Smoking and Colorectal Cancer Risk, Overall and by Molecular Subtypes

Smoking and Colorectal Cancer Risk, Overall and by Molecular Subtypes: A Meta-Analysis

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INTRODUCTION: **The aim of this study was to provide the most comprehensive and up-to-date evidence on the association between cigarette smoking and colorectal cancer (CRC) risk.**

METHODS: **We conducted a systematic review and meta-analysis of epidemiological studies on the association between cigarette smoking and CRC risk published up to September 2018. We calculated relative risk (RR) of CRC according to smoking status, intensity, duration, pack-years, and time since quitting, with a focus on molecular subtypes of CRC.**

RESULTS: **The meta-analysis summarizes the evidence from 188 original studies. Compared with never smokers, the pooled RR for CRC was 1.14 (95% confidence interval [CI] 1.10–1.18) for current smokers and 1.17 (95% CI 1.15–1.20) for former smokers. CRC risk increased linearly with smoking intensity and duration. Former smokers who had quit smoking for more than 25 years had significantly decreased risk of CRC compared with current smokers. Smoking was strongly associated with the risk of CRC, characterized by high CpG island methylator phenotype (RR 1.42; 95% CI 1.20–1.67; number of studies [n] = 4), *BRAF* mutation (RR 1.63; 95% CI 1.23–2.16; n = 4), or high microsatellite instability (RR 1.56; 95% CI 1.32–1.85; n = 8), but not characterized by *KRAS* (RR 1.04; 95% CI 0.90–1.20; n = 5) or *TP53* (RR 1.13; 95% CI 0.99–1.29; n = 5) mutations.**

DISCUSSION: **Cigarette smoking increases the risk of CRC in a dose-dependent manner with intensity and duration, and quitting smoking reduces CRC risk. Smoking greatly increases the risk of CRC that develops through the microsatellite instability pathway, characterized by microsatellite instability-high, CpG island methylator phenotype positive, and *BRAF* mutation.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/AJG/B607, http://links.lww.com/AJG/B608

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common types of cancer, with over 1.8 million new CRC cases and 881,000 deaths from CRC worldwide in 2018 (1). The risk of CRC can be substantially reduced by participating in the CRC screening program (2) and by following a favorable lifestyle, specifically by being physically active, minimizing excess body fatness, and avoiding tobacco smoking, alcoholic beverages, and a diet rich in red and processed meats (3,4).

The role of tobacco smoking in CRC risk has been unclear until recently. Only in 2009, the International Agency for Research on Cancer ascertained the link between carcinogenicity of tobacco smoking and risk of CRC (5). Although cigarette smoking considerably increases the risk of cancers in many organs, including lung, oral cavity, pharynx, esophagus, bladder, kidney, cervix, and pancreas, previous estimates suggest that cigarette smoking has a marginal effect on CRC risk, increasing risk by 15%–20% (6). Although early studies considered CRC a

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single disease, in recent years, different molecular subtypes have been defined, characterized by different driver mutations including in *BRAF* and *KRAS* and by epigenetic modifications including hypermethylation. Little is known about how cigarette smoking affects the risk of CRC and whether it selectively affects the risk of specific subtypes of CRC.

To provide up-to-date estimates of the effect of cigarette smoking on CRC risk, we conducted a comprehensive review and meta-analysis of epidemiological studies published until 2018. In this meta-analysis, we performed new dose-response analyses to investigate how smoking duration and smoking intensity impact CRC risk. For the first time in the literature, we systematically pooled together the evidence on the effect of smoking cessation on CRC risk. Finally, we calculated the risk estimates according to tumor characteristics, such as site within the colorectum, and CRC molecular characteristics to investigate hypotheses on the molecular mechanisms behind the association between cigarette smoking and risk of CRC.

METHODS

This meta-analysis on CRC risk is part of a series of systematic reviews and meta-analyses on the association between cigarette smoking (from now on simply referred to as smoking) and the risk of cancer at any site (7–11). This review takes advantage of an innovative methodology, which combines umbrella and traditional reviews (7,10). Through the umbrella review, all systematic reviews and meta-analyses on the association of interest are identified. All original studies published after the last review or meta-analysis are identified through the traditional review. The full search strategy, eligibility criteria, and data extraction are summarized in Annex 1 (see Supplementary Digital Content 1, http://links.lww.com/AJG/B608). The protocol of the present study has been registered in the International Prospective Register of Systematic Reviews (registration number: CRD42017063991).

Statistical methods

Pooled relative risks (RRs) for current, former, and ever smokers were estimated for CRC and, separately, for colon cancer and rectal cancer, overall and by study design (i.e., cohort and case control). These estimates were obtained using random-effects meta-analytic models to take into account the heterogeneity of risk estimates (12). Heterogeneity between studies was assessed using the χ^2 test, and inconsistency was measured using the I^2 statistic, which represents the proportion of total variation attributable to between-study variance (13). We conducted stratified analyses based on various study and population characteristics. Moreover, we conducted stratified analyses according to CRC molecular subtypes.

To evaluate publication bias, we examined the funnel plots (14) and applied the Egger test for funnel plot asymmetry (15).

Study quality was assessed independently by 2 authors (E. Borroni and G.P.) using the Newcastle-Ottawa Scale (16). Discrepancies were solved with the help of 2 other authors (S.G. and A.L.). Newcastle-Ottawa Scale score ranges between 0 (poor quality) and 9 (good quality) and considers information on the following 3 broad categories: selection (4 points), comparability (2 points), and outcome for case-control or exposure for cohort studies (3 points). For comparability, we identified the following 4 essential confounders: age, sex, body mass index, and alcohol. In this meta-analysis, high-quality studies were defined as those with score $\geq$7.

We investigated both linear and nonlinear associations between smoking intensity (for current and ever vs never smokers), smoking duration (for current and ever vs never smokers), pack years (for current and ever vs never smokers), time since quitting (for former vs current smokers), and the log RR of CRC. For each exposure variable, we tested the log-linearity using the Wald test. Dose-response relationships between smoking variables and log RR of CRC, either linear or not, were evaluated using a 1-stage random-effects dose-response model (17). The observed nonlinear relationships were modeled using restricted cubic spline with 3 knots at fixed percentiles of exposure (10%, 50%, and 90%) (7,18). For each category, the level of exposure was assigned as the midpoint between the upper and the lower bounds; for open-ended upper categories, the level of exposure was determined as 1.2 times the lower bound (10,19,20). When the number of cases and/or controls in one or more exposure categories was not provided in the original study publication, we estimated the covariance among the log RR by considering the total number of cases and/or controls in the study weighted by the average percent distribution of subjects pooled from all other studies (21).

All statistical analyses were performed using the R-software version 3.4.1 (R Development Core Team, 2017) and, in particular, the "meta" and "dosresmeta" packages (21).

The main findings of this meta-analysis will be published in a dedicated website (www.epideuro.eu/scp), where additional data could be provided to keep the meta-analysis updated.

RESULTS

Study selection and description

We identified 225 eligible original articles; 37 of them were excluded because their results were already reported in other publications (see Figure 1 and Table 1, Supplementary Digital Content 2, http://links.lww.com/AJG/B607). Thus, a total of 188 original articles met the eligibility criteria and were included in the present meta-analysis. Included studies were published between 1958 and 2018 and described a total of 383,154 CRC cases. The main characteristics of the included case control (n = 106) and cohort (n = 82) studies are summarized in Tables 2 and 3 (see Supplementary Digital Content 2, http://links.lww.com/AJG/B607), respectively. Publications containing data that were partially excluded from the meta-analysis are described in Table 4 (see Supplementary Digital Content 2, http://links.lww.com/AJG/B607), with the corresponding reasons of exclusion.

Quantitative data synthesis

The pooled RR of CRC was 1.14 (95% confidence interval [CI] 1.10–1.18) for current compared with never smokers, based on 88 studies (Figure 1), 1.17 (95% CI 1.15–1.20) for former compared with never smokers, based on 79 studies (see Figure 2, Supplementary Digital Content 2, http://links.lww.com/AJG/B607), and 1.18 (95% CI 1.15–1.22) for ever compared with never smokers, based on 131 studies (see Figure 3, Supplementary Digital Content 2, http://links.lww.com/AJG/B607).

Compared with never smokers, the RR for cancer located within the colon was 1.05 (95% CI 0.99–1.10; n = 54) for current smokers, 1.15 (95% CI 1.11–1.19; n = 49) for former smokers, and 1.11 (95% CI 1.07–1.15; n = 67) for ever smokers (Table 1 and see Figures 4–6, Supplementary Digital Content 2, http://links.lww.com/AJG/B607). The RR for rectal cancer were 1.16 (95% CI 1.09–1.23; n = 50) for current smokers, 1.17 (95% CI 1.12–1.22; n = 46) for former smokers, and 1.15 (95%

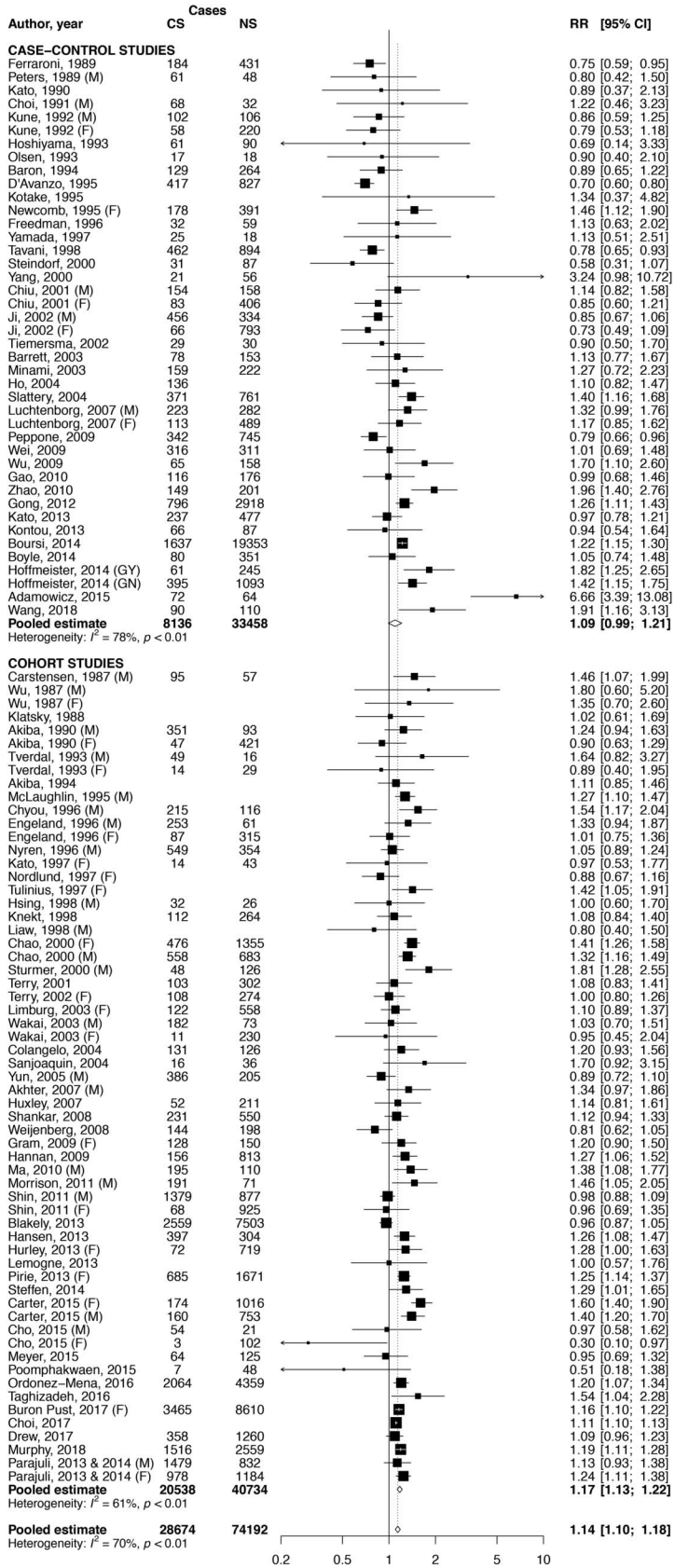

Figure 1. Forest plot of study-specific and pooled relative risk (RR) of colorectal cancer for current cigarette smokers (CS) vs never smokers (NS). CI, confidence interval; F, female; GN, gastroscopy no; GY, gastroscopy yes; M, male.

Table 1. Pooled relative risk (RR) and corresponding 95% confidence interval (CI) for colorectal cancer risk for current, former, and ever cigarette smokers vs never cigarette smokers, overall and in strata of selected characteristics

	Current smokers				Former smokers				Ever smokers			
Strata	No. of studies	Pooled RR (95% CI)	P^a	P^b	No. of studies	Pooled RR (95% CI)	P^a	P^b	No. of studies	Pooled RR (95% CI)	P^a	P^b
Total	88	1.14 (1.10–1.18)		<0.01	79	1.17 (1.15–1.20)		<0.01	131	1.18 (1.15–1.22)		<0.01
Cancer site												
Rectum	50	1.16 (1.09–1.23)	0.02	<0.01	46	1.17 (1.12–1.22)	0.62	0.03	58	1.15 (1.10–1.21)	0.20	<0.01
Colon	54	1.05 (0.99–1.10)		<0.01	49	1.15 (1.11–1.19)		<0.01	67	1.11 (1.07–1.15)		<0.01
Distal	11	1.04 (0.97–1.11)	0.05	0.21	10	1.23 (1.18–1.28)	<0.01	0.91	15	1.12 (1.07–1.17)	0.41	0.64
Proximal	12	1.15 (1.07–1.23)		0.07	10	1.11 (1.06–1.15)		0.42	15	1.14 (1.10–1.19)		0.50
Sex												
Men	37	1.19 (1.11–1.21)	0.63	<0.01	34	1.22 (1.18–1.26)	0.03	0.46	46	1.17 (1.11–1.22)	0.47	<0.01
Women	34	1.17 (1.09–1.25)		<0.01	30	1.16 (1.13–1.19)		0.90	39	1.14 (1.09–1.19)		<0.01
Geographic area[c]												
North America	26	1.24 (1.15–1.33)	0.01	<0.01	25	1.20 (1.17–1.24)	0.28	0.52	31	1.19 (1.14–1.24)	0.08	<0.01
Europe	32	1.12 (1.04–1.21)		<0.01	29	1.15 (1.09–1.21)		<0.01	40	1.17 (1.11–1.23)		<0.01
Asia	24	1.06 (0.99–1.14)		0.03	20	1.18 (1.16–1.20)		0.89	49	1.18 (1.12–1.25)		<0.01
Oceania	4	1.00 (0.86–1.16)		0.15	4	1.10 (0.96–1.27)		0.01	4	1.07 (0.93–1.23)		0.01
Others[d]	0	—		—	0	—		—	5	2.46 (1.39–4.37)		0.01
Income group												
High income	79	1.15 (1.11–1.20)	0.15	<0.01	72	1.18 (1.15–1.21)	0.15	<0.01	99	1.15 (1.12–1.19)	<0.01	<0.01
Middle income	7	1.01 (0.85–1.20)		0.04	6	1.05 (0.90–1.22)		0.83	30	1.34 (1.23–1.46)		<0.01
Type of study												
Cohort	51	1.17 (1.13–1.22)	0.18	<0.01	46	1.17 (1.15–1.20)	0.84	0.14	51	1.17 (1.14–1.20)	0.10	<0.01
Case-control	37	1.09 (0.99–1.21)		<0.01	33	1.16 (1.09–1.25)		<0.01	80	1.22 (1.17–1.28)		<0.01
Type of controls[e]												
Hospital	17	1.10 (0.91–1.34)	0.77	<0.01	14	1.06 (0.94–1.19)	0.04	0.06	42	1.36 (1.20–1.53)	0.03	<0.01
Population	20	1.14 (1.04–1.24)		<0.01	19	1.23 (1.14–1.32)		0.03	37	1.18 (1.12–1.24)		<0.01
End point[f]												
Incidence	37	1.13 (1.09–1.18)	<0.01	<0.01	36	1.16 (1.14–1.19)	0.08	0.11	40	1.15 (1.11–1.18)	<0.01	<0.01
Mortality	20	1.32 (1.27–1.38)		0.19	16	1.21 (1.17–1.25)		0.84	18	1.24 (1.20–1.28)		0.88
Year of publication												
≤2002	39	1.06 (0.98–1.16)	0.06	<0.01	35	1.12 (1.06–1.20)	0.06	0.01	44	1.06 (1.00–1.13)	<0.01	<0.01
2003–2010	22	1.16 (1.06–1.27)		<0.01	20	1.24 (1.17–1.30)		0.50	36	1.23 (1.16–1.30)		<0.01

Table 1. (*continued*)

Strata	Current smokers				Former smokers				Ever smokers			
	No. of studies	Pooled RR (95% CI)	P^a	P^b	No. of studies	Pooled RR (95% CI)	P^a	P^b	No. of studies	Pooled RR (95% CI)	P^a	P^b
≥2011	27	1.20 (1.14–1.26)		<0.01	24	1.17 (1.14–1.20)		0.01	51	1.24 (1.19–1.29)		<0.01
Adjustments												
Nonadequate	60	1.11 (1.06–1.16)	0.07	<0.01	53	1.15 (1.11–1.18)	0.02	<0.01	100	1.18 (1.14–1.23)	0.68	<0.01
Adequate[g]	28	1.20 (1.12–1.28)		<0.01	26	1.21 (1.17–1.25)		0.26	31	1.20 (1.14–1.26)		<0.01
Study quality												
Low (NOS <7)	58	1.10 (1.05–1.16)	0.03	<0.01	51	1.15 (1.11–1.18)	0.01	<0.01	101	1.19 (1.15–1.23)	0.57	<0.01
High (NOS ≥7)	30	1.20 (1.14–1.26)		<0.01	28	1.21 (1.18–1.24)		0.61	30	1.20 (1.16–1.24)		0.15

NOS, Newcastle-Ottawa Scale.
[a]*P* value for heterogeneity across strata.
[b]*P* value for heterogeneity within strata.
[c]Studies conducted in multiple countries from different geographic areas were not included.
[d]South America based on 4 studies and Africa on 1 study only.
[e]Type of controls for case-control studies only. Pooled analyses considering both studies with hospital and with population controls were not included.
[f]End point for cohort studies only. Studies providing RRs for both incidence and mortality were considered in both categories.
[g]Estimates adjusted for, at least, age, sex, body mass index, and alcohol consumption.

CI 1.10–1.21; n = 58) for ever smokers (see Table 1 and Figures 7–9, Supplementary Digital Content 2, http://links.lww.com/AJG/B607).

We conducted stratified analyses to investigate possible sources of heterogeneity for current, former, and ever smokers (Table 1). Among current smokers, significant differences were observed according to geographic area (RRs of CRC were higher in North America compared with Europe, Asia, and Oceania; $P = 0.01$) and endpoint (among cohort studies; RRs 1.32 for mortality, and 1.13 for incidence; $P < 0.01$). Among former smokers, men (RR 1.22) had significantly higher risk of CRC compared with women (RR 1.16; $P = 0.03$) and RRs of CRC were higher in population-based (RR 1.23) compared with hospital-based case-control studies (RR 1.06; $P = 0.04$). Among ever smokers, significant differences were observed according to the income group (RRs were 1.34 for middle- and 1.15 for high-income countries; $P < 0.01$), type of controls (RRs were 1.18 in population-based and 1.36 in hospital-based case-control studies; $P = 0.03$), endpoint (RRs were 1.24 among cohort studies investigating mortality and 1.15 incidence of CRC; $P < 0.01$), and year of publication (RRs of CRC were higher in studies published after 2003 compared with previous studies; $P < 0.01$). RRs of CRC were higher in high-quality studies in both current (RR 1.20 in high- and 1.10 in low-quality studies; $P = 0.03$) and former smokers (RR 1.21 in high- and 1.15 in low-quality studies; $P = 0.01$), compared with never smokers. Similar results were observed when stratifying by adequacy of adjustments.

No clear differences emerged for the effect of smoking on cancer in proximal vs distal colon (Table 1). However, when stratifying according to sex and cancer site simultaneously, we found that women who were current, former, and ever smokers had a significantly increased risk of proximal colon cancer compared with never smokers (RR 1.22, 95% CI 1.14–1.30; RR 1.14, 95% CI 1.03–1.26, and RR 1.17, 95% CI 1.10–1.26, respectively), whereas men did not (RR 1.03, 95% CI 0.92–1.16; RR 1.04, 95% CI 0.91–1.19; and RR 1.07, 95% CI 0.99–1.16). The difference between men and women was significant among current smokers ($P = 0.01$) but not among former and ever smokers ($P = 0.29$ and 0.09, respectively). By contrast, no clear trend or significant difference emerged between men and women when analyzing distal colon cancer or rectal cancer (data not shown).

Dose-response analysis

Sixty-one studies reported RR estimates for smoking intensity (34 among current and 27 among ever smokers), 45 for smoking duration (9 among current and 36 among ever smokers), and 19 for time since quitting smoking. Figure 2 shows the dose-response relationships among current smokers between smoking intensity (panel a), duration (panel b), pack-years (panel c), and time since quitting (panel d) and the risk of CRC. CRC risk increased linearly with intensity of smoking: RR were 1.14 (95% CI 1.06–1.23) for 20 and 1.31 (95% CI 1.12–1.52) for 40 cigarettes per day (Figure 2A, estimated using the curve functions in Supplement Box 1, see Supplementary Digital Content 2, http://links.lww.com/AJG/B607). The RR of CRC also increased linearly with increasing duration of smoking: RRs were 1.09 (95% CI 1.04–1.15) for 20 and 1.20 (95% CI 1.09–1.32) for 40 years (Figure 2B). The RR of CRC also increased linearly with increasing number of pack years: RRs were 1.10 (95% CI 1.05–1.14) for 20 and 1.20 (95% CI 1.05–1.31) for 40 (Figure 2C). The risk of CRC started decreasing at 10 years

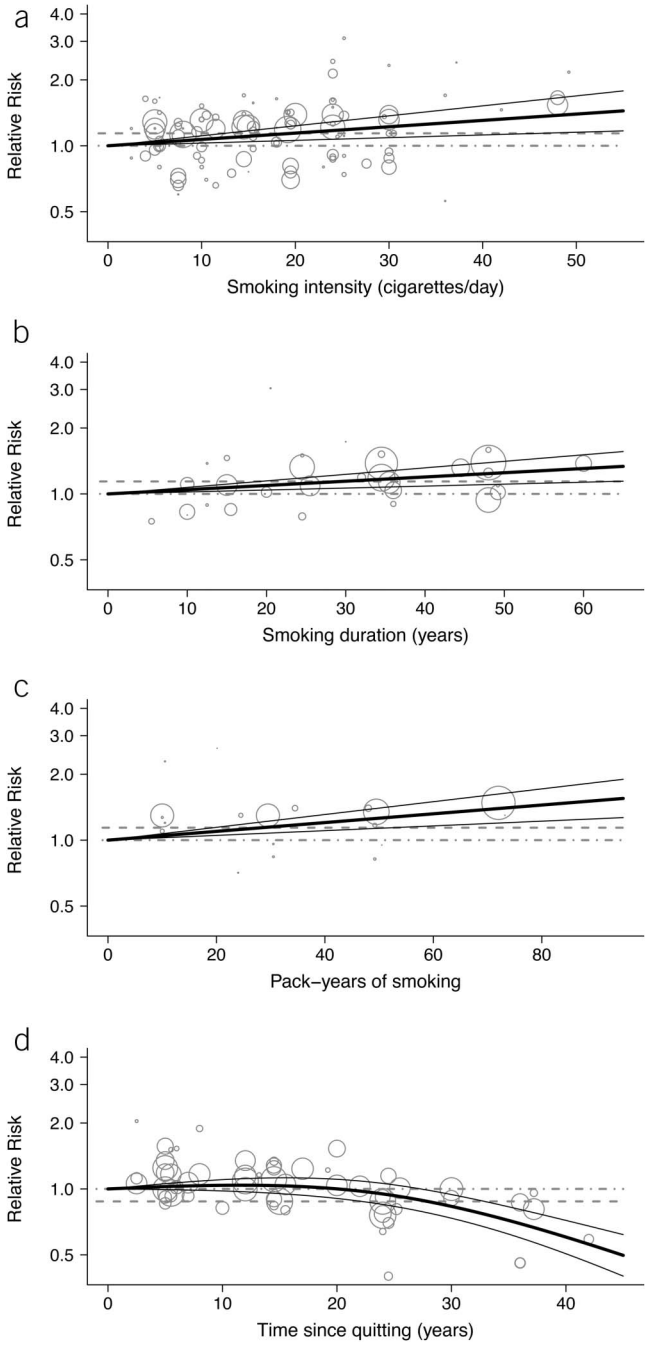

Figure 2. Relative risk (RR) for the dose-response relationships between cigarette smoking intensity, duration, pack-years, and time since quitting and colorectal cancer. (**a**) Cigarette smoking intensity (based on 34 studies). (**b**) Cigarette smoking duration (based on 9 studies). (**c**) Pack years of smoking (based on 6 studies). (**d**) Time since quitting (based on 19 studies). Linear model (**a**, **b**, and **c**), or restricted cubic spline from a random-effects dose-response model (**d**); 95% confidence interval (CI) of the linear model (**a**, **b**, and **c**) or spline model (**d**); RR for the reference category (never smokers in **a**, **b**, and **c**, current smokers in **d**); RR for current vs never cigarette smokers (**a**, **b**, and **c**) never vs current cigarette smokers (**d**); RR for various exposure categories in each study included in the analysis. The area of the circle is proportional to the precision (i.e., to the inverse variance) of the RR.

after quitting smoking, and at 26 years after cessation, it was significantly lower in former smokers than current smokers (RR 0.88; 95% CI 0.79–0.98; Figure 2D).

In ever smokers, the risk of CRC increased nonlinearly with increasing smoking intensity (RRs for 20 and 40 cigarettes per day were 1.17 and 1.24, respectively) and pack years (RRs for 20 and 40 were 1.15 and 1.22, respectively) and increased linearly with increasing smoking duration (RRs for 20 and 40 years of smoking were 1.09 and 1.19, respectively; see Figure 10, Supplementary Digital Content 2, http://links.lww.com/AJG/B607).

Analysis by molecular subtypes

Fifteen studies reported the association between ever smoking and the risk of CRC stratified by molecular features of CRC including CpG island methylator phenotype (CIMP) (n = 4 studies), *BRAF* mutation status (n = 4), microsatellite instability (MSI) phenotype (n = 8), *KRAS* mutation status (n = 5), and *TP53* mutation status (n = 5). Smoking was strongly associated with the risk of CIMP-positive CRC (RR 1.42; 95% CI 1.20–1.67) and MSI-high CRC (RR 1.56; 95% CI 1.32–1.85), but not with CIMP-negative CRC (RR 1.08; 95% CI 0.98–1.19) and microsatellite stable/MSI-low CRC (RR 1.08; 95% CI 1.00–1.16; Figure 3). Smoking was also strongly associated with the risk of mutated *BRAF* CRC (RR 1.63; 95% CI 1.23–2.16) and marginally with the risk of wildtype *BRAF* CRC (RR 1.12; 95% CI 1.02–1.22). Smoking was significantly associated with wildtype *KRAS* CRC (RR 1.17; 95% CI 1.04–1.31) and wildtype *TP53* CRC (RR 1.19; 95% CI 1.02–1.39), and not with mutated *KRAS* CRC (RR 1.04; 95% CI 0.90–1.20) and mutated *TP53* CRC (RR 1.13; 95% CI 0.99–1.29). The risk estimates for molecular features were significantly different according to CIMP status ($P = 0.01$), MSI status ($P < 0.01$), and *BRAF* mutation ($P = 0.01$), but not according to *KRAS* ($P = 0.24$) and *TP53* mutations ($P = 0.62$).

Publication bias

No evidence of publication bias emerged for current and former smokers in CRC either from the visual inspection of the funnel plots (see Figure 11, Supplementary Digital Content 2, http://links.lww.com/AJG/B607; panels A and B) or from the Egger test ($P = 0.46$ and $P = 0.15$ for current and former smokers, respectively). Evidence for publication bias was found for ever smokers (P value for the Egger test = 0.01; see Figure 11, Supplementary Digital Content 2, http://links.lww.com/AJG/B607; panel C).

DISCUSSION

This meta-analysis provides the most comprehensive and up-to-date evidence on the effect of cigarette smoking on the risk of CRC, summarizing risk estimates from 188 original studies published from 1958 to 2018. It shows that smoking increases the risk of CRC in a dose-dependent manner with duration and intensity of smoking and provides evidence that quitting smoking reduces CRC risk. Findings indicate that smoking largely increases the risk of CRC that develops through the MSI pathway, characterized by MSI, CIMP, and *BRAF* mutations.

The findings show that smoking increases the risk of CRC by 15%–20%, both in men and in women, confirming previous estimates (6) and provide strong evidence that the risk increases

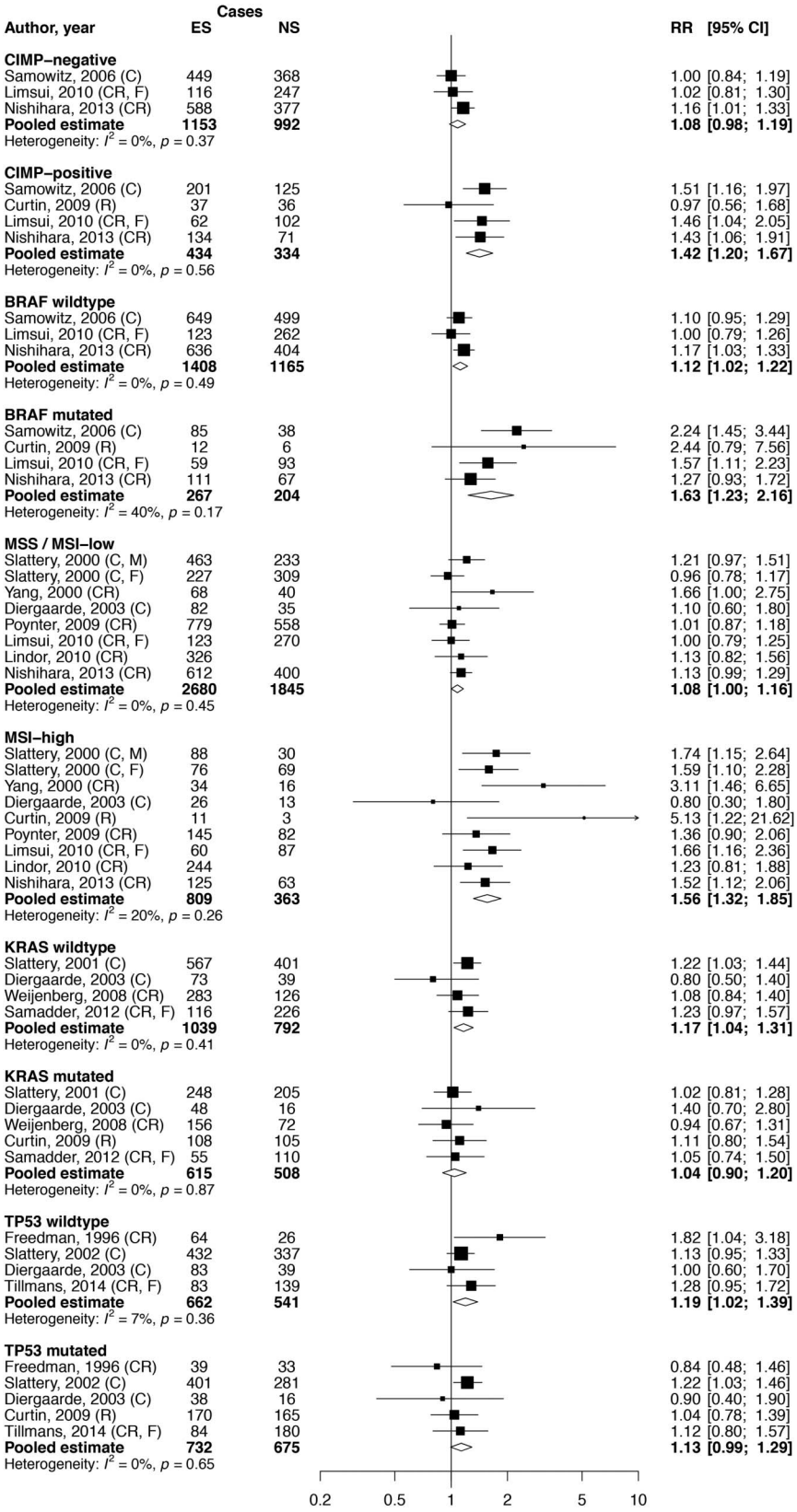

Figure 3. Forest plot of study-specific and pooled relative risk (RR) of colorectal cancer for ever cigarette smokers (ES) vs never smokers (NS) according to major molecular subtypes of colorectal cancer. C, colon cancer; CI, confidence interval; CIMP, CpG island methylator phenotype; CR, colorectal cancer; F, female; M, male; MSI, microsatellite instability; MSS, microsatellite stable; R, rectal cancer.

with intensity and duration of smoking. For example, the risk of CRC is increased by 25%–30% in smokers of 40 cigarettes per day or in those who smoke for 50–60 years. Evidence for the impact of smoking duration also comes from heterogeneity analyses that found higher risk estimates of CRC mortality than incidence; studies investigating mortality had longer follow-up, hence longer exposure to smoking, compared with studies investigating incidence (19 vs 13 years on average, respectively). The findings provide new evidence that quitting smoking reduces the risk of CRC. Former smokers had a significantly higher CRC risk than never smokers for 20 years after quitting and the effect of smoking cessation was significant only after 25 years since quitting, suggesting that former smokers maintain an elevated risk of CRC for many years after cessation. Elevated risk after quitting has been noted for other cancer types including lung and esophageal cancer (22). Surprisingly, those who had recently quit (<10 years) showed a slightly higher CRC risk compared with current smokers; this finding may reflect the tendency of people with undiagnosed cancer to quit smoking, possibly because of the initial appearance of symptoms (23).

As diverse molecular mechanisms of CRC tumorigenesis and development have been characterized, CRC is increasingly treated as a heterogeneous disease. In consideration of that, we investigated the association between smoking and CRC according to key molecular characteristics. According to the current knowledge, colorectal carcinogenesis follows 2 major pathways: the MSI pathway, which accounts for approximately 15% of the CRCs, and the chromosomal instability (CIN) pathway, which accounts for the remaining 85% of the CRC (24). The MSI pathway is characterized by a positive CIMP that induces hypermethylation and inactivation of genes including DNA mismatch repair gene *MHL1* (25,26). The resulting genetic hypermutability leads to MSI and mutation of genes including the *BRAF* oncogene (27). In accordance to previous observations (28,29), the findings show that ever smokers had a much higher risk of CRC that was CIMP-positive (RR 1.42), MSI-high (RR 1.56), or *BRAF* mutated (RR 1.63), compared with never smokers, indicating a strong effect of smoking on the risk of CRC that develops through the MSI pathway. Consistent with these findings, a study was excluded from the meta-analysis because it did not report estimates according to single molecular characteristics and found that current smokers had a much higher risk of CRC positive for any of *BRAF* mutation, MSI-high, and CIMP positive (30,31). Smoking induces DNA methylation at CpG islands (32,33), identifying a plausible mechanism linking smoking with the hypermethylator phenotype and accumulation of mutations in microsatellite sequences and in driver genes such as *BRAF* (27).

MSI-high CRC occurs in the proximal part of the colon and with increased frequency in women (34). However, we did not observe higher risk estimates in women than in men. A possible explanation is that, among smokers, men smoke more and for longer time than women, counterbalancing the higher risk of MSI-high CRC in women than in men. In addition, we did not find a clear higher risk for proximal compared with distal CRC. This may be due to the fact that not only men smoke more and for longer, but they are at higher risk of MSI-high cancer in the distal colon than women (35). When we stratified the risk estimates by sex and by site simultaneously, we found that smoking was associated with increased risk of proximal colon cancer in women but not in men, lending additional support to the hypothesis that smoking selectively effects MSI-high CRC. Accordingly, in a cohort of 546 healthy women, authors reported that the age-dependent DNA hypermethylation was accelerated by long-term smoking in the proximal colon, and not in the distal colon (36). This specific relationship between smoking and risk of proximal CRC in women highlights the need to address public health concerns of increased smoking prevalence rates in women in a growing number of countries (37), particularly in younger women and girls (38).

A large subset of CRC are microsatellite stable (or MSI-low) but have CIN and develop through the canonical adenoma-carcinoma model proposed by Fearon and Vogelstein in 1990 (39). CIN CRC tumors are initiated by mutations in the adenomatous polyposis coli gene and driven by mutations in *KRAS* oncogene and subsequently in the *TP53* tumor suppressor gene (26). *KRAS* and *BRAF* mutations are almost mutually exclusive, indicating the independence of MSI and CIN pathways (40,41). We found no significant association between smoking and the risk of CRC with mutated *KRAS* or *TP53*, suggesting that smoking does not increase the risk for CRC that develops through the CIN pathway. This raises an apparent paradox: in a previous meta-analysis, we found that smoking increases the risk of adenoma, the recognized precursor of CIN carcinoma (42). MSI CRC, by contrast, originates from sessile serrated polyps or adenomas, characterized by CIMP and MSI (43). Given that observation, one would expect that smoking also increases the risk of CRC following the CIN pathway, but our current findings suggest no association. Because most adenomas do not go on to acquire mutations in *TP53* or undergo malignant conversion to CRC, one possible explanation is that smoking increases the risk of adenomas that are less susceptible to malignant transformation. In support to this hypothesis, smoking was associated with adenomas that did not overexpress p53, but not with adenomas that overexpressed p53 (44); overexpression of p53 is highly correlated with *TP53* mutation (45), an event that occurs late in colorectal carcinogenesis, immediately before the transition of an adenoma to a carcinoma (39). In summary, we hypothesize first that smoking increases the risk of adenomas and sessile serrated polyps through independent pathways and second that smoking is involved in the canceration of sessile serrated polyps, but not of adenomas.

Strengths of the meta-analysis are inclusion of a large number of studies that allowed robust dose-response analyses linking smoking intensity and duration with CRC risk and provided new evidence on the effect of smoking cessation on CRC risk. The magnitude of the study allowed reporting on risk estimates according to various population and tumor characteristics, including sex and cancer site and geographic area. By combining the sparse evidence on the effect of smoking on CRC risk according to CRC molecular features, the study provides fundamental insights into the molecular mechanisms of smoking on CRC. A limitation of the study is the heterogeneity of the analyzed studies, especially in quality of the reports and adjustments of the estimates for potential confounders, including alcohol consumption and body mass index. To determine the impact of this heterogeneity, analyses were stratified according to both quality of the studies and adequacy of the adjustments of the estimates. Interestingly, we observed that high-quality studies reported higher risk estimates of CRC compared with low-quality studies. Similar results were observed for the adequacy of the adjustments. Another limitation is that we found evidence of publication bias for the estimate of ever vs never smokers, indicating a possible overestimation of the effect of smoking. A possible failure in detecting

some studies reporting low, null, or nonsignificant risk estimates might have contributed to the observed publication bias. Because information on smoking was self-reported in all the included studies, recall and reporting biases might have played a role in this meta-analysis. In cohort studies, where exposure was assessed before CRC occurrence, this might have led to a nondifferential misclassification toward the null (i.e., underestimation of the risk). In case-control studies, the direction of the bias is less predictable because the misclassification might differ between cases and controls. Regarding time since smoking cessation, we acknowledge that few studies attempted to adjust the analysis for smoking history measures, such as smoking duration and intensity. Thus, we cannot quantify how much the estimates for smoking cessation are influenced by those factors, and our findings should be considered with caution. Finally, although risk stratification by molecular characteristics of CRC relied on a small number of studies, the results were consistent among those studies, supporting the finding of a differential effect of smoking on the 2 major molecular pathways.

In conclusion, cigarette smoking is significantly associated with CRC risk. The association is driven by the selective effect of smoking on the risk of CRC developing through the MSI pathway. Our findings support smoking cessation to reduce the risk of CRC. Further evaluations of the molecular mechanisms through which smoking affects colorectal carcinogenesis are warranted.

CONFLICTS OF INTEREST

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Specific author contributions: Silvano Gallus, ScD, and Alessandra Lugo, PhD, contributed equally to this work. S.G. and A.L. had the original idea of the work and designed the innovative methodology for the identification of original publications. E. Borroni and C. Santucci identified the articles, screened them for eligibility, and extracted the data, with the help of S.G. and A.L.E. Borroni, G.P., and A.L. performed the statistical analyses. E. Botteri, E.K.S., E. Borroni, A.L., and S.G. drafted the manuscript. C.B., C. Specchia, V.B., and P.v.d.B. provided statistical and epidemiological supervision. All authors contributed to critical review, editing, and revision of the manuscript draft, and approval of the final version.

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