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Original Article

Genetic and lifestyle influence on telomere length and subsequent risk of colon cancer in a case control study

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Abstract: Background: Telomeres cap the ends of chromosomes and help maintain genomic stability and integrity. Telomere length (TL) has been linked to a number of diseases, including a variety of cancers; however, the association between TL and risk for colorectal cancer is unclear. Methods: We investigate the association between genetic, diet, and lifestyle factors and TL and the association between TL and colorectal cancer using data from a population-based case-control study of colon (249 cases and 371 controls) and rectal cancer (276 cases and 372 controls) conducted in Utah. DNA samples came from immortalized cell lines for colon cancer and directly from whole blood for rectal cancer. We genotyped 11 single nucleotide polymorphisms in five genes associated with telomeres, *TERT*, *MEN1*, *MRE11A*, *RECQL5*, and *TNKS.* Results: TL was measured using quantitative PCR. *TERT* rs2853676 (p=0.044) and *RECQL5* rs820152 (p=0.001) were associated with TL at <0.05 level of significance. After adjusting for age and sex, BMI and cigarette smoking were significantly inversely associated with TL among controls. Use of aspirin/NSAIDs interacted significantly with *TERT* rs10069690 and rs2242652 to alter TL. Longer TL was significantly associated with reduced colon cancer risk after adjusting for age and sex (OR = 0.94 95% confidence intervals 0.89-0.99 per decile of TL). Further adjustment for BMI and cigarette smoking attenuated the association so that it was no longer significant. Conclusions: In summary several genetic and lifestyle factors were observed to influence TL. These factors also appear to confound associations between TL and colon cancer.

Keywords: *TERT, MEN1, RECQL5, MRE11A, TNKS*, Colorectal cancer, telomere length

Introduction

Telomeres are located on the ends of chromosomes, and play a role in maintaining genomic integrity and stability [1]. Telomeres carry out this function in two ways. First they are responsible for keeping chromosomes from shortening and losing genetic data with each chromosomal replication. Second, telomeres keep chromosomes from being recognized as double strand breaks that are targeted for repair causing ligation of chromosome ends. Telomeres consist of tandem $(TTAGGG)_n$ nucleotide repeats and in most cells shorten with aging. Telomere length (TL) is influenced by many factors; TL has been associated with several human diseases, including cardiovascular diseases, diabetes, and certain types of cancer [2-8].

The maintenance of telomeres requires a number of telomere associated factors. One such complex is the telomerase enzyme, which contains TERT, a protein, and a RNA component, TERC. TERT, Telomerase Reverse Transcriptase, uses the RNA subunit of telomerase as a template for the synthesis of single stranded DNA in the telomeric region of the chromosome, resulting in tandem nucleotide repeats that prevent the chromosome from shortening. However, telomerase activity is absent in most differentiated cells so that telomeres shorten over time. When telomeres are reduced to a certain length cells undergo senescence or apoptosis. Telomerase activity, present in most cancer cells, may block cancer cells from senescence or apoptosis [9, 10].

An earlier study found an association between certain single nucleotide polymorphisms (SNPs) within certain telomere related protein coding

genes and TL [1]. These SNPs are found in the *MEN1, MRE11A, RECQL5*, and *TNKS* genes. *MEN1* associates with the *TERT* promoter. *MRE11A* is involved in modulating t-loop formation, a structure within telomeres that helps prevent the telomere from being targeted for repair. *RECQL5* is a helicase that helps to protect genomic integrity. *TNKS* is a polymerase thought to positively regulate TL. Others have reported an association between TL and genetic variations in *TERC* [11], although the study by Mirabello and colleagues did not observe an association between *TERC* and TL [1].

In addition to genetic factors regulating TL, diet and lifestyle factors have been examined with TL in a few studies. These studies have indicated that nutrition, along with other lifestyle factors, may affect TL [12, 13]. In general, factors associated with healthier lifestyles, such as performing high levels of physical activity, not smoking cigarettes, maintaining a normal body mass index (BMI), and eating a healthy diet correspond to longer TL [8]. This information suggests that lifestyle factors associated with chronic disease may in part be from their influence on TL.

In this study we look at how genetic and lifestyle factors affect TL. Previous studies have proposed possible factors which affect TL and we have built upon these to determine which factors actually have an effect on TL. In this study we evaluate five genes, *TERT*, *MEN1*, *MRE11A*, *RECQL5*, and *TNKS*, that we hypothesize influence TL. We also evaluate *TERT-CLPTM1L* rs2853668 which is located 5.1kb upstream of *TERT* and has been associated with colon cancer in GWAS [14]. We examine dietary and lifestyle factors, including physical activity, cigarette smoking, hormone replacement therapy (HRT), and BMI with TL. We also evaluate the interaction between diet and lifestyle factors with genetic factors to determine their combined effect on TL. We examine the correlation between TL and colorectal cancer accounting for diet, lifestyle and genetic factors that may influence this association.

Methods

This study was approved by the University of Utah Institutional Review Board (IRB_00002335). All participants signed an informed consent.

Study population

The study population comes from the Diet, Activity, and Lifestyle Study (DALS) that was initiated in 1991 to study colorectal cancer [15, 16]. The DALS Study is a case-control study, drawing subjects from the Twin Cities Metropolitan Area, Kaiser Permanente Medical Care Program of Northern California (KPMCP), and seven counties in the state of Utah; the current study only used the data obtained from individuals from Utah. Two sample populations were used; one consisting of 249 colon cancer cases and 374 five-year group age-matched and sex-matched controls who were interviewed between 1991 and 1994, the other consisting of 276 rectal cancer cases and 372 five-year age group matched controls who were interviewed between 1997 and 2002. Cases were identified from the Utah Cancer registry using a rapid reporting system that allowed most cases to be identified within 30 days of diagnosis. Controls were randomly selected from driver's license and social security lists based on five-year age distribution of cases. Driver's license and social security lists provided a sampling framework that targeted the population from which the cases derived. The majority of cases were interviewed and had a blood sample drawn within 90 days of diagnosis. To be eligible for inclusion in the study participants could not have a previous history of colorectal cancer. Additionally, eligible participants were English speaking and mentally competent to give informed consent, and between 30 and 79 years of age at the time of diagnosis or selection. Cases were ruled ineligible if there was a known history of familial adenomatous polyposis, ulcerative colitis, or Crohn's disease. The study population was 96% non-Hispanic white.

Data ascertainment

Data for the study were collected by trained and certified interviewers using laptop computers [17]. Interviewers ascertained physical activity [18], weight history, medical history including use of aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), and cigarette smoking history using a health and lifestyle history questionnaire. The referent year for which exposures were ascertained was the calendar year two years prior to diagnosis for cases or selection for controls. Regular use of aspirin or NSAIDs was defined as use of these substances

at least three times per week for one month during the referent year. A detailed history of diet was obtained using a modified version of the CARDIA diet history questionnaire [19]. Dietary data were converted to nutrient values using the University of Minnesota Nutrition Coordinating Center nutrient database. Alcohol intake was ascertained as a component of the diet history questionnaire and included detailed questions on consumption patterns of beer, wine, and liquor on weekdays and weekend days. Along with recalled weight history, height and weight were measured in duplicate at the time of the interview. Current weight was based on the average of the two measurements. Body mass index (BMI) was calculated based on measured height and weight using the formula $wt(kg)/ht(m)^2$.

Genotyping analysis

Previous studies have indicated that certain Single Nucleotide Polymorphisms (SNPs) could possibly be associated with TL. From previous reports, four SNPs were selected for further investigation with TL. In this study we genotyped four SNPs; rs670358 from gene *MEN1*, rs820152 from gene *RECQL5*, rs13447720 from gene *MRE11A*, and rs11991621 from gene *TNKS*. Additionally, six SNPs associated with *TERT* were genotyped to investigate their association with TL: rs10069690, rs2242652, rs2736100, rs2736118, rs2853676, and rs4246742. The SNPs from *MEN1, RECQL5, MRE11A*, and *TNKS* were genotyped using Taqman assays. Since data regarding functionality of *TERT* SNPs are mixed, *TERT* SNPs were selected to provide coverage of the gene based on LD blocks using a Caucasian LD map and an r^2=0.8 based on hapmap data; minor allele frequency (MAF) >0.1; range=-1500 bps from the initiation codon to +1500 bps from the termination codon; and 1 SNP/LD bin and genotyped on Illumina's Golden Gate platform. We also analyzed *TERT-CLPTM1L* rs2853668 which is 5.1kb upstream of *TERT* and has been associated with colon cancer in GWAS [14].

Telomere length analysis

TL was measured using a multiplexed quantitative PCR (qPCR) method previously described by Cawthon [20]. This method modified earlier qPCR methods in which telomere signals were measured separately from single copy gene (scg) signals in order to produce a T/S ratio corresponding to TL. The multiplexed PCR analysis uses a single dye and measures both the telomere signals and single copy gene signals in the same well. This is achieved by using CG clamps to stabilize the single copy gene giving it a higher melting point. The telomere amplification signal is collected early in the thermal cycling, while the scg amplification signal is still at baseline; and the scg amplification signal is collected at later cycles at a high temperature that completely melts the telomere amplification product, sending its fluorescent signal to baseline. This design allows a single qPCR to determine the T/S ratio. Eleven samples yielded a T/S ratio greater than three and were excluded. These samples were excluded because we concluded, through gel electrophoresis, that the DNA was degraded and therefore the T/S ratio was unreliable. DNA was obtained from immortalized cell lines from study participants for the colon cancer study and from whole blood for the rectal cancer study. To assure the quality of telomere assay for these samples from different DNA origins we evaluated associations with TL separately for cases and controls from each study. However, results were similar for both populations, suggesting that different sources of DNA did not influence results. Additionally, case/control comparisons for associations with cancer were done with study-specific samples where DNA was analyzed in the same manner for both cases and controls.

Statistical analysis

Statistical analyses were performed using SAS® version 9.2 (SAS Institute, Cary, NC). The minor allele frequency (MAF) and test for Hardy-Weinberg Equilibrium (HWE) were calculated using the allele procedure among the white control populations. All SNPs assessed were in HWE among colon controls except for *TERT* rs2853668. While this SNP was out of HWE among controls, it was in HWE among cases. Since cases and controls were run intermixed in the analysis, we believe that the slight difference in HWE among controls could be from random sample variation. Adjusted multivariable linear regression models were used to estimate the association with TL for each SNP. The SNP inheritance models were selected based on similarities/differences in TL distribution by genotype. Haplotypes based on *TERT* tagSNPs that were important for TL were constructed

using the EM algorithm. Generalized linear models weighted by haplotype probabilities were used to estimate the regression coefficients.

Associations between continuous lifestyle and dietary factors and TL were measured using Spearman's partial-rank order correlation coefficient, adjusting for age, gender, and study. Generalized linear models were used to assess the age, gender, and study-adjusted means for interactions between genes and diet and lifestyle factors. All regression models including dietary variables were adjusted for total caloric intake as a continuous covariate to account for the direct correlation between calories and nutrients. The continuous dietary variables were categorized into sex-specific quartiles, collapsing the middle two levels to form the intermediate group. The p values testing the difference in mean $TL^{1/2}$ among the different SNP*exposure levels are adjusted for multiple comparisons at the gene level using the Bonferroni correction method. Associations between colon and rectal cancer and TL were assessed using multiple logistic regression models. The odds ratios (OR) and 95% confidence intervals for the various models are presented and represent the risk associated per decile of TL. Additionally we present risk per quintile of TL length for colon, rectal, and colorectal cancer.

Results

Three *TERT* SNPs were associated with TL in the controls or the combined case/control sample (**Table 1**). For *TERT* rs2736100, a borderline significant association with TL was observed in the control population (p=0.09); in the larger population of cases and controls (adjusted for disease status) this SNP became significantly associated with TL (p=0.006). *TERT* rs4246742 (p=0.04) showed a significant association in the control population, while *TERT* rs2853676 (p=0.080) was borderline significantly associated in the control population. We evaluated the haplotype of the three *TERT* SNPs that were associated with TL: rs4246742, rs2736100, and rs2853676. The most common haplotype in these three SNPs, T-G-A (41% frequency), showed a non-significant inverse association with TL (p=0.10) while the rare (present in 1.79% of the population) G-A-T haplotype was significantly directly associated with TL (p=0.010) (**Table 2**). *TERT-CLPTM1L* rs2853668 was not associated with TL in this study.

In addition to the associations observed with *TERT*, we observed that the SNP rs820152 from *RECQL5* (p=0.001) was inversely associated with TL (**Table 1**). The strength of association between *RECQL5* rs820152 and TL was strongest when cases and controls were analyzed simultaneously, rather than separately, possibly due to the larger sample size. We did not replicate previous reports that showed associations between *MEN1, MRE11A, and TNKS* and TL.

In addition to the SNPs predicted to have an association with TL, we showed that BMI (p=0.02 among control and <0.01 among cases and controls) and smoking (p = 0.03 among controls and p=<0.01 among cases and controls) are inversely associated with TL (**Table 3**), although the magnitude of the association was modest. We also showed an inverse association between aspirin/NSAID use (p=0.03 among cases and controls) and TL in the total population.

We also assessed the interaction between SNPs and lifestyle factors that could influence TL (data not shown in table). We determined that *TERT* SNPs interacted with a variety of diet and lifestyle factors to affect TL in the control population after adjustment for multiple comparisons. *TERT* rs2736118 interacted with total fat (p = 0.003 unadjusted and 0.02 adjusted), beta carotene (0=0.001 unadjusted; 0.007 adjusted), and linoleic acid (p = 0.004 unadjusted; 0.028 adjusted) to affect TL. Recent aspirin/NSAID interacted with both *TERT* rs2242652 and rs10069690 (p = 0.003 and 0.008 respectively unadjusted; 0.02 and 0.056 adjusted. Among recent aspirin users, longer TL were observed among those with the variant *TERT* rs10069690 and rs2242652 genotypes (adjusted p for interaction was 0.049 and 0.022 respectively). However, among non-aspirin/NSAID use people with these genotypes had shorter TL. Among consumers of low dietary fat, TL was lower for those with the rs2736118 GG genotype (adjusted p value 0.032). Among those who consumed high levels of beta carotene, TL was higher if the GG genotype was present (adjusted p value 0.029).

The unadjusted mean TL was 1.233 in controls, 1.156 in colon cancer cases, and 1.203 in rectal cancer cases. We found an inverse associa-

Table 1. Description of genes and telomere length.

Gene	Location	SNP	Major/Minor Allele	MAF[1]	Controls						M[2]	Controls[3]		Cases and Controls[4]	
					Wildtype		Heterozygote		Variant						
					Median	(Q1,Q3)	Median	(Q1,Q3)	Median	(Q1,Q3)		Estimate (StdErr)	*P*	Estimate (StdErr)	*P*
TERT	5p15.33	rs2736118	A/G	0.27	1.19	(0.98, 1.44)	1.15	(0.97, 1.41)	1.13	(0.93, 1.39)	A	-0.018 (0.025)	0.42	-0.024 (0.019)	0.21
		rs4246742	A/T	0.15	1.15	(0.96, 1.38)	1.21	(0.98, 1.55)	1.41	(1.07, 1.45)	A	0.069 (0.032)	0.04	0.031 (0.023)	0.19
		rs10069690	C/T	0.25	1.15	(0.94, 1.44)	1.20	(1.00, 1.44)	1.15	(0.80, 1.36)	R	-0.092 (0.066)	0.13	-0.066 (0.047)	0.13
		rs2242652	C/T	0.19	1.15	(0.95, 1.44)	1.18	(0.99, 1.43)	1.21	(0.99, 1.45)	A	-0.008 (0.031)	0.81	-0.018 (0.022)	0.45
		rs2736100	T/G	0.50	1.14	(0.93, 1.39)	1.19	(0.98, 1.45)	1.18	(0.97, 1.44)	D	0.058 (0.037)	0.09	0.069 (0.028)	<.01
		rs2853676	G/A	0.26	1.14	(0.93, 1.42)	1.20	(0.99, 1.45)	1.26	(1.01, 1.47)	A	0.038 (0.026)	0.08	0.025 (0.020)	0.14
TERT-CLPTM1L		rs2853668	C/A	0.28	1.17	(0.99, 1.43)	1.16	(0.95, 1.44)	1.10	(0.87, 1.38)	R	-0.075 (0.059)	0.16	-0.047 (0.047)	0.29
MEN1	11q13	rs670358	A/G	0.18	1.16	(0.97, 1.44)	1.19	(0.95, 1.44)	1.03	(0.98, 1.24)	R	-0.059 (0.139)	0.80	0.107 (0.099)	0.26
MRE11A	11q21	rs13447720	C/T	0.26	1.16	(0.98, 1.39)	1.19	(0.96, 1.47)	1.18	(0.98, 1.46)	D	0.043 (0.034)	0.20	0.014 (0.025)	0.58
RECQL5	17q25	rs820152	C/T	0.1	1.19	(0.98, 1.49)	1.16	(0.96, 1.42)	1.15	(0.98, 1.31)	A	-0.040 (0.024)	0.11	-0.059 (0.018)	<.01
TNKS	8p23.1	rs11991621	C/T	0.37	1.15	(0.95, 1.44)	1.19	(0.99, 1.45)	1.18	(1.06, 1.38)	D	0.022 (0.036)	0.46	0.011 (0.027)	0.67

[1]Minor Allele Frequency (MAF) based on white control population; [2]Model (M) for estimate is based on Additive (A), Recessive (R), or Dominant (D); [3]Adjusted for age, gender, and study; [4]Adjusted for age, gender, study, and disease status.

Table 2. Association between *TERT* haplotypes and TL among controls.

Haplotype	Frequency	Beta (StdErr)[1]	P Value
T-G-A	0.41	-0.030 (0.020)	0.10
G-G-A	0.22	-0.030 (0.023)	0.18
G-A-A	0.20	0.017 (0.024)	0.31
T-G-T	0.08	0.038 (0.037)	0.35
G-G-T	0.04	0.054 (0.048)	0.30
T-A-A	0.02	0.025 (0.063)	0.63
G-A-T	0.02	0.188 (0.074)	0.01

[1]Adjusted for age, gender, and study; [2]*TERT* haplotypes are based on rs4246742, rs2736100, and rs2853676.

tion between TL and risk of colon based on a linear model but not for rectal cancer (**Table 4**), despite the rectal cancer study having a slightly larger sample. However, after adjustment for identified potential confounding variables of BMI, aspirin/NSAID use, and cigarette smoking the association was no longer statistically significant. Evaluation of risk associated by quintile of TL suggested a U-shaped association that was most pronounced for rectal cancer. However, this association was statistically significant when combining colon and rectal cancer cases, where those at highest risk had the lowest TL after adjusting for possible confounders.

Discussion

Previous data found that *MEN, MRE11A, RECQL5*, and *TNKS* are all associated with TL [1]; we only observed an association with *RECQL5* and TL. It has been suggested that there is telomerase activity in cancer cells unlike normal differentiated cells where telomerase activity is negligible [21]. We found that certain SNPs in *TERT*, a subunit of the telomerase enzyme, are associated with TL and that TL is associated with colon cancer in a linear fashion. Additionally, we confirmed some earlier reports that diet and lifestyle factors are related to TL and that the interaction between these factors and *TERT* may further influence TL. However, we did not observe an association between *TERT-CLPTM1L* rs2853668 and TL; a lack of association with TL has previously been reported for *TERT-CLPTM1L* rs401681 [22]. Given this SNP is 5.1 kb upstream of *TERT*; our findings would suggest that the reported association with colon cancer is independent of TL. Additionally, two earlier studies found no association between leukocyte TL and the risk of colorectal cancer [23, 24]. The differences in findings between our study and other reports could be due to the limited power of the previous studies due to small population sizes or the fact that colon and rectal cancer cases were combined in their analyses.

Our identified association between *RECQL5* and TL has biological plausibility. *RECQL5* is a helicase that interacts with *RAD51,* an enzyme involved in the repair of DNA double strand breaks (DSBs), to inhibit inappropriate homologous recombination of chromosomes [25]. It has been suggested that incorrect homologous recombination and defective DSB repair is associated with TL [26-28], indicating a possible reason for the association between *RECQL5* and TL. Additionally, it has been suggested that topoisomerase type 1 poisoning can lead to DNA replication stress and a collapse of DNA replication forks, and *RECQL5* is necessary to reactivate replication forks [25]. It is possible that rs820152 is in linkage disequilibrium with causes a missense mutation in *RECQL5* that does not allow the protein to facilitate movement of replication forks, causing a decrease in chromosome length and TL.

Consumption of fruits and vegetables, meat, fiber, trans-fatty acids, tea, alcohol, Vitamin E, lycopene, Vitamin D, selenium, beta carotene, and aspirin/NSAID use have all previously been associated with TL; BMI, exercise levels and overall diet pattern also have been linked to TL [8, 12, 13, 29, 30]. We were only able to confirm the associations between aspirin/NSAID use, BMI, and cigarette smoking. Oxidative stress, which is associated with increased inflammation, has been shown to be inversely associated with TL [31]. However, our finding of an inverse association between aspirin use, which could be related to inflammation, and TL

Table 3. Associations between diet and lifestyle factors and telomere length.

			Spearman's Rank Correlation			
	Controls		Controls[1]		Cases and Controls[2]	
Exposure	Median	(Q1,Q3)	ρ	P Value	ρ	P Value
Age	66	(56, 73)	-0.149	<.0001	-0.183	<.0001
BMI	26.58	(23.78, 29.76)	-0.090	0.02	-0.080	<0.01
Number of Cigarettes	0	(0, 10)	-0.081	0.03	-0.080	<0.01
Alcohol (g)	0.05	(0.01, 0.26)	0.016	0.66	0.003	0.91
Tea (serv.)	0	(0.00, 0.08)	-0.039	0.28	-0.014	0.63
Total Fats (g)	78.51	(55.25, 113.12)	-0.051	0.17	-0.037	0.19
Polyunsaturated Fatty Acids (g)	15.58	(10.18, 23.64)	-0.033	0.38	-0.018	0.53
Monunsaturated Fatty Acids (g)	27.95	(19.41, 41.52)	-0.050	0.17	-0.038	0.18
Saturated Fatty Acids (g)	28.02	(19.31, 40.89)	-0.048	0.19	-0.041	0.15
Trans-Fatty Acids (g)	5.19	(3.26, 8.28)	-0.047	0.20	-0.048	0.09
Omega-3 Fatty Acids (g)	1.65	(1.15, 2.49)	-0.034	0.35	-0.024	0.40
Protein (g)	81.13	(60.16, 111.47)	-0.067	0.07	-0.025	0.37
Dietary Fiber (g)	22.92	(16.91, 30.60)	-0.049	0.18	0.004	0.88
Red Meat (serv.)	0.69	(0.42, 1.19)	-0.046	0.21	-0.021	0.46
Fruit (serv.)	1.87	(1.08, 2.98)	-0.030	0.42	0.014	0.62
Vegetables (serv.)	2.8	(1.72, 4.21)	-0.053	0.15	-0.011	0.70
Carbohydrates (g)	287.14	(218.14, 391.19)	-0.029	0.43	0.011	0.70
Prudent Diet Factor Score	-0.34	(-0.73, 0.18)	-0.021	0.56	0.019	0.49
Western Diet Factor Score	-0.21	(-0.62, 0.39)	-0.027	0.47	-0.028	0.33
Lifetime Activity Score	8	(5, 10)	0.038	0.30	0.031	0.27
Alpha-Tocopherol (mg)	9.86	(6.82, 13.89)	-0.020	0.58	-0.002	0.93
Lycopene (mcg)	6793.72	(4003.73, 12339.10)	-0.038	0.30	-0.017	0.55
Lutein+Zeaxanthin (mcg)	2209.56	(1478.44, 3233.87)	-0.053	0.15	-0.016	0.57
Vitamin D (mcg)	7.14	(4.43, 10.76)	-0.054	0.14	-0.019	0.50
Selenium (mcg)	122.38	(89.42, 174.75)	-0.037	0.32	-0.003	0.92
Beta-Carotene (mcg)	3792.27	(2428.07, 6530.77)	-0.032	0.39	0.011	0.69
Iron (mg)	15.67	(11.61, 20.73)	-0.044	0.23	-0.006	0.84
Vitamin C (mg)	150.95	(100.57, 212.00)	0.005	0.89	0.025	0.37
Folic Acid (mcg)	344.65	(249.36, 452.98)	-0.043	0.25	-0.001	0.98
Calcium (mg)	1060.7	(714.33, 1550.94)	-0.059	0.11	-0.017	0.55
Linoleic Acid (g)	13.82	(8.96, 21.17)	-0.032	0.38	-0.016	0.57
Calories (kcal)	2166.24	(1667.30, 2987.10)	-0.046	0.21	-0.018	0.53
		Estimate (StdErr)	*P* Value	Estimate (StdErr)		*P* Value
% Female	46	0.022 (0.033)	0.36	0.035 (0.025)		0.09
% Using NSAID recently	47	-0.03 (0.034)	0.22	-0.050 (0.025)		0.03
% Taking Post-Menopausal HRT	44	-0.059 (0.054)	0.30	-0.002 (0.041)		0.95

[1]Adjusted for age, gender and study; [2]Adjusted for age, gender, study, and disease status.

has been shown by others [8]. The direction of the inverse association between aspirin and TL is not what would be expected, although it could arise from interaction with specific genotypes. We found that aspirin/NSAID use increases TL among individuals with higher risk *TERT* genotypes. Cigarette smoking and BMI also can increase oxidative stress and inflammation which could lead to telomere shortening.

Studies have shown that diet and lifestyle factors can interact with genes to influence dis-

Table 4. Associations between TL and colon and rectal cancer.

	N		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
	Controls	Cases	Unadjusted	Model 1[1]	Model 2[2]	Model 3[3]
Colon[4]	374	249	0.94 (0.89,1.00)	0.94 (0.89,1.00)	0.96(0.91,1.02)	0.96 (0.91,1.02)
Quintile 1	75	65	1.00 (referent)	1.00 (referent)	1.00(referent)	1.00 (referent)
Quintile 2	75	61	0.94 (0.58,1.51)	0.95 (0.59,1.52)	0.97(0.60,1.57)	1.00 (0.62,1.64)
Quintile 3	77	40	0.60 (0.36,0.99)	0.61 (0.37,1.01)	0.68(0.41,1.15)	0.70 (0.41,1.18)
Quintile 4	73	38	0.60 (0.36,1.00)	0.60 (0.36,1.01)	0.70(0.41,1.19)	0.69 (0.41,1.19)
Quintile 5	74	45	0.70 (0.43,1.15)	0.70 (0.43,1.16)	0.80(0.48,1.34)	0.82 (0.49,1.38)
Rectal[4]	372	276	0.98 (0.93,1.04)	0.97 (0.92,1.03)	0.97(0.92,1.03)	0.97 (0.91,1.02)
Quintile 1	78	64	1.00 (referent)	1.00 (referent)	1.00(referent)	1.00 (referent)
Quintile 2	77	62	0.98 (0.61,1.57)	0.95 (0.59,1.53)	0.96(0.59,1.55)	0.87 (0.53,1.43)
Quintile 3	74	40	0.66 (0.40,1.09)	0.63 (0.38,1.06)	0.68(0.40,1.15)	0.67 (0.39,1.14)
Quintile 4	69	53	0.94 (0.58,1.52)	0.89 (0.54,1.47)	0.92(0.55,1.54)	0.84 (0.50,1.41)
Quintile 5	74	57	0.94(0.58,1.51)	0.83 (0.50,1.39)	0.84(0.50,1.41)	0.78 (0.46,1.33)
Colorectal[4]	746	525	0.95 (0.93,1.00)	0.96 (0.92,1.00)	0.97(0.93,1.01)	0.96 (0.93,1.00)
Quintile 1	153	129	1.00 (referent)	1.00 (referent)	1.00(referent)	1.00 (referent)
Quintile 2	152	123	0.96 (0.69,1.34)	0.95 (0.68,1.33)	0.97(0.69,1.36)	0.94 (0.67,1.33)
Quintile 3	151	80	0.63 (0.44,0.90)	0.62 (0.43,0.89)	0.68(0.47,0.99)	0.67 (0.46,0.98)
Quintile 4	142	91	0.76 (0.53,1.08)	0.75 (0.52,1.07)	0.82(0.57,1.17)	0.77 (0.53,1.11)
Quintile 5	148	102	0.82 (0.58,1.15)	0.78 (0.55,1.12)	0.84(0.58,1.20)	0.81 (0.56,1.17)

[1]Model 1 is adjusted for age and sex; [2]Model 2 is adjusted for age, sex, BMI, and cigarette smoking; [3]Model 3 is adjusted for age, sex, BMI, cigarette smoking, aspirin/NSAID use and trans-fatty acid intake; [4]Odds ratio (OR) and 95% confidence intervals (CI) are per decile of TL.

ease risk. While it is logical that similar interaction could influence TL, others have not attempted to evaluate the combined effect of lifestyle factors with genetic factors. Although we had limited power to detect interactions, we did observed that key lifestyle factors associated with colorectal cancer, such as aspirin/NSAID use, interacted with *TERT* genotypes to influence TL length after adjustment for multiple comparisons. Use of aspirin/NSAID was associated with longer TL among those with high-risk genotypes, while overall aspirin/NSAIDs had an inverse association with TL. Likewise, those consuming high levels of beta carotene with a variant *TERT* genotype had longer telomeres. This illustrates the importance of evaluating diet and lifestyle factors together with genetic factors to obtain a better understanding of TL biology.

We observed that shorter TL was associated with a greater likelihood of developing colon cancer. However, this association appears to be confounded by lifestyle factors that are associated both with TL and colon cancer. Others have not accounted for confounding factors when evaluating the association between TL and colon cancer [5, 6, 32, 33]. The study by Pooley and colleagues found an increased risk for colorectal cancer after adjusting for age and gender in the retrospective portion of their study, but not in the prospective component of their study [5]. It is unclear if these differences in risk could be due to lack of adjustment for confounding factors, time of sample collection, or differences in proportion of colon and rectal cancer for the two studies. Additionally, there were considerable differences in sample size between the two studies, with 185 cases and 406 controls in the prospective study while the retrospective portion of the study had 2,249 cases and 2,161 controls. It is also possible that samples from the prospective component of the study were drawn many years before diagnosis and therefore changes in diet and lifestyle factors over time could have influenced TL, or conversely TL was influenced by diagnosis and treatment in

the retrospective portion of the study. A large BMI and smoking cigarettes have been shown to increase risk of colon cancer [15, 34, 35]; these factors were inversely associated with TL in our study. Likewise, aspirin/NSAIDs have been shown consistently to reduce risk of colon cancer [36-38], and may further confound the association between TL and colon cancer. Additionally, our study suggests that linear associations with TL are more pronounced for colon cancer while a quadratic association with the greatest risk for those with the lowest and highest quintiles more appropriately fits the rectal cancer data. The sample sizes for colon and rectal cancer were similar. The distribution of colon and rectal cases in Pooley's study was not given. However, if in our study the association was purely an artifact from having cancer, one would expect similar associations with rectal cancer which we did not observe.

This study is not without limitations. First, although our sample size is adequate to evaluate associations with TL, we were limited in our ability to evaluate interactions and to look at survival or other clinical features of interest. Likewise, our sample size was too small to evaluate tumor molecular phenotype which may be important to better understand the association between TL and colorectal cancer. Some have suggested that methylation may influence TL [39], therefore larger studies with extensive tumor molecular phenotype data are needed. Our colon study DNA was obtained from immortalized cell lines which could influence TL and distort findings. To assess this possibility we evaluated TL associations with genotype data for colon and rectal controls separately. Since we did not observe meaningful differences in these two sets of samples, we felt that combining them and adjusting for study was appropriate. Additionally our assessment of cancer outcome is by study, where DNA ascertainment for cases and controls was handled in the same manner. Likewise, our population of controls comes from the population, although age matched to our population-based sample of cases. We present data from controls only and cases and controls adjusting for disease status. The gain of power from combining the cases and controls was obvious for some factors where the beta coefficients were similar for all groups assessed but became significant with the larger sample. Despite these limitations, our diet and lifestyle data are comprehensive and were collected in a rigorous manner, allowing for the evaluation of several factors that could influence TL. Our sample size was adequate to evaluate associations with colon and rectal cancer separately.

Conclusions

TL has been linked to risk for a number of diseases, including osteosarcoma, breast cancer, prostate cancer, pancreas cancer, and coronary heart disease [5, 12, 22, 40]. However, the role TL has not been well established in colorectal cancer [41]. This study provides evidence for a linear association between TL and risk for colon cancer and a possible U-shaped association with rectal cancer. Our data suggest that lifestyle factors and genetic factors significantly interact to affect TL. Assessment of TL and disease should consider confounding effects of lifestyle factors.

Authors' contributions

AP performed TL assays and assisting in genotyping *TERT* SNPs. He was responsible for writing and editing the manuscript. MLS oversaw data collection from case and control participants, obtained funding, designed the study, and assisted in writing and editing the manuscript. AL conducted the statistical analysis. RC oversaw TL assays and provided input into data interpretation and reviewed and approved the manuscript. RW was responsible for genetic analysis, assisted in data interpretation, and reviewed and approved the manuscript.

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