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Telomere maintenance variants and survival after colorectal cancer: Smoking- and sex-specific associations

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Abstract

Background: Telomeres play an important role in colorectal cancer (CRC) prognosis. Variation in telomere maintenance genes may be associated with survival after CRC diagnosis but evidence is limited. In addition, possible interactions between telomere maintenance genes and prognostic factors such as smoking and sex also remain to be investigated.

Methods: We conducted gene-wide analyses of CRC prognosis in 4,896 invasive CRC cases from the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO).1871 common variants within 13 telomere maintenance genes were included. Cox models were fit to estimate associations of these variants individually with overall and CRC-specific survival. Likelihood ratio tests were used to test for interaction by smoking and sex. P-values were adjusted using Bonferroni correction.

Results: The association between minor allele of rs7200950 (*ACD*) with CRC-specific survival varied significantly by smoking pack-years (corrected p-value=0.049), but no significant trend was observed. By sex, minor alleles for rs2975843 (*TERF1*), rs75676021 (*POT1*), and rs74429678 (*POT1*) were associated with decreased overall and/or CRC-specific survival in women but not in men.

Conclusions: Our study reported a gene-wide statistically significant interaction with sex (*TERF1, POT1*). Although significant interaction by smoking pack-years (ACD) was observed, there was no evidence of a dose-response. Validation of these findings in other large studies and further functional annotation on these SNPs are warranted.

Impact: Our study found a gene-smoking and gene-sex interaction on survival after CRC diagnosis, providing new insights into the role of genetic polymorphisms in telomere maintenance on CRC prognosis.

Keywords

telomere; telomere maintenance genes; colorectal cancer; survival

Introduction

Telomeres are comprised of repetitive nucleotide sequences that cap the ends of eukaryotic chromosomes (1) and protect chromosomes from deterioration or end-to-end fusion with neighboring chromosomes (2). Telomeres thus prevent aberrant chromosomal replication and help maintain chromosomal stability and genomic integrity. Telomere replication is regulated by telomerase complex, which is made up of telomerase reverse transcriptase (encoded by *TERT*), an RNA component (encoded by *TERC*), shelterin complex (encoded

by *TERF1, TERF2, TINF2, TERF2IP, ACD,* and *POT1*) (3,4) and other associated proteins (encoded by *TNKS, TNKS2, TNKS1BP1,TEP1* and *PINX1*) (5). Over time, telomeres shorten with each cell division, partly due to incomplete replication of the 3'-end of the chromosomes (1). Personal and lifestyle factors such as age, sex and cigarette smoking may also impact telomere function (6). Dysfunction in telomere replication mechanisms may result in accelerated genetic changes and cellular senescence. Hence, telomeres are considered to be a hallmark of aging.

Telomeres and telomerases may also play an integral role in cancer progression through overexpression of the telomerase enzyme. Indeed, genetic variation in telomere maintenance genes has been associated with overall and cancer-specific survival in cancers of the lung, glioma, liver, ovaries and breast (7–10). The relationship between telomere maintenance genes and CRC prognosis, however, is less clear. Further understanding of the prognostic role of telomeres and telomerases in CRC carcinogenesis may also help provide important insights into CRC treatment.

To date, no published studies have investigated whether telomere maintenance genes are specifically associated with survival after CRC diagnosis. To evaluate this association, we utilized data from the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) (11) to elucidate the relationship between single nucleotide polymorphism (SNP) variation in 13 telomere maintenance genes (*TERT, TERC, TERF1, TERF2, TINF2, TERF2IP, ACD, POT1, TNKS, TNKS2, TNKS1BP1, TEP1* and *PINX1*) and both overall and disease-specific survival after CRC diagnosis. We also considered whether such associations may be modified by host characteristics, such as smoking and sex, which are both involved in telomere erosion.

Methods

Study participants:

Study participants were drawn from 12 case-control and cohort studies, including data from seven cohort studies in the United States: The Seattle site of the Colon Cancer Family Registry (SCCFR), Health Professionals Follow-up Study (HPFS), Physicians' Health Study (PHS), VITamins And Lifestyle study (VITAL); Women's Health Initiative (WHI); Nurses' Health Study (NHS), Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) and Diet, Activity and Lifestyle Study (DALS). GECCO study population and details of the participating studies have been described in detail previously (11).

For the current analysis, study subjects were restricted to participants with self-reported European descent, primary invasive CRC, and available genotype and survival information. CRC diagnosis was confirmed by medical records and pathology reports. The primary outcomes were death from any cause as well as CRC-specific deaths. Active follow-up was used to ascertain vital status in HPFS, PHS, NHS, PLCO, WHI; dates and causes of deaths were confirmed using death certificates and/or medical records. For VITAL, DALS and SCCFR, vital status was confirmed through National Death Index, cancer registries, state death records, or population registries, with cause of death verified by information from death certificates. All participants gave written informed consent and studies were approved

by their Institutional Review Board (IRB) respectively. Studies were conducted in accordance with the Declaration of Helsinki. Characteristics of included studies are described in Table 1.

Data collection:

Data on demographic, lifestyle and environmental characteristics were collected through self-report using questionnaires and/or in-person or telephone interviews, details of which have been described previously (11). Data elements considered in the current analyses were age at diagnosis, sex, research study, cancer site, disease stage at diagnosis, smoking status, pack-years smoked and age at quitting smoking. Survival data included data on deaths from any cause, CRC-specific deaths, and time from diagnosis to death or last follow-up. Principal components analysis (PCA) was used for population stratification, to account for ancestry. Details of genotyping, quality assurance and quality control (QA/QC) and imputation are described in supplementary materials. Genomic DNA was extracted from blood samples or buccal cells by conventional methods. Genotyping platform used for each study is summarized in Table 1. Before imputation, genotyped SNPs were excluded based on call rate (< 98%), lack of Hardy-Weinberg Equilibrium in controls (HWE, $P < 1\times10^{-4}$), and minor allele frequency (MAF < 5% for WHI Set 1, DALS Set 1; MAF < 5/number of samples for all other studies). All autosomal SNPs were imputed to a reference panel generated from WHI whole genome sequencing (WGS).

Based on a literature search conducted through 31st December 2018, we included in our analysis genes encoding proteins that participate in telomere length regulation. Genetic variation in some of the included genes have previously been associated with risk of cancer, including CRC, (12–16) as well as with survival after cancers of lung, liver, brain, and ovary (7,8,10,17). Details on genes selected for the current analysis can be found in Supplementary Table S1. Data was available for 6,578 SNPs within our genes of interest. After focusing on common SNPs (MAF≥5%) (details can be found in supplementary materials), a total of 1,871 SNPs were included in the final analyses. All genotyping data have been published and deposited in dbGaP with accession numbers (18).

Statistical analysis:

Data from individual studies were combined for pooled statistical analyses. Within each gene being evaluated in the current study, Pearson's correlation coefficients were computed to determine the correlations between every pair of SNPs within the gene, and principal components analysis (PCA) was performed to obtain the effect number of independent tests (M_{eff_G}) (19). M_{eff_G} was used for type I error control in Bonferroni correction in single-SNP model in survival analysis (19). Multivariate Cox proportional hazard regression models were used to estimate hazard ratios (HR) and 95% confidence intervals (95% CI) for the associations between single SNPs and survival. Separate models were constructed for overall and CRC-specific survival. Schoenfeld residuals were computed to check for the proportional hazards assumption. The dosage scaling from 0 to 2 represented the estimated number of copies of the count allele. All models were adjusted for age at diagnosis, sex, research study, and the first three principal components of genetic ancestry. Estimates from single-SNP models were considered to reach statistical significance if the adjusted p-value

was <0.05 (adjusted for M_{eff_G} of each gene). Host-related factors such as cigarette smoking history (yes/no), smoking status at the time of completing the questionnaire (former/current/never smoker), and categorical smoking pack-years (<12, 12–24, 25–44, ≥45, as dummy variable) were assessed for gene-environment (GxE) interaction. An interaction with sex was also assessed for the SNPs under study. Likelihood ratio tests were used to evaluate whether the interaction terms were significant. For any associations and/or interactions that had a raw p-value <0.05, we chose the SNPs with the smallest p-value as the representative SNP in the region. For those SNPs that were statistically significant in the main and interaction analyses, further sensitivity analyses were performed to evaluate potential heterogeneity by age at diagnosis, sex and smoking status across studies (more details in supplementary material). All analyses were conducted using R Version 3.4.3.

Results

This study included a total of 4,896 invasive CRC cases. After a mean follow-up of 5.5 years, a total of 1,681 deaths occurred, of which 1,098 (65.3%) were attributed to CRC. The demographic and clinical characteristics of our study participants are summarized in Table 2. The majority of the participants were women (62.4%) aged 65 years and older at diagnosis (72.5%). Smoking was common, with 56.3% reporting ever having smoked, but only 10.1% were current smokers. Most cases were colon (87.6%) versus rectal (12.4%) cancers.

Associations between selected SNPs (with P <0.05) and overall and CRC-specific survival are presented in Table 3. Although SNPs located within *TERT*, *TERF1*, *TNKS*, *TNKS1BP1*, *TEP1* and *TERF2* were nominally associated with survival after CRC diagnosis (P <0.05), none of these associations remained significant after gene-level multiple comparison correction.

Next, we evaluated if genetic associations between telomere maintenance SNPs and survival differed by smoking status (Table 4). Associations with SNPs in *TERT*, *TERF1*, *TERF2, PINX1*, *TEP1, TNKS* and *ACD* showed suggestive differences by smoking status, but these differences were not statistically significant after correction for multiple testing. When evaluated by pack-years of smoking (Table 5), rs7200950 (*ACD*) was differentially associated with CRC-specific survival (adjusted P =0.049 for interaction), however, no clear dose-response was observed with increasing pack years of smoking. Therefore, this finding needs to be interpreted with caution. Additionally, comparing lowest vs. highest exposure groups (0 vs. ≥45) for pack-years of smoking suggested reduced CRC deaths with variants in *TERF2IP* (rs1865493) and *TNKS* (rs73202875). (Supplementary Table S2).

Then, we evaluated the role of genetic variants located in telomere maintenance genes with survival after CRC, according to sex (Table 6). Two SNPs in *POT1*, rs75676021 and rs74429678, were differentially associated with sex, such that women had a poorer overall and CRC-specific survival (adjusted P =0.023 and 0.019 for interaction, respectively) compared to men. rs2975843 within the *TERF1* gene also showed a gene-wide significant interaction with sex for the association with overall as well as CRC-specific survival (adjusted P=0.002 and 0.004 for interaction) such that women had a significantly poorer survival for both overall and CRC-specific survival than men.

Finally, we evaluated if CRC sties differentiated the association between telomere maintenance gene and survival. Some SNPs located within *TEP1*, *TNKS2*, *PINX1*, *TERT*, *TNKS1BP1* and *POT1* showed some suggestive association with survival (P<0.05, Supplementary Table S3), but none of them remain statistically significant after multiple comparison adjustment.

In sensitivity analyses, we did not observe any significant heterogeneity (P>0.05, Supplementary Table S6) by study in covariates including age at diagnosis, sex, and smoking. Therefore, it is reasonable to assume that the effects of age at diagnosis, sex and smoking status are common across studies.

Discussion

In this large candidate gene study of 4,896 colorectal cancer patients and variation in 13 telomere maintenance genes, including *TERT, TERC, TERF1, TERF2, TINF2, TERF2IP, ACD, POT1*, *TNKS, TNKS2, TNKS1BP1, TEP1*, *PINX1*, we found differential associations of CRC-specific survival with smoking pack-years (*ACD)* and sex (*POT1* and *TERF1).* Specifically, rs2975843 (*TERF1*), rs75676021 (*POT1*), and rs74429678 (*POT1*) showed statistically significant interaction with sex, while rs7200950 (*ACD*) showed a suggestive association of smoking pack-years with CRC-specific survival but there was a lack of trend for dose-response. These SNPs decreased both overall and CRC-specific survival in women but not in men. Thus, the current analyses suggest that multiple variants in telomere maintenance genes may play a simultaneous role in progression of cancer, and that these variants may interact with lifestyle factors, including smoking and sex.

The dual role of telomeres and the enzyme telomerase in carcinogenesis and cancer progression is complex (20). Briefly, telomere shortening may lead to carcinogenesis but induce cell death in cancer cell lines (20); telomerase may promote tumor growth and aid in tumor progression. Indeed, several in vitro and in vivo studies have demonstrated an association between high levels of telomerase/TERT and poorer survival (21,22). Telomerase also participates in gene expression regulation, particularly NF-κB signaling, cell growth and migration, thus suggesting that telomerase may also act as a tumor-promoting factor (23,24). Taken together, it is biologically plausible that telomere maintenance genes may impact cancer prognosis, but the current evidence from existing population studies is limited. Previous reports have shown that variants in telomere maintenance genes may be associated with survival after ovarian (9,17) and breast cancer (25); however, the evidence for CRC-specific survival is lacking. There is evidence of a statistically significant association between variants in telomere maintenance genes and risk of developing CRC. SNPs within *TERT (*rs2736100, rs2736098) (13,16,26), and *TERC* (rs10936599) (15) have been associated with increased CRC susceptibility, but we did not detect a statistically significant association between these SNPs and survival in the current study. Variants within *TERT, TERF2, PINX1* and *TNKS* have previously shown suggestive associations with overall mortality across multiple cancers, including glioblastoma, bladder, lung, breast, and ovary (8–10,25,27), although none of those associations remained statistically significant after multiple comparison corrections. These previous studies were moderately sized and, therefore, had limited power to investigate interactions with smoking

and sex (where applicable). We were able to examine associations within subgroups of smoking and sex in our study, owing to the much larger sample size of our study.

In the current study, we observed a statistically significant interaction between rs7200950 (*ACD*) and smoking pack-years, although with no trend for dose-response. Cigarette smoking appears to accelerate telomere length shortening by oxidative stress (28) and methylation (29,30). Circulating telomere length is inversely associated with ever smoking (31) and the number of packs smoked per day (6,32) among current smokers. Short telomeres and smoking have been previously shown to jointly affect the risk of CRC (33). None of the previous studies have looked at joint associations of these genes with CRC-specific survival and smoking status. Recently, a study among non-smoking Asian women demonstrated that variants in telomere maintenance genes associated with longer telomere lengths are also associated with progression of lung cancer (34). These polymorphisms might be interacting similarly among patients with colorectal cancer.

Our study found a statistically significant association for variants within *POT1* and *TERF1* and decreased overall and CRC-specific survival in women, but not in men. Another study found similar results with *RAP1,* another telomere maintenance gene in lung cancer patients (35). These results can be partially explained by the effect of regulation of sex hormones on telomerase activity. Estrogen activates telomerase via upregulating the telomerase catalytic subunit or activating c-Myc/Max that then binds to *TERT* promoter to increase its activity (36). Furthermore, telomere length in men has been shown to be shorter compared to that in similarly aged women (37), and telomere length and sex are both associated with CRC risk (33).

To the best of our knowledge, this is the first study investigating the association between genetic variants involved in telomere maintenance and survival after CRC diagnosis. Our study has a large sample size with long-term follow-up and validated survival outcomes data, which permitted a robust assessment of a gene-wide main effect and GxE interactions. We had access to detailed data on smoking status allowing us to study the effect of smoking quantity, increasing the sensitivity and specificity of our analyses. We acknowledge some limitations of our work. We only included common variants in telomere maintenance genes in our analyses and, therefore, we may have missed any associations with low-frequency and rare variants. Larger sample sizes will be required to analyze such low-frequency variants. We included a comprehensive list of telomere maintenance genes, but it is possible that we missed additional genes contributing to telomere length regulation. Further, all autosomal SNPs were imputed and we used the expected number of copies of the minor allele in our analyses. However, we restricted SNPs with high imputation accuracy and previous reports show that imputed SNPs provide unbiased inference (38).

In conclusion, our large gene-wide study observed suggestive associations between genetic variation related to telomere maintenance function and overall as well as CRC-specific survival. We also observed statistically significant interactions between genes involved in telomere maintenance, smoking pack-years (*ACD*) and sex (*POT1, TERF1*) on their association with survival after CRC diagnosis. Current results need to be verified in larger

studies and further functional annotation of the identified variants in this study may be of interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Characteristics of included studies in Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO)

Study	Case (N)	Male/Female (N /N)	Age (years)[a]	Smoking[c]			N. deaths, all-cause[b]	N. deaths, CRC[b]	Median follow-up time (days)	Platform*
				Current (%)	Former (%)	Never (%)				
DALS 1	710	403/307	65.1 (32–79)	101 (14.2)	326 (45.9)	283 (39.9%)	244 (34.4%)	135 (19.0%)	1917.56	610K, 550K
DALS 2	415	220/195	65.1 (31–79)	47 (11.4)	168 (40.8)	197 (47.8%)	115 (27.7%)	81 (19.5%)	1674.1	300K
HPFS	168	168/0	71.5 (50–90)	6 (3.6)	89 (53.9)	70 (42.4%)	82 (48.8%)	47 (28.0%)	2007.5	730K
NHS	296	0/296	68.1 (46–85)	37 (12.5)	132 (44.7)	126 (42.7%)	118 (39.9%)	89 (30.1%)	2296.45	730K
PHS	324	324/0	70.6 (44–92)	34 (10.5)	160 (49.4)	130 (40.1%)	200 (61.7%)	131 (40.4%)	2062.25	730K
PLCO 1	531	301/230	68.9 (55–82)	54 (10.2)	242 (45.6)	235 (44.3%)	180 (33.9%)	108 (20.3%)	2430	300/240S, 610K
PLCO 2	478	275/203	70.4 (55–86)	46 (9.6)	221 (46.2)	211 (44.1%)	103 (21.6%)	75 (15.7%)	1237.5	300K
SCCFR	279	0/279	64.4 (47–74)	38 (13.6)	116 (41.6)	125 (44.8%)	99 (35.5%)	54 (19.4%)	3374	300K
VITAL	285	150/135	69.9 (51–83)	28 (9.9)	152 (53.9)	102 (36.2%)	117 (41.1%)	70 (24.6%)	1847	300K
WHI 1	304	0/304	71.0 (52–86)	21 (7.0)	147 (49.0)	132 (44%)	103 (33.9%)	77 (25.3%)	1868	550K, 550K duo, and 610K
WHI 2	618	0/618	71.9 (50–91)	45 (7.4)	265 (43.5)	299 (49.1%)	177 (28.6%)	132 (21.4%)	1163.5	300K
WHI WGS	488	0/488	71.4 (52–89)	37 (7.7)	227 (47.2)	217 (45.1%)	143 (29.3%)	99 (20.3%)	1337.5	Whole genome sequencing

Abbreviation: N: number; CRC: colorectal cancer

[a] mean (range)

[b] number (percentage of cases)

[c] number (percentage of cases except for 30 missing value on smoking status

* All genotyping platform, except for WHI WGS, were illumina assay

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Table 2.

Patients characteristics and clinical features for eligible participants from GECCO

Characteristics	Cases		Deaths, number (percentage of cases, %)	
	N	%	all-cause, N (%)	CRC, N (%)
Age (years)				
<65	1345	27.5	414 (30.8)	321(23.9)
65–69	1059	21.6	335 (31.6)	218 (20.6)
70–74	1251	25.6	461 (36.9)	274 (21.9)
≥75	1241	25.3	471 (38.0)	285 (23.0)
Sex				
Male	1841	37.6	727 (39.5)	438 (23.8)
Female	3055	62.4	954 (31.2)	660 (21.6)
Cancer site				
Proximal	2428	49.6	851 (35.0)	556 (22.9)
Distal	1596	32.6	493 (30.9)	307 (19.2)
Rectal	726	14.8	255 (35.1)	181(24.9)
Other[a]	146	3.0	82 (56.2)	54 (37.0)
Cancer stage[b]				
In situ	41	0.9	5 (12.2)	1 (2.4)
Local	1563	34.5	278 (17.8)	74 (4.7)
Regional	2366	52.2	745 (31.5)	461 (19.5)
Distant	563	12.4	489 (86.0)	460 (81.7)
Smoking status[c]				
Never	2127	43.7	644 (30.3)	453 (21.3)
Former	2245	46.1	823 (36.7)	522 (23.3)
Current	494	10.1	204 (41.3)	116 (23.5)
Ever smoker[c]				
Yes	2739	56.3	1027 (37.5)	638 (23.3)
No	2127	43.7	644 (30.3)	453 (21.3)
Smoking pack-years[d]				
<12	612	24.4	184 (30.1)	128 (20.9)
12–<25	645	25.7	197 (30.5)	128 (19.8)
25–<45	613	24.5	232 (37.8)	145 (23.7)
≥45	635	25.4	287 (45.2)	156 (24.6)
Age quit smoking[e]				
<35	517	23.5	154 (29.8)	111 (21.5)
35–<45	516	23.5%	165 (32.0)	115 (22.3)
45–<55	596	27.1%	231 (38.8)	147 (24.7)
≥55	569	25.9%	262 (46.0)	134 (23.6)

[a]Other sites include those cancer sites that cannot be classified as proximal or distal colon or a rectal site

[b] 363 participants did not have data on stage at cancer diagnosis

[c] 30 study participants did not report on their smoking status

[d] 234 study participants did not report on the frequency or duration of smoking and therefore had missing data on smoking pack-years

[e] 47 former smokers did not report on the age at which they quit smoking

Table 3.

Hazard ratios and 95% confidence intervals for the association between selected SNPs (with unadjusted p-value <0.05) involved in telomere maintenance and survival after colorectal cancer diagnosis

Outcome	SNP	Gene	HR[a] (95% CI)	P-value	adj. P-value[b]	minor/ major allele	MAF
Overall survival	rs2075785	*TERT*	0.86 (0.76–0.98)	0.018	0.736	T/C	0.123
	rs2981096	*TERF1*	0.84 (0.70–0.99)	0.046	0.274	G/A	0.053
	rs10102030	*TNKS*	1.09 (1.01–1.18)	0.036	>0.99	T/A	0.230
	rs4939134	*TNKS1BP1*	1.08 (1.02–1.17)	0.017	>0.99	G/C	0.471
	rs1760894	*TEP1*	1.15 (1.04– 1.26)	0.004	0.224	C/T	0.222
CRC-specific survival	rs2075785	*TERT*	0.85 (0.73–0.99)	0.043	>0.99	T/C	0.123
	rs2981096	*TERF1*	0.79 (0.63–0.99)	0.040	0.2418	G/A	0.053
	rs10088969	*TNKS*	1.13 (1.02–1.25)	0.018	>0.99	C/A	0.226
	rs2229101	*TEP1*	0.78 (0.64–0.95)	0.013	0.6783	C/A	0.064
	rs251796	*TERF2*	1.13 (1.02–1.25)	0.015	0.2156	G/A	0.301

Abbreviation: CRC: colorectal cancer; HR: hazard ratio; CI: confidence intervals; MAF: minor allele frequency

[a] adjusted for age at diagnosis, sex, study center and the first three principal components (pc)

[b] p-value is adjusted using Bonferroni method using M_{eff_G}

Table 4.

Hazard ratios and 95% confidence intervals for the association between selected SNPs (with unadjusted p-value <0.05) involved in telomere maintenance and survival after colorectal cancer diagnosis—stratified by cigarette smoking

Outcome	SNP	Gene	HR [a] (95% CI)			$P_{.int}$	adj. P-value [b]	minor/major allele
			Non-smoker	Former smoker	Current smoker			
Overall survival	rs56963355	*TERT*	1.27 (0.98–1.64)	0.98 (0.75–1.22)	0.48 (0.12–0.84)	0.024	>0.99	T/G
	rs2975842	*TERF1*	0.86 (0.77–0.96)	1.03 (0.93–1.14)	1.06 (0.86–1.27)	0.031	0.0942	T/C
	rs2409652	*PINX1*	0.90 (0.79–1.03)	1.04 (0.92–1.16)	1.23 (0.97–1.49)	0.036	>0.99	T/C
	rs1760899	*TEP1*	0.85 (0.71–1.01)	1.09 (0.93–1.25)	0.64 (0.41–0.87)	0.007	>0.99	C/T
CRC-specific survival	rs6420019	*TERT*	1.23 (0.98–1.54)	1.49 (0.45–1.63)	0.85 (0.85–1.22)	0.013	0.52	A/C
	rs56106543	*TERF2*	0.74 (0.51–1.09)	1.08 (0.40–1.67)	1.49 (0.61–1.46)	0.014	0.196	C/T
	rs13259648	*PINX1*	1.27 (1.07–1.50)	0.78 (0.75–1.32)	0.99 (0.87–1.19)	0.024	0.696	T/G
	rs35656875	*TNKS*	0.63 (0.42–0.98)	1.28 (0.09–1.97)	1.43 (0.51–1.56)	0.015	>0.99	G/C
	rs1760901	*TEP1*	0.80 (0.65–0.99)	0.48 (0.78–1.29)	1.12 (0.83–1.24)	0.002	0.102	G/C
	rs6979	*ACD*	0.96 (0.83–1.11)	0.69 (0.84–1.23)	1.07 (0.89–1.18)	0.023	0.069	G/A

Abbreviation: CRC: colorectal cancer; HR: hazard ratio; CI: confidence intervals; P_{int}: P-value of likelihood ratio test for testing interaction by smoking

[a] adjusted for age at diagnosis, sex, study center and the first three principal components (pc)

[b] p-value is adjusted using Bonferroni method using M_{eff_G}

Table 5.

Hazard ratios and 95% confidence intervals for the association between selected SNPs (with unadjusted p-value <0.05) involved in telomere maintenance and survival after colorectal cancer diagnosis—stratified by pack-years of smoking

Outcome	SNP	Gene	HR[a] (95% CI), smoking pack-years					P_{int}
			0	<12	12–24	25–44	≥45	
Overall survival	rs556947195	*TERT*	1.00 (0.75–1.34)	1.48 (0.87–2.08)	0.82 (0.39–1.25)	0.76 (0.33–1.19)	1.90 (1.47–2.33)	0.013
	rs2975842	*TERF1*	0.86 (0.77–0.96)	1.13 (0.89–1.36)	1.05 (0.83–1.27)	1.16 (0.94–1.38)	0.98 (0.76–1.20)	0.033
	rs251797	*TERF2*	0.99 (0.88–1.12)	1.08 (0.84–1.33)	1.06 (0.82–1.30)	1.25 (1.01–1.49)	0.78 (0.54–1.02)	0.019
	rs67456872	*TNKS*	0.68 (0.48–0.97)	2.42 (1.00–3.84)	0.58 (0.17–0.99)	1.59 (1.18–2.00)	0.96 (0.55–1.37)	0.002
	rs76990680	*TNKS*	1.15 (1.02–1.30)	0.82 (0.63–1.02)	1.24 (0.99–1.49)	0.97 (0.72–1.22)	0.95 (0.70–1.20)	0.096
CRC-specific survival	rs2242652	*TERT*	0.89 (0.73–1.07)	0.76 (0.48–1.03)	1.40 (0.93–1.87)	0.82 (0.35–1.29)	1.29 (0.82–1.76)	0.025
	rs2853690	*TERT*	1.12 (0.93–1.33)	1.17 (0.81–1.53)	0.65 (0.41–0.89)	1.30 (1.06–1.54)	1.05 (0.81–1.29)	0.042
	rs153045	*TERF2*	1.10 (0.95–1.28)	1.16 (0.83–1.48)	1.18 (0.85–1.51)	0.74 (0.41–1.07)	1.41 (1.08–1.74)	0.024
	rs10091836	*PINX1*	0.88 (0.77–1.00)	0.78 (0.59–0.98)	1.19 (0.90–1.48)	1.00 (0.71–1.29)	1.26 (0.97–1.55)	0.011
	rs67456872	*TNKS*	0.63 (0.40–0.97)	2.67 (0.91–4.43)	0.81 (0.16–1.46)	2.16 (1.51–2.81)	0.95 (0.30–1.60)	0.002
	rs7200950	***ACD***	**1.11 (0.83–1.48)**	**1.65 (0.91–2.39)**	**0.69 (0.24–1.14)**	**1.45 (1.00–1.90)**	**0.52 (0.07–0.97)**	**0.016**[*]

Abbreviation: CRC: colorectal cancer; HR: hazard ratio; CI: confidence intervals; P_{int}: P-value of likelihood ratio test

[a] adjusted for age at diagnosis, sex, study center and the first three principal components (pc)

[*] adjusted p-value is 0.0495; other adjusted p-values are not significant (>0.05)

Table 6.

Hazard ratios and 95% confidence intervals for the association between selected SNPs (with unadjusted p-value <0.05) involved in telomere maintenance and survival after colorectal cancer diagnosis—stratified by sex

Outcome	SNP	Gene	HR [a] (95% CIs)		P_{int}	adj. P-value [b]
			Female	Male		
Overall survival	**rs75676021**	***POT1***	**1.21 (1.01–1.45)**	**0.77 (0.60–0.95)**	**0.002**	**0.023***
	rs2853685	*TERT*	1.14 (1.03–1.26)	0.89 (0.79–1.00)	0.002	0.096
	rs2975843	***TERF1***	**1.08 (0.99–1.18)**	**0.84 (0.75–0.92)**	**3.00×10^{-4}**	**0.002***
	rs73615082	*TERF2IP*	0.76 (0.62–0.93)	1.08 (0.85–1.30)	0.019	0.154
	rs4840518	*PINX1*	1.21 (1.02–1.43)	0.90 (0.72–1.08)	0.026	0.748
	rs77103162	*TNKS1BP1*	0.93 (0.80–1.08)	1.17 (0.99–1.34)	0.0337	0.506
	rs35259162	*TEP1*	0.83 (0.73–0.94)	1.02 (0.88–1.16)	0.0293	>0.99
	rs3950296	*TERC*	0.91 (0.82–1.02)	1.09 (0.96–1.22)	0.0278	0.139
CRC-specific survival	**rs74429678**	***POT1***	**1.33 (1.07–1.65)**	**0.75 (0.52–0.97)**	**0.0019**	**0.019***
	rs2736115	*TERT*	1.21 (1.07–1.37)	0.90 (0.76–1.04)	0.0027	0.108
	rs2975843	***TERF1***	**1.12 (1.00–1.25)**	**0.83 (0.71–0.94)**	**6.00×10^{-4}**	**0.004***
	rs73615082	*TERF2IP*	0.77 (0.60–0.98)	1.12 (0.83–1.42)	0.0391	0.313
	rs10503412	*PINX1*	1.21 (1.00–1.46)	0.78 (0.58–0.97)	0.0051	0.148
	rs4416825	*TNKS*	0.97 (0.83–1.14)	1.24 (1.01–1.47)	0.0482	>0.99
	rs1760895	*TEP1*	1.19 (1.02–1.40)	0.86 (0.67–1.06)	0.0195	0.995
	rs9876206	*TERC*	0.89 (0.78–1.01)	1.12 (0.95–1.28)	0.0252	0.126

Abbreviation: CRC: colorectal cancer; HR: hazard ratio; CI: confidence intervals; P_{int}: P-value of likelihood ratio test

[a] adjustment for age at diagnosis, study center and the first three principal components (pc)

[b] p-value is adjusted using Bonferroni method using M_{eff_G}