(0.91-1.28)
TERT	RS2736098	1.06	(0.90-1.25)	1.10	(0.93-1.30)	1.07	(0.91-1.27)	1.13	(0.95-1.34)	1.11	(0.93-1.34)	1.18	(0.98-1.42)
TERT	RS7726159[c]	1.00	(0.79-1.27)	1.09	(0.86-1.37)	1.04	(0.82-1.33)	1.14	(0.89-1.44)	1.01	(0.80-1.29)	1.10	(0.86-1.39)
p_{gene}[d]		0.07		0.13		0.06		0.06		0.11		0.12	

[a]Adjusted for age (continuous), enrollment phase (1, 2, 3), study center (Massachusetts, New Hampshire), tubal ligation (yes, no), smoking (never, former/current), OC use (never, <2 years, 2-5 years, 5+ years), hormone replacement therapy (ever use, never), menopausal status (premenopausal/dubious, postmenopausal). All analyses are restricted to white women. [b]Adjusted for covariates above plus histology (serous invasive, mucinous, endometrioid, clear cell, other/undifferentiated) and grade (1/2, 3, missing/unknown/indeterminable). [c]RS6882077 and RS2242652 were not genotyped in phase 3 and RS7726159 was not genotyped in phases 1 and 2. [d]Principal components analyses were used to determine gene-level associations with survival accounting for linkage disequilibrium between SNPS SNPs.

Liz Tilberis Scholarship.

Abbreviations: genome wide association study, GWAS; body mass index, BMI; New England Case-Control Study, NECC; coefficient of variation, CV; hazard ratio, HR; confidence interval, CI; relative telomere length, RTL

Address correspondence to: Dr. Holly Harris, Ob/Gyn Epidemiology Center, Brigham and Women's Hospital, 221 Longwood Avenue, Boston, MA 02115-5804 Tel: 617-732-4895; Fax: 617-732-4899; E-mail: hharris3@partners.org

References

[1] Stewart SA and Weinberg RA. Telomeres: cancer to human aging. Annu Rev Cell Dev Biol 2006; 22: 531-557.

[2] Wong J and Collins K. Telomere maintenance and disease. Lancet 2003; 2003: 983-988.

[3] Savage SA, Stewart BJ, Eckert A, Kiley M, Liao JS and Chanock SJ. Genetic variation, nucleotide diversity, and linkage disequilibrium in seven telomere stability genes suggest that these genes may be under constraint. Hum Mutat 2005; 26: 343-350.

[4] Bakaysa SL, Mucci LA, Slagboom PE, Boomsma DI, McClearn GE, Johansson B and Pedersen NL. Telomere length predicts survival independent of genetic influences. Aging Cell 2007; 6: 769-774.

[5] Willeit P, Willeit J, Kloss-Brandstätter A, Kronenberg F and Kiechl S. Fifteen-Year Follow-up of Association Between Telomere Length and Incident Cancer and Cancer Mortality. JAMA 2011; 306: 42-44.

[6] Willeit P, Willeit J, Mayr A, Weger S, Oberhollenzer F, Brandstätter A, Kronenberg F and Kiechl S. Telomere Length and Risk of Incident Cancer and Cancer Mortality. JAMA 2010; 304: 69-75.

[7] Bisoffi M, Heaphy CM and Griffith JK. Telomeres: Prognostic markers for solid tumors. Int J Cancer 2006; 119: 2255-2260.

[8] Andrew T, Aviv A, Falchi M, Surdulescu GL, Gardner JP, Lu X, Kimura M, Kato BS, Valdes AM and Spector TD. Mapping Genetic Loci That Determine Leukocyte Telomere Length in a Large Sample of Unselected Female Sibling Pairs. Am J Hum Genet 2006; 78: 480-486.

[9] Slagboom PE, Droog S and Boomsma DI. Genetic determination of telomere size in humans: a twin study of three age groups. Am J Hum Genet 1994; 55: 876-882.

[10] Johnatty SE, Beesley J, Chen X, Macgregor S, Duffy DL, Spurdle AB, deFazio A, Gava N, Webb PM, Rossing MA, Doherty JA, Goodman MT, Lurie G, Thompson PJ, Wilkens LR, Ness RB, Moysich KB, Chang-Claude J, Wang-Gohrke S, Cramer DW, Terry KL, Hankinson SE, Tworoger SS, Garcia-Closas M, Yang H, Lissowska J, Chanock SJ, Pharoah PD, Song H, Whitemore AS, Pearce CL, Stram DO, Wu AH, Pike MC, Gayther SA, Ramus SJ, Menon U, Gentry-Maharaj A, Anton-Culver H, Ziogas A, Hogdall E, Kjaer SK, Hogdall C, Berchuck A, Schildkraut JM, Iversen ES, Moorman PG, Phelan CM, Sellers TA, Cunningham JM, Vierkant RA, Rider DN, Goode EL, Haviv I and Chenevix-Trench G. 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.

[11] McKay JD, Hung RJ, Gaborieau V, Boffetta P, Chabrier A, Byrnes G, Zaridze D, Mukeria A, Szeszenia-Dabrowska N, Lissowska J, Rudnai P, Fabianova E, Mates D, Bencko V, Foretova L, Janout V, McLaughlin J, Shepherd F, Montpetit A, Narod S, Krokan HE, Skorpen F, Elvestad MB, Vatten L, Njolstad I, Axelsson T, Chen C, Goodman G, Barnett M, Loomis MM, Lubinski J, Matyjasik J, Lener M, Oszutowska D, Field J, Liloglou T, Xinarianos G, Cassidy A, Vineis P, Clavel-Chapelon F, Palli D, Tumino R, Krogh V, Panico S, Gonzalez CA, Ramon Quiros J, Martinez C, Navarro C, Ardanaz E, Larranaga N, Kham KT, Key T, Bueno-de-Mesquita HB, Peeters PH, Trichopoulou A, Linseisen J, Boeing H, Hallmans G, Overvad K, Tjonneland A, Kumle M, Riboli E, Zelenika D, Boland A, Delepine M, Foglio M, Lechner D, Matsuda F, Blanche H, Gut I, Heath S, Lathrop M and Brennan P. Lung cancer susceptibility locus at 5p15.33. Nat Genet 2008; 40: 1404-1406.

[12] Petersen GM, Amundadottir L, Fuchs CS, Kraft P, Stolzenberg-Solomon RZ, Jacobs KB, Arslan AA, Bueno-de-Mesquita HB, Gallinger S, Gross M, Helzlsouer K, Holly EA, Jacobs EJ, Klein AP, LaCroix A, Li D, Mandelson MT, Olson SH, Risch HA, Zheng W, Albanes D, Bamlet WR, Berg CD, Boutron-Ruault MC, Buring JE, Bracci PM, Canzian F, Clipp S, Cotterchio M, de Andrade M, Duell EJ, Gaziano JM, Giovannucci EL, Goggins M, Hallmans G, Hankinson SE, Hassan M, Howard B, Hunter DJ, Hutchinson A, Jenab M, Kaaks R, Kooperberg C, Krogh V, Kurtz RC, Lynch SM, McWilliams RR, Mendelsohn JB, Michaud DS, Parikh H, Patel AV, Peeters PH, Rajkovic A, Riboli E, Rodriguez L, Seminara D, Shu XO, Thomas G, Tjonneland A, Tobias GS, Trichopoulos D, Van Den Eeden SK, Virtamo J, Wactawski-Wende J, Wang Z, Wolpin BM, Yu H, Yu K, Zeleniuch-Jacquotte A, Fraumeni JF, Jr., Hoover RN, Hartge P and Chanock SJ. A genome-wide association study identifies pancreatic cancer susceptibility loci on chromosomes 13q22.1, 1q32.1 and 5p15.33. Nat Genet 2010; 42: 224-228.

[13] Rafnar T, Sulem P, Stacey SN, Geller F, Gudmundsson J, Sigurdsson A, Jakobsdottir M,

Helgadottir H, Thorlacius S, Aben KK, Blondal T, Thorgeirsson TE, Thorleifsson G, Kristjansson K, Thorisdottir K, Ragnarsson R, Sigurgeirsson B, Skuladottir H, Gudbjartsson T, Isaksson HJ, Einarsson GV, Benediktsdottir KR, Agnarsson BA, Olafsson K, Salvarsdottir A, Bjarnason H, Asgeirsdottir M, Kristinsson KT, Matthiasdottir S, Sveinsdottir SG, Polidoro S, Hoiom V, Botella-Estrada R, Hemminki K, Rudnai P, Bishop DT, Campagna M, Kellen E, Zeegers MP, de Verdier P, Ferrer A, Isla D, Vidal MJ, Andres R, Saez B, Juberias P, Banzo J, Navarrete S, Tres A, Kan D, Lindblom A, Gurzau E, Koppova K, de Vegt F, Schalken JA, van der Heijden HF, Smit HJ, Termeer RA, Oosterwijk E, van Hooij O, Nagore E, Porru S, Steineck G, Hansson J, Buntinx F, Catalona WJ, Matullo G, Vineis P, Kiltie AE, Mayordomo JI, Kumar R, Kiemeney LA, Frigge ML, Jonsson T, Saemundsson H, Barkardottir RB, Jonsson E, Jonsson S, Olafsson JH, Gulcher JR, Masson G, Gudbjartsson DF, Kong A, Thorsteinsdottir U and Stefansson K. Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. Nat Genet 2009; 41: 221-227.

[14] Shete S, Hosking FJ, Robertson LB, Dobbins SE, Sanson M, Malmer B, Simon M, Marie Y, Boisselier B, Delattre JY, Hoang-Xuan K, El Hallani S, Idbaih A, Zelenika D, Andersson U, Henriksson R, Bergenheim AT, Feychting M, Lonn S, Ahlbom A, Schramm J, Linnebank M, Hemminki K, Kumar R, Hepworth SJ, Price A, Armstrong G, Liu Y, Gu X, Yu R, Lau C, Schoemaker M, Muir K, Swerdlow A, Lathrop M, Bondy M and Houlston RS. Genome-wide association study identifies five susceptibility loci for glioma. Nat Genet 2009; 41: 899-904.

[15] Stacey SN, Sulem P, Masson G, Gudjonsson SA, Thorleifsson G, Jakobsdottir M, Sigurdsson A, Gudbjartsson DF, Sigurgeirsson B, Benediktsdottir KR, Thorisdottir K, Ragnarsson R, Scherer D, Hemminki K, Rudnai P, Gurzau E, Koppova K, Botella-Estrada R, Soriano V, Juberias P, Saez B, Gilaberte Y, Fuentelsaz V, Corredera C, Grasa M, Hoiom V, Lindblom A, Bonenkamp JJ, van Rossum MM, Aben KK, de Vries E, Santinami M, Di Mauro MG, Maurichi A, Wendt J, Hochleitner P, Pehamberger H, Gudmundsson J, Magnusdottir DN, Gretarsdottir S, Holm H, Steinthorsdottir V, Frigge ML, Blondal T, Saemundsdottir J, Bjarnason H, Kristjansson K, Bjornsdottir G, Okamoto I, Rivoltini L, Rodolfo M, Kiemeney LA, Hansson J, Nagore E, Mayordomo JI, Kumar R, Karagas MR, Nelson HH, Gulcher JR, Rafnar T, Thorsteinsdottir U, Olafsson JH, Kong A and Stefansson K. New common variants affecting susceptibility to basal cell carcinoma. Nat Genet 2009; 41: 909-914.

[16] Turnbull C, Ahmed S, Morrison J, Pernet D, Renwick A, Maranian M, Seal S, Ghoussaini M, Hines S, Healey CS, Hughes D, Warren-Perry M, Tapper W, Eccles D, Evans DG, Hooning M, Schutte M, van den Ouweland A, Houlston R, Ross G, Langford C, Pharoah PD, Stratton MR, Dunning AM, Rahman N and Easton DF. Genome-wide association study identifies five new breast cancer susceptibility loci. Nat Genet 2010; 42: 504-507.

[17] Landi MT, Chatterjee N, Yu K, Goldin LR, Goldstein AM, Rotunno M, Mirabello L, Jacobs K, Wheeler W, Yeager M, Bergen AW, Li Q, Consonni D, Pesatori AC, Wacholder S, Thun M, Diver R, Oken M, Virtamo J, Albanes D, Wang Z, Burdette L, Doheny KF, Pugh EW, Laurie C, Brennan P, Hung R, Gaborieau V, McKay JD, Lathrop M, McLaughlin J, Wang Y, Tsao MS, Spitz MR, Krokan H, Vatten L, Skorpen F, Arnesen E, Benhamou S, Bouchard C, Metsapalu A, Vooder T, Nelis M, Valk K, Field JK, Chen C, Goodman G, Sulem P, Thorleifsson G, Rafnar T, Eisen T, Sauter W, Rosenberger A, Bickeboller H, Risch A, Chang-Claude J, Wichmann HE, Stefansson K, Houlston R, Amos CI, Fraumeni JF, Jr., Savage SA, Bertazzi PA, Tucker MA, Chanock S and Caporaso NE. A genome-wide association study of lung cancer identifies a region of chromosome 5p15 associated with risk for adenocarcinoma. Am J Hum Genet 2009; 85: 679-691.

[18] Terry KL, Tworoger SS, Vitonis AF, Wong J, Titus-Ernstoff L, De Vivo I and Cramer DW. Telomere Length and Genetic Variation in Telomere Maintenance Genes in Relation to Ovarian Cancer Risk. Cancer Epidemiol Biomarkers Prev 2012; 21: 504-512.

[19] Catarino R, Araújo A, Coelho A, Gomes M, Nogueira A, Lopes C and Medeiros RM. Prognostic Significance of Telomerase Polymorphism in Non–Small Cell Lung Cancer. Clin Cancer Res 2010; 16: 3706-3712.

[20] Simon M, Hosking FJ, Marie Y, Gousias K, Boisselier B, Carpentier C, Schramm J, Mokhtari K, Hoang-Xuan K, Idbaih A, Delattre J-Y, Lathrop M, Robertson LB, Houlston RS and Sanson M. Genetic Risk Profiles Identify Different Molecular Etiologies for Glioma. Clin Cancer Res 2010; 16: 5252-5259.

[21] Harris HR, Cramer DW, Vitonis AF, DePari M and Terry KL. Folate, vitamin B6, vitamin B12, methionine and alcohol intake in relation to ovarian cancer risk. Int J Cancer 2011; doi: 10.1002/ijc.26455.

[22] Vitonis A, Titus-Ernstoff L and Cramer D. Assessing ovarian cancer risk when considering elective oophorectomy at the time of hysterectomy. Obstet Gynecol 2011; 117: 1042-1050.

[23] Gauderman WJ, Murcray C, Gilliland F and Conti DV. Testing association between disease and multiple SNPs in a candidate gene. Genet Epidemiol 2007; 31: 383-395.

[24] Landi MT, Chatterjee N, Yu K, Goldin LR, Goldstein AM, Rotunno M, Mirabello L, Jacobs

K, Wheeler W, Yeager M, Bergen AW, Li Q, Consonni D, Pesatori AC, Wacholder S, Thun M, Diver R, Oken M, Virtamo J, Albanes D, Wang Z, Burdette L, Doheny KF, Pugh EW, Laurie C, Brennan P, Hung R, Gaborieau V, McKay JD, Lathrop M, McLaughlin J, Wang Y, Tsao M-S, Spitz MR, Wang Y, Krokan H, Vatten L, Skorpen F, Arnesen E, Benhamou S, Bouchard C, Metsapalu A, Vooder T, Nelis M, Välk K, Field JK, Chen C, Goodman G, Sulem P, Thorleifsson G, Rafnar T, Eisen T, Sauter W, Rosenberger A, Bickeböller H, Risch A, Chang-Claude J, Wichmann HE, Stefansson K, Houlston R, Amos CI, Fraumeni Jr JF, Savage SA, Bertazzi PA, Tucker MA, Chanock S and Caporaso NE. A Genome-wide Association Study of Lung Cancer Identifies a Region of Chromosome 5p15 Associated with Risk for Adenocarcinoma. The American Journal of Human Genetics 2009; 85: 679-691.

[25] Gates MA, Rosner BA, Hecht JL and Tworoger SS. Risk Factors for Epithelial Ovarian Cancer by Histologic Subtype. Am J Epidemiol 2010; 171: 45-53.

[26] Purdie DM, Webb PM, Siskind V, Bain CJ and Green AC. The Different Etiologies of Mucinous and Nonmucinous Epithelial Ovarian Cancers. Gynecol Oncol 2003; 88: S145-S148.

[27] Soegaard M, Jensen A, Høgdall E, Christensen L, Høgdall C, Blaakær J and Kjaer SK. Different Risk Factor Profiles for Mucinous and Nonmucinous Ovarian Cancer: Results from the Danish MALOVA Study. Cancer Epidemiol Biomarkers Prev 2007; 16: 1160-1166.

[28] Song H, Ramus SJ, Tyrer J, Bolton KL, Gentry-Maharaj A, Wozniak E, Anton-Culver H, Chang-Claude J, Cramer DW, DiCioccio R, Dork T, Goode EL, Goodman MT, Schildkraut JM, Sellers T, Baglietto L, Beckmann MW, Beesley J, Blaakaer J, Carney ME, Chanock S, Chen Z, Cunningham JM, Dicks E, Doherty JA, Durst M, Ekici AB, Fenstermacher D, Fridley BL, Giles G, Gore ME, De Vivo I, Hillemanns P, Hogdall C, Hogdall E, Iversen ES, Jacobs IJ, Jakubowska A, Li D, Lissowska J, Lubinski J, Lurie G, McGuire V, McLaughlin J, Medrek K, Moorman PG, Moysich K, Narod S, Phelan C, Pye C, Risch H, Runnebaum IB, Severi G, Southey M, Stram DO, Thiel FC, Terry KL, Tsai Y-Y, Tworoger SS, Van Den Berg DJ, Vierkant RA, Wang-Gohrke S, Webb PM, Wilkens LR, Wu AH, Yang H, Brewster W, Ziogas A, Houlston R, Tomlinson I, Whittemore AS, Rossing MA, Ponder BAJ, Pearce CL, Ness RB, Menon U, Kjaer SK, Gronwald J, Garcia-Closas M, Fasching PA, Easton DF, Chenevix-Trench G, Berchuck A, Pharoah PDP and Gayther SA. A genome-wide association study identifies a new ovarian cancer susceptibility locus on 9p22.2. Nat Genet 2009; 41: 996-1000.

[29] Kurman RJ and Shih I-M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer—Shifting the paradigm. Hum Pathol 2011; 42: 918-931.

[30] Seidman J, Kurman R and Ronnett B. Primary and metastatic mucinous adenocarcinomas in the ovaries: incidence in routine practice with a new approach to improve intraoperative diagnosis. Am J Surg Pathol 2003; 27: 985-993.

[31] Shebzukhov Y, Lavrik I, Karbach J, Khlgatian S, Koroleva E, Belousov P, Kashkin K, Knuth A, Jager E, Chi N, Kuprash D and Nedospasov S. Human tankyrases are aberrantly expressed in colon tumors and contain multiple epitopes that induce humoral and cellular immune responses in cancer patients. Cancer Immunol Immunother 2008; 57: 871-881.

[32] Gelmini S, Poggesi M, Pinzani P, Mannurita S, Cianchi F, Valanzano R and Orlando C. Distribution of Tankyrase-1 mRNA expression in colon cancer and its prospective correlation with progression stage. Oncol Rep 2006; 16: 1261-1266.

[33] Codd V, Mangino M, van der Harst P, Braund PS, Kaiser M, Beveridge AJ, Rafelt S, Moore J, Nelson C, Soranzo N, Zhai G, Valdes AM, Blackburn H, Mateo Leach I, de Boer RA, Goodall AH, Ouwehand W, van Veldhuisen DJ, van Gilst WH, Navis G, Burton PR, Tobin MD, Hall AS, Thompson JR, Spector T and Samani NJ. Common variants near TERC are associated with mean telomere length. Nat Genet 2010; 42: 197-199.

[34] Jones AM, Beggs AD, Carvajal-Carmona L, Farrington S, Tenesa A, Walker M, Howarth K, Ballereau S, Hodgson SV, Zauber A, Bertagnolli M, Midgley R, Campbell H, Kerr D, Dunlop MG and Tomlinson IPM. TERC polymorphisms are associated both with susceptibility to colorectal cancer and with longer telomeres. Gut 2012; 61: 248-254.

[35] Prescott J, Kraft P, Chasman DI, Savage SA, Mirabello L, Berndt SI, Weissfeld JL, Han J, Hayes RB, Chanock SJ, Hunter DJ and De Vivo I. Genome-Wide Association Study of Relative Telomere Length. PLoS ONE 2011; 6: e19635.