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Original Article

Correlation between smoking history and molecular pathways in sporadic colorectal cancer: a meta-analysis

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Abstract: Background: Epidemiological studies have shown that smoking increases the risk for colorectal cancer (CRC). Evidence of the guiding significance of smoking history for molecular classification and molecular targeted anti-tumor therapy is not well established. Aims: To provide indirectly evidence, we conducted a systematic meta-analysis of association between smoking history and different molecular classification. Methods: We searched in multiple databases up to January 2014, and identified 27 eligible studies. All studies were divided into seven groups based on different molecular alteration categories, which are MSI, CIMP, and three molecular pathway-associated gene alterations (APC, KRAS, P53, BRAF mutation, and APC methylation). Crude odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated to evaluate the association. Results: Smoking showed a significantly positive correlation with P53 mutation (exons 4 to 8), BRAF (codon 600) mutation, MSI positivity, and CIMP positivity, with ORs of 1.25 (95% CI: 1.07-1.45), 1.41 (95% CI: 1.18-1.68), 1.28 (95% CI: 1.12-1.47), and 1.23 (95% CI: 1.01-1.50), respectively. However, smoking was not positively correlated with APC (mutation cluster region) and KRAS (codons 12 and 13) mutation in sporadic CRC patients. Conclusions: These findings suggested smoking history occurred with P53 mutation, BRAF mutation, MSI positivity, and CIMP positivity in sporadic CRCs; and could guide those specifically therapeutic designs when molecular classification with genetic test was infeasible. More associated studies should be conducted for strengthening and renewing the current result.

Keywords: Smoking, molecular pathways, sporadic colorectal cancer, genetic, therapy

Introduction

Compared with the traditional treatment, molecular targeted therapies play a dominant position in the clinical practice of colorectal cancer (CRC), with the better understanding of its molecular features. Generally, stepwise accumulated genetic and epigenetic alterations drive the normal glandular epithelium into invasive adenocarcinoma through at least three diametrically exclusive molecular pathways [1]. Furthermore, genetic alterations can be classified as chromosomal instability (CIN) and microsatellite instability (MSI) [2, 3]. Meanwhile, the most common epigenetic alteration is the DNA methylation phenotype of CpG islands named as CpG island methylation phenotype (CIMP) [4]. CIN, MSI, and CIMP are the major molecular pathways involved in colorectal carcinogenesis [3].

These alterations inactivate or activate tumor suppressor genes and oncogenes, such as APC, KRAS, P53, and BRAF [5]. KRAS mutation in codons 12 and 13 or BRAF mutation in codon 600 aberrantly regulates the RAS/RAF/MAPK signal pathway and disturbs the balance between cell proliferations. However, KRAS and BRAF mutations exist in CRC separately; BRAF shows high association with CIMP [6]. APC mutation exists in most cases of sporadic CRC, in which mutation cluster regions (MCRs) from codons 1286 to 1513 are the most common sites [7]. Similarly, upregulation of the downstream target gene cyclin-dependent kinase inhibitor P21 leads to alterations in CRC genes, including the tumor suppressor P53, which acts as guardian of the genome and responds to DNA strand breaks [8]. Published data have robustly proved that different molecular characteristics did not respond similar to chemotherapy of patient with CRC [9-11]. The most classical practice was the worse response to cetuximab, a monoclonal antibody that inhibit the epidermal growth factor receptor, due to the

KRAS mutation status. Hence, routine molecular examines have been recommended in clinical practice to design specific therapeutic strategies based on molecular classification [12].

The International Agency for Research on Cancer has recently reviewed epidemiological and evidence-based studies of human carcinogens and smoking, and concluded that smoking is a deleterious risk for human health and a cause of colon and ovarian cancers [13]. Animal experiments showed that many carcinogens in cigarette smoking are processed by the metabolic activation pathway and are covalently bound to DNA. The final DNA adducts might lead to miscoding, resulting in permanent mutations of tumor suppressor genes and oncogenes, such as APC, KRAS, and P53 [14]. Several large-scale case-control studies have focused on determining the association between lifestyle and diet and genetic alterations in CRC, but their results were discrepant and ambiguous. Therefore, deriving a comprehensive estimation of this association is important. In this study, we quantitatively assessed the association between smoking history and several major molecular features of sporadic CRC to study its referential value for molecular classification and targeted therapies.

Materials and methods

Literature search strategy

We searched MEDLINE, EMBASE, and Cochrane Library to identify eligible studies until January 2014. Keywords related to smoking (e.g., "smoking" or "cigarette") in combination with words related to CRC (e.g., "colorectal" combined with "cancer", "carcinoma", "tumor" or "neoplasms") and gene alteration (e.g., "alteration", "mutation" or "variation") were used during the search. All relevant reports identified were included with no restriction.

The inclusion criteria were as follows: (1) original studies on sporadic CRC published in English; (2) CRC diagnosis was based on histological or cytological findings; (3) samples for mutation, methylation, or MSI analysis was obtained from biopsy or surgical tumor tissue specimens; (4) repetitive studies were unified based on the largest edition; (5) case-control, nested case-control, or cohort studies.

The exclusion criteria were as follows: (1) reviews, case reports or meeting abstracts were excluded; (2) papers with insufficient or duplicated data were excluded; (3) studies about hereditary colorectal cancers like Lynch syndrome or inflammatory bowel diseases were excluded.

Data extraction

Two authors (KC, GX) independently extracted data of suitable articles and collected the following information: first author (publication year), country, study design, age, sex ratio, histological types, number of smokers, number of mutation/methylation/MSI cases, source and people distribution. Discrepancies were settled by discussion among the four researchers. Cigarette status was classified as nonsmokers and smokers (former or current). Smokers were those who had more than 100 cigarettes in their lifetime regardless of the smoking status at the enrollment time.

Molecular classification

APC status, KRAS (codons 12 and 13), P53 (exon 4 to 8), and BRAF (codon 600) were classified as either mutant or wild type. The difference between MSI-L and MSS is merely quantitative; thus, tumors with more than 30% unstable markers were defined as MSI positive when the MSI status was sorted into three categories (MSI-high, low, and MSS) [15]. Papers with information only about MSI negativity or positivity were pooled directly. Tumors with promoter hypermethylation in at least three genes of the CACNA1G, IGF2, NEUROG1, RUNX3, and SOCS1 gene panel or in at least two genes of another five-gene panel (p16, MLH1, MINT1, MINT2, and MINT31) were defined as CIMP positive as the blurred boundary between CIMP-L and CIMP-0 [3].

Statistical analysis

In this meta-analysis, crude odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated to measure the association between smoking and gene alteration based on the alteration frequencies in sporadic CRC cases. Adjusted ORs from some parts of articles were rejected because different covariates were considered in the multivariate regression model. Heterogeneity was assessed with the Q and I^2 statistics. Results with substantial heterogeneity (I^2 > 50%) were pooled with a random-effect model (the DerSimonian and

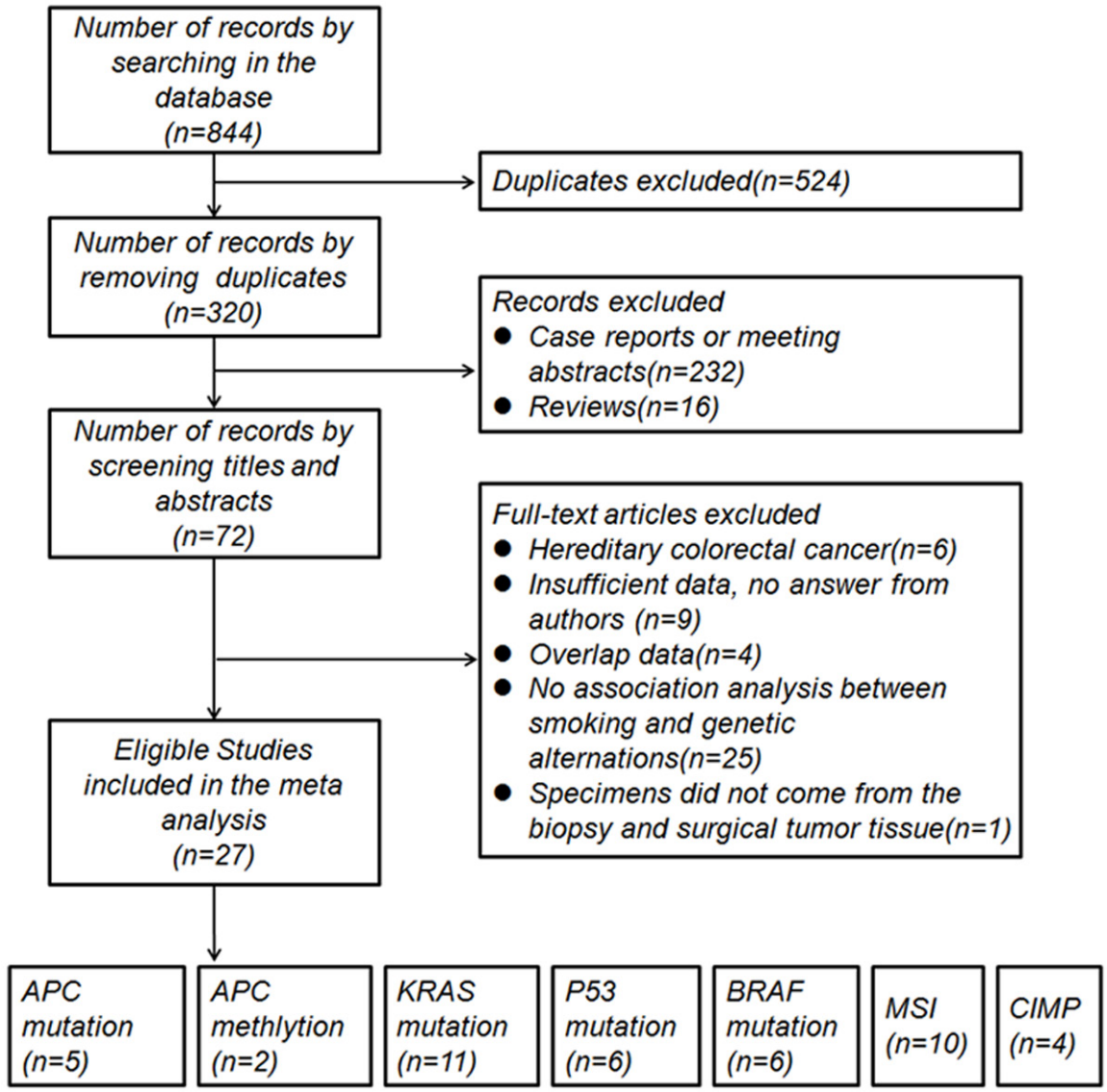


Figure 1. Flow chart of the study selection.

Laird method) [16]; otherwise, a fix-effect model (the Mantel-Haenszel method) was applied [17]. For publication bias assessment, both Begg's and Egger's funnel plots were used. All statistical analyses were performed using a commercial statistical software package (STATA 12.0; STATA Corporation, College Station, TX, US). Statistical significance was considered at $P < 0.05$.

Results

Characteristics of studies

A total of 844 items were found after considering the search criteria described above. After carefully reviewing, 27 eligible studies [18-44] were finally included in this analysis (**Figure 1**). A total of 22 studies described the three molecular pathway-associated gene alterations. Among these studies, 5 investigated APC mutation, 2 described APC methylation, 11 researched KRAS mutation, and 6 investigated P53 and BRAF mutations. Moreover, MSI was analyzed in 9 articles, and the CIMP was found in 4 articles. The characteristics of the included studies are summarized in **Table 1**.

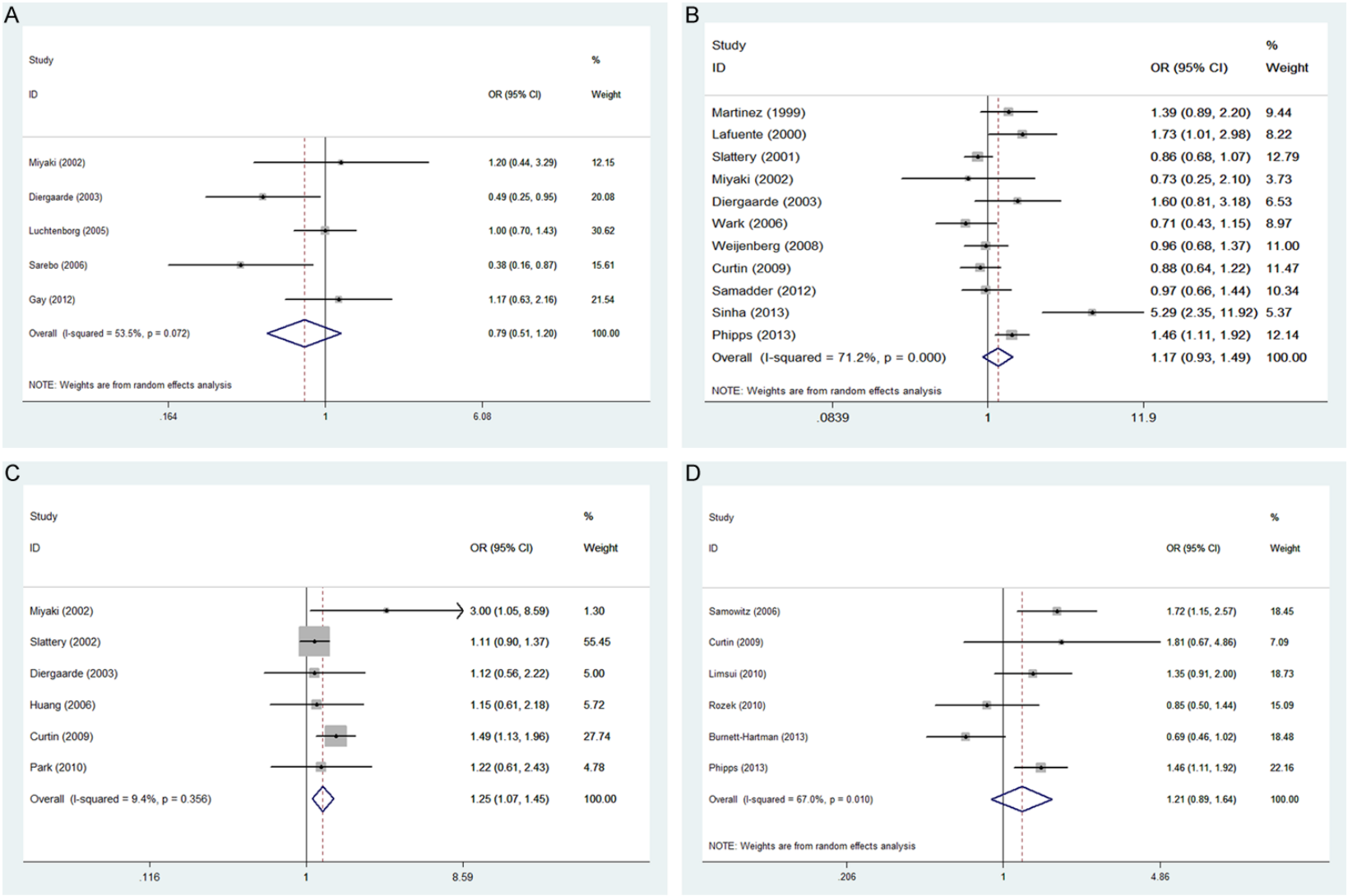
Cigarette smoking and gene mutation in CRC

APC mutation was not affected by the smoking habit of CRC patients. The combined OR esti-

Table 1. Main characteristics of studies included in the meta-analysis

First Author	Country	Study design	Study Period	Age	Gender (M/F)	No. of cases number	No. of molecular cases	Histological types	Source	People distribution	Molecular features reported
Martinez (1999)	USA	Case-case	1990-1995	65.7	451/227	723	678	Colorectal adenoma	WBF	Hospital based	KRAS mutation
Lafuente (2000)	Spain	Case-control	1996-1997	40-90	61/56	247	117	colorecal cancer	N/A	Hospital based	KRAS mutation
Yang (2000)	USA	Case-control Case-case	1996-1997	> 50	66/95	161	161	Colorectal adenocarcinoma	N/A	Hospital based	MSI mutation
Slattery (2001)	USA	Case-control Case-case	1991-1994	30-79	821/689	1,510	1,510	Colon carcinoma	KPMCP	Population based	MSI mutation
Slattery (2001)	USA	Case-control Case-case	1991-1994	30-79	774/654	1,510	1,428	Colon carcinoma	KPMCP	Population based	KRAS mutation
Wu (2001)	USA	Case-case	1995-1996	N/A	146/130	276	276	Colon adenocarcinoma	CAP	Population based	MSI mutation
Miyaki (2002)	Japan	Case-case	N/A	N/A	27/34	61	61	Colon adenocarcinoma	N/A	Hospital based	APC, P53, KRAS mutation
Slattery (2002)	USA	Case-control	1991-1994	30-79	793/665	1,510	1,458	Colon carcinoma	KPMCP	Population based	P53 mutation
Diergaarde (2003)	Netherlands	Case-control Case-case	1989-1993	61.7	93/83	176	176	Colon carcinoma	N/A	Population based	APC, P53, KRAS mutation MSI
Luchtenborg (2005)	Netherlands	Case-subcohort Case-case	1986-1994	55-69	330/264	594	594	colorecal cancer	NCLS	Population based	APC mutation
Huang (2006)	TaiWan	Case-case	2000-2005	26-95	80/73	153	153	colorecal cancer	N/A	Hospital based	P53 mutation
Sarebo (2006)	Norway	Case-control Case-case	1999-2001	50-64	82/51	133	133	Colorectal adenoma/ carcinoma	NORCCAP	Population based	APC mutation
Wark (2006)	Netherlands	Case-control Case-case	1997-2000	18-75	292/242	658	534	Colorectal adenoma	N/A	Hospital based	KRAS mutation
Samowitz (2006)	USA	Case-control Case-case	1991-1994	30-79	717/598	1,315	1143/1271	Colon carcinoma	KPMCP	Population based	BRAF mutation CIMP
Weijenberg (2008)	Netherlands	Case-subcohort Case-case	1986-1994	55-69	367/281	648	648	colorecal cancer	NCLS	Population based	KRAS mutation
Curtin (2009)	USA	Case-control Case-case	1997-2001	30-79	Both	750	750	Rectosigmoid junction or rectum cancer	KPMCP	Population based	KRAS, P53 mutation MSI, CIMP
Poynter (2009)	USA	Sibling Case-control	1998-2005	54.8	821/743	1,564	1,564	colorecal cancer	N/A	Population based	MSI
Limsui (2010)	USA	Case-case	1986-2004	55-69	0/540, 527, 537	1,233	540/527/537	colorecal cancer	IWHS	Population based	BRAF mutation MSI, CIMP
Park (2010)	UK	Case-case	1993-1997	45-79	92/93	185	138	colorecal cancer	EPIC	Population based	P53 mutation
Rozek (2010)	Northern Israel	Case-case	1998-2004	N/A	651/618	1,297	1,269	colorecal cancer	MECC	Population based	BRAF mutation MSI
Gay (2011)	UK	Case-case	1993-1997	45-79	84/85	185	169	colorecal cancer	EPIC	Population based	MSI
Gay (2012)	UK	Case-case	1993-1997	45-79	88/87, 90/88	185	175/178	colorecal cancer	EPIC	Population based	APC mutation and methylation
Naghibalhossaini (2012)	Southern Iran	Case-case	2003-2005	N/A	71/38	112	109	colorecal cancer	N/A	Hospital based	APC methylation
Samadder (2012)	USA	Case-case	1986-2004	55-69	0/507	507	507	colorecal cancer	IWHS	Population based	KRAS mutation
Burnett-Hartman (2013)	USA	Case-case	1998-2007	24-80	209/210	419	419	Serrated lesions	N/A	Population based	BRAF mutation CIMP
Sinha (2013)	India	Case-case	N/A	55.4	47/15	62	62	Colorectal adenocarcinoma	N/A	Hospital based	KRAS mutation
Phipps (2013)	USA	Case-case	2004-2009	N/A	1024/935	1,959	1,959	Colon adenocarcinoma	NCCTG	Hospital based	KRAS, BRAF mutation

Note: Molecular case is the subgroup of CRC patients that participated in the molecular study with smoking information. In the study by Samowitz (2006), the total number of molecular cases for BRAF, MSI and CIMP are 540, 527and 537, respectively. In the study by Limsui (2010), the total number of molecular cases for BRAF and CIMP are 1271 and 1143, respectively. In the study by Gay (2012), the total number of molecular cases for APC mutation and APC methylation are 175 and 178, respectively. WBF, the Wheat Bran Fiber trial; KPMCP, the Kaiser Permanente Medical Care Program; CAP, the los Angeles County cancer center surveillance program; NCLS, the prospective Netherlands Cohort Study; NORCCAP, the Norwegian Colorectal Cancer Prevention study; IWHS, the prospective Iowa Women's Health Study; EPIC, the European Prospective Investigation of Cancer study; MECC, the Molecular Epidemiology of Colorectal Cancer study; NCCTG, the North Central Cancer Treatment Group; N/A, not available.


A
Study
ID
OR (95% CI)
%
Weight
Miyaki (2002) 1.20 (0.44, 3.29) 12.15
Diergaarde (2003) 0.49 (0.25, 0.95) 20.08
Luchtenborg (2005) 1.00 (0.70, 1.43) 30.62
Sarebo (2006) 0.38 (0.16, 0.87) 15.61
Gay (2012) 1.17 (0.63, 2.16) 21.54
Overall (I-squared = 53.5%, p = 0.072) 0.79 (0.51, 1.20) 100.00
NOTE: Weights are from random effects analysis
.164
1
6.08
B
Study
ID
OR (95% CI)
%
Weight
Martinez (1999) 1.39 (0.89, 2.20) 9.44
Lafuente (2000) 1.73 (1.01, 2.98) 8.22
Slattery (2001) 0.86 (0.68, 1.07) 12.79
Miyaki (2002) 0.73 (0.25, 2.10) 3.73
Diergaarde (2003) 1.60 (0.81, 3.18) 6.53
Wark (2006) 0.71 (0.43, 1.15) 8.97
Weijenberg (2008) 0.96 (0.68, 1.37) 11.00
Curtin (2009) 0.88 (0.64, 1.22) 11.47
Samadder (2012) 0.97 (0.66, 1.44) 10.34
Sinha (2013) 5.29 (2.35, 11.92) 5.37
Phipps (2013) 1.46 (1.11, 1.92) 12.14
Overall (I-squared = 71.2%, p = 0.000) 1.17 (0.93, 1.49) 100.00
NOTE: Weights are from random effects analysis
.0839
1
11.9
C
Study
ID
OR (95% CI)
%
Weight
Miyaki (2002) 3.00 (1.05, 8.59) 1.30
Slattery (2002) 1.11 (0.90, 1.37) 55.45
Diergaarde (2003) 1.12 (0.56, 2.22) 5.00
Huang (2006) 1.15 (0.61, 2.18) 5.72
Curtin (2009) 1.49 (1.13, 1.96) 27.74
Park (2010) 1.22 (0.61, 2.43) 4.78
Overall (I-squared = 9.4%, p = 0.356) 1.25 (1.07, 1.45) 100.00
.116
1
8.59
D
Study
ID
OR (95% CI)
%
Weight
Samowitz (2006) 1.72 (1.15, 2.57) 18.45
Curtin (2009) 1.81 (0.67, 4.86) 7.09
Limsui (2010) 1.35 (0.91, 2.00) 18.73
Rozek (2010) 0.85 (0.50, 1.44) 15.09
Burnett-Hartman (2013) 0.69 (0.46, 1.02) 18.48
Phipps (2013) 1.46 (1.11, 1.92) 22.16
Overall (I-squared = 67.0%, p = 0.010) 1.21 (0.89, 1.64) 100.00
NOTE: Weights are from random effects analysis
.206
1
4.86

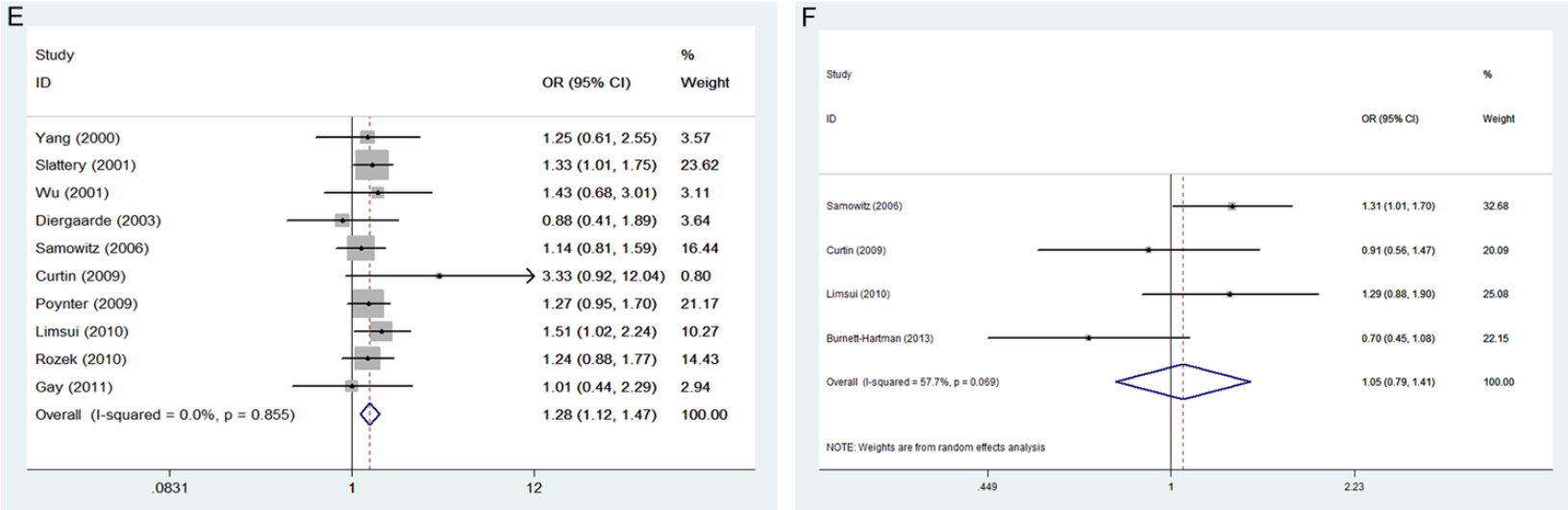


Figure 2. Forest plots of association between sporadic CRC patients' smoking habit and several major molecular features. A. APC mutation; B. KRAS mutation; C. P53 mutation; D. BRAF mutation; E. MSI; F. CIMP.

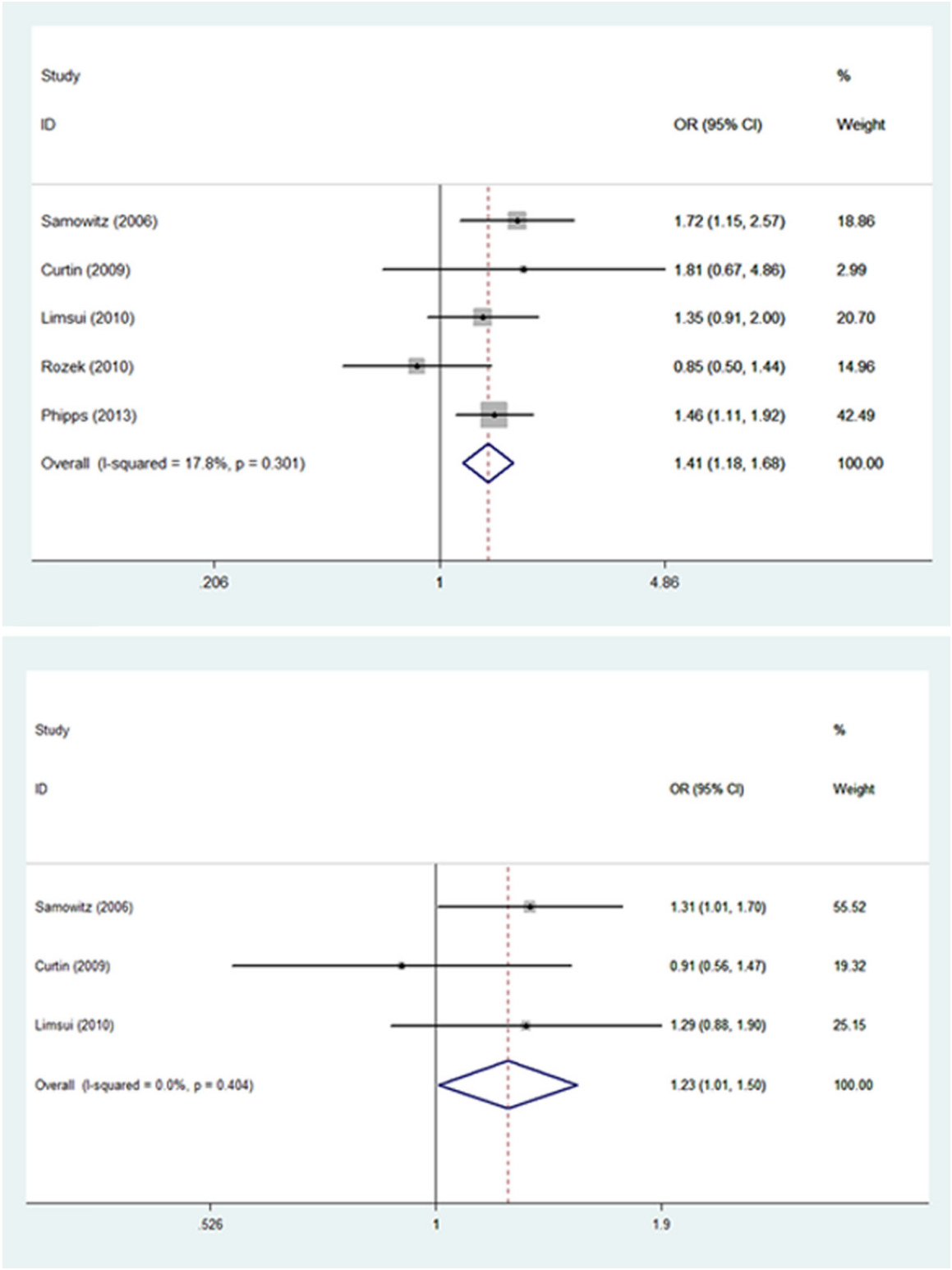


Figure 3. Meta-analysis of smoking and BRAF mutation or CIMP in sporadic CRC without Burnett-Hartman's study. A. BRAF mutation; B. CIMP.

mate was 0.79 (95% CI: 0.51-1.20) (**Figure 2A**) with a statistically significant heterogeneity (I^2 = 53.5%, P = 0.072). We analyzed two eligible studies that included 287 patients to complete-

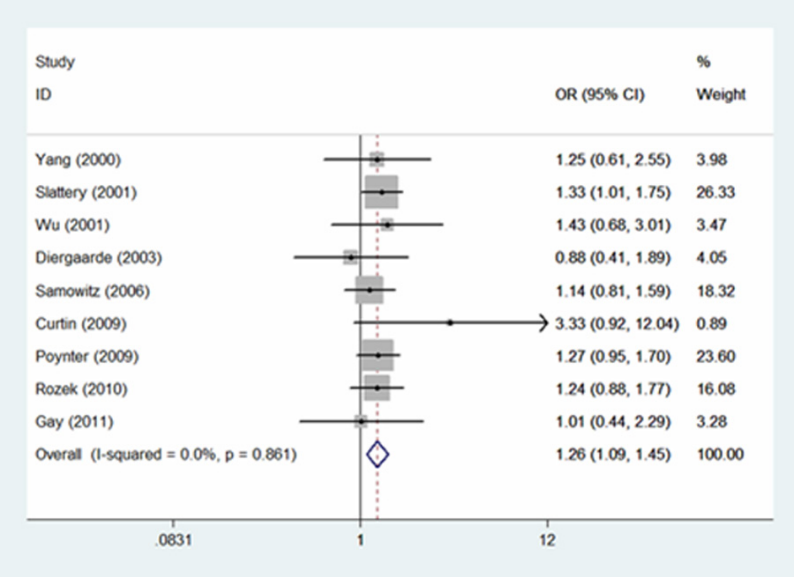


Figure 4. Meta-analysis of smoking and MSI in male sporadic CRC patients.

ly understand the relationship between APC status and smoking; results showed no statistically significant association between smoking and APC methylation [22, 23]. We combined the two OR estimates and found a negative relationship (OR = 0.94, 95% CI: 0.79-1.12) (data not shown).

A total of 6920 CRC patients from 1 case-control and 10 case-case studies were enrolled for KRAS (codons 12 and 13) mutation [18, 19, 21, 29, 30, 32, 36, 38, 41, 43, 44]. Only 30.9% cases showed significant association between KRAS mutation and smoking among CRC patients. The results of the remaining studies were the opposite. The pooled OR estimate was 1.17 (95% CI: 0.93-1.49) (**Figure 2B**) with the random-effect model, suggesting that smoking is not associated with KRAS mutation in CRC. We also combined 4 of these 11 studies that included patients suffering from colon cancer and detected no associations.

Six studies investigated P53 mutation frequency in smokers vs. nonsmokers with 2736 CRC patients [26, 29, 34, 36-38]. Two of these studies found a significant relationship between smoking and P53 mutation. Furthermore, an increased P53 mutation risk was found in patients who had smoking habits when all the six studies were pooled for the meta-analysis (pooled OR = 1.25, 95% CI: 1.07-1.45) (**Figure 2C**) without significant heterogeneity.

Four studies focusing on BRAF mutation and smoking in CRC patients had diverse conclusions, with OR ranging from 0.69 (95% CI: 0.46-1.02) to 1.72 (95% CI: 1.15-2.57) [18, 20, 25, 27, 29, 31]. In summary, the results from the combined analyses of all six studies indicate that cigarette smoking has no effect on BRAF mutation among CRC patients (OR = 1.21, 95% CI: 0.89-1.64) (**Figure 2D**). After revising the articles, we removed the serrated lesion cases and Burnett-Hartman's result [20], which is different from sporadic CRC to some degree [45]. This revision yielded a summary OR of 1.41 (95% CI: 1.18-1.68) (**Figure 3A**).

Cigarette smoking and MSI in CRC

Ten studies revealed that an association exists between cigarette smoking and MSI in CRC patients [24, 25, 27-29, 31, 36, 39, 40, 42]. OR estimates ranged from 0.88 (95% CI: 0.41-1.89) to 3.33 (95% CI: 0.92-12.04). The OR estimates from the studies of Slattery et al. [40] and Limsui et al. [27] reached statistical significance. **Figure 2E** shows a forest plot for studies that examined the association between tobacco smoking and MSI in CRC patients. A fixed-effect model was used to pool these data by I^2 < 50%. Their results indicated that smoking is strongly associated with MSI positivity (pooled OR = 1.28, 95% CI: 1.12-1.47). To exclude the effects produced by gender, we pooled the data by excluding the study (Limsui et al.) where patients were all women and then obtained a combined OR estimate of 1.26 (95% CI: 1.09-1.45) (**Figure 4**). Moreover, stratification by the tumor histological type revealed that smoking has a greater effect on increasing risk in the CRC group (pooled OR = 1.32, 95% CI: 1.10-1.59) than in the colon cancer group (pooled OR = 1.24, 95% CI: 1.01-1.50) (**Figure 5**).

Cigarette smoking and CIMP in CRC

Four studies [20, 27, 29, 31] reported an association between smoking and CIMP in CRC

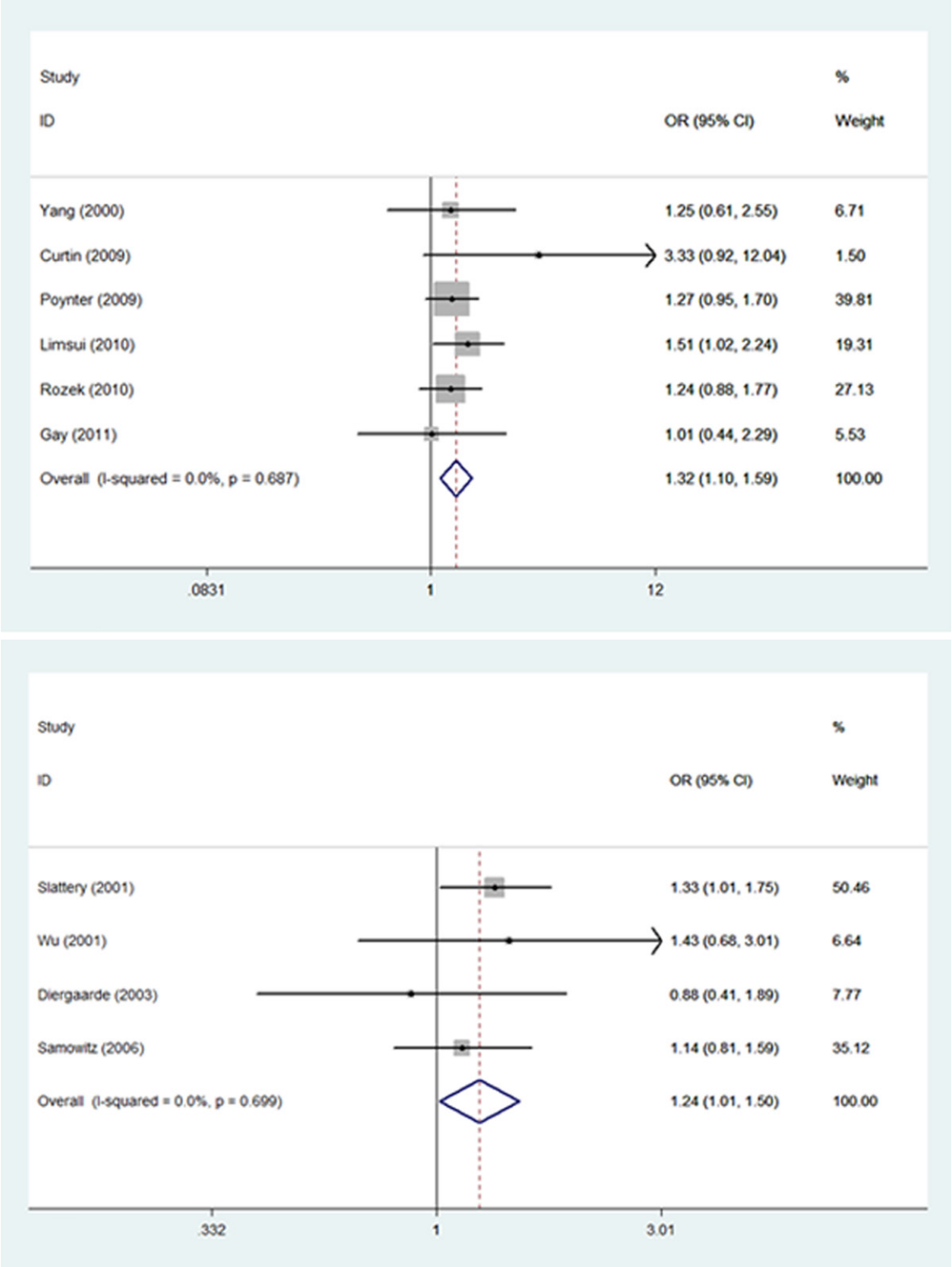
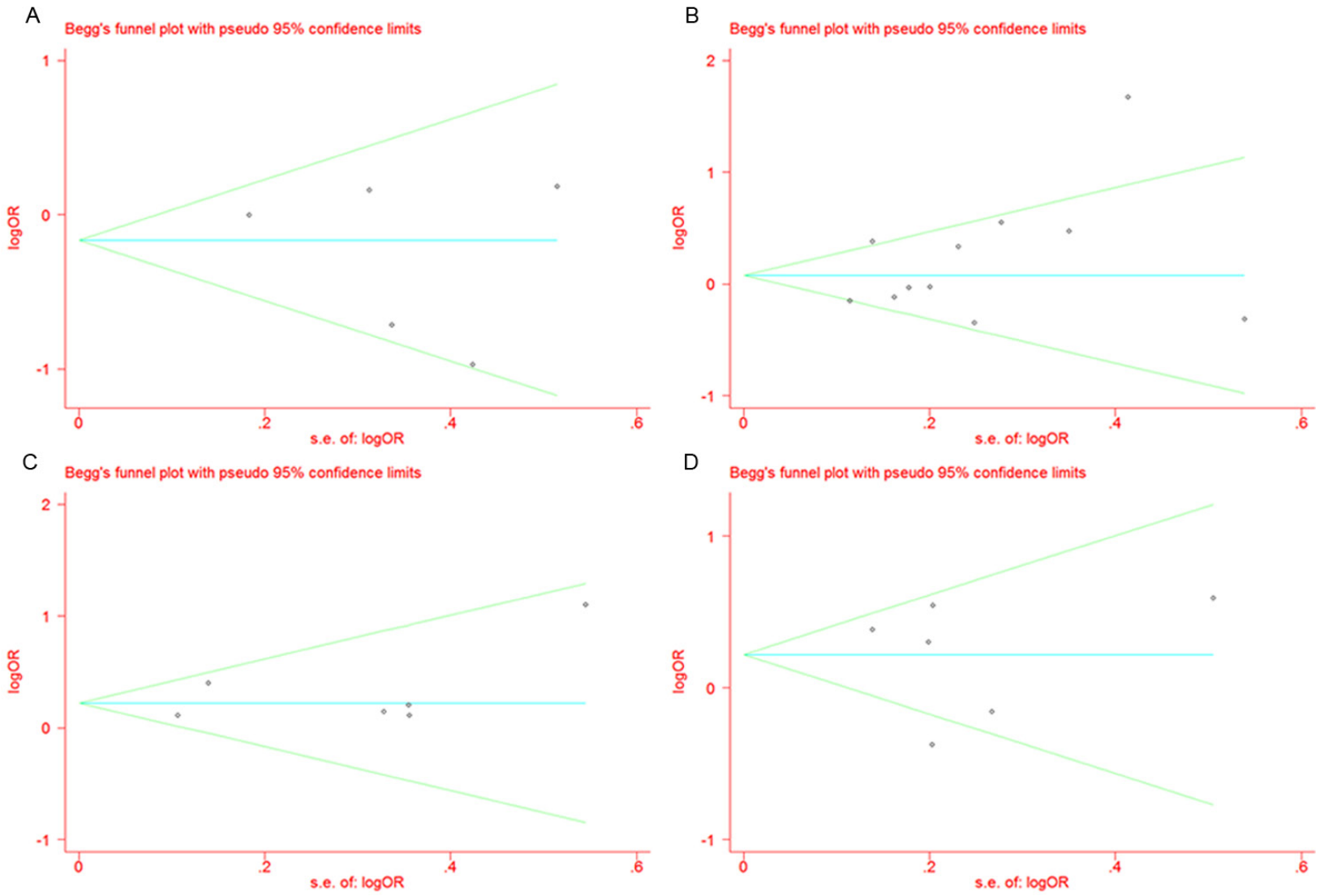


Figure 5. Meta-analysis of smoking and MSI subgroups stratified by tumor histological type in sporadic CRC. A. Colorectal tumor; B. colon tumor.

patients, and OR estimates ranged from 0.88 (95% CI: 0.70-1.41) to 1.33 (95% CI: 1.01-1.70).

A meta-analysis combining the CIMP OR estimates for CRC patients in these four studies


A
Begg's funnel plot with pseudo 95% confidence limits
logOR
s.e. of: logOR
B
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s.e. of: logOR
C
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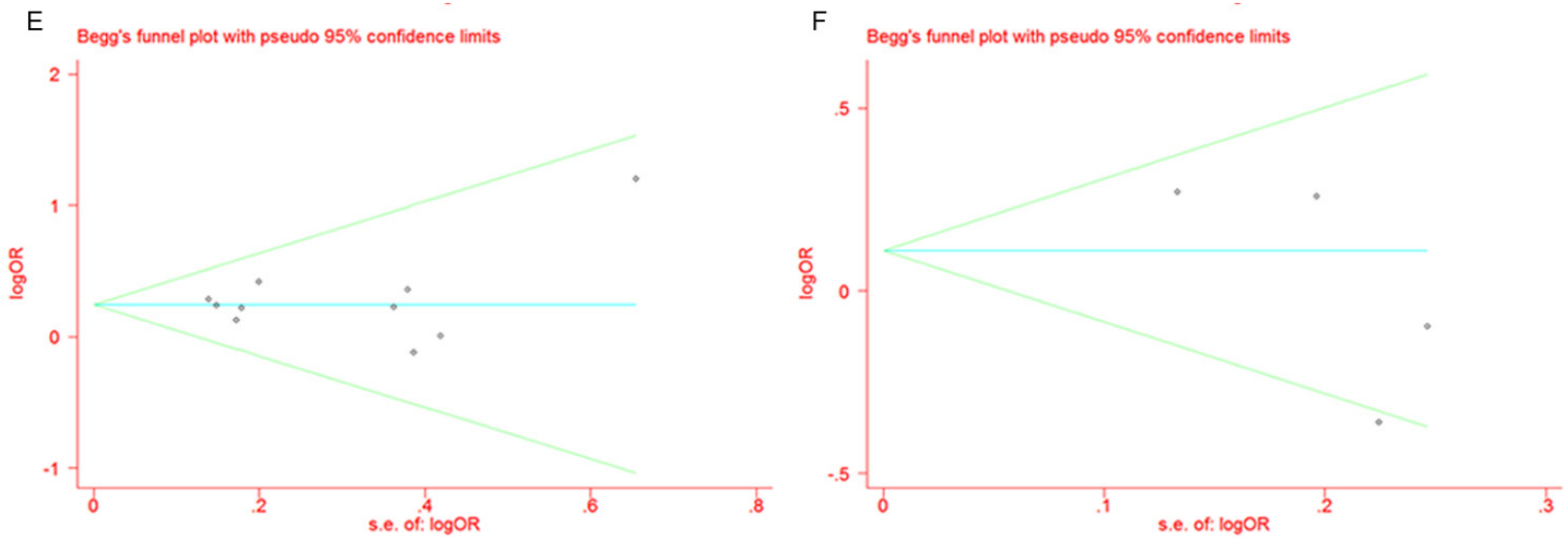

Figure 6. Begg's and Egger's funnel plots for association between sporadic CRC patients' smoking habit and several major molecular features. A. APC mutation; B. KRAS mutation; C. P53 mutation; D. BRAF mutation; E. MSI; F. CIMP.

Table 2. Summary results of association between sporadic CRC patients' smoking habit and several major molecular features

Gene	No. of sudies	Effect model	OR (95% CI)	Heterogeneity		Publication bias (*P* value)	
				I^2 (%)	P	Begg's test	Egger's test
APC mutation	5	Random	0.79 (0.51-1.20)	53.5	0.072	0.221	0.485
APC methylation	2	Fixed	0.94 (0.79-1.12)	0	0.660	N/A	N/A
KRAS mutation	11	Random	1.17 (0.93-1.49)	71.2	0	0.213	0.2
P53 mutation	6	Fixed	1.25 (1.07-1.45)	9.4	0.356	0.452	0.453
BRAF mutation	6	Random	1.21 (0.89-1.64)	67	0.010	1	0.783
MSI	10	Fixed	1.28 (1.12-1.47)	0	0.855	1	0.711

N/A, not avaliable.

obtained a summary OR estimate of 1.05 (95% CI: 0.79-1.41) with a significant heterogeneity (I^2 = 57.7%, *P* = 0.069). With BRAF mutation, the obtained pooled OR was 1.23 (95% CI: 1.01-1.50) without Burnett-Hartman's study 20. The results are shown in **Figures 2F** and **3B**.

Assessment of publication bias

Begg's and Egger's tests were performed to predict the publication bias; neither of the tests provided significantly statistical publication bias. Nevertheless, some funnel plots seemed slightly asymmetrical. All funnel plots are displayed in **Figure 6**, and data are illustrated in **Table 2**.

Discussion

This meta-analysis indicated the different correlations between smoking and several critical gene alterations among CRC patients. The results showed higher rates of P53 (exons 4 to 8) mutation, BRAF (codon 600) mutation, MSI positivity, and CIMP positivity in the smoking patients than in the nonsmoking patients. The rest did not show any significant correlations. The complete summary about the OR estimate, heterogeneity, and publication bias is provided in **Table 2**. All studies did not show any publication bias. The positive results showed minimal heterogeneity, whereas the negative results did not. Eliminating biased influence from the pooled negative results that originated from the discrepancy among these studies was difficult. Inversely, the positive results should be more convincible. To the best of our knowledge, this meta-analysis is the first to explore the key molecular features of sporadic CRC in smokers. Porta et al. [46] researched the relation between cigarette smoking and KRAS mutations in the pancreas, lung, and colorectal adenocarcinomas. Some of their results agreed with the present findings.

In 85% of sporadic CRC patients, mutation events of oncogenes and tumor suppressor genes, such as APC, KRAS, and P53, followed by CIN promote carcinogenesis via the classical adenoma-carcinoma sequence [47]. The well-known APC cluster region mutation and promoter A1 methylation, which lead to APC inactivation and sequentially WNT signal pathway over activity, could be found in aberrant crypt foci (ACF) in the early stage of adenoma-carcinoma sequence. The downstream event is KRAS mutation, which is usually located in codons 12 and 13. Low-grade dysplasia was also observed. P53 (exons 4 to 8) mutation promotes the progression toward malignancy. Several studies focused on the causality between lifestyle and gene mutations in CRC patients. The most common lifestyles studied include smoking, alcohol, meat consumption, and body mass index. The present meta-analysis revealed that smoking habit increases the P53 mutation rate (pooled OR = 1.25, 95% CI: 1.07-1.45) in sporadic CRC patients. However, the effects for APC and KRAS were uncertain. These results suggested that smoking is associated with the malignant transition instead of the early ACF formation and further epithelial dysplasia in sporadic CRC formation.

The germline mutation of MMR systems (MLH1, MSH2, MSH6, or PMS2 gene) and the promoter methylation of MLH1 gene lead to another molecular pathway called MSI. The former is the major genetic mechanism in hereditary nonpolyposis colorectal cancer or Lynch syndrome, which was excluded in our meta-analysis. Nevertheless, the latter makes up the 64%

of the MSI pathway in sporadic CRC patients [48]. According to the NCI consensus panel, five robust microsatellite markers (D2S123, D5S346, D17S250, BAT25, and BAT26) had been recommended to standardize the assessment. In addition, researchers might test additional markers to improve accuracy. With the markers described above, MSI could be classified into MSI positive or negative (more than 30% unstable markers were defined as MSI positive). Slattery et al. [40] first reported that smoking significantly contributes to MSI (adjusted OR = 1.50, 95% CI: 1.20-2.00) in colon tumors in a large population-based study of colon adenocarcinoma. However, studies that followed could not strongly support the result, except for the recent one conducted by Limsui et al. [27]. The present meta-analysis found that smoking is strongly associated with MSI positivity (pooled OR = 1.28, 95% CI: 1.12-1.47) with no heterogeneity (I^2 = 0, P = 0.855). Sample size, tumor histological type, gender ratio, and mean age might account for the discrepant results among these published studies. Subgroup analysis in CRC and colon cancer showed that rectal cancer suffers from the influence of smoking to MSI. However, the interpretation of our subgroup analysis remains challenging because MSI-positive tumors are usually located in the proximal colon [24].

MSI-positive tumors are manifested differently from MSI-negative sporadic CRCs, especially in terms of anatomical location and tumor's Dukes stage. MSI-positive tumors are usually located in the proximal colon and have a Dukes stage of A/B; MSI-negative tumors show the opposite property [24, 40, 49]. Gay et al. [24] found that MLH1 promoter methylation prefers poor differentiation, except for the characteristics showed in MSI positive tumors. Furthermore, we speculated that smoking interacts with sporadic CRC before further metastasis. Former results showing a relationship between smoking and P53 mutation events before the malignant transition strengthened our speculation. The influence of smoking in different stages of carcinogenesis should be validated with more clinical and basic studies. Both types of tumor had some degree of overlaps in clinical features and predisposing factors because two-thirds of MSI were produced by MLH1 promoter methylation as a consequence of CIMP [50, 51]. Obviously, data from our study also established that smoking exposure may be the collective and momentous factor for these tumors.

Aside from genetic alterations, epigenetic alterations have also received increasing attention. A subset of sporadic CRCs is accompanied by the CIMP of tumor suppressor genes, such as BRAF mutation at codon 600 [50, 51]. Experiments have proven that smoking exposure is associated with CIMP at the tumor suppressor gene p16 promoter in nonsmall cell lung cancer [52, 53]. Several clinical studies attempted to discover this relationship in CRC patients. Their results showed no significant associations with a summary OR estimate of 1.05 (95% CI: 0.79-1.41). Moreover, with the associated molecular events, BRAF mutation did not show definite correlation with smoking (pooled OR = 1.21, 95% CI: 0.89-1.64). We speculated that Burnett-Hartman's study, which focused on the newly founded serrated pathway, might account for this phenomenon. The rare serrated pathway should be classified into hyperplastic polyp, sessile serrated adenoma/polyp, or traditional serrated adenoma according to the WHO recommendation [45]. Hence, we deleted this article and obtained a significantly positive correlation between smokers with CIMP or BRAF mutation and minimal heterogeneity. Nevertheless, only four eligible studies were included, and insufficient cases for the CIMP pooled analysis should be noticed.

Previous studies reported CRC patients harbored MSI-positive and CIMP-negative might not to suffer the benefit of adjuvant 5-FU regimes [54, 55]. Recently, Chen's meta-analysis indicated P53 mutation was associated with improved good and complete response, decreased poor response in neoadjuvant radiation-based treatment [10]. The latest data shows FOLFOXIRI plus bevacizumab might be a reasonable option for the first-line treatment of BRAF mutant metastatic CRC patients [11]. In those studies, high throughput methods such as RELP, ARMS and DMH based on PCR were utilized for molecular alternation detection [12]. However, high cost or low sensitivity always limited their large-scale extension. Reliable and convenient referential factors should be recommended, when those genetic methods are infeasible, aiming to carry out a better molecular classification and furthermore therapeutic design. With support of our study, smoking history might be effective to indicate a

worse response to MSI, CIMP, P53, and BRAF specific therapy.

The correlation analysis between smoking and gene alteration has several limitations. First, standardized and precise gene panel for the assessment of MSI and CIMP positivity is unavailable. In CIMP, two sets of marker panels (described above) are accepted [50, 56, 57]. Both sets certify the correlation between CIMP and MSI positivity and BRAF mutation, whereas the diagnostic value or prognostic power is discriminating among the two panels [58]. This finding emphasizes the need for further subgroup analysis for CIMP detected by different panels with sufficient data. Moreover, in KRAS mutation analysis, substantial heterogeneity (I^2 = 71.2%, P = 0.000) was generated. Heterogeneity could not be eliminated when we attempted to remove studies with less than 100 cases or those with a large scope of mutation sites, such as exon 1 (data not shown). Other factors such as quality grade, race, and histological type might contribute to the observed disparities. Apparently, we could not exclude the unsuspected bias from the final result.

In conclusion, results of this meta-analysis suggested that smoking increases the rates of P53 (exons 4 to 8) mutation, BRAF (codon 600) mutation, MSI positivity, and CIMP positivity in sporadic CRC patients. However, it had no obvious correlation with APC (MCR) mutation, APC promoter methylation, and KRAS (codons 12 and 13). MSI, CIMP, and partial CIN pathways suffered the effects of smoking that probably occurred in tumor formation before further metastasis. Efforts are necessary for the clinical molecular screening of P53, BRAF, MSI, and CIMP events among smokers with precancerous lesions and sequential smoking cessation. Besides, smoking histories could be treated as an important referential factor to guide the P53, BRAF, MSI, and CIMP specifically therapeutic design when molecular classification with genetic test is infeasible. More associated studies should be conducted for strengthening and renewing the current result.

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Disclosure of conflict of interest

None.

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