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A Prospective Study of Smoking and Risk of Synchronous Colorectal Cancers

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Abstract

Objective—Cigarette smoking has been linked to somatic genetic and epigenetic aberrations, including CpG island methylator phenotype (CIMP)-high, microsatellite instability (MSI)-high, and *BRAF* mutation. These molecular features have been associated with synchronous primary colorectal cancers (CRCs). Thus, we examined the hypothesis that smoking might be associated with the risk of synchronous CRCs.

Design—Within the Health Professionals Follow-up Study and Nurses' Health Study, we examined the relationship of smoking and incidence of CRC according to tumor synchronicity, using duplication-method Cox proportional hazards regression analysis.

Results—We confirmed 1,981 solitary CRC and 45 synchronous CRC cases during follow up of 134,305 individuals. CRC risk associated with smoking differed significantly by tumor synchronicity status ($P_{\text{heterogeneity}} < 0.001$). When comparing current smokers with never smokers, multivariable hazard ratios (HR) were 5.27 (95% confidence interval [CI], 2.08–13.40) for synchronous CRCs and 0.97 (95% CI, 0.83–1.14) for solitary CRC. Similarly, differential associations were observed when examining cumulative pack-years smoked ($P_{\text{heterogeneity}} = 0.006$). Smoking cessation for 10 years relative to current smoking might reduce the risk of synchronous CRCs (multivariable HR=0.42; 95% CI, 0.19–0.95), but not solitary CRC (multivariable HR=1.10; 95% CI, 0.94–1.29) ($P_{\text{heterogeneity}} = 0.001$). Comparing current and former smokers with never smokers, multivariable HRs for synchronous CRCs were significantly higher than those of solitary CRC positive for either CIMP-high, MSI-high, or *BRAF* mutation ($P_{\text{heterogeneity}} = 0.002$).

Conclusion—Smoking is associated with an elevated risk of synchronous CRCs. Our data support a model where smoking contributes to an etiologic field effect that favors these somatic molecular alterations and the development of multiple primary tumors.

Keywords

molecular pathologic epidemiology; colon cancer; smoking; synchronous cancers

INTRODUCTION

Although colorectal cancers (CRCs) across a population demonstrate considerable genetic and epigenetic heterogeneity, the “field effect” theory posits that neoplasms that develop in a background of common etiologic (genetic, lifestyle, or environmental) factors within a single individual may share molecular features (1, 2). Cigarette smoking, an established CRC risk factor (3, 4), serves as a possible exposure which may contribute to field cancerization. Although the precise carcinogenic mechanism of cigarette smoking in CRC is unclear, cigarette smoking appears to associate most consistently with specific somatic alterations in CRC, including aberrant promoter methylation pattern (termed high-level CpG island methylator phenotype [CIMP-high]) (5–7), microsatellite instability (MSI) (5, 7–11), and *BRAF* mutation (5–7, 12). Importantly, experimental evidence suggests that cigarette

smoking can directly drive the epigenomic instability associated with these molecular alterations through the induction of hypermethylation at CpG islands (13–15).

Synchronous CRCs, which indicates the presence of two or more primary carcinomas separated by normal mucosa and identified within a single individual at the time of cancer diagnosis (16–18), are perhaps the most convincing clinical manifestation of a putative field effect. Importantly, previous studies have demonstrated that somatic epigenetic signatures across synchronous CRCs are highly concordant (1, 19). Furthermore, synchronous CRCs are associated with CIMP-high, MSI-high, and *BRAF* mutation, molecular markers that, as previously discussed, are also associated with smoking-induced carcinogenesis (19–24). Therefore, we hypothesized that cigarette smoking might contribute directly to a field effect that promotes multifocal cancer development. However, to our knowledge, no previous studies have prospectively examined the association of smoking with incidence of CRC according to tumor synchronicity status.

In this study, we tested this hypothesis by examining the relationship between cigarette smoking and incidence of CRCs according to tumor synchronicity status, utilizing the databases of two well-characterized, large, prospective cohorts: the Nurses' Health Study I (NHS) and the Health Professionals Follow-up Study (HPFS).

METHODS

Study population

The NHS is a prospective U.S. cohort of 121,701 female registered nurses, who were 30–55 years of age at enrollment in 1976. The HPFS is a prospective cohort of 51,529 U.S. male professionals, who were 40–75 years of age at enrollment in 1986. In both studies, biennial questionnaires have been mailed to participants in order to update information on lifestyle factors including smoking status, as well as diagnoses of diseases, including CRC. Participants have also provided self-reported average food intake using previously described and validated food frequency questionnaires (25, 26). This study was approved by the Harvard T.H. Chan School of Public Health and Brigham and Women's Hospital Institutional Review Boards. Completion and return of the questionnaire was considered to imply informed consent. In the present study, we excluded men and women with a baseline history of cancer (except non-melanoma skin cancer), and those who did not provide dietary or smoking information on the baseline questionnaire (Supplemental Figure 1). A total of 45,691 men and 88,614 women were eligible for inclusion in the analysis.

Assessment of smoking status

Collection of data on smoking habits within the two cohorts has been detailed previously (27). Briefly, current smoking status and the number of cigarettes smoked per day were reported by participants in biennial questionnaires, beginning in 1980 for NHS and 1986 for HPFS. At baseline, participants provided information on their age when they started smoking, age when they stopped (for former smokers), and the number cigarettes/day they smoked in the past. Based on this information, we calculated the duration of smoking cessation, cumulative pack-years smoked [(cumulative average of packs per day) multiplied

by (number of years during which smoking occurred)], and the number of pack-years smoked before age 30.

Assessment of incident colorectal cancer (CRC) and tumor synchronicity status

Identification of incident CRC within the prospective cohorts has been detailed previously (27). On each biennial questionnaire, participants were asked whether they had received a new or past diagnosis of colon or rectal cancer. Once a participant reported a diagnosis of CRC they were contacted to obtain consent to obtain and review medical records. For non-responders, we used the National Death Index and next-of-kin to identify diagnosis of CRC that contributed to death or that were a secondary diagnosis and then contacted next of kin to obtain permission to review medical records. At the time of this analysis, CRC incidence data were available up to January 31, 2010 for men and June 1, 2012 for women. All reported cases were confirmed through medical record review. When available, formalin-fixed paraffin-embedded CRC tissue blocks were retrieved from hospitals where surgical resections of the cancer had occurred. The criteria defining synchronous CRCs have been described previously; all cases were reviewed by two study physicians (including S.O.), both of whom agreed on the synchronicity of each case (1). Synchronous CRC cases were strictly defined as the presence of two or more colorectal carcinomas with at least submucosal invasion (i.e., stage pT1) that were clearly separated by normal intestinal mucosa at the first diagnosis of cancer according to pathology reports or available tissue specimens. Any potential metastases mimicking synchronous tumors were excluded.

Assessment of tumor molecular characteristics

Detailed methods for the assessment of tumor molecular characteristics have been detailed previously (27). Briefly, DNA was extracted from formalin-fixed paraffin-embedded tumor and normal colon specimens. We conducted pyrosequencing of the *BRAF* (codon 600) oncogene (28), MSI analysis (29), and methylation analysis for 8 CIMP-specific CpG islands (30) using a validated bisulfite DNA treatment and real-time polymerase chain reaction (MethyLight assay) (31). Tissue for molecular analyses was available from at least one of the synchronous tumors in the majority of cases. Our previous studies have demonstrated significant correlation between available synchronous tumor pairs' epigenetic profiles (1, 19). Therefore, we classified individuals with synchronous CRC according to the molecular characteristics of the larger tumor among synchronous CRCs.

Statistical analysis

All statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina). All statistical tests were two-sided. Our primary hypothesis test was for heterogeneity between 'the association of smoking with synchronous CRC' and 'the association of smoking with solitary CRC'. To account for multiple hypothesis testing for each of the primary hypotheses associated with the five smoking exposure variables [smoking status (never, former, current), cumulative pack-years smoked, pack-years smoked before age 30, age at initiation of smoking habit, and years of cessation (if applicable)], we corrected the standard two-sided significance value to $\alpha=0.05/5=0.01$ by Bonferroni correction. All other assessments, including evaluation of individual hazard ratio (HR) estimates, were secondary analyses.

To compare differential associations of smoking exposures with CRC risk by tumor synchronicity status, we conducted initial duplication-method Cox proportional hazards models and assessed for heterogeneity among the combined cohort (32). This allows for the estimation of separate regression coefficients for smoking status stratified by multiple outcomes (e.g. synchronous vs. solitary). The Anderson-Gill data structure was used to handle left truncation and time-varying covariates in an efficient manner (33). To control for confounding by age as finely as possible, we stratified our analysis jointly by age at start of follow-up, calendar year of a given questionnaire cycle and sex. No violation of the proportionality of hazards assumption was observed for any of the smoking-related variables. For the primary hypothesis test, a likelihood ratio test was used to assess whether smoking exposures conferred differential risk for solitary vs. synchronous tumors. Tests for linear trend were performed using the ordinal categorical values (i.e. status) or the median value for continuous variable categories (i.e. pack-years). In addition to sex and age-adjusted models, we utilized multivariable-adjusted models including multiple potential confounders. All covariates used the most up-to-date questionnaire data collected prior to each follow-up cycle. Follow-up ended at diagnosis of CRC, death from other causes, or Jan 31, 2010 for men and June 1, 2012 for women, whichever occurred first. Models were stratified for time of questionnaire return, sex and age. Models were further adjusted for body mass index (BMI; <25 vs. 25–30 vs. 30 kg/m²) prior to diagnosis, family history of CRC in any first-degree relative, regular use of aspirin [≥2 tablets (325 mg) per week], physical activity level [quintiles of mean metabolic equivalent task (MET) score per week], red meat intake (quintiles of servings per day), total caloric intake (quintiles), alcohol consumption (0 or quartile of grams (g) per day), folate intake (quintiles of µg per day), calcium intake (quintiles of mg per day), current multivitamin use, and postmenopausal hormone use (women only). Consistent with prior analyses (27), the cumulative average of BMI, physical activity, and red meat, caloric, alcohol, folate, and calcium intake was used to reduce within-individual variability and better represent long-term exposure. Prior to pooling the cohorts, we performed tests of heterogeneity using the Q statistic. Incidence rates of CRC for participants in a specific category of smoking status were calculated by dividing the number of incident cases by the number of person-years of follow-up. The age-adjusted incidence rate ratio (IRR) was calculated by dividing the incidence rate in one category by the incidence rate in the reference category and further stratifying by age. Age-standardized incidence rates were calculated based on the IRRs.

RESULTS

The age-adjusted baseline characteristics of the NHS and HPFS cohorts are shown in Table 1. Using up to 32 years of follow-up of 88,614 women and 45,691 men with available smoking history data (encompassing 3,463,102 person-years), we documented 3,203 cases of incident CRC. Overall, compared with never smokers (incidence rate: 81 cases/100,000 person years), the multivariable hazard ratios (HRs) for incident CRC were 1.17 (95% confidence intervals [CI] 1.08–1.26; age-standardized incidence rate: 100 cases/100,000 person years; Incidence Rate Ratio [IRR]=1.23; 95% CI, 1.14–1.32) for former smokers and 1.09 (95% CI, 0.96–1.23; age-standardized incidence rate: 101 cases/100,000 person years; IRR=1.25; 95% CI 1.11–1.41) for current smokers ($P_{\text{trend}}=0.005$) (Supplemental Table 1).

Additionally, compared to never smokers, the risk of CRC increased according to cumulative pack-years smoked (40 pack-years, multivariable HR=1.26; 95% CI, 1.14–1.39; $P_{\text{trend}} < 0.0001$), and pack-years smoked before age 30 (10 pack-years, multivariable HR=1.15; 95% CI, 1.04–1.26; $P_{\text{trend}} = 0.005$) confirming that smoking is a modest risk factor for CRC overall (Supplemental Table 1). Among these CRC cases, we were able to assess synchronicity status of 2,026 cases. From these 2,026 cases, we identified 45 individuals with synchronous CRCs. Supplemental Tables 2–4 show cohort-specific results for smoking exposures and incident CRC risk by tumor synchronicity. No substantial heterogeneity was observed between the associations of smoking status with risk of CRC according to tumor synchronicity status by sex ($P_{\text{heterogeneity}} = 0.05$). Thus, we pooled data from the two cohorts in subsequent analyses.

Smoking habit and CRC risk by tumor synchronicity status

We tested our primary hypothesis, that there was a differential association of smoking with CRC incidence according to tumor synchronicity, in the combined cohort (with the adjusted α -level of 0.01). The association between smoking status (never, former, current) and CRC incidence differed significantly according to tumor synchronicity (Table 2; $P_{\text{heterogeneity}} < 0.001$). Compared with never smoking, current smoking was associated with a significantly higher risk for synchronous tumors (multivariable HR=5.27, 95% CI, 2.08–13.4; $P_{\text{trend}} < 0.001$), but not risk of solitary tumors (multivariable HR=0.97; 95% CI, 0.83–1.14; $P_{\text{trend}} = 0.37$). Smoking duration (measured in cumulative pack-years) was associated with an increased risk of both synchronous and solitary CRCs, but this association was significantly stronger for synchronous CRC ($P_{\text{heterogeneity}} = 0.006$) (Table 2). For example, compared to never smokers, those who smoked 40 pack-years had a multivariable HR for synchronous CRCs of 4.01 (95% CI, 1.67–9.65; $P_{\text{trend}} = 0.001$) compared to the corresponding multivariable HR of 1.20 (95% CI, 1.05–1.37; $P_{\text{trend}} = 0.007$) for solitary cancers.

These longitudinal measures account for the smoking exposures most proximate to the time of diagnosis. However, given the time that may be required for CRC to develop, we also examined the association of early life smoking exposures with incidence of CRC. The association of pack-years smoked before age 30, or age of initiation of smoking with CRC risk, did not significantly differ by tumor synchronicity status (Table 2). However, smoking status as assessed at baseline (1980 for NHS and 1986 for HPFS), showed similar, yet slightly attenuated, associations as updated status. Compared with never smokers, current smokers at baseline had multivariable HRs of 1.11 (95% CI, 0.86–1.45; $P_{\text{trend}} = 0.11$) for solitary CRC and 4.31 (95% CI, 1.13–16.45; $P_{\text{trend}} = 0.03$; $P_{\text{heterogeneity}} = 0.05$) for synchronous CRC.

Smoking cessation and colorectal cancer synchronicity

We further examined whether the risks associated with smoking were diminished after smoking cessation (Table 3). Duration of smoking cessation (compared to current smoking) appeared to be significantly associated with a lower risk of synchronous CRCs but not with the risk of solitary CRC ($P_{\text{heterogeneity}} = 0.001$). Relative to current smokers, the multivariable HRs for synchronous CRCs were 0.53 (95% CI, 0.18–1.58) after smoking cessation of 1–9

years and 0.42 (95% CI, 0.19–0.95) after cessation 10 years ($P_{\text{trend}} = 0.001$). As expected, the strongest inverse association was observed among never smokers (multivariable HR=0.19; 95% CI, 0.07–0.48).

Smoking status and colorectal cancer synchronicity stratified by tumor molecular subtypes

Our previous studies have each independently associated synchronous CRCs and, separately, cigarette smoking with specific tumor molecular features that have been implicated in the serrated pathway of neoplasia, including CIMP-high, MSI-high, and *BRAF* mutation irrespective of tumor synchronicity (1, 27). In the current analysis, we confirmed that synchronous CRCs commonly exhibit these molecular alterations, with 47% (16/34) of synchronous cancers positive for at least one of these markers compared to 27% (321/1,207) of solitary CRC ($P = 0.01$) (Table 4). Next, we attempted to examine the association of smoking with the risk of CRC according to synchronicity in context of their molecular subtypes. The total number of synchronous CRC cases was too small to be further stratified by any or all of these molecular markers. However, we had sufficient power to model the association of smoking with CRC according to subtypes defined as solitary cancers positive for CIMP-high, MSI-high, or *BRAF* mutation, solitary cancers negative for these markers, or synchronous cancer (Table 5). Current smoking, compared with never smoking, was associated with a multivariable HR of 1.07 (95% CI, 0.84–1.36) for solitary CRC that was *BRAF* wild-type, MSI-low or microsatellite stable, and CIMP-low or negative; 1.47 (95% CI, 1.02–2.12) for solitary CRC positive for any of *BRAF* mutation, MSI-high, and CIMP-high; and 5.35 (95% CI, 2.11–13.5) for synchronous CRC. Thus, the association of smoking with synchronous CRC appeared significantly more pronounced compared to the association with solitary CRC positive for at least one of these serrated pathway-related markers ($P_{\text{heterogeneity}} = 0.002$).

DISCUSSION

In this prospective study of cigarette smoking and incidence of CRC according to tumor synchronicity, we showed that, compared to never smokers, a history of smoking and increasing number of pack-years smoked were associated with an increased risk of synchronous CRCs, which was significantly stronger than that of solitary CRC. In contrast, smoking cessation compared to current smoking was specifically associated with a reduction in risk for synchronous CRCs. Moreover, given these associations and the absence of substantial heterogeneity according to early life-exposures, it appears that smoking habit more proximate to the time of diagnosis may be strongly associated with the development of synchronous neoplasms. Nonetheless, our data supports that smoking is a moderate risk factor for CRC overall.

Furthermore, as in our prior studies (1), we have demonstrated that synchronous CRCs more frequently possess molecular features characteristic of the serrated neoplasia pathway (*BRAF* mutant, MSI-high, or CIMP-high). Our results appear consistent with a previous study demonstrating that synchronous advanced neoplasms may be more likely to arise from multiple sessile serrated adenomas (34). The association of smoking with sessile serrated

adenomas and the challenges of endoscopically detecting and removing these lesions, especially within the proximal colon, have been well documented (35–43). Thus, both of these factors could significantly contribute to the formation of multiple primary cancers and may explain the observed association between smoking and synchronous CRCs. While we were unable to perform analyses further stratified by anatomical subsite due to limited statistical power, we were able to stratify solitary CRC cases according to their molecular subtype to examine the possible mediation by these pathways. While smoking was associated with a significantly increased risk for solitary CRCs that are *BRAF* mutant, MSI-high, or CIMP-high, the risk of synchronous CRC regardless of molecular subtype was significantly more pronounced. Combined, our observations suggest that these molecular pathways may partially, but not completely, mediate the link between smoking and the formation of multiple primary tumors. This incomplete mediation suggests that additional studies focused on identifying additional markers of synchronous CRC risk are warranted; especially those that may influence serrated carcinogenesis. However, the link between smoking and traditional dysplastic adenoma incidence established by other studies suggests that these lesions may also contribute to multifocal tumor development (42, 44, 45). Thus, examination of the genetic markers associated with traditional carcinogenesis (e.g. *APC* or *KRAS* mutations) may also provide valuable insight (46).

In addition, previous studies have linked other demographic and lifestyle factors with synchronous CRC incidence including age, family history and male sex (47, 48). Our study, to our knowledge, is the first to link smoking directly to synchronous CRC incidence. The current analyses were stratified for age and sex and adjusted family history to minimize confounding by these potential factors. No significant interaction was observed for smoking and sex on the incidence of CRC overall or by tumor synchronicity. Taken together our data suggest that smoking in addition to other factors, significantly contributes to a field effect that encourages the formation of synchronous CRCs, in part due to the presence of epigenetic dysregulation and the formation of multiple serrated neoplasms (Figure 1).

There are several key strengths in our study. Our prospective design minimizes recall bias on smoking data and other relevant CRC risk factor data. Questionnaire data were updated every two years allowing for the estimation of duration of smoking exposures and cessation as well as the ability to carefully control for potential confounding variables. Furthermore, our tumor data, carefully annotated for clinicopathologic and molecular features, enabled us to use an integrative molecular pathologic epidemiology approach (32, 49) to test heterogeneity between different outcome cases (synchronous vs. solitary).

Our study has limitations. First, despite our large population, synchronous cancers are relatively rare events, comprising less than 3% of all CRC cases. Thus, we had a limited number of synchronous CRC cases for molecular analysis, which limited our ability to conduct more detailed mediation analyses. While the association between smoking and synchronous cancer appears relatively strong (~five-fold increased relative risk), other factors are likely involved in the pathogenesis of synchronous CRC. Other risk factors for CRC (e.g. alcohol, BMI, physical activity, germline genetics) that are present throughout the life-course have not yet been associated with the presence of synchronous CRC. This may be explained by a distinct set of carcinogenic mechanisms associated with cigarette smoking

that preferentially leads to “field carcinogenesis” effect defined by aberrant promoter methylation that leads to microsatellite instability and somatic *BRAF* mutations that favor serrated carcinogenesis. Nonetheless, further studies examining other CRC risk factors with tumor multiplicity (e.g. family history, age, gender) (48) are warranted. Second, we were unable to obtain tumor tissue from all CRC cases. However, baseline features of participants without available tissue did not differ from those with tissue (27). Last, our cohorts represent a selected population of largely Caucasian health professionals. Thus, our findings require validation in additional populations.

In summary, this prospective study suggests that smoking is associated with the risk of synchronous CRCs; this association is significantly stronger than that with the risk of solitary CRC. Furthermore, our results suggest that, compared to current smoking, smoking cessation is associated with a reduced risk of synchronous CRCs, implying that the field effect associated with smoking may be paused or reversed in the absence of exposure. Our results provide insight into the mechanisms of colorectal carcinogenesis and add further scientific support for the field effect theory of carcinogenesis. Moreover, these findings also highlight the importance of smoking cessation and abstinence as a component of a CRC prevention strategy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Study Highlights

What is current knowledge

- Synchronous colorectal cancers (CRCs) may arise as a result of a “field effect” and/or shared etiologic factors.
- Synchronous CRCs have been linked to somatic epigenetic aberrations.
- Cigarette smoking, an established CRC risk factor, has been associated with altered tumor DNA methylation patterns.

What are the new findings

- Current cigarette smoking status and cumulative pack-years smoked are associated with elevated risk for synchronous CRCs.
- Compared with current smoking, smoking cessation was associated with a reduction of risk for synchronous CRCs.
- The association of smoking with synchronous CRC may be partially mediated by field carcinogenesis that can lead to molecular alterations including CIMP-high, MSI-high, and *BRAF* mutation.

Figure 1. Proposed model for the association of smoking with synchronous colorectal cancer
 Smoking contributes to a field effect that strongly favors the formation of multiple primary colorectal cancers. This field effect may be further influenced through other etiologic factors such as family history, genetics, or dietary and lifestyle factors. The smoking-induced field effect, at least partially, favors the formation of serrated neoplasms with markers of epigenomic dysregulation, which contributes to the formation of multiple primary cancers. However, other pathways may also specifically promote multiple cancerization.

Table 1Age-adjusted demographic characteristics according to smoking status at study baseline^a

	Women (NHS)			Men (HPFS)		
	Never (N=38,576)	Former (N=24,447)	Current (N=25,591)	Never (N=21,235)	Former (N=19,876)	Current (N=4,580)
Age ^b	46.6 (7.3)	46.9 (7.1)	46.7 (7.0)	52.9 (9.7)	56.0 (9.7)	54.2 (9.3)
Body mass index (BMI, kg/m ²), %						
<25	66	69	73	50	44	47
25–29.9	24	22	20	43	47	46
30	10	9	7	7	9	8
Family history of colorectal cancer in any first-degree relative, %	8	8	7	9	8	8
Regular use of aspirin, %	34	36	37	28	33	32
Physical activity, METs/week ^c	13.9 (19.2)	15.6 (22.5)	12.5 (19.5)	21.6 (29.8)	21.0 (29.1)	15.4 (22.2)
Red meat intake, servings/day	1.4 (0.8)	1.3 (0.8)	1.4 (0.8)	1.1 (0.8)	1.1 (0.8)	1.4 (0.8)
Alcohol consumption, g/day	4.0 (7.8)	7.4 (10.6)	8.9 (13.1)	7.9 (12.2)	13.5 (16.3)	17.5 (20.4)
Total calories, Kcal/day	1589 (503)	1551 (489)	1548 (512)	1991 (618)	1974 (615)	2023 (640)
Folate intake, µg/day	372 (280)	382 (285)	338 (253)	489 (278)	482 (278)	434 (261)
Calcium intake, mg/day	739 (320)	747 (306)	708 (315)	923 (433)	886 (423)	839 (403)
Current multivitamin use, %	34	36	31	42	43	42
Menopausal hormone therapy (ever), %	42	45	43	–	–	–

BMI, body mass index; HPFS, Health Professionals Follow-up Study; MET, metabolic equivalent task; NHS, Nurses' Health Study.

Values are means (standard deviation) or percentages and are standardized to the age distribution of the study population.

^aData are from the questionnaire in 1980 for the Nurses' Health Study (NHS) and in 1986 for the Health Professionals Follow-up Study (HPFS).^bNot age-adjusted.^cMET calculated according to the frequency of a range of physical activities in 1986 for both women and men.

Table 2

Smoking and risk of solitary or synchronous tumors in colorectal cancer in the combined cohorts.

	Solitary tumor				Synchronous tumor				$P_{\text{heterogeneit}}^b$
	Person-years	No. of cases	Sex and age stratified HR (95% CI)	Multivariable HR (95% CI) ^a	No. of cases	Sex and age stratified HR (95% CI)	Multivariable HR (95% CI) ^a		
Smoking status									
Never	1,557,648	800	1 [referent]	1 [referent]	10	1 [referent]	1 [referent]	<0.001	
Former	1,459,798	963	1.17 (1.06–1.28)	1.13 (1.03–1.25)	25	2.29 (1.06–4.95)	2.28 (1.06–4.93)		
Current	458,773	218	1.07 (0.92–1.25)	0.97 (0.83–1.14)	10	5.64 (2.23–14.27)	5.27 (2.08–13.40)		
P_{trend}^c			0.03	0.37		<0.001	<0.001		
Cumulative pack-years									
Never	1,557,648	800	1 [referent]	1 [referent]	10	1 [referent]	1 [referent]	0.006	
1–19	948,258	460	1.03 (0.92–1.16)	1.00 (0.89–1.13)	11	1.83 (0.75–4.45)	1.80 (0.74–4.39)		
20–39	562,910	345	1.12 (0.99–1.28)	1.06 (0.93–1.21)	11	3.09 (1.26–7.55)	2.99 (1.22–7.33)		
40	374,638	334	1.31 (1.15–1.49)	1.20 (1.05–1.37)	12	4.32 (1.79–10.4)	4.01 (1.67–9.65)		
P_{trend}^c			<0.0001	0.007		<0.001	0.001		
Pack-years smoked before age 30									
Never	1,638,337	834	1 [referent]	1 [referent]	13	1 [referent]	1 [referent]	0.13	
1–9	1,209,229	637	1.07 (0.96–1.19)	1.02 (0.91–1.13)	17	2.41 (1.06–5.48)	2.35 (1.03–5.34)		
10	624,393	459	1.23 (1.09–1.38)	1.15 (1.02–1.30)	12	2.46 (1.00–6.03)	2.35 (0.95–5.79)		
P_{trend}^c			<0.001	0.03		0.05	0.06		
Age at start of smoking									
Never	1,557,648	800	1 [referent]	1 [referent]	10	1 [referent]	1 [referent]	0.08	
20	826,229	519	1.06 (0.95–1.19)	1.00 (0.90–1.12)	18	3.02 (1.35–6.78)	2.86 (1.27–6.42)		
<20	109,0668	632	1.18 (1.06–1.31)	1.11 (1.00–1.24)	16	2.41 (1.06–5.52)	2.37 (1.04–5.41)		
P_{trend}^c			0.002	0.07		0.04	0.04		

CI, confidence interval; HR, hazard ratio.

^aModels were stratified by sex, age, and time of questionnaire return. Multivariable models were adjusted for body mass index (<25 vs. 25–30 vs. 30 kg/m²), family history of colorectal cancer in any first-degree relative, regular use of aspirin, physical activity level [quintiles of mean metabolic equivalent task (MET) score per week], red meat intake (quintiles of servings/day), total calorie intake (quintiles), alcohol consumption (0 or quartile of g/day), folate intake (quintiles of µg/day), calcium intake (quintiles of mg/day) and current multivitamin intake.

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^d Multivariable tests for heterogeneity shows significance ($p < 0.01$) of differential smoking effects on colorectal cancer risk by tumor synchronicity. The threshold for statistical significance ($\alpha = 0.01$) was adjusted using conservative Bonferroni correction.

^e Based on the linear trend test by using the ordinal or median continuous value for each of the categories as shown.

Duration of smoking cessation (years) and risk of solitary or synchronous tumors in colorectal cancer.

Table 3

Person-years	Solitary tumor			Synchronous tumor			$P_{\text{heterogeneity}}^b$
	No. of cases	Sex and age stratified HR (95% CI)	Multivariable HR (95% CI) ^d	No. of cases	Sex and age stratified HR (95% CI)	Multivariable HR (95% CI) ^d	
Current	460,651	1 [referent]	1 [referent]	10	1 [referent]	1 [referent]	0.001
1–9	336,233	1.29 (1.07–1.55)	1.34 (1.11–1.61)	5	0.55 (0.19–1.65)	0.53 (0.18–1.58)	
10	1,107,921	1.03 (0.88–1.20)	1.10 (0.94–1.29)	20	0.38 (0.17–0.86)	0.42 (0.19–0.95)	
Never	1,558,296	0.93 (0.80–1.08)	1.02 (0.90–1.19)	10	0.18 (0.07–0.44)	0.19 (0.07–0.48)	
P_{trend}^c		0.70	0.61		<0.001	0.001	

CI, confidence interval; HR, hazard ratio.

^aModels were stratified by sex, age and time of questionnaire return. Multivariable models were adjusted for body mass index (<25 vs. 25–30 vs. 30 kg/m²), family history of colorectal cancer in any first-degree relative, regular use of aspirin, physical activity level [quintiles of mean metabolic equivalent (MET) task score per week], red meat intake (quintiles of servings/day), total calorie intake (quintiles), alcohol consumption (0 or quartile of g/day), folate intake (quintiles of µg/day), calcium intake (quintiles of mg/day), current multivitamin intake and postmenopausal hormone use (NHS only).

^bMultivariable tests for heterogeneity shows significance ($p < 0.01$) of differential smoking effects on colorectal cancer risk by tumor synchronicity. The threshold for statistical significance ($\alpha = 0.01$) was adjusted using conservative Bonferroni correction.

^cBased on the linear trend test by using the ordinal categories among current and former smokers by additionally adjusting for never vs. ever smoking status.

Table 4

Frequency of molecular markers according to tumor synchronicity

	<u>Solitary CRC</u>		<u>Synchronous CRC</u>		<i>P</i> *
	n	(%)	n	(%)	
CIMP-negative or CIMP-low	1033	(83)	22	(65)	0.01
CIMP-high	215	(17)	12	(35)	
Microsatellite stable or MSI-low	1084	(84)	23	(66)	0.008
MSI-high	200	(16)	12	(34)	
<i>BRAF</i> wild-type	1117	(86)	21	(62)	<0.001
<i>BRAF</i> mutant	188	(14)	13	(38)	
CIMP-negative/low & microsatellite stable/MSI-low & <i>BRAF</i> wild-type (all negative)	886	(73)	18	(53)	0.01
CIMP-high OR MSI-high- OR <i>BRAF</i> mutant (at least one positive)	321	(27)	16	(47)	

* Two-tailed Fisher's exact test.

Table 5

Smoking status and risk of solitary CRC stratified by molecular subtype or synchronous CRC

	Solitary CRC		Synchronous CRCs	$P_{\text{heterogeneity}}^b$
	CIMP-low or negative, MSI-low or microsatellite stable, and <i>BRAF</i> wild-type (all negative) Multivariable HR (95% CI) ^a	CIMP-high or MSI-high or <i>BRAF</i> mutant (at least one positive) Multivariable HR (95% CI) ^a	Multivariable HR ^a (95% CI)	
Never	1 [referent]	1 [referent]	1 [referent]	<0.001
Past	1.20 (1.04-1.39)	1.18 (0.93-1.49)	2.33 (1.08-5.03)	
Current	1.07 (0.84-1.36)	1.47 (1.02-2.12)	5.35 (2.11-13.54)	
P_{trend}^c	0.10	0.03	<0.001	
$P_{\text{heterogeneity}}^d = 0.002$				

^a Models were stratified by sex, age, and time of questionnaire return. Multivariable models were adjusted for body mass index (<25 vs. 25–30 vs.

30 kg/m²), family history of colorectal cancer in any first-degree relative, regular use of aspirin, physical activity level [quintiles of mean metabolic equivalent task (MET) score per week], red meat intake (quintiles of servings/day), total calorie intake (quintiles), alcohol consumption (0 or quartile of g/day), folate intake (quintiles of µg/day), calcium intake (quintiles of mg/day) and current multivitamin intake. Solitary CRC cases with unknown molecular status were also included in the model as a separate outcome (*data not shown*).

^b Multivariable test for heterogeneity shows significance ($p < 0.05$) of differential smoking effects between solitary CRC (stratified by molecular subtype) and synchronous CRCs overall.

^c Based on the linear trend test by using the ordinal values for each of the categories shown.

^d Pair-wise test for heterogeneity of differential smoking effects between solitary CRC cases positive for CIMP-H, MSI-H or *BRAF* mutant and synchronous CRC cases.