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Cigarette Smoking and Colorectal Cancer Risk by *KRAS* Mutation Status Among Older Women

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Abstract

OBJECTIVES—Existing data support a modest association between cigarette smoking and incident colorectal cancer (CRC) overall. In this study, we evaluated associations between cigarette smoking and CRC risk stratified by *KRAS* mutation status, using data and tissue resources from the Iowa Women's Health Study (IWHS).

METHODS—The IWHS is a population-based cohort study of cancer incidence among 41,836 randomly selected Iowa women, ages 55–69 years of age at enrollment (1986). Exposure data,

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including cigarette smoking, were obtained by self-report at baseline. Incident CRCs (n = 1,233) were ascertained by annual linkage with the Iowa Cancer Registry. Archived tissue specimens from CRC cases recorded through 2002 were recently requested for molecular epidemiology investigations. Tumor *KRAS* mutation status was determined by direct sequencing of exon 2, with informative results in 507/555 (91%) available CRC cases (342 mutation negative and 165 mutation positive). Multivariate Cox regression models were fit to estimate relative risks (RRs) and 95% confidence intervals (CIs) for associations between cigarette smoking variables and *KRAS*-defined CRC subtypes.

RESULTS—Multiple smoking variables were associated with increased risk for *KRAS* mutation-negative tumors, including age at initiation (P = 0.02), average number of cigarettes per day (P = 0.01), cumulative pack-years (P = 0.05), and induction period (P = 0.04), with the highest point estimate observed for women who smoked ≥ 40 cigarettes per day on average (RR = 2.38; 95% CI = 1.25–4.51; compared with never smokers). Further consideration of CRC subsite suggested that cigarette smoking may be a stronger risk factor for *KRAS* mutation-negative tumors located in the proximal colon than in the distal colorectum. None of the smoking variables were significantly associated with *KRAS* mutation-positive CRCs (overall or stratified by anatomic subsite).

CONCLUSIONS—Data from this prospective study of older women demonstrate differential associations between cigarette smoking and CRC subtypes defined by *KRAS* mutation status, and are consistent with the hypothesis that smoking adversely affects the serrated pathway of colorectal carcinogenesis.

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common incident and second most common fatal malignancy in the United States, with ~140,000 new diagnoses recorded and nearly 50,000 deaths attributed to CRC each year (1). Given this burden of disease, further understanding of common, potentially modifiable CRC risk factors represents an important public health priority. Despite widespread tobacco control efforts, about one in five US adults still characterize themselves as active cigarette smokers (2). Pooled analyses of data from numerous observational studies demonstrate increased risks for both benign and malignant colorectal neoplasia among cigarette smokers, as compared with non-smokers (3,4). Interestingly, our group (5) and others (6–15) have reported that cigarette smoking appears to be associated with distinct, molecularly defined CRC subtypes, although the spectrum of genetic alterations and epigenetic modifications involved in smoking-related CRC risk remains incompletely described.

The *KRAS* oncogene has been commonly implicated in colorectal carcinogenesis, with somatic mutations identified in 30–40% of sporadic CRCs (16,17). Tumor testing for *KRAS* mutations has been endorsed as an adjunct to chemotherapy planning (specifically, to inform the addition of anti-epidermal growth factor receptor agents, such as cetuximab or panitumumab, among patients with metastatic CRC) (18,19), emphasizing the relevance of this molecular marker in clinical practice. Since *KRAS* mutations are thought to occur at the early adenoma stage (17), it seems biologically plausible that exposures associated with both invasive and preinvasive disease might differentially modulate CRC risks based on the *KRAS* mutation status. Laboratory experiments have also shown that carcinogens found in tobacco smoke can induce cancer-related base substitutions, such as G:C→A:T transitions, in *ras* oncogenes (16,20). However, to date, relatively few epidemiological studies have examined associations between cigarette smoking and *KRAS*-defined CRC risks (9–13), including only one previous report from a prospective, population-based cohort study (13).

For the current report, we utilized data and tissue resources from the Iowa Women's Health Study (IWHS), a prospective cohort study of cancer end points among older women, to

examine associations between smoking habits and incident CRC by *KRAS* mutation status (negative or positive). *KRAS*-defined CRC risks were further evaluated with respect to anatomic subsite (proximal colon and distal colorectum), as another potential indicator of subtype-specific associations. These data extend prior analyses of cigarette smoking and CRC risk within the IWHS cohort (5,21) by including additional follow-up time and novel molecular marker associations.

METHODS

Approvals for the present study were obtained from the Institutional Review Boards for Human Research at Mayo Clinic Rochester, the University of Minnesota, and the University of Iowa.

Subjects

Details regarding the methods used for recruitment and enrollment of IWHS participants have been previously reported (22). Briefly, a 16-page baseline questionnaire was mailed out in January 1986 to 99,826 randomly selected women, ages 55 –69 years, who resided in Iowa and held a valid driver's license. A total of 41,836 women (42%) returned the baseline questionnaire and these subjects constitute the parent IWHS cohort. As reported by Bisgard *et al.* (23), demographic characteristics and CRC rates were similar for the initial survey responders and non-responders. Vital status and state of residence were determined by mailed follow-up questionnaires in 1987, 1989, 1992, 1997, and 2004, as well as through linkage to Iowa death certificate records. Non-respondents were checked via the National Death Index to identify descendents. For the current analyses, women with a history of malignancy other than skin cancer (n = 3,830), unable to be followed longitudinally for at least 1 day (n = 10), or incomplete characterization of cigarette smoking at baseline (n = 660) were excluded (not mutually exclusive), leaving 37,399 women in the final analytic cohort.

Risk factor assessment

Cigarette smoking patterns among IWHS participants were ascertained at baseline in 1986, including smoking status (never, ever (former or current)), age at smoking initiation (years), smoking duration (years), average number of cigarettes smoked per day, cumulative pack-years, and induction period (difference between the baseline date and age at smoking initiation). Potential confounding factors were also derived from the baseline questionnaire, including body mass index; waist-to-hip ratio; physical activity level; exogenous estrogen use; and daily intake of total calories, total fat, red meat, calcium, folate, methionine, vitamin E, sucrose, and alcohol. Family history of CRC and non-steroidal anti-inflammatory drug use were not systematically recorded at baseline and therefore were not included in this study. However, since neither of these factors has been associated with smoking status (to our knowledge), the potential for confounding from these unmeasured variables seems remote.

Case ascertainment

Incident CRC cases were identified through the Iowa Cancer Registry, which participates in the National Cancer Institute's SEER (Surveillance, Epidemiology, and End Results) program (24). Annual matching between a computer-generated list of all IWHS cohort members and SEER registry data was completed using combinations of first, last, and maiden names; zip code; birth date; and social security number. Data from follow-up surveys indicate that the migration rate out of the IWHS cohort is < 1% annually, allowing for near-complete follow-up of cancer-related end points (25). Incident CRC cases were identified by ICD-O codes, with cancers located in the cecum, ascending colon, hepatic

flexure, transverse colon, and splenic flexure (ICD-O codes 18.0, 18.2–18.5) categorized as proximal colon and cancers located in the descending colon, sigmoid colon, rectosigmoid junction, and rectum (ICD-O codes 18.6, 18.7, 19.9, 20.9) categorized as distal colorectum (26,27). Beginning in 2006, archived, paraffin-embedded tissue specimens were requested from incident CRC cases diagnosed among IWHS participants through 31 December 2002. Tissue specimens were subsequently retrieved for 732/1,255 cases (58 %). For the present study, 22 incident CRC cases were excluded due to incomplete smoking data. To assess the possibility of selection bias introduced by tissue availability status, general demographics, smoking patterns, and tumor characteristics (size and stage) were compared between incident CRC cases with retrieved vs. non-retrieved tissue specimens; no statistically significant differences were observed ($P > 0.05$ for any comparison; data not shown). All incident CRC cases were histologically confirmed by a single gastrointestinal pathologist. Following tissue processing (including DNA extraction), high-quality, usable samples were obtained for 555 CRC cases.

Tissue selection and DNA extraction

Paraffin blocks were serially sectioned in 5 or 10 μm increments. One slide was stained with hematoxylin and eosin, and areas of normal and neoplastic (defined as ≥50% dysplastic cells in the field of view) tissue were identified. Tumor and normal tissue samples were scraped from unstained slides and placed into separate tubes for DNA extraction, according to manufacturer's instructions (Qiagen, Valencia, CA).

Characterization of *KRAS* mutation status

The tumor DNA was PCR amplified with primers for exon 2 (codons 12 and 13). Thermocycler conditions were 95 ° C for 10 min, followed by 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30s. There was a final extension for 10 min at 72 °C. The PCR product was cleaned using Shrimp Alkaline Phosphatase and Exonuclease I. Next, the product was sequenced using the Applied Biosystems PRISM BigDye Terminator v1.1 cycle sequencing kit per directions on an ABI PRISM 3730 DNA analyzer (ABI, Carlsbad, CA). Data analysis was performed using the Mutation Surveyor software (SoftGenetics, State College, PA). *KRAS* mutation status (wild-type vs. specific mutation) was determined for 507 (91.4 %) of the available 555 CRC cases. Positive results for 30 of the 555 cases were confirmed using the DxS *KRAS* Mutation Test Kit (Qiagen), which utilizes fluorescent, allele specific real-time quantitative PCR to detect seven point mutations in the *KRAS* oncogene on the LC 480 (Roche, Indianapolis, IN) instrument.

Statistical analysis

Data were descriptively summarized using frequencies and percentages for categorical variables and means and standard deviations for continuous variables. Measures of agreement across molecularly defined tumor subtypes of CRC were examined by use of kappa coefficients. Follow-up was calculated as age at completion of the baseline survey until age at first CRC diagnosis, age at move from Iowa, or age at death. If none of these events occurred, a woman was assumed to be alive, cancer free, and living in Iowa through 31 December 2002. Cox proportional hazard regression analysis was used to estimate relative risks (RRs) and 95% confidence intervals (CIs) for associations between cigarette smoking exposures and incident CRC outcomes.

All eligible IWHS participants were included in the Cox regression analyses, regardless of eventual cancer status. Incidence was modeled as a function of age because age is a better predictor of cancer risk in our cohort than follow-up time (28). We assessed the effects of smoking status (never, ever, former, or current), age at smoking initiation (>30 or ≤ 30 years), total smoking duration (1–19, 20–39, or ≥ 40 years), average number of cigarettes

smoked per day (1–19, 20–39, or ≥ 40 cigarettes per day), cumulative cigarette pack-years (1–19, 20 –39, or ≥ 40 pack-years), and smoking induction period (<35, 35–39, 40–44, or ≥ 45 years). For all such analyses, never smokers were modeled as the reference group. Tests for trend were carried out for each smoking variable by ordering the categorized values from lowest to highest category and including the resulting variable as a linear term with 1 df in a Cox proportional hazards model. The Cox regression proportionality assumption was formally evaluated by fitting and testing a smoking-by-time interaction term.

Smoking associations were examined with respect to CRC subsets defined by *KRAS* mutation status (mutation negative or mutation positive) and anatomic subsite (proximal colon or distal colorectum). The outcome variable was incident CRC with the *KRAS* mutation status of interest; all other CRC cases (including those with missing or unknown *KRAS* mutation status) were considered censored observations at the date of first diagnosis. We also examined associations between cigarette smoking and incident CRC, based on the tissue availability status (available or not available) by using the same multi-outcome analytic approach as described above to determine whether incomplete tissue collection introduced any possible association biases. Two sets of Cox regression models were fit, one accounting for age and one adjusting for age and other potential confounding factors were body mass index (quartiles); waist-to-hip ratio (quartiles); physical activity level (low, moderate, or high); exogenous estrogen use (never or ever); and daily intake (quartiles) of total calories (kcal/day), total fat (g/day), red meat (g/day), calcium (mg/day), folate (μg/day), methionine (g/day), vitamin E (mg/day), sucrose (g/day), and alcohol (0, 0–3.4, or >3.4g/day). We also formally determined whether risk estimates for the smoking-related variables differed across *KRAS*-defined CRC subtypes using a competing risk form of Cox proportional hazards regression (29). This approach allowed us to specifically model and test the interaction between smoking (modeled as a covariate) and CRC subtype (included as a Cox regression stratum variable). All statistical tests were two-sided, and all analyses were carried out with the SAS (SAS Institute, Cary, NC) and S-Plus (Insightful, Seattle, WA) software systems (SAS[r] proprietary software, release 8.2 [TS2MO]; Splus, version 8.0.1 for Sun SPARC; and Sun OS, version 5.8, 32-bit:2006).

RESULTS

Smoking status was characterized as never, former, and current for 24,638 (66 %), 7,208 (19 %), and 5,553 (15 %) IWHS subjects, respectively. At baseline, never smokers were slightly older than either former or current smokers (mean ages 62.4, 61.9, and 61.4 years; $P<0.01$). Current smokers had a lower body mass index (25.3kg/m^2) compared with never (27.3 kg/m^2) or former (27.2kg/m^2) smokers. Other baseline characteristics also differed by smoking status including waist-to-hip ratio, physical activity level, exogenous estrogen use, and daily intakes of total energy, total fat, red meat, calcium, folate, methionine, vitamin E, sucrose, and alcohol ($P<0.01$ for each variable, except $P=0.03$ for vitamin E; Table 1).

Among the 507 CRC cases for which *KRAS* status could be classified, the molecular subtype distribution was 342 (67 %) mutation-negative and 165 (33 %) mutation-positive tumors. The agreement between *KRAS* mutation status and other molecular subtypes was MSH-H, $\kappa=0.25$; CpG island methylator phenotype positive, $\kappa=0.27$; and *BRAF* mutation positive, $\kappa=0.31$. Multivariate-adjusted risk estimates for associations between cigarette smoking and incident CRC, stratified by *KRAS* mutation status, are presented in Table 2 (age-adjusted risk estimates were generally similar; data not shown). In general, smoking-related risk estimates were higher for *KRAS* mutation-negative than for *KRAS* mutation-positive tumors. Tests for trend across age at initiation (P trend=0.02), average number of cigarettes per day (P trend=0.01), cumulative pack-years (P trend=0.05), and induction period (P trend=0.04) exposure levels were statistically significant for *KRAS* mutation-

negative tumors. Positive associations were also observed for *KRAS* mutation-negative tumors with smoking status (*P* trend=0.08) and total smoking duration (*P*=0.06), but the trend tests were not statistically significant. Women who smoked an average of ≥40 cigarettes per day were at the highest risk for *KRAS* mutation-negative tumors (RR=2.38; 95% CI=1.25–4.51, compared with never smokers). In contrast, none of the smoking variables were significantly associated with *KRAS* mutation-positive CRCs. Of note, tests for heterogeneity in the smoking-related risk estimates across levels of *KRAS* mutation status were not statistically significant (*P*>0.05 for each comparison), likely due in part to power limitations imposed by the available sample size.

Further analyses were conducted to explore smoking-related associations with incident CRCs defined by both *KRAS* mutation status and anatomic subsite (Table 3). For *KRAS* mutation-negative tumors located in the proximal colon, statistically significant risks were observed with smoking status (*P* trend=0.04), age at initiation (*P*=0.03), and average number of cigarettes per day (*P* trend=0.01). Conversely, null associations were observed between the analyzed smoking variables and *KRAS* mutation-negative tumors located in the distal colorectum, as well as *KRAS* mutation-positive tumors located in either the proximal colon or distal colorectum. Of note, relatively small event rates minimized our ability to obtain robust risk estimates for some of the *KRAS*-defined, subsite-specific CRC associations. In analyses defining cases as only those with available tissue, results comparing ever smokers with never smokers (RR=1.19; 95% CI=1.00–1.42, *P*=0.05) were similar to those based on all incident cases (RR = 1.20, 95 % CI=1.07–1.35, *P*=0.003), supporting a low likelihood of selection bias introduced by tissue availability status.

DISCUSSION

In this large prospective cohort study of older women, cigarette smoking was more closely associated with incident CRCs characterized by *KRAS* mutation-negative, rather than *KRAS* mutation-positive, status. Consistent (though not always statistically significant) trends were observed across all categories of cigarette smoking exposure, with intensity (i.e., average number of cigarettes per day), duration, and induction period demonstrating the strongest associations with *KRAS* mutation-negative tumors. Findings from the current study complement our previous report of differential associations between cigarette smoking and CRC subtypes defined by microsatellite instability, CpG island methylator phenotype, or *BRAF* mutation status in the IWHS cohort (5). Together, these data support the hypothesis that cigarette smoking affects CRC risk through the serrated pathway of carcinogenesis (30).

Existing data on *KRAS*-defined CRC risks among cigarette smokers and non-smokers are limited and inconsistent across studies (9–13,15). Consistent with our findings, Slattery *et al.* (11) reported no statistically significant associations between cigarette smoking and *KRAS* mutation-positive colon cancers among men or women in a multicenter case–control study. However, smoking exposure of ≥ 20 cigarettes per day was reportedly associated with a 50 % increased risk for *KRAS* mutation-negative tumors among men (OR=1.5; 95% CI=1.2–1.9), although a null association was observed among women (OR=1.1; 95% CI= 0.8–1.4) (11). A subsequent report from the Slattery group (9) demonstrated no apparent association between active smoking and *KRAS* mutation-positive rectal cancer, although subjects who described long-term exposure to environmental tobacco smoke of >10 h per week were at increased risk for this *KRAS*-mutated rectal cancer (OR=1.50; 95% CI=1.04–2.20). In the only other prospective study reported to date, Weijenberg *et al.* (13) analyzed CRC specimens from a subset of Netherlands Cohort Study participants (*n*=648 cases and 4,083 subcohort controls). Ex-smokers were found to be at increased risk for *KRAS* mutation-negative tumors (RR=1.79; 95% CI=1.00–3.20), but not for *KRAS* mutation-positive tumors

(RR=1.20; 95% CI=0.61–2.33). Current smokers were not at significantly increased risk for either of the *KRAS*-defined CRC subtypes.

Other previous observational studies have described slightly different *KRAS*-specific CRC risk associations than we observed in the IWHS cohort. Diergaarde *et al.* (15) conducted a Dutch population-based, case–control study, which showed no significant difference between risks for *KRAS* mutation-positive or *KRAS* mutation-negative colon cancers among ever vs. never smokers (OR=1.4; 95% CI=0.7–2.8 and OR=0.8; 95% CI=0.5– 1.4, respectively). Using a different study design, Miyaki *et al.* (14) compared the prevalence of *KRAS* mutations in CRCs analyzed from a small group of cigarette smokers (n=28) and non-smokers (n=33), with no statistically significant difference detected between groups (32 vs. 39 % ; P=0.38). In an attempt to clarify the relationship between cigarette smoking, *KRAS* mutation, and colorectal neoplasia risk, Porta *et al.* (16) performed a meta-analysis of available observational data (including two studies with adenoma rather than adenocarcinoma end points) (10,12). The summary risk estimate for the association between tobacco use and *KRAS* mutation-positive tumors was not statistically significant (RR=0.96; 95% CI=0.83–1.13). However, no risk estimate for *KRAS* mutation-negative tumors was reported. Although not conclusive, data from the Netherlands Cohort Study and the IWHS (at least) suggest that further evaluation of cigarette smoking effects on *KRAS* -independent pathways of colorectal carcinogenesis may be informative.

Data from our study revealed statistically significant associations between smoking indicators and *KRAS*-defined, subsite-specific CRC risks. Conversely, in the Netherlands Cohort Study, smoking-related risk estimates for *KRAS* mutation-positive and *KRAS* mutation-negative tumors were reportedly similar when analyzed by colon, rectosigmoid, and rectal subsites (13). However, as with our study, small case numbers limited accurate risk assessment for some of the subgroup analyses. Interestingly, neoplasms arising through the serrated pathway of carcinogenesis (30) are typically characterized by a proximal colonic location, micro-satellite instability-high, CpG island methylator phenotype-high and *BRAF* mutation-positive status, and absence of *KRAS* mutations. These clinical and molecular features are consistent with the smoking-related associations reported in the current study, as well as in previous IWHS reports (5,31). Of note, other studies have also described limitations in colonoscopy-based screening and surveillance algorithms for reducing CRC risk in the proximal colon (32,33) and among active smokers (34). Coupled with our observations, further consideration of modified CRC early detection strategies tailored specifically to cigarette smokers seems indicated.

Major strengths of our study include the prospective design, detailed exposure data, prolonged follow-up time, near-complete case ascertainment, CRC tissue availability, and high-quality *KRAS* mutation analyses. Potential limitations should also be acknowledged. First, the reported findings cannot be directly extrapolated to other demographic subgroups (e.g., younger women, men, and non-Caucasian subjects), which will require further investigation in more diverse subject populations. Second, we were unable to retrieve adequate tissue specimens from all IWHS subjects with incident CRC for the planned molecular analyses. However, as noted above, tissue availability biases did not appear to influence the smoking-related, molecularly defined CRC risk estimates. Third, our sample sizes were relatively low in some of the CRC subsets defined by combinations of *KRAS* mutation status and anatomic subsite. Although we did find a statistically significant association between cigarette smoking and *KRAS* mutation-negative tumors arising from the proximal colon, it remains possible that associations based on other subtype/subsite combinations went undetected.

In summary, data from this prospective cohort study of older women suggest that cigarette smoking is associated with molecularly distinct CRC subtypes, which can be defined, in part, by *KRAS* mutation-negative status. These findings support a possible causative effect from tobacco exposure on *KRAS*-independent mechanisms of colorectal carcinogenesis, perhaps through methylation-induced silencing of DNA mismatch repair genes (and/or other growth regulating genes), resulting in tumors with the serrated pathway phenotype. Further investigation of smoking-related CRC risks based on other molecular markers and integrated pathways is ongoing in the IWHS cohort, which should yield additional insights regarding the mechanisms of carcinogenesis induced by this common, potentially modifiable exposure.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- Cigarette smoking is associated with a moderately increased risk for incident colorectal cancer (CRC) overall.
- Smoking appears to be linked to higher risk for select, molecularly defined CRC subtypes.
- To date, relatively few studies have examined smoking-related CRC risks stratified by *KRAS* mutation status, with mixed results.

WHAT IS NEW HERE

- In this prospective, population-based study of older women, cigarette smoking was more strongly associated with *KRAS* mutation-negative tumors, particularly in the proximal colon.
- These findings suggest that smoking is a greater risk factor for specific colorectal cancer (CRC) subtypes, and are consistent with the hypothesis that smoking adversely affects the serrated pathway of colorectal carcinogenesis.
- If confirmed in other subject populations, then these observations may have important clinical implications with respect to CRC early detection and perhaps chemoprevention/chemotherapy strategies for cigarette smokers.

Table 1

Baseline characteristics of participants, by cigarette smoking status

Variable	Cigarette smoking status		
	Never (*N*=24,638)	Former (*N*=7,208)	Current (*N*=5,553)
Age at enrollment, years	62.4 (4.24)	61.9 (4.2)	61.4 (4.2)
Body mass index, kg/m^2	27.3 (5.02)	27.2 (5.4)	25.3 (4.7)
Waist-to-hip ratio	0.837 (0.1)	0.839 (0.1)	0.843 (0.1)
Physical activity, N *(%)*			
Low	10,874 (45.1)	3,204 (45.1)	3,302 (60.3)
Medium	6,957 (28.9)	1,906 (26.8)	1,266 (23.1)
High	6,279 (26.0)	2,000 (28.1)	910 (16.6)
Estrogen use, N *(%)*			
Never	15,521 (63.7)	4,111 (57.6)	3,281 (59.7)
Ever	8,848 (36.3)	3,024 (42.4)	2,217 (40.3)
Alcohol consumption, g/day	2.1 (5.7)	5.5 (10.2)	8.3 (14.8)
Total energy, kcal/day	1,807.8 (712.4)	1,755.1 (764.6)	1,770.8 (760.0)
Total fat, g/day	68.4 (31.1)	66.3 (33.5)	69.3 (33.6)
Red meat, g/day	91.6 (73.8)	83.5 (77.7)	91 (71.6)
Calcium, mg/day	1,101.7 (561.6)	1,120.9 (590.7)	1,008.6 (582.8)
Folate, μg/day	432.7 (259.9)	438.3 (271.3)	398.5 (268.1)
Methionine, g/day	1.9 (0.9)	1.8 (0.9)	1.8 (0.9)
Vitamin E, mg/day	67.7 (149.7)	68.4 (150.6)	62.2 (148.1)
Sucrose, g/day	42.7 (23.5)	39 (26.1)	38.1 (26.2)
Age at smoking initiation, years	N/A	21.7 (6.6)	22.2 (7.5)
Duration smoked, years	N/A	25 (12.9)	39.2 (8.0)
Average number of cigarettes per day	N/A	15.5 (10.4)	18.4 (8.8)
Cumulative pack-years	N/A	21.4 (19.7)	36.1 (19.1)
Induction period, years	N/A	40.3 (7.3)	39.2 (8.0)
Time since smoking cessation, years	N/A	15.7 (12.1)	N/A

Data presented as mean value (s.d.), unless otherwise indicated; calcium, folate, and vitamin E intake includes supplements.

Table 2

Associations between cigarette smoking and incident colorectal cancer (CRC), by *KRAS* mutation status

Smoking variable	Person-years[a]	*KRAS* mutation negative (*n*=342)			*KRAS* mutation positive (*n*=165)		
		No. of cases[a]	Median years to diagnosis	RR (95% CI)[b]	No. of cases[a]	Median years to diagnosis	RR (95% CI)[b]
Never smokers	375,486	226	12.42	1.00 (ref.)	110	11.93	1.00 (ref.)
Ever smokers	180,409	116	10.90	1.23 (0.97–1.57)	55	10.69	1.05 (0.74–1.50)
Former	104,111	68	10.75	1.20 (0.91–1.59)	34	10.77	1.06 (0.70–1.60)
Current	76,297	48	11.69	1.29 (0.92–1.79)	21	10.69	1.04 (0.63–1.71)
P trend				0.083			0.81
Age at initiation							
> 30 years	17,795	4	9.32	0.38 (0.14–1.02)	7	12.71	1.09 (0.48–2.50)
≤30 years	161,711	111	10.90	1.35 (1.06–1.72)	46	10.05	1.01 (0.70–1.46)
P trend				0.024			0.95
Total duration							
1–19 years	40,381	23	11.65	1.15 (0.74–1.77)	14	11.09	1.14 (0.62–2.09)
20–39 years	87,073	48	10.83	1.14 (0.82–1.58)	20	11.73	0.86 (0.52–1.42)
≥40 years	50,848	43	10.06	1.40 (0.99–1.97)	18	9.20	1.09 (0.65–1.83)
P trend				0.06			0.99
Average number of cigarettes							
1–19 per day	95,965	58	10.99	1.13 (0.84–1.52)	28	10.05	0.99 (0.64–1.53)
20–39 per day	73,546	48	10.90	1.29 (0.93–1.80)	22	11.73	1.00 (0.61–1.65)
≥40 per day	9,022	10	10.96	2.38 (1.25–4.51)	3	12.45	1.37 (0.43–4.34)

Smoking variable	Person-years[a]	*KRAS* mutation negative (*n*=342)			*KRAS* mutation positive (*n*=165)		
		No. of cases[a]	Median years to diagnosis	RR (95% CI)[b]	No. of cases[a]	Median years to diagnosis	RR (95% CI)[b]
P trend				0.01			0.83
Cumulative pack-years							
1–19	74,225	46	11.19	1.20 (0.87–1.67)	18	11.65	0.78 (0.46–1.34)
20–39	59,187	32	10.29	1.03 (0.70–1.51)	24	10.25	1.45 (0.91–2.31)
≥40	42,566	35	11.71	1.55 (1.07–2.25)	9	12.13	0.72 (0.36–1.44)
P trend				0.05			0.98
Induction period							
< 35 years	34,086	8	11.39	0.51 (0.25–1.03)	11	12.22	1.21 (0.63–2.34)
35–40 years	46,825	28	11.46	1.55 (1.03–2.35)	10	11.05	0.94 (0.47–1.90)
40–44 years	52,266	41	11.81	1.64 (1.16–2.32)	14	13.42	0.95 (0.53–1.71)
≥45 years	46,331	38	10.09	1.12 (0.79–1.60)	18	8.58	1.02 (0.61–1.71)
P trend				0.04			0.95

CI, confidence interval; RR, relative risk.

[a] Do not always sum to total due to missing smoking data.

[b] Adjusted for age, body mass index, waist-to-hip ratio, physical activity level, alcohol consumption, exogenous estrogen use, and daily intake of total calories, fat, sucrose, red meat, calcium, folate, vitamin E, and methionine.

Table 3

Associations between cigarette smoking and incident CRC, by *KRAS* mutation status and anatomic subsite

Smoking variable	Person- years[a]	CRC subtype							
		KRAS mutation negative				*KRAS* mutation positive			
		Proximal colon		Distal colon/rectum		Proximal colon		Distal colon/rectum	
		No. of cases[a]	RR (95% CI)[b]	No. of cases[a]	RR (95% CI)[b]	No. of cases[a]	RR (95% CI)[b]	No. of cases[a]	RR (95% CI)[b]
Never smokers	375,486	129	1.00 (ref.)	95	1.00 (ref.)	57	1.00 (ref.)	53	1.00 (ref.)
Ever smokers	180,409	72	1.37 (1.00–1.86)	40	0.98 (0.66–1.45)	30	1.19 (0.74–1.92)	25	0.91 (0.54–1.53)
Former	104,111	41	1.30 (0.90–1.88)	24	0.98 (0.62–1.55)	19	1.23 (0.71–2.13)	15	0.88 (0.47–1.65)
Current	76,297	31	1.47 (0.97–2.23)	16	0.98 (0.56–1.72)	11	1.13 (0.57–2.23)	10	0.95 (0.47–1.95)
P trend			0.04		0.93		0.57		0.80
Age at initiation									
> 30 years	17,795	4	0.66 (0.24–1.79)	0	—	4	1.40 (0.50–3.89)	3	0.77 (0.19–3.19)
≤30 years	161,711	67	1.45 (1.06–2.00)	40	1.11 (0.75–1.65)	25	1.13 (0.68–1.88)	21	0.88 (0.51–1.52)
P trend			0.03		0.71		0.61		0.63
Total duration									
1–19 years	40,381	17	1.56 (0.93–2.60)	6	0.66 (0.29–1.52)	10	1.89 (0.95–3.75)	4	0.38 (0.09–1.57)
20–39 years	87,073	28	1.19 (0.78–1.83)	16	0.87 (0.51–1.50)	10	0.84 (0.41–1.73)	10	0.88 (0.44–1.77)
≥40 years	50,848	25	1.37 (0.88–2.16)	18	1.44 (0.85–2.44)	8	0.95 (0.44–2.05)	10	1.21 (0.60–2.47)
P trend			0.12		0.48		0.85		0.88
Average number of cigarettes									
1–19 per day	95,965	36	1.24 (0.84–1.81)	20	0.91 (0.55–1.49)	17	1.23 (0.70–2.16)	11	0.74 (0.37–1.48)
20–39 per day	73,546	31	1.50 (0.99–2.28)	17	1.05 (0.61–1.79)	10	0.94 (0.45–1.95)	12	1.06 (0.54–2.10)

Smoking variable	Person- years[a]	CRC subtype							
		KRAS mutation negative				*KRAS* mutation positive			
		Proximal colon		Distal colon/rectum		Proximal colon		Distal colon/rectum	
		No. of cases[a]	RR (95% CI)[b]	No. of cases[a]	RR (95% CI)[b]	No. of cases[a]	RR (95% CI)[b]	No. of cases[a]	RR (95% CI)[b]
≥40 per day	9,022	5	2.29 (0.93–5.66)	3	1.46 (0.46–4.65)	2	2.00 (0.48–8.35)	1	0.84 (0.11–6.15
P trend			0.01		0.77		0.64		0.87
Cumulative pack-years									
1–19	74,225	30	1.41 (0.94–2.12)	14	0.84 (0.47–1.49)	10	0.97 (0.49–1.92)	8	0.59 (0.25–1.39)
20–39	59,187	18	0.99 (0.59–1.67)	14	1.09 (0.61–1.93)	13	1.53 (0.80–2.92)	11	1.35 (0.69–2.66)
≥40	42,566	21	1.67 (1.03–2.70)	12	1.20 (0.65–2.24)	4	0.66 (0.23–1.86)	5	0.78 (0.30–2.00)
P trend			0.08		0.63		0.94		0.91
Induction period									
< 35 years	34,086	6	0.71 (0.31–1.61)	2	0.28 (0.07–1.13)	6	1.50 (0.64–3.52)	5	0.94 (0.34–2.64)
35–40 years	46,825	16	1.69 (0.97–2.95)	10	1.17 (0.60–2.29)	6	1.38 (0.58–3.29)	4	0.56 (0.17–1.84)
40–44 years	52,266	31	2.31 (1.53–3.48)	10	0.88 (0.45–1.70)	7	0.95 (0.40–2.24)	7	0.93 (0.41–2.09)
≥45 years	46,331	18	0.88 (0.53–1.46)	18	1.36 (0.81–2.29)	10	1.07 (0.53–2.14)	8	0.97 (0.45–2.09)
P trend			0.09		0.42		0.83		0.75

CI, confidence interval; CRC, colorectal cancer; RR, relative risk.

[a] Do not always sum to total due to missing smoking and/or anatomic subsite data.

[b] Adjusted for age, body mass index, waist-to-hip ratio, physical activity level, alcohol consumption, exogenous estrogen use, and daily intake of total calories, fat, sucrose, red meat, calcium, folate, vitamin E, and methionine.