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Telomere length and genetic analyses in population-based studies of endometrial cancer risk

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Abstract

Background—Telomeres are protective structures at the ends of linear chromosomes, regulated by a host of associated proteins. When telomeres become dysfunctional genomic instability ensues. The vast majority of cells undergo apoptosis, though a rare cell may survive and become tumorigenic.

Methods—We used conditional logistic regression to examine relative telomere length in peripheral blood leukocytes, genetic variants at telomere maintenance gene loci (*TERT, TNKS2, POT1, TERF1, TERF2*), and endometrial cancer risk in case-control studies nested within the Nurses' Health Study and the Women's Health Study.

Results—Relative telomere length was significantly inversely correlated with BMI and weight gain since age 18. We did not observe a relationship between relative telomere length and endometrial cancer risk. Women in the shortest quartile had a multivariate-adjusted odds ratio (OR) = 1.20 (95% confidence interval (CI) = 0.73 – 1.96, P_{trend} = 0.37) compared with women in the longest quartile. We found an elevation in endometrial cancer risk among women carrying at least one minor allele of rs2736122 (*TERT*; OR = 1.18, 95% CI = 1.01 – 1.38) or RS12412538 (*TNKS2*; OR = 1.16, 95% CI = 1.00 – 1.34).

Conclusion—Relative telomere length was not associated with endometrial cancer risk. Other aspects of telomere maintenance remain to be explored.

Keywords

telomere length; polymorphism; uterus; cancer; leukocytes

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Relative telomere length was not associated with endometrial cancer risk in this prospective population-based study. Elevated endometrial cancer risk associated with polymorphisms at telomere maintenance gene loci suggests other aspects of telomere dynamics may be involved in endometrial carcinogenesis.

Introduction

Telomeres are structures at the ends of linear chromosomes composed of proteins complexed to long hexameric $(TTAGGG)_n$ DNA repeats charged with the critical role of maintaining structural integrity [1]. Due to limitations in lagging strand synthesis, human telomeres shorten by 50-100 bp per mitotic division [2]. Therefore, telomeres are analogous to a "molecular clock" reflecting the number of divisions a cell has undergone [3]. When telomeres shorten to a critical length the cell enters replicative senescence. However, according to the telomere hypothesis of carcinogenesis, if the Rb and p53 signaling pathways have been inactivated, cell division continues further eroding telomeres with a concurrent increase in genomic instability. Upon reaching crisis, a second proliferation block characterized by gross chromosomal aberrations, the vast majority of these cells undergo apoptosis. A rare cell may escape by re-expressing telomerase reverse transcriptase (TERT), which may facilitate tumorigenesis. Reactivation of telomerase is detected in >90% of human tumors, making it one of the most common abnormalities in cancer cells [1].

Endometrial cancer, the most common gynecologic malignancy among women in the US, arises from the epithelial lining of the uterine corpus. Younger age at menarche, older age at menopause, and nulliparity have been associated with endometrial cancer risk [4] suggesting a higher number of total lifetime menstrual cycles may lead to accelerated telomere erosion in endometrial cells. To date, data to support this hypothesis are limited. A few small studies found shortened telomere length in endometrial tumor tissue compared to adjacent normal [5-7], whereas another did not find significant differences in length between adjacent normal, endometrial hyperplasia and endometrial cancer [8]. It is difficult for such studies to distinguish whether short telomeres trigger early carcinogenic events or are a byproduct of the cancer cell's high proliferative rate.

Telomere length in peripheral blood leukocytes (PBL) has emerged as a potential biomarker for chronic disease risk. Short telomere length in PBL has been associated with increased risk of some cancers (e.g. head and neck, esophageal, lung, renal cell, and bladder) [9-13], but not all (melanoma and breast) [14-18]. Our study is the first to examine the relationship between relative telomere length (RTL) and endometrial cancer risk using 2 nested case-control studies, the Nurses' Health Study and the Women's Health Study. We also investigated whether common variation at 5 telomere maintenance gene loci (*TERT, TNKS2, POT1, TERF1, TERF2*) were associated with endometrial cancer risk.

Materials and Methods

Study populations

The Nurses' Health Study (NHS) is a prospective cohort study of 121,700 female registered nurses in 11 US states who were 30-55 years of age at enrollment. In 1976 and biennially thereafter, self-administered questionnaires gather detailed information on lifestyle, menstrual and reproductive factors, and medical history. During 1989-90, blood samples were collected from 32,826 women. From 2000-2002, buccal cell samples were collected from 33,040 women who did not provide a blood sample. Eligible cases consisted of women with biospecimen samples diagnosed with pathologically confirmed invasive endometrial cancer anytime after cohort inception up to June 1, 2004 with no prior cancer diagnosis except nonmelanoma skin cancer. Controls were randomly selected women who had not had a hysterectomy and were free of cancer (except non-melanoma skin cancer) up to and including the questionnaire cycle in which the case was diagnosed. Controls were matched to cases according to age, menopausal status and postmenopausal hormone use (current versus not current) at biospecimen collection, and type of biospecimen. The complete nested case-control study consisted of 551 endometrial cancer cases and 1320 matched controls.

For this analysis, participants were restricted to Caucasian women: 544 cases (300 blood, 244 buccal) and 1296 controls (817 blood, 479 buccal). Completion of the self-administered questionnaire and submission of the biospecimen was considered to imply informed consent. The Nurses' Health Study protocol was approved by the Human Research Committee of Brigham and Women's Hospital, Boston, MA.

The Women's Health Study (WHS) is a completed randomized, double-blind, placebo-controlled trial investigating the benefits and risks of aspirin and vitamin E in the primary prevention of cancer and cardiovascular disease among 39,876 female health professionals, aged 45 or older without a history of cancer (except non-melanoma skin cancer), coronary heart disease, or cerebral vascular disease. Enrollment began in September 1992 and randomization in April 1993. Prior to randomization, blood samples were collected from 28,345 women. WHS participants completed a detailed baseline questionnaire including information on endometrial risk factors including smoking, menopausal status, PMH use, age at menarche, and BMI. Every 6 months for the first year and annually thereafter, participants were sent follow-up questionnaires. Eligible cases consisted of women with pathologically confirmed invasive endometrial cancer diagnosed after blood collection (1993-1995) and prior to June 1, 2002. Controls were randomly selected participants who had given a blood sample, had not had a hysterectomy and were free of cancer. Controls were matched to cases according to age at randomization, menopausal status and postmenopausal hormone use (current versus not current) at time of blood draw. The complete nested case-control study consisted of 137 endometrial cancer cases and 411 matched controls. After restricting to Caucasian women, 130 cases and 389 controls were available for analysis. Written informed consent was obtained from all women before entry into the trial. The Women's Health Study protocol was approved by the Human Research Committee of Brigham and Women's Hospital, Boston, MA.

Single nucleotide polymorphism (SNP) selection

We used the International Hapmap project (www.HapMap.org) to identify SNPs that effectively cover genes. Some SNPs are in linkage disequilibrium; therefore, a more efficient set of tagging SNPs captures the same genetic variation [19]. Using Haploview program version 3.12 and a minimum r^2 threshold of 0.8, we identified parsimonious intragenic tagging SNPs across telomerase reverse transcriptase catalytic subunit (*TERT*); tankyrase, TERF1-interacting ankyrin-related ADP-ribose polymerase 2 (*TNKS2*); protection of telomeres 1 homolog (*POT1*); telomeric repeat binding factor 1 (*TERF1*); and telomeric repeat binding factor 2 (*TERF2*). In addition, we genotyped a putative functional *TERT* promoter SNP, RS2735940, and a SNP further upstream, RS401681, recently associated with basal cell carcinoma, lung, bladder, prostate, and cervical cancer [20].

Genotyping Methods

Genomic DNA was extracted from peripheral blood leukocyte and buccal cell samples using the QIAmp (Qiagen, Chatsworth, CA) 96-spin blood protocol. DNA was whole-genome amplified with GE Healthcare Genomiphi (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). Genotyping was performed at the Dana Farber/Harvard Cancer Center High-Throughput Genotyping Core on whole genome amplified DNA using the 5' nuclease assay (Taqman) either on the Applied Biosystems 7900HT Sequence Detection System (Foster City, CA) or the Biotrove OpenArray® Real-Time qPCR system (Woburn, MA). Laboratory personnel were blinded to case-control status, and 5% blinded quality control samples were inserted to validate genotyping procedures; concordance for blinded samples was 100%. The percentage of missing genotyping data was <6%.

Relative Telomere Length (RTL)

Currently, it is unknown whether buccal cells are a valid DNA source for the RTL assay. Thus, we restricted measurement of RTL to participants who donated blood specimens. RTL was measured for all controls (n=1206) that provided a blood sample and for incident cases diagnosed after blood collection (n=313). PicoGreen quantitation of genomic DNA was performed using a Molecular Devices 96-well spectrophotometer. The ratio of telomere repeat copy number to a single gene copy number (T/S) was determined by a previously described modified, high-throughput version [10] of the quantitative real-time PCR telomere assay [21]. Triplicate reactions of each assay were performed on each sample. RTL is reported as the exponentiated sample T/S ratio corrected for a reference sample. Telomere and single-gene assay coefficients of variation (CV) for triplicates were 0.87% and 1.09%, respectively. The CV for RTL of quality control samples was 14%.

Statistical Analysis

Chi-square tests determined whether polymorphisms were in Hardy-Weinberg equilibrium among controls within each study population. Participants with outlier RTL values, identified using an extreme studentized deviate many-outlier procedure [22] (1 case, 6 controls), or missing RTL (6 cases, 19 controls) were excluded from RTL analyses. The Wilcoxon rank sum test did not find a significant difference in RTL distribution between study populations (P = 0.07). Subsequent analyses used the distribution of all controls to categorize RTL by median or quartile values. Linear regression was used to examine age- and study-adjusted associations between RTL and endometrial cancer risk factors (Table I). Midpoints within categories of usage (1-4, 5-14, 15-24, 25-34, 35-44, and 45 or more) were used for number of cigarettes per day. We used conditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CI). For RTL and endometrial cancer risk analyses, we excluded women missing information on smoking, age at first birth, parity, or duration of PMH use. Gene dosage effects were modeled by assigning a value of 0, 1, or 2 to a genotype trend variable according to a participant's number of minor alleles. Individuals homozygous for the common variant comprised the reference category. A Wald test was used to calculate the p-value for trend. Covariates included in the multivariate models are listed in Tables II and III. DerSimonian and Laird random effects models combined results from the cohorts after testing for heterogeneity. As an exploratory analysis, we used the Wald test to test for additive interactions between polymorphisms and smoking status (never vs. ever), age at menarche (< 13 vs. ≥ 13 years), and BMI (≤ 25 vs. > 25 kg/m^2). We used linear regression models adjusted for age and study to examine associations between polymorphisms and natural logarithm transformed RTL values. All *P* values are two-sided; *P* values < 0.05 were considered statistically significant. We used SAS Version 9.1 software (SAS Institute, Cary, NC).

Results

Descriptive characteristics of the study populations have been published [23]. Briefly, mean age at biospecimen collection was similar among cases and controls as expected from the matched design. In both cohorts, cases had greater mean BMI at diagnosis and were more likely to have never smoked than controls. Most participants were postmenopausal at diagnosis. Among postmenopausal women, cases were more likely to have used postmenopausal hormones.

Based on 1181 controls with blood specimens that were successfully assayed, we examined the relationship of RTL with several endometrial cancer risk factors (Table I). RTL showed significant inverse relationships with age (P_{trend} = 0.03) and BMI at blood draw (P_{trend} = 0.003). Weight gain from age 18 until blood draw among NHS participants showed a

significant inverse association with RTL (P_{trend} = 0.001). Among all controls, we found a highly significant positive association with age at menarche (P_{trend} < 0.001), where women who had a later age at menarche tended to have longer RTL. RTL distributions did not differ by smoking status (p=0.41). RTL was also not associated with cigarettes per day among ever smokers, oral contraceptive use, or age at menopause ($P_{trend} \geq 0.34$).

To minimize the potential for reverse causation, we restricted RTL analyses to 279 women with incident endometrial cancer diagnosed after blood collection and their 791 matched controls. We did not observe a significant relationship between RTL and endometrial cancer risk (P_{trend} = 0.37; Table II). Women in the shortest quartile of RTL (1st quartile) had a multivariate adjusted OR = 1.20 (95% CI = 0.73 – 1.96) compared to women in the longest quartile of RTL (4th quartile). This risk was attenuated when restricted to women postmenopausal at diagnosis (OR = 1.04, 95% CI = 0.61 – 1.79).

Using blood and buccal cell DNA samples from all women (674 cases, 1685 controls), we assessed whether endometrial cancer risk was associated with single nucleotide polymorphisms (SNPs) at telomere maintenance gene loci. Based on a Bonferroni corrected p-value of 0.0012, departure from Hardy-Weinberg equilibrium in the control populations was evident for 6 of the 41 SNPs genotyped [RS2242652 (*TERT*), rs2736098 (*TERT*), rs11972248 (*POT1*), rs929365 (*POT1*), rs1530941 (*TNKS2*), rs166134 (*TERF2*)] and thus were excluded from analyses. We observed positive multivariate-adjusted per allele increases in endometrial cancer risk with rs2736122 (*TERT*; OR = 1.18, 95% CI = 1.01 – 1.38) and RS12412538 (*TNKS2*; OR = 1.16, 95% CI = 1.00 – 1.34) (Table III). Too few minor allele carriers of RS6882077 (*TERT*), RS6989159 (*TERF1*), and RS6989493 (*TERF1*) were available for analysis. In multivariate analyses, p-values for tests of heterogeneity comparing NHS and WHS results were >0.05 for all except one SNP, RS153045 (*TERF2*). No association with endometrial cancer risk was observed for RS153045 (OR = 0.94, 95% CI = 0.80 – 1.11) in the NHS, whereas a positive association with risk was observed (OR = 1.45, 95% CI = 1.02 – 2.08) in the WHS.

Oxidative stress preferentially damages telomeres, contributing to telomere shortening [24]. Obesity and smoking are associated with an increase in systemic oxidative stress [25, 26] and, as discussed below, age at menarche may serve as a proxy for adolescent adiposity. Thus, we conducted exploratory analyses of SNP-environment interactions with smoking, BMI, and age at menarche. One additive interaction was observed at p < 0.05 between RS401681 (*TERT*) and BMI. However, when stratified by BMI, RS401681 was not associated with endometrial cancer risk.

Our rationale for investigating common variation at these loci was based on the notion that genetic variants tagged by these markers influence protein expression levels and therefore would have an effect on telomere length. To test our assumption, we examined the relationship between the SNPs in our study and RTL. We did not find significant associations between the SNPs analyzed and RTL, adjusted for age and study.

Discussion

Since the endometrial surface is a highly proliferative tissue, according to the telomere hypothesis of carcinogenesis, one might expect that women with shorter RTL would be at greater risk of endometrial cancer. We did not find evidence to support an association with RTL as measured in peripheral blood leukocytes among 279 incident invasive endometrial cancer cases and 791 matched controls nested within the Nurses' Health Study and Women's Health Study. Women in the shortest RTL quartile were not at significantly greater risk compared to women in the longest RTL quartile (OR = 1.20, 95% CI = 0.73 – 1.96; P_{trend} =

0.37). Although, our power was limited by the small sample size having only 35% power to detect a significant trend.

DNA from 674 endometrial cancer cases and 1685 matched controls were genotyped for tag SNPs across gene loci containing the core proteins that localize to telomeres in order to shape and tightly regulate the length of telomeres[27, 28]. An elevation in risk was observed among women carrying variant alleles of RS2736122 at the *TERT* locus (P=0.03) and RS12412538 at the *TNKS2* locus (P=0.05). However, after adjusting the significance level using either a Bonferroni correction or the less conservative False Discovery Rate procedure [29], neither of these SNPs reached significance. RS153045 at the *TERF2* locus displayed significant heterogeneity between the studies. This SNP was not associated with endometrial cancer risk in NHS, but showed a 45% increased risk per variant allele in WHS. We believe the heterogeneity was due to chance. After adjustment for multiple comparisons, Rafnar et al observed that RS401681 at the *TERT-CLPTM1L* locus was significantly associated with basal cell carcinoma (OR=1.25), lung (OR=1.15), bladder (OR=1.12), prostate (OR=1.07), and cervical cancer (1.31), but not with breast (OR=0.98) or endometrial cancer (OR=1.21) [20]. We had 80% power to detect an OR=1.21 between RS401681 and endometrial cancer risk. Our study supports a null association between RS401681 and endometrial cancer risk. RTL was not associated with the SNPs analyzed in this study, which is not entirely surprising given the constraint on nucleotide diversity observed in these genes [30].

This is the first population-based investigation of the relationship between RTL, telomere-related gene SNPs and endometrial cancer risk. Whereas some studies found evidence for telomere attrition within endometrial tumors compared to matched normal adjacent tissue[5-7], a recent report on telomere dynamics during uterine carcinogenesis did not find consistent telomere shortening among endometrial cancer samples compared to surrounding normal tissue. An increase in telomere length was observed in roughly a third of patients [8]. Additionally, Type I endometrial cancers, which make up the majority of endometrial tumors, generally lack defects in the p53 gene and associated chromosomal instability [31], two key components of the telomere hypothesis of carcinogenesis [1]. Over 95% of cases in our study were classified as Type I endometrial tumors. Our results and observations from these prior studies suggest telomere shortening may not be a major mechanism of Type I endometrial carcinogenesis.

We observed statistically significant inverse correlations with age, BMI, and weight gain from age 18. Telomere length decreases with age as a result of the end replication problem. Obesity, a state of chronic inflammation and oxidative stress [25], is believed to contribute to telomere attrition [32], which has been observed in some prior studies of women [33-36]. We also observed a significant association between longer RTL and later age at menarche ($P_{trend} < 0.001$). We do not have a plausible biological reason to believe the onset of menarche would have a direct effect on telomere length or vice versa. The positive correlation between RTL and age at menarche was attenuated slightly, but still significant after adjusting for BMI at diagnosis (data not shown). Since adolescent body fatness has been inversely associated with age at menarche [37, 38], the observed RTL association with age at menarche may actually reflect an association with adolescent body size.

We cannot be certain that RTL measured in blood reflects telomere length in endometrial tissue. However, telomere lengths are highly synchronized in fetal tissues [39] and at birth among white blood cells, umbilical artery cells, and skin cells[40, 41]. Though variation in telomere length increases with age, studies that have compared telomere length of blood DNA with that of matched skin [42, 43], synovial tissue [43], or fibroblasts [44] in older participants have found significant correlations between the pairs of tissues. Interindividual variation in telomere length far exceeds the variation between different tissues from the

same individual[40, 41]. This suggests blood serves as an adequate proxy for non-malignant endometrial tissue.

Our analyses benefit from the nested case-control design as cases and controls were drawn from well-characterized relatively homogeneous populations limiting selection bias. To prevent invalid risk estimates due to the limitations of retrospective case-control studies, such as cancer treatment and/or the disease itself influencing telomere length, we restricted telomere length analyses to endometrial cancer cases diagnosed after blood collection. Cases were followed for a median of 5.9 years prior to diagnosis (range: 1 month to 14.3 years). Estimates were similar after excluding cases diagnosed within 12 months of blood draw (n=30).

Due to recent evidence suggesting racial differences in telomere length dynamics [40], we restricted our analyses to white women as most women in the NHS and WHS are Caucasian, limiting the generalizability of our results. Recent studies have also demonstrated differences in telomere attrition rates between individuals, which were mainly dictated by baseline telomere length[40, 45-47]. We were not able to assess telomere attrition rate as a risk factor since we were only able to measure telomere length at one point in time. Nevertheless, Nordfjäll et al. [47] found comparable telomere attrition rates among individuals who later developed cancer and those who did not. Since RTL distributions were similar among cases and controls within our study, we have reason to believe attrition rates between these two groups would be alike as well.

In summary, we did not observe a significant elevation in endometrial cancer risk associated with shorter telomere lengths. We observed a nominal elevation in endometrial cancer risk associated with genetic variants at the *TERT* and *TNKS2* loci. Overall, our data provide little support for leukocyte RTL as a biomarker of endometrial cancer risk among white women. Additional prospective epidemiologic studies are needed to confirm our findings as well as explore the relationship of telomere length dynamics in other racial and ethnic groups.

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Table 1

Age and Study Standardized Characteristics by Relative Telomere Length Quartiles among Controls

Characteristic	Quartile 1* (0.19-0.84)	Quartile 2 (0.844-1.189)	Quartile 3 (1.19-1.57)	Quartile 4 (1.58-7.7)	P-values†
Age at blood draw (years)	59.4	61.0	58.6	57.0	0.03
Cigarettes/day among ever smokers	17.7	17.5	16.4	16.7	0.58
BMI at blood draw (kg/m^2)	25.9	25.6	25.3	24.8	0.003
Weight gain from age 18 to blood draw (kg)‡	12.7	11.6	10.3	9.1	0.001
Age at menarche (years)	12.5	12.4	12.7	12.8	<0.001
Ever oral contraceptive use (%)	48	49	50	50	0.76
Age at menopause (years)	48.4	48.5	48.8	48.5	0.34

*Quartile 1 represents shortest quartile of relative telomere length

†Linear regression p-values for quartiles of RTL, adjusted for age and study

‡NHS controls only

Table 2

Association Between Relative Telomere Length and Endometrial Cancer Risk

RTL	Cases, n (%)	Controls, n (%)	OR (95% CI)*	OR (95% CI)†
4th quartile‡	65 (23.3)	203 (25.7)	1.00	1.00
3rd quartile	68 (24.4)	203 (25.7)	1.05 (0.70 - 1.58)	1.05 (0.67 - 1.64)
2nd quartile	77 (27.6)	192 (24.3)	1.35 (0.76 - 2.41)	1.40 (0.81 - 2.39)
1st quartile	69 (24.7)	193 (24.4)	1.23 (0.80 - 1.89)	1.20 (0.73 - 1.96)
			P_{trend} = 0.24	P_{trend} = 0.37
Above median	133 (47.7)	406 (51.3)	1.00	1.00
Below median	146 (52.3)	385 (48.7)	1.24 (0.77 - 1.98)	1.24 (0.81 - 1.89)

RTL, relative telomere length; OR, odds ratio; CI, confidence interval

*Conditional logistic regression analysis adjusted for matching factors.

†Conditional logistic regression analysis additionally adjusted for age at menarche, age at first birth and parity, smoking status at diagnosis, BMI at diagnosis, age at menopause, recent PMH use, and first-degree family history of colon cancer.

‡4th quartile represents longest quartile of relative telomere length

Table 3

Association Between Telomere Maintenance Gene SNPs and Endometrial Cancer Risk

Gene	RS number	N‡	MAF	Per allele* OR	95% CI	Per allele† OR	95% CI
	RS401681	2198	0.45	1.04	(0.91 - 1.19)	1.03	(0.89 - 1.18)
	RS2735940	2153	0.50	0.97	(0.77 - 1.21)	0.95	(0.80 - 1.13)
	RS2853676	2256	0.26	1.08	(0.93 - 1.26)	1.06	(0.91 - 1.24)
	RS2736100	2076	0.45	1.09	(0.91 - 1.31)	1.10	(0.95 - 1.29)
TERT	RS10069690	2229	0.25	1.09	(0.94 - 1.28)	1.08	(0.92 - 1.26)
	RS4975605	2142	0.47	0.93	(0.81 - 1.07)	0.95	(0.83 - 1.09)
	RS4246742	2259	0.16	0.93	(0.78 - 1.12)	0.94	(0.78 - 1.13)
	RS2075786	2216	0.36	0.94	(0.82 - 1.08)	0.96	(0.83 - 1.11)
	RS2736122	2282	0.25	1.15	(0.99 - 1.34)	1.18	(1.01 - 1.38)
	RS3802650	2278	0.48	1.19	(0.86 - 1.65)	1.17	(0.86 - 1.59)
	RS10509637	2237	0.18	1.00	(0.74 - 1.34)	1.00	(0.73 - 1.37)
	RS1772180	2263	0.43	0.86	(0.68 - 1.10)	0.87	(0.68 - 1.12)
TNKS2	RS10509639	2274	0.09	1.03	(0.81 - 1.31)	1.05	(0.82 - 1.34)
	RS1772186	2237	0.17	0.99	(0.79 - 1.24)	0.98	(0.76 - 1.27)
	RS10881982	2198	0.03	1.25	(0.35 - 4.44)	0.99	(0.34 - 2.94)
	RS12412538	2244	0.30	1.13	(0.98 - 1.30)	1.16	(1.00 - 1.34)
	RS7087365	2249	0.30	1.11	(0.96 - 1.27)	1.12	(0.97 - 1.29)
	RS4360236	2224	0.12	0.97	(0.79 - 1.20)	0.94	(0.76 - 1.16)
POT1	RS12532038	2247	0.33	0.99	(0.78 - 1.25)	0.99	(0.78 - 1.27)
	RS7801661	2218	0.28	1.01	(0.87 - 1.18)	1.02	(0.87 - 1.18)
	RS2896361	2246	0.39	1.04	(0.90 - 1.19)	1.04	(0.90 - 1.20)
	RS2975842	2260	0.42	1.05	(0.92 - 1.20)	1.05	(0.92 - 1.21)
	RS2975852	2243	0.27	1.03	(0.89 - 1.19)	1.02	(0.88 - 1.18)
	RS12334686	2243	0.34	1.02	(0.88 - 1.18)	1.02	(0.88 - 1.18)
TERF1	RS6982126	2242	0.26	0.94	(0.81 - 1.10)	0.93	(0.80 - 1.09)
	RS2981096	2280	0.05	0.94	(0.70 - 1.26)	0.85	(0.53 - 1.37)
	RS10107605	2250	0.13	0.96	(0.78 - 1.17)	0.98	(0.80 - 1.20)
	RS1545827	2283	0.45	1.01	(0.89 - 1.15)	1.01	(0.88 - 1.15)

Gene	RS number	N‡	MAF	Per allele* OR	95% CI	Per allele† OR	95% CI
	RS8061382	2207	0.03	0.73	(0.49 - 1.08)	0.74	(0.49 - 1.12)
TERF2	RS3785074	2226	0.27	0.95	(0.82 - 1.11)	0.95	(0.81 - 1.11)
	RS251796	2252	0.30	1.04	(0.84 - 1.28)	1.09	(0.80 - 1.48)

OR, odds ratio; CI, confidence interval

*Conditional logistic regression analysis adjusted for matching factors.

†Conditional logistic regression analysis additionally adjusted for age at menarch, BMI at diagnosis, and first-degree family history of colon cancer.

‡Numbers do not sum due to missing genotypes.