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REVIEW

Lifestyle factors and risk of sporadic colorectal cancer by microsatellite instability status: a systematic review and meta-analyses

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Introduction: The association of lifestyle factors with molecular pathological subtypes of colorectal cancer (CRC), such as microsatellite instability (MSI), could provide further knowledge about the colorectal carcinogenic process. The aim of this review was to evaluate possible associations between lifestyle factors and risk of sporadic CRC by MSI status.

Methods: PubMed and Web of Science were searched for studies investigating the association between alcohol, body mass index, dietary fiber, hormone replacement therapy (HRT), non-steroidal anti-inflammatory drugs, physical activity, red meat, smoking, or statin use, with MSI-high (MSI-H) and microsatellite stable (MSS) CRC. Meta-analyses were carried out to calculate summary relative risks (sRR).

Results: Overall, 31 studies reporting on the association between lifestyle factors and CRC according to MSI status were included in this review. Ever smoking was associated with MSI-H (sRR = 1.62; 95% CI: 1.40–1.88) and MSS/MSI-low CRC (sRR = 1.10; 95% CI: 1.01–1.20), but the association was significantly stronger for MSI-H CRC. The use of HRT was associated with a 20% decrease (sRR = 0.80; 95% CI: 0.73–0.89) in the risk of MSS CRC, but was not associated with MSI-H CRC. An increase in body mass index per 5 kg/m^2 was equally associated with MSS and MSI-H CRC (sRR = 1.22, in both cases), but was statistically significant for MSS CRC only (95% CI: 1.11–1.34 and 0.94–1.58, respectively). Limited evidence for associations between other lifestyle factors and CRC by MSI status exists.

Conclusions: Lifestyle factors, such as HRT and smoking are differentially associated with the risk of MSI-H and MSS CRC. Further research on associations of lifestyle factors and CRC subtypes is necessary to provide a better understanding of the CRC disease pathway.

Key words: colorectal cancer, lifestyle factors, microsatellite instability, incidence, molecular pathological epidemiology

Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of cancer related death worldwide [1, 2]. According to the World Cancer Research Fund International [3], the major risk factors for CRC include consumption of red and processed meat [4, 5], alcoholic drinks [6], body fatness [7] and smoking [8]. On the other hand, protective factors for CRC include physical activity, foods containing dietary fiber [9], hormone replacement therapy (HRT) [10, 11], and long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) [12]. Despite the well-recognized importance of these lifestyle factors in relation to CRC incidence, many of the underlying mechanisms by which they contribute to the development of CRC remain uncertain [3–8, 10–14].

CRC is a heterogeneous disease that can arise via different molecular pathological pathways [15–17]. Chromosomal instability,

epigenetic alterations, and defects in the DNA mismatch repair (MMR) system are recognized as possible pathways by which CRC can develop. Microsatellite instability (MSI) is the phenotype by which alterations in the MMR systems are expressed and it is present in ~15% of all CRC. Also, there is evidence to suggest that patients with MSI-high (MSI-H) CRC have a better prognosis compared with patients with low MSI or microsatellite stable (MSI-L/MSS) CRC [18].

In recent years, several studies have assessed the association between lifestyle factors and molecular pathological subtypes of CRC to better understand the etiology of this heterogeneous disease. However, only one systematic literature review has summarized the findings on smoking and molecular pathological subtypes of CRC, using studies of mixed quality published up to January 2014 [19]. The associations of other lifestyle factors with molecular subtypes of CRC, specifically MSI status have not yet been summarized in a systematic review.

Consideration of the different molecular pathological pathways when examining lifestyle factors in relation to CRC could provide a clearer distinction of CRC and provide insights into the carcinogenic process, if previously unknown associations are yet to be found. Therefore, the aim of this systematic literature review was to evaluate whether established lifestyle factors show differential associations with the risk of MSI-L/MSS and MSI-H CRC.

Methods

Data source and literature search

This systematic review was conducted according to PRISMA and MOOSE guidelines [20, 21]. PubMed and Web of Science were searched from inception until July 2017 without language or publication date restrictions. Search strategies were built based on terms related to CRC, major lifestyle factors [alcohol, body mass index (BMI), dietary fiber, HRT, NSAIDs, physical activity, red meat, smoking and statins] and MSI status (supplementary Table S1, available at *Annals of Oncology* online). After removal of duplicates, records were screened by title and abstract for inclusion according to pre-specified criteria. Records identified as potentially relevant for this review were assessed for eligibility in full-text review. Reference lists of included studies were screened for additional potentially relevant articles.

Eligibility criteria/study selection

The focus of this study was to evaluate the association of major lifestyle factors (alcohol, BMI, dietary fiber, HRT, NSAIDs, physical activity, red meat, smoking and statins) with MSI-H or MSS CRC in unselected CRC patients with sporadic disease. All studies that exclusively focused on hereditary CRC were excluded. All clinical trials, cohort studies, case–control studies, and case–case analyses reporting relative risk estimates [odds ratios, hazard ratios (incidence) rate ratios and risk ratios] with corresponding 95% confidence intervals or enough data to calculate them were eligible for inclusion. Case series, case reports and expert opinion articles were not eligible for inclusion. Only studies performing multivariable analyses that adjusted for at least age and sex in the statistical analyses were included.

Data extraction

The study information was extracted independently by two reviewers (SB, JW) and included information on first author, year, country, study design, sex, age, location of tumor, study size, effect size, proportion of MSI cases (MSI-H, MSS/MSI-L), exposure assessment, tumor analysis (immunohistochemistry, genetic), major exposure categories, results (effect size, 95% CI) and adjustment variables. Several studies reported multiple effect sizes for the relationship of interest; in such cases the most comprehensive model was included in this review.

Quality and risk of bias assessment

The included articles were assessed for quality and ranked with a scale up to 6.5 points adapted from the Newcastle-Ottawa Scale (NOS). This scale consisted of six criteria which evaluate the description of study population and case definition (1 point); selection of non-exposed controls (1 point); study design (1 point); ascertainment of exposure (1 point); assessment of MSI status (1 point); and adjustment of the model (1.5 points). Studies had to score at least three points to be eligible for inclusion in a possible meta-analysis.

Data synthesis and analysis

The eligibility criterion for a meta-analysis was to have at least three studies investigating the relationship between the exposure (using similar assessment) and CRC. Summary relative risks (sRR) were calculated using categorization of exposures in discrete groups for smoking (ever versus never smoker), BMI (per 5 kg/m^2 increase), and HRT (ever versus never use) for the risk of MSI or MSS CRC. Because of the currently limited evidence available in this field, risk estimates from both case–control and cohort studies were included. In studies where estimates were reported only by subgroup (e.g. former or current smokers) the risk estimates were first pooled using a fixed effect model and then included as ever smoking in the meta-analysis [22–24]. Similarly, past and current use of HRT were pooled to 'ever use' of HRT in one study [25] and estrogen replacement therapy and compounded hormone replacement therapy were pooled to 'ever use' of any HRT in another study [26]. Furthermore, the ratios of the pooled sRR comparing MSI-H versus MSS/MSI-L of the meta-analyzed summary estimates and their 95% CIs were calculated as suggested by Altman and Bland [27].

For the studies included in the meta-analyses, random-effects models were carried out according to the DerSimonian and Laird methodology [28]. Statistical heterogeneity was assessed using the I^2 test [29, 30]. Funnel plots were applied to investigate publication bias. Data analyses were conducted using the R package 'meta' (version 4.5-0) for the R statistical programming software [R version 3.3.1 (2016-06-21)].

Table 1. Characteristics of included studies

Author	Year	Country	Design	Cancer	MSI-H (%)	MSS/MSI-L (%)	Lifestyle factors	Quality score
Poynter et al. [22]	2009	USA, Canada, Australia	Case–control	Colorectal	227 (14.5)	1337 (85.5)	Smoking, alcohol	3
Nishihara et al. [23]	2013	USA	Cohort	Colorectal	188 (16.0)	1012 (84)	Smoking	5.5
Luchtenborg et al. [24]	2005	The Netherlands	Case cohort	Colorectal	56 (8.6)	594 (91.4)	Smoking	5
Chia et al. [31]	2006	USA	Case–control	Colorectal	197 (16.4)	1005 (83.6)	Smoking, NSAIDs	5.5
Curtin et al. [32]	2009	USA	Case–control	Rectal	16 (2.1)	734 (97.9)	Smoking	5
Diergaarde et al. [33]	2003	The Netherlands	Case–control	Colon	39 (22.2)	137 (77.8)	Smoking	5
Limsui et al. [34]	2010	USA	Cohort	Colorectal	147 (27.2)	393 (72.8)	Smoking	5
Slattery et al. [35]	2000	USA	Case–control	Colon	266 (17.6)	1244 (82.4)	Smoking, BMI, NSAIDs, PA	4
Wu et al. [36]	2001	USA	Cross-sectional	Colon	35 (12.7)	241 (87.3)	Smoking, red meat	5
Yang et al. [37]	2000	USA	Case–control	Colorectal	51 (31.7)	110 (66.3)	Smoking	3
Lin et al. [25]	2012	USA	Cohort	Colorectal	118 (21.7)	425 (78.3)	HRT	5.5
Brandstedt et al. [26]	2014	Sweden	Cohort	Colorectal	44 (17.3)	210 (82.7)	HRT	5
Limsui et al. [38]	2012	USA	Cohort	Colorectal	145 (26.9)	394 (73.1)	HRT	5
Newcomb et al. [39]	2007	USA	Case–control	Colorectal	83 (26.7)	228 (73.3)	HRT	5.5
Slattery et al. [40]	2001	USA	Case–control	Colon	129 (20.9)	487 (79.1)	HRT	5
Campbell et al.[41]	2010	USA, Canada, Australia	Case–control	Colorectal	188 (15.0)	1062 (85.0)	BMI	4
Hughes et al. [42]	2012	The Netherlands, Australia	Cohort	Colorectal	171 (14.1)	1039 (85.9)	BMI	5
Brandstedt et al. [43]	2013	Sweden	Cohort	Colorectal	71 (14.6)	416 (85.4)	BMI	6
Hoffmeister et al. [44]	2013	Germany	Case–control	Colorectal	115 (9.5)	1100 (90.5)	BMI	5.5
Hanyuda et al. [45]	2016	USA	Cohort	Colorectal	210 (16.1)	1093 (83.9)	BMI	5.5
Slattery et al. [46]	2001	USA	Case–control	Colon	266 (17.6)	1244 (82.4)	Alcohol, red meat, dietary fiber	5
Diergaarde et al. [47]	2003	The Netherlands	Case–control	Colon	40 (22.0)	144 (78.0)	Alcohol, dietary fiber, red meat	5
Satia et al. [48]	2005	USA	Case–control	Colon	49 (10.1)	437 (89.9)	Alcohol, dietary fiber, red meat	5.5
Bongaerts et al. [49]	2007	The Netherlands	Cohort	Colorectal	32 (5.6)	541 (94.4)	Alcohol	5
De Vogel et al. [50]	2008	The Netherlands	Cohort	Colorectal	84 (12.7)	578 (87.3)	Alcohol	5.5
Mrkonjic et al. [51]	2009	Canada	Case–control	Colorectal	112 (14.9)	640 (85.1)	Alcohol, red meat	2
Razzak et al. [52]	2011	USA	Cohort	Colorectal	148 (27.0)	400 (73)	Alcohol	5
Joshi et al. [53]	2015	USA, Canada	Case–control	Colorectal	243 (21.7)	876 (78.3)	Red meat	3
Carr et al. [54]	2017	Germany	Case–control	Colorectal	236 (10.7)	1966 (89.2)	Red meat	5.5
Cao et al. [55]	2016	USA	Cohort	Colorectal	215 (16.2)	1110 (83.8)	NSAIDs	4.5
Lee et al. [56]	2011	USA	Cohort	Colorectal	124 (15.2)	692 (84.8)	Statins	5.5

BMI, body mass index; HRT, Hormone replacement therapy; MSI-H, high microsatellite instability; MSS/MSI-L, microsatellite stable or MSI low; NSAIDs, non-steroidal anti-inflammatory drugs; PA, physical activity.

Results

Search process and study selection

After removal of duplicates, 1148 records were screened for eligibility, of which 94 articles were assessed in full-text and 31 were included in the final review (supplementary Figure S1, available at *Annals of Oncology* online). Table 1 shows the characteristics of the included studies, lifestyle factors analyzed, and the score obtained according to the adapted NOS scale. Full details of the study characteristics are provided in supplementary Table S2, available at *Annals of Oncology* online. The majority of studies had a quality score of 5 or more points, and only four studies had a score of 3 or fewer points. The detailed results of the quality evaluation for all studies are presented in supplementary Table S3, available at *Annals of Oncology* online.

Results of individual studies/synthesis of results

Smoking. Ten studies (7 case–control, 2 cohort, 1 cross-sectional) evaluated the association of smoking with MSI-H or MSS CRC [22–24, 31–37]. Two studies reported only case–case results and were not included in the meta-analysis [35, 36]. One study was excluded because it did not provide 95% CIs [37] and another because it combined never and former smokers as the reference group for the analysis [32]. The results of the six remaining studies were summarized in a meta-analysis (Figure 1). Ever smoking was more strongly associated with MSI-H CRC (sRR= 1.62; 95% CI: 1.40–1.88) than with MSS (sRR = 1.10; 95% CI: 1.01–1.20) CRC. The results were significantly different when comparing MSI-H with MSS CRC (sRR = 1.47; 95% CI: 1.24–1.75) (Table 2). No between study heterogeneity was observed for the association with MSI-H CRC but low heterogeneity was observed for MSS CRC (MSI $I^2 = 0\%$; MSS $I^2 = 33\%$). Funnel plots did not indicate publication bias for studies on MSI CRC or for

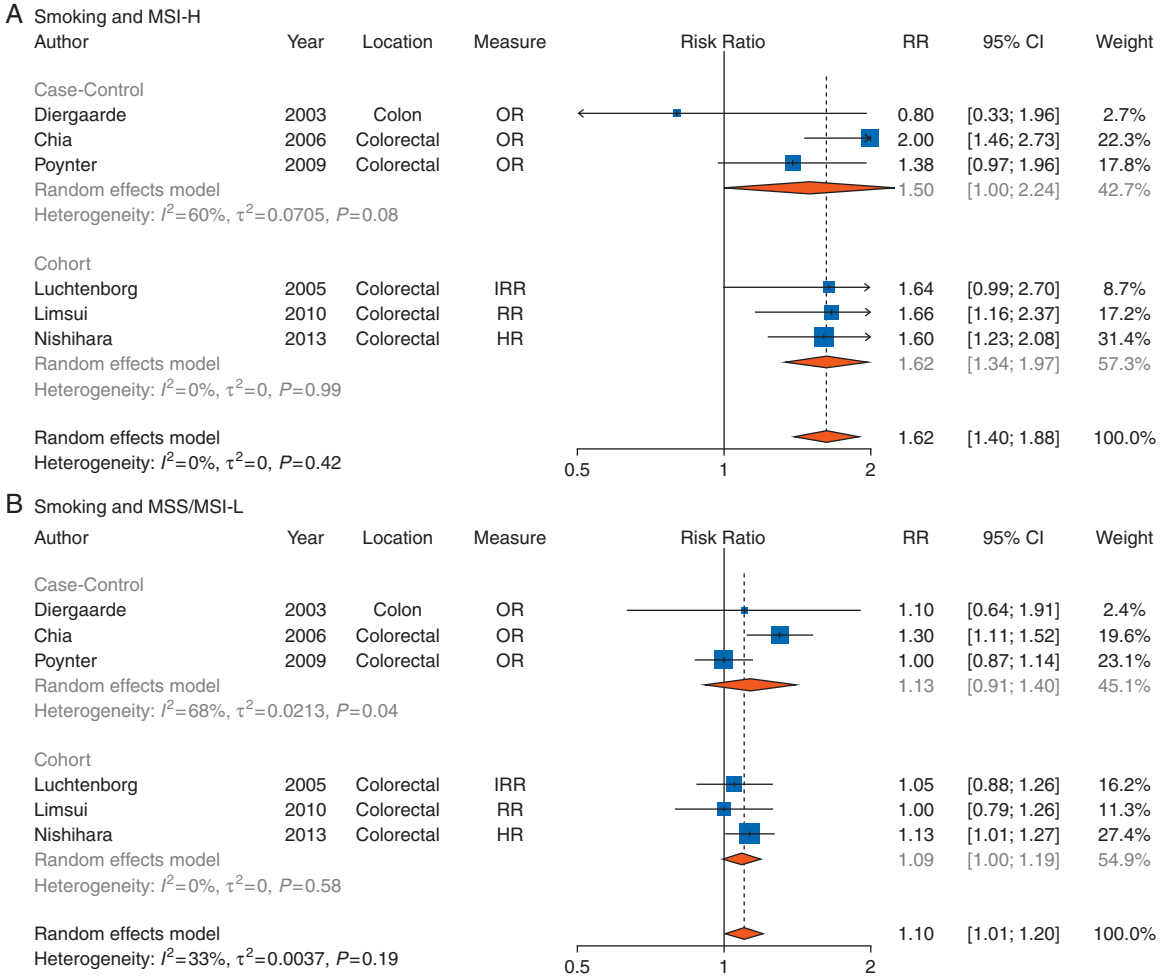

Figure 1. Meta-analysis for the association of smoking (ever versus never smoker) with risk of colorectal cancer.

Table 2. Summary relative risks of MSI-H and MSS in meta-analyses of smoking, HRT, and BMI

Lifestyle factor	Result from MA for MSI-H sRR (95% CI)	Results from MA for MSS/MSI-L sRR (95% CI)	Ratio of sRR (MSI-H versus MSS/MSI-L) sRR (95% CI)
Smoking (ever versus never)	1.62 (1.40–1.88)	1.10 (1.01–1.20)	1.47 (1.24–1.75)
HRT (ever versus never)	1.02 (0.85–1.21)	0.80 (0.73–0.89)	0.78 (0.64–0.96)
BMI (per 5 kg/m^2 increase)	1.22 (0.94–1.58)	1.22 (1.11–1.34)	1.00 (0.76–1.32)

MA, meta-analysis; MSI-H, high microsatellite instability; MSS/MSI-L, microsatellite stable or MSI low; sRR, summary rate ratio; HRT, hormone replacement therapy; BMI, body mass index.

MSS/MSI-L CRC (supplementary Figure S2, available at *Annals of Oncology* online). Similarly, the results of the two studies not included in the meta-analysis reporting on smoking and MSI-H or MSS CRC, support these findings [32, 37]. Both studies showed a 50% increased risk of MSI-H colon cancer compared with MSS colon cancer for 'ever smokers' versus never smokers.

Hormone replacement therapy. Five studies (two case–control, three cohort) described the association between the use of post-menopausal HRT and MSI-H or MSS CRC [25, 26, 38–40]. A meta-analysis of results showed that ever use of HRT was associated with lower risk of MSS CRC (sRR = 0.80; 95% CI: 0.73–0.89) but no association was observed with MSI-H CRC (sRR = 1.02; 95% CI: 0.85–1.21) (Figure 2). No between study heterogeneity was observed (MSI: $I^2 = 0\%$; MSS $I^2 = 0\%$). The ratio of sRR for MSS/MSI-L compared with MSI indicated a differential effect (sRR = 0.78; 95% CI: 0.64–0.96) (Table 2). No publication bias was detected in funnel plots (supplementary Figure S2, available at *Annals of Oncology* online). In a case–case analysis, one study [40] found significantly higher risk of MSI-H

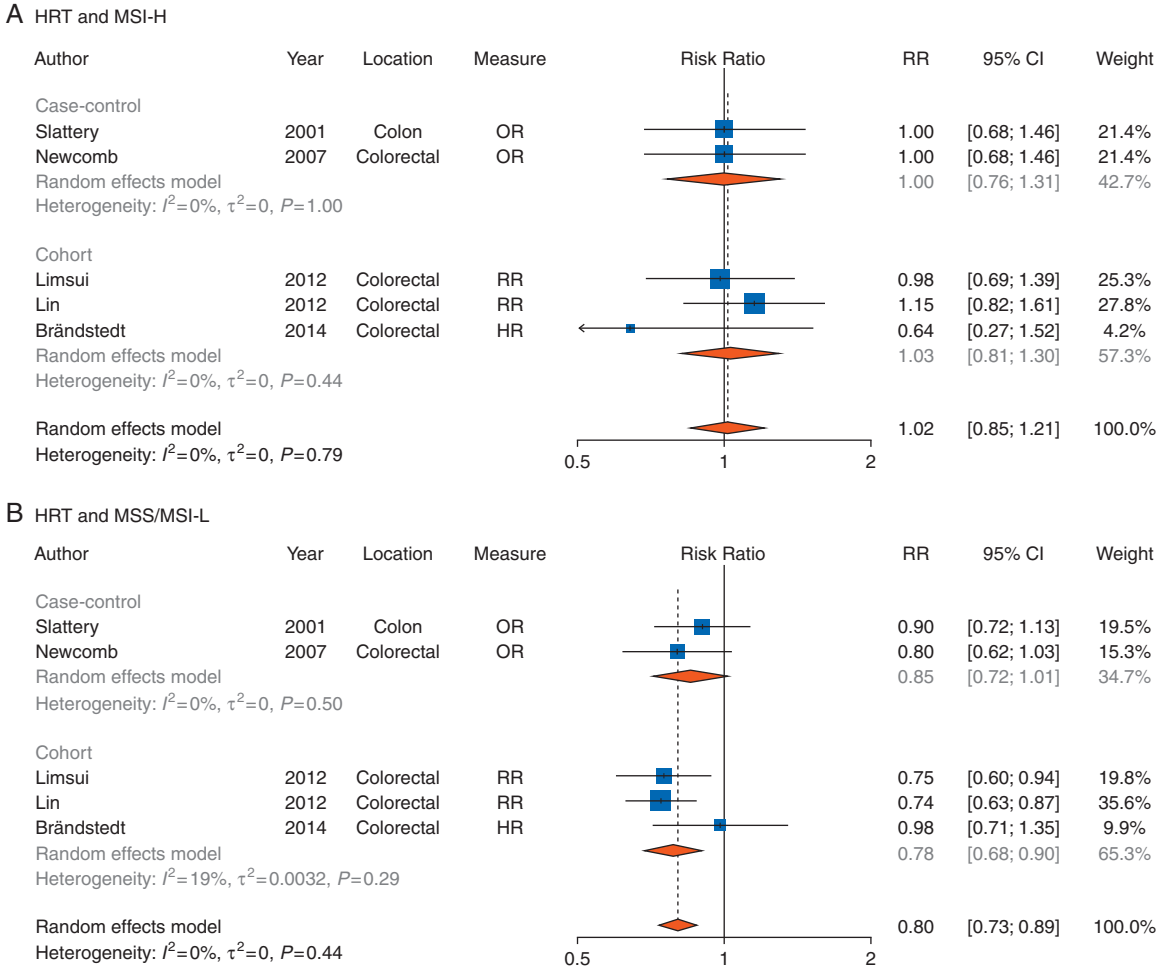

Figure 2. Meta-analysis for the association of HRT (ever versus never use) with risk of colorectal cancer.

colon cancer compared with MSS colon cancer (OR = 2.0; 95% CI: 1.1–3.5) for former HRT users, although no association was observed overall for 'ever' or 'recent' HRT users.

Body mass index. Six studies (three cohort, three case–control) reported on the association of BMI and CRC risk according to MSI status [35, 41–45]. Four studies (reported in three publications) described results per 5 kg/m^2 increase and were summarized in a meta-analysis (Figure 3). Higher BMI was equally associated with MSS CRC (sRR per 5 kg/m^2 increase = 1.22; 95% CI: 1.11–1.34) and with MSI-H CRC (sRR = 1.22; 95% CI: 0.94–1.58), but the association was statistically significant for MSS CRC only. The heterogeneity between studies was high (MSI: I^2 = 77%; MSS I^2 = 68%). The ratio of sRR for MSI-H compared with MSS/MSI-L CRC indicated no difference in effect between the two subgroups (sRR = 1.00; 95% CI: 0.76–1.32) (Table 2). Results of the three studies not included in the meta-analysis also showed significant associations of BMI with increased risk of MSS CRC. One case–control study found significantly higher risk of MSS colon cancer in the highest BMI categories for both men and women, but no significant associations with MSI-H colon cancer; although results were only adjusted for age [35]. A multivariable analysis of the US Health Professionals Follow-up Study and the Nurses' Health Study found increasing risk of MSS CRC with increasing BMI, but no significant associations for MSI CRC [45]. Similarly, a Swedish cohort study found higher risk of MSS CRC but this was for women in the higher quartiles of BMI only. No significant associations were observed for men or for MSI CRC [43]. Case–case comparisons in one study [35] found no significant associations between BMI and MSI-H compared with MSS CRC; however, another study [44] found that a 5 kg/m^2 increase in BMI was associated with an increased risk of MSI-H CRC compared with MSS/MSI-L CRC (OR = 1.44; 95% CI: 1.13–1.82).

Alcohol. Eight (three cohort, five case–control) studies investigated the association between alcohol intake and CRC according to MSI status [22, 46–52]. These studies categorized alcohol consumption in different groups, including 'drinkers', 'intermediate/high consumption', and intake in 'grams per day' or 'drinks per week'. Due to this heterogeneity in the definition of exposure categories, a meta-analysis of results was not conducted. Overall, most studies did not find a significant association between increased alcohol consumption and MSI-H or MSS CRC. In a case–case comparison, one study [46] found intermediate consumption and high consumption of alcohol to be associated with higher risk of MSI-H colon cancer (OR = 1.5; 95% CI:

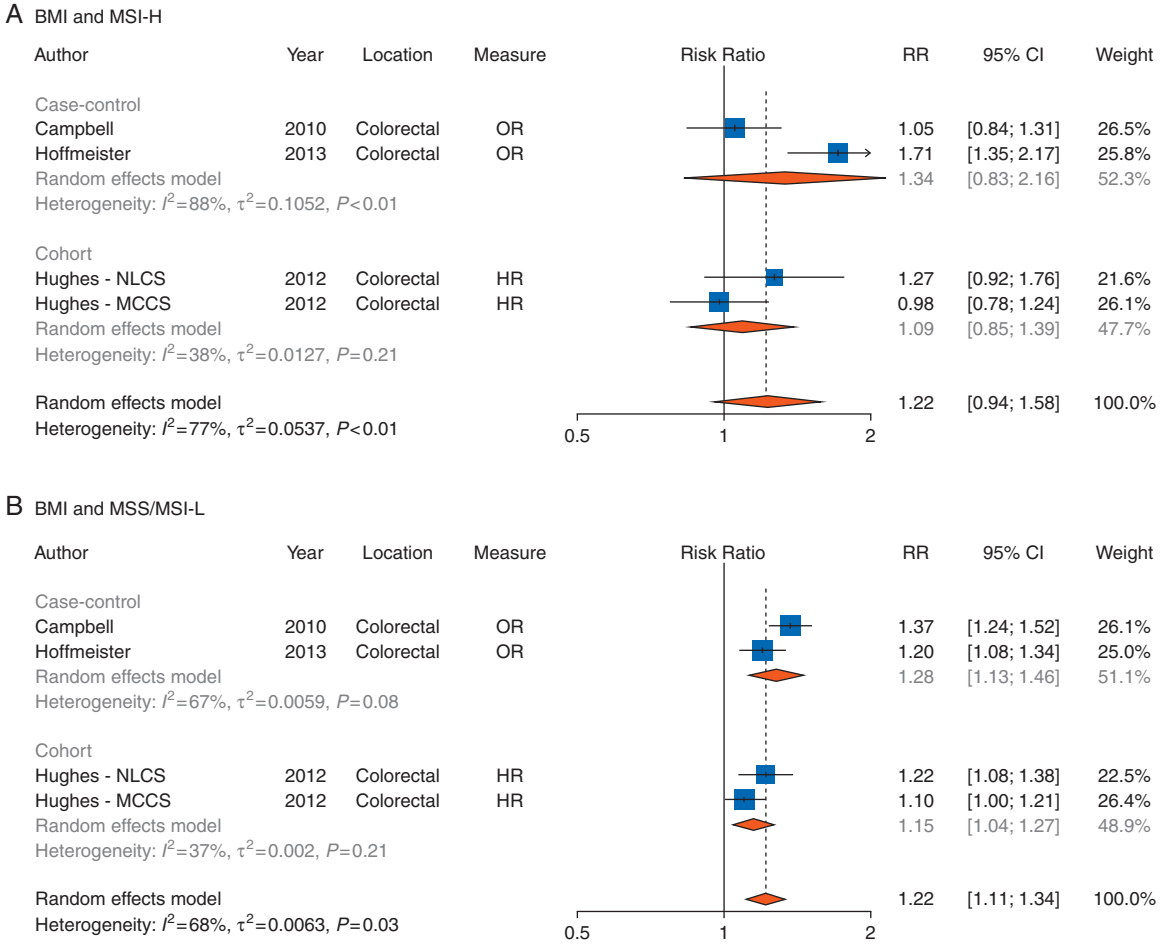

Figure 3. Meta-analysis for the association of BMI (per 5 kg/m^2 increase) with risk of colorectal cancer.

1.1–2.0 and OR = 1.6; 95% CI: 1.0–2.5, respectively) compared with MSS.

Red meat. Seven studies (six case–control, one cross-sectional) described the association between red meat consumption and CRC according to MSI status [36, 46–48, 51, 53, 54]. Different exposure categories and reference groups for the analyses prevented a summary of results with a meta-analysis. Overall, in most studies higher intake of red meat was not associated with MSI-H or MSS CRC. However, two studies [51, 54] found higher consumption of red meat to be associated with higher risk of MSS, but no association was found for MSI-H CRC. A case–case analysis reported by one of these studies [54] did not find a significant difference when comparing MSI-H and MSS CRC.

Dietary fiber. Three case–control studies evaluated the association between dietary fiber and colon cancer according to MSI status [46–48]. Given the different exposure categories used in these studies, a meta-analysis of results was not carried out. One study [47] found increased consumption of dietary fiber to be associated with lower risk of MSS colon cancer (OR = 0.7; 95% CI: 0.5–0.9), and one study [48] reported lower risk of both MSI-H and MSS colon cancer. On the contrary, one study [46] reported increased risk of MSS/MSI-L colon cancer for patients with low (≤16 g/day) fiber intake (OR = 1.4; 95% CI: 1.1–1.9). Case–case analyses in all three studies revealed no significant difference in the association of dietary fiber intake and MSI-H colon cancer compared with MSS/MSI-L colon cancer.

Non-steroidal anti-inflammatory drugs. Three studies (one cohort, two case–control) investigated the relationship between use of NSAIDs and MSI-H or MSS CRC [31, 35, 55]. In one study of colon cancer patients [35], non-use of NSAIDs was associated with an increased risk of both MSI-H and MSS colon cancer among both males and females. Case–case analyses did not reveal significant differences in the association of NSAIDs with MSI-H colon cancer compared with MSS colon cancer. Two other studies found significantly reduced risk of MSS/MSI-L CRC for current NSAID use (OR = 0.7; 95% CI: 0.6–0.9) [31] or regular aspirin use (RR = 0.78; 95% CI: 0.69–0.88) [55], compared with never/non-regular use but no associations were observed for MSI-H CRC.

Physical activity. One case–control study [35] investigated the association between physical activity and colon cancer by MSI status. For males, intermediate and low levels of physical activity were risk factors for both MSI-H and MSS CRC. Among females, low levels of physical activity were associated with an increased risk of MSS/MSI-L colon cancer but no association was observed

for MSI colon cancer. In a case–case analysis comparing MSI-H with MSS colon cancer, no difference was identified.

Statins. One study [56] investigated the association between statin use and CRC by MSI-status and found no association between current statin use and MSI-H or MSS CRC.

Discussion

This systematic review aimed to comprehensively summarize the current evidence on the relationship between lifestyle factors and risk of MSI-H or MSS CRC. Overall, the majority of studies focused on reporting associations with smoking, alcohol, red meat intake, BMI and HRT. For the other factors (dietary fiber, NSAID use, physical activity and statins) only few studies were available. In the meta-analyses, the association of smoking was stronger with MSI-H CRC than with MSS/MSI-L CRC, while the use of HRT was associated only with reduced risk of MSS/MSI-L CRC. Higher BMI was associated with higher risk of both MSI-H and MSS CRC, although the association was statistically significant for MSS only. To the best of our knowledge, this is the first systematic review and meta-analysis to summarize the evidence of the relationship between major lifestyle factors and CRC by MSI status.

One meta-analysis has summarized studies reported up to January 2014, investigating the relationship between smoking and MSI status in CRC [19]. This study found a significant association between smoking and MSI CRC, but did not report on the association with MSS CRC. The authors did not apply any quality assessment criteria, which led to the inclusion of studies that would not have fulfilled our quality criteria of minimum adjustment for age and sex [57, 58]; therefore, it is difficult to compare our results with this meta-analysis. A previous meta-analysis that summarized the evidence between smoking and CRC risk overall found a relative risk of 1.18 (95% CI 1.11–1.25) [8] for ever-smokers. Combining the risks of MSI-H CRC (assuming 15% of CRCs) and MSS CRC observed in our meta-analysis would reveal a very similar overall CRC risk associated with smoking. About 75%–80% of MSI-H CRC occurs because of methylation-induced silencing of the *MLH1* gene which is similar to the process of CpG methylation leading to the CpG island methylator phenotype (CIMP-high) [59]. Studies have shown that DNA methylation can be induced by cigarette smoking [60], leading to CIMP-H or MSI-H tumors. This could potentially explain the stronger association observed with MSI-H CRC. However, knowledge about underlying epigenetic mechanisms explaining the role of smoking in the development of MSI-H CRC is still limited.

Several observational studies and two randomized controlled trials have reported that women who use HRT have a reduced risk of developing CRC [10, 11, 61, 62]. We found no association between ever use of HRT and MSI-H CRC, but our findings suggest that the protective effect of HRT on CRC incidence might be restricted to MSS tumors. Estrogen binds to the estrogen receptor beta (estrogen receptor 2, ESR2) in the large bowel and there is some evidence from biological studies relating the ESR2 pathway with the function of the MMR system, suggesting that estrogen induces MLH1 expression [63]. Thus, the protective effect of estrogen might be restricted to tumors in which the MMR system is normally expressed [25]. However, given the numerous downstream pathways that are regulated by the estrogen receptors, there are several mechanisms by which estrogen may exert its effect in CRC [64] and more studies are needed to completely elucidate these complex, potentially differential associations [65] between MSI-H and MSS CRC.

The combined evidence from our meta-analysis along with the evidence from the studies not included in the current meta-analysis suggests that excess body weight is a risk factor for both MSS and MSI-H CRC. Although available epidemiologic evidence suggests that BMI is associated with CRC, the exact biologic mechanisms are not fully understood. Mechanisms hypothesized to play a role include insulin and the insulin-like growth factors, sex steroids, adipokines such as leptin and adiponectin, inflammation (e.g. C-reactive protein) and oxidative stress [66, 67]. More recent evidence has suggested that obesity can alter DNA methylation [68], however, due to the limited number of studies and this mostly speculative hypothesis, additional large population based studies are warranted to investigate the obesity-CRC relationship according to relevant molecular tumor features. Although it has been found that the association between obesity and CRC may vary by sex, cancer site [7], or use of HRT [44], we were not able to further investigate subgroups in our meta-analysis. Other studies limited to patients with Lynch syndrome have found associations between obesity and risk of CRC [69] or colorectal adenomas [70], potentially differing by sex. Further investigation of potential sex and cancer site differences regarding tumor MSI status, may provide further understanding of the etiology of MSS and MSI-H CRC.

The relationship between alcohol consumption and MSI status was investigated in eight studies and overall, most studies did not find a significant association between increased alcohol consumption and MSI-H or MSS CRC. However, studies were inconsistent in their exposure definition and their findings, and mostly too small to detect significant associations. No clear associations were observed between the other lifestyle factors and MSI status of CRC, but further research is required to clarify the possible role that red meat consumption or NSAID use might have on the development of MSS or MSI-H tumors.

Further knowledge of the relationship between common lifestyle factors and molecular pathological subtypes will provide a more comprehensive understanding of the heterogeneity of CRC, which may allow clinicians, pathologists and epidemiologists to develop more personalized prevention strategies for CRC. Also, previous studies have shown associations between genetic variants and risk of specific subtypes of CRC, which reflects the importance of taking genetic factors into account in the risk prediction of CRC [71]. Results from our meta-analyses support the suggestion that since smoking is associated with MSI-H CRC, measures for prevention and early detection may differ in their effectiveness between smokers and non-smokers [72]. For example, colonoscopy screening may not be as effective in smokers as in non-smokers, as smokers are more likely to develop MSI-H CRC (through the serrated-adenoma pathway), whose precursors are more difficult to detect by colonoscopy [73–75]. Furthermore, accumulating evidence suggests that response to treatment or other interventions depends on cancer subtypes, therefore, further research and understanding is fundamental to provide insights into targeted treatment strategies [15].

This systematic review has a number of limitations which require discussion. Our meta-analyses combined results from case–

control and cohort studies due to the small number of available studies. However, we used stringent quality criteria in order to provide a first overview of studies in this field. In studies analyzing effect heterogeneity between MSI-H CRC and MSS CRC it is crucial to account for tumor location, since the reported relationship might merely be based on the relationship between risk factors and tumor location [8, 76]. Yet, not all studies adjusted for tumor location, which could have biased the estimates. Furthermore, for each of the risk factors dietary fiber, physical activity, NSAIDs and statins, less than four studies were identified, which could not be combined in a meta-analysis. Also, each study used slightly different methodology for tumor collection, analysis and the definition of MSI, which can contribute to limited comparability. Some studies only measured the MSI status in a subsample of the original study population, which could have led to selection bias if the choice of the sample was not random. A recent publication discussed the use of inverse probability weighting (IPW) to reduce possible selection bias caused by nonrandom tissue availability [77]. Although none of the studies have incorporated this method to date, the application of IPW in future studies may have potential to advance the field of molecular pathological epidemiology by reducing selection bias. Furthermore, the included studies used varying covariate adjustment, therefore residual confounding may have occurred. Several studies included in this review had a limited sample size of cases with measured MSI status and about one third of the studies assessed the relationship with colon cancer cases only. Based on the limited studies identified, it was not possible to conduct subgroup analyses and compare findings separately depending on location or tumor marker combinations. Moreover, assessment of the individual risk factors was heterogeneous in the included studies and may have affected our meta-analyses and interpretation. Finally, results regarding the associations of different lifestyle factors with the MSI status of a tumor should be interpreted with caution, since the MSI phenotype can arise via diverse pathogenic mechanisms that may not be causally linked to lifestyle factors [78].

Despite the limitations, this first comprehensive systematic literature review and meta-analysis investigating the association between various risk and protective factors and MSI status in CRC provides important insights for future research. In particular, this review supports a stronger association of smoking with MSI-H CRC, and a reduced risk of MSS CRC only with HRT use. Due to the limited number of studies and the heterogeneity of results from existing studies, further large-scale population-based studies as well as meta-analyses are warranted. Also, additional studies are needed to further elucidate the potential mechanisms underlying the differential associations observed between lifestyle factors and MSS and MSI-H CRC as well as for other molecular pathological subtypes of CRC.

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