Intake of Dietary Fruit, Vegetables, and Fiber and Risk of Colorectal Cancer According to Molecular Subtypes: A Pooled Analysis of 9 Studies

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ABSTRACT

Protective associations of fruits, vegetables, and fiber intake with colorectal cancer risk have been shown in many, but not all epidemiologic studies. One possible reason for study heterogeneity is that dietary factors may have distinct effects by colorectal cancer molecular subtypes. Here, we investigate the association of fruit, vegetables, and fiber intake with four well-established colorectal cancer molecular subtypes separately and in combination. Nine observational studies including 9,592 cases with molecular subtypes for microsatellite instability (MSI), CpG island methylator phenotype (CIMP), and somatic mutations in *BRAF* and *KRAS* genes, and 7,869 controls were analyzed. Both case-only logistic regression analyses and polytomous logistic regression analyses (with one control set and multiple case groups) were used. Higher fruit intake was associated with a trend toward decreased risk of *BRAF*-mutated tumors [OR 4th vs. 1st quartile = 0.82 (95% confidence interval, 0.65–1.04)] but not *BRAF*-wildtype tumors [1.09 (0.97–1.22); *P* difference as shown in case-only analysis = 0.02]. This difference was observed in case–control studies and not in cohort studies. Compared with controls, higher fiber intake showed negative association with colorectal cancer risk for cases with microsatellite stable/MSI-low, CIMP-negative, *BRAF*-wildtype, and *KRAS*-wildtype tumors (P_{trend} range from 0.03 to 3.4e-03), which is consistent with the traditional adenoma-colorectal cancer pathway. These negative associations were stronger compared with MSI-high, CIMP-positive, *BRAF*-mutated, or *KRAS*-mutated tumors, but the differences were not statistically significant. These inverse associations for fruit and fiber intake may explain, in part, inconsistent findings between fruit or fiber intake and colorectal cancer risk that have previously been reported.

Significance: These analyses by colorectal cancer molecular subtypes potentially explain the inconsistent findings between dietary fruit or fiber intake and overall colorectal cancer risk that have previously been reported.

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Introduction

Colorectal cancer is the third most commonly diagnosed cancer worldwide, and the number of cases is predicted to increase to 2.2 million new cases per year by 2030 (1). To date, various dietary factors have been investigated in relation to colorectal cancer risk in many epidemiologic studies. Meta-analyses report that higher intake of fruit (2), vegetables (2), and fiber (3) is associated with decreased colorectal cancer risk. However, the World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) concluded that there is no "convincing strong evidence" for these dietary factors. High intake of foods containing dietary fiber only has "probable strong evidence" for decreasing the risk of colorectal cancer, and low intake of fruit and vegetables only has "limited-suggestive evidence" for increasing risk (https://www.wcrf.org/dietandcancer/colorectal-cancer; ref. 4) because these preventive associations have been shown in many, but not all epidemiologic studies (5–7). One possible reason for study heterogeneity is differential effects of diet on distinct colorectal cancer molecular subtypes.

Four molecular markers in particular have been well-studied with regard to colorectal cancer heterogeneity: microsatellite instability (MSI), the CpG island methylator phenotype (CIMP), and oncogenic mutations in *BRAF* and *KRAS* genes (8). Increasing evidence showed that some lifestyle risk factors can be differentially associated with these molecular markers (9). Probably the evidence is currently strongest for smoking, which is more strongly associated with MSI-high, CIMP-positive, and *BRAF*-mutated tumors (10, 11). Furthermore, there is some evidence that body mass index and hormone replacement therapy are associated with MSI status (12). These findings provide a strong rationale to investigate if dietary risk factors may also be differentially associated with colorectal cancer molecular markers. Although several epidemiologic studies have evaluated the association of fruit (13–19), vegetables (13–20), and fiber (13, 16–22) intake with risk of colorectal cancer molecular subtypes, results from these studies have been inconsistent. In addition, there has been no study to this point that has investigated all four characteristic molecular markers together despite the fact that these can point to differential pathways. Specifically, three different pathways to colorectal cancer development may be affected by the combination of molecular subtypes: (i) a serrated pathway, (ii) an alternate pathway, and (iii) a traditional pathway (23, 24). Only one meta-analysis reported the association of fiber intake with MSI status of colorectal cancer (12). However, this meta-analysis of three case–control studies used heterogeneous fiber definitions across studies (12). The direction of the association between fiber intake and MSI status in each study was different because one study found higher fiber intake was associated with decreased risk of MSS tumors (13), but another study reported decreased risk of both MSI-high and MSS/MSI-low tumors (16). On the contrary, one study found increased risk of MSS/MSI-low tumors with low fiber intake (17). To better address the hypothesis that these dietary factors may affect risk of colorectal cancer molecular subtypes differently, here we pooled nine population-based studies with individual-level data harmonized in a consistent manner.

We analyzed data from 9,592 colorectal cancer cases and 7,869 controls within the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) and the Colon Cancer Family Registry (CCFR) to assess the association of fruit, vegetables, and fiber intake with colorectal cancer risk by molecular subtypes using data on MSI, CIMP, *BRAF* mutation, and *KRAS* mutation status. Identification of associations with specific molecular subtypes may point to specific diet–colorectal cancer associations and help inform underlying biological mechanisms relevant to colorectal cancer risk.

Patients and Methods

Study participants

This study population consisted of 8,783 colorectal cancer cases and 7,869 controls from 9 observational studies within GECCO and the CCFR with available tumor marker, fruit, fiber, vegetables, and total energy intake data. In addition, 809 population-based colorectal cancer cases from the Mayo Clinic CCFR, which did not enroll population-based controls, were included in case-only analyses. Among study participants, 3,258 colorectal cancer cases and 3,984 controls were from cohort studies, and 6,334 colorectal cancer cases and 3,885 controls were from case–control studies. Descriptive characteristics of each study and mean intake of quartile cut points of fruits, vegetables, and fiber in each study are shown in Supplementary Tables S1 and S2. In addition, details regarding each observational study are described in Supplementary Text. All colorectal cancer cases were defined by colorectal adenocarcinoma and confirmed by pathological records, medical records, and/or death certificate information. All study participants provided written-informed consent, and each study was approved by their relevant research ethics committee or Institutional Review Board.

Definition of tumor subtypes

Testing for MSI, CIMP, and mutations in the *BRAF* and *KRAS* gene was conducted previously by each study and according to individual study protocols. Details regarding marker testing in each study are described in Supplementary Text. Especially, Supplementary Table S3 shows study-specific markers used to assess MSI and definition of MSI status. In addition, Supplementary Table S4 also shows study-specific panels used to assess CIMP status. We defined marker combinations for molecular subtypes, consistent with previously suggested classifications (23, 25): Types 1–10. Details of the defined marker combinations are given in **Fig. 1** and Supplementary Text. Subtype

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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classifications with fewer than 50 cases were excluded from analyses. With regard to colorectal carcinogenic pathways, previous studies reported that three different pathways to colorectal cancer development may be affected by the combination of molecular subtypes: (i) a serrated pathway (Type 1: MSI-high, CIMP-positive, *BRAF*-mutated, *KRAS*-wildtype and Type 2: MSS/MSI-low, CIMP-positive, *BRAF*-mutated, *KRAS*-wildtype), (ii) an alternate pathway (Type 3: MSS/MSI-low, CIMP-negative, *BRAF*-wildtype, *KRAS*-mutated), and (iii) a traditional pathway (Type 4: MSS/MSI-low, CIMP-negative, *BRAF*-wildtype, *KRAS*-wildtype and Type 5: MSI-high, CIMP-negative, *BRAF*-wildtype, *KRAS*-wildtype; refs. 23, 24).

Exposure data

In each study, demographic and lifestyle risk factor information was assessed via in-person interviews or structured self-administered questionnaires, as described in the Supplementary Text. Dietary variables were ascertained using food frequency questionnaires or diet history. Data were collected at study entry, at blood draw, or 1 to 2 years prior to sample ascertainment. A multistep, iterative data harmonization procedure was applied, reconciling each study's unique protocols and data collection instruments (26). Multiple quality-control checks were performed, and outlying values of variables were truncated to the minimum or maximum value of an established range for each variable. Variables were combined into a single dataset using common definition, standardized coding, and standardized permissible values.

In our study analysis, we selected dietary intake variables for fruit (servings/day), vegetables (servings/day), and fiber (measured as g/day). Sex- and study-specific quartiles for dietary variables were created based on the distribution in the controls. Data harmonization was performed using SAS and T-SQL.

Statistical analyses

We used the χ^2 and Mann–Whitney *t* tests to compare baseline characteristics between cases and controls. To test if the association of fruit, vegetables, and fiber intake with each molecular subtype differed, we conducted case-only logistic regression analysis. We also estimated the strength of the association between the dietary variables and risk for the specific molecular subtypes using polytomous logistic regression models with two case groups and one control set. The ORs and their corresponding 95% confidential intervals (CI) for the quartiles of dietary variables were compared with the lowest intake category as a reference. *P* values for linear trends were calculated by treating quartiles of intake as a continuous variable. Participants with missing values of each dietary intake for fruit (n = 1,933), vegetables (n = 1,775), and fiber (n = 5,422) were excluded from the analyses. In our primary analyses, minimally adjusted models (minimally adjusted OR) included study site, age at diagnosis, total energy consumption (kcal/day), and sex as covariates. To determine confounding factors for multivariate-adjusted models (multivariate-adjusted OR), we evaluated the association of the following colorectal cancer–related parameters: tobacco smoking, alcohol, body mass index, physical activity, history of diabetes mellitus, red meat intake, processed meat intake, and aspirin/nonsteroidal anti-inflammatory drugs use, with dietary factors on the risk of colorectal cancer overall. We included those factors that changed the beta estimate of the dietary factors by more than 10% when compared with the minimally adjusted models (27). Based on this, we included tobacco smoking, red meat intake, and processed meat intake in the multivariate adjusted models. Missing covariates were imputed by sex-specific mean for each study for age at diagnosis (n = 8 missing), total energy consumption (n = 5,473 missing), tobacco smoking (n = 1,375 missing), red meat intake (n = 1,709 missing), and processed meat intake (n = 6,565 missing). Processed meat was not imputed for NFCCR because it was missing for all subjects (n = 1,045 missing). We conducted a subgroup analysis stratified by study design (case–control or cohort study) as sensitivity analyses to evaluate differences in effects between study designs.

All *P* values are two-sided. A *P* value <0.05 was considered statistically significant for association with colorectal cancer risk. For assessing the heterogeneous association with molecular subtypes, a Bonferroni-corrected *P* value <0.05/10 was considered statistically significant to account for the 10 subtypes being tested. All statistical analyses were performed using R version 3.6.0.

Results

Cases were more likely to be men, younger, past or current smokers, have a higher intake of red meat, processed meat, and energy, and have a lower intake of fruits and vegetables (**Table 1**). In a case–control analysis of all colorectal cancer cases combined, we observed an inverse association between fiber intake and overall colorectal cancer risk [multivariate-adjusted OR $_{\text{4th vs. 1st quartile}}$ = 0.85 (95% CI, 0.76–0.97); P_{trend} = 6.2e-03], but no statistically significant association between colorectal cancer risk with fruit intake [multivariate-adjusted OR $_{\text{4th vs. 1st quartile}}$ = 1.04 (95% CI, 0.93–1.15); P_{trend} = 0.99] and vegetable intake [multivariate-adjusted OR $_{\text{4th vs. 1st quartile}}$ = 0.92 (95% CI, 0.82–1.03); P_{trend} = 0.09; Supplementary Table S5]. Among colorectal cancer cases, 15.6% were MSI-high, 18.9% CIMP-positive, 12.8% *BRAF*-mutated, and 32.8% *KRAS*-mutated.

When we analyzed the association of dietary variables with risk of each molecular colorectal cancer subtype in a case-only analysis (**Table 2**), we observed that fruit intake was differentially associated with *BRAF*-mutated compared with *BRAF*-wildtype tumors [multivariate-adjusted OR $_{\text{4th vs. 1st quartile}}$ = 0.75 (95% CI, 0.60–0.94), *P* difference = 0.02]. In addition, a polytomous logistic regression analysis (**Table 3**) comparing *BRAF*-mutated and *BRAF*-wildtype tumors with controls showed that a trend toward inverse association between fruit intake and colorectal cancer risk was limited to *BRAF*-mutated tumors [multivariate-adjusted OR $_{\text{4th vs. 1st quartile}}$ = 0.82 (95% CI, 0.65–1.04), P_{trend} = 0.06] as compared with controls, whereas there was no association between fruit intake and *BRAF*-wildtype tumors [multivariate-adjusted OR $_{\text{4th vs. 1st quartile}}$ = 1.09 (95% CI, 0.97–1.22), P_{trend} = 0.54] as compared with controls. In subgroup analyses, these associations were found statistically significant in case–control studies, but not in cohort studies (**Table 4** and Supplementary Table S6). Fruit intake did not show a statistically significant differential association with other molecular subtypes. Neither vegetable nor fiber intake showed significant differential associations for any of the molecular markers as shown by the case-only analysis (**Table 2**). However, in the subgroup analyses, we found that fiber intake was differentially associated with CIMP-negative tumors as compared with CIMP-positive tumors in cohort studies (**Table 4**).

When we combined markers to define subtypes in polytomous logistic regression analyses (**Fig. 1**; Supplementary Table S7), we observed a linear trend for increased fiber intake in relation to colorectal cancer risk among Type 4 tumors, MSS/MSI-low, CIMP-negative, *BRAF*-wildtype, and *KRAS*-wildtype [multivariate-adjusted OR = 0.94 (95% CI, 0.88–0.99), P_{trend} = 0.03)] compared with controls, although the difference between Type 4 and each of the other Types was not statistically significant. This finding was consistent with results in the single-marker analyses (**Table 3**), where the same trend

Table 1. Baseline characteristics of cases and controls.

Characteristics	Cases[a]	Controls	*P* value[b]
n	9,592	7,869	
Age, mean (SD)[c]	58.6 (11.5)	60.9 (10.3)	<2.2e-16
Sex (%)			
Men	4,883 (50.9)	3,713 (47.2)	
Women	4,709 (49.1)	4,156 (52.8)	9.9e-07
Tobacco smoking (%)[d]			
Never smoker	3,790 (41.8)	3,292 (46.9)	
Past or current smoker	5,273 (58.2)	3,731 (53.1)	1.6e-10
Dietary intake			
Red meat, servings/day, mean (SD)[e]	0.77 (0.68)	0.73 (0.65)	5.0e-06
Processed meat, servings/day, mean (SD)[f]	0.29 (0.34)	0.25 (0.29)	1.8e-08
Total energy, kcal/day, mean (SD)[g]	2118.3 (851.1)	2024.2 (768.0)	5.4e-07
Fruits, servings/day, mean (SD)[h]	1.82 (1.63)	2.09 (1.75)	<2.2e-16
Vegetables, servings/day, mean (SD)[i]	2.51 (1.98)	2.95 (2.15)	<2.2e-16
Fiber, g/day, mean (SD)[j]	22.9 (10.7)	23.1 (10.4)	0.30
Location of colorectal cancer (%)			
Proximal	3.670 (38.3)	–	
Distal	2,975 (31.0)	–	
Rectum	2,526 (26.4)	–	
Missing	421 (4.4)	–	–
Colorectal cancer stage (%)			
I	2,038 (21.2)	–	
II	1,780 (18.6)	–	
III	1,847 (19.3)	–	
IV	817 (8.5)	–	
Missing	3,110 (32.4)	–	–
Microsatellite instability (%)			
High	1,417 (15.6)	–	
Stable/low	7,639 (84.4)	–	–
CpG island methylator phenotype (%)			
High	1,298 (18.9)	–	
Low	5,569 (81.1)	–	–
BRAF (%)			
Mutated	1,109 (12.8)	–	
Wildtype	7,566 (87.2)	–	–
KRAS (%)			
Mutated	2,355 (32.8)	–	
Wildtype	4,831 (67.2)	–	–

[a]The number of cases includes 809 colorectal cancer cases from the Mayo Clinic CCFR, which does not include controls.
[b]Based on χ^2 test or Mann–Whitney *t* test.
[c]Age is missing for 4 cases and 4 controls.
[d]Tobacco smoking is missing for 529 cases and 846 controls.
[e]Red meat intake is missing for 743 cases and 966 controls.
[f]Processed meat intake is missing for 4,606 cases and 1,959 controls.
[g]Total energy intake is missing for 4,067 cases and 1,406 controls.
[h]Fruit intake is missing for 850 cases and 1,083 controls.
[i]Vegetables intake is missing for 731 cases and 1,044 controls.
[j]Fiber intake is missing for 4,038 cases and 1,384 controls.

was seen for MSS/MSI-low tumors (P_{trend} = 3.4e-03), CIMP-negative tumors (P_{trend} = 9.0e-03), *BRAF*-wildtype tumors (P_{trend} = 3.5e-03), and *KRAS*-wildtype tumors (P_{trend} = 0.03) compared with controls.

Discussion

This is the largest analysis using individual level of dietary data to investigate their association with well-described molecular subtypes for colorectal cancer. Although higher fruit intake was not associated with overall colorectal cancer risk, we observed that fruit intake was statistically significantly associated with a decreased risk for *BRAF*-mutated tumors but not *BRAF*-wildtype tumors. This finding was observed only among case–control studies. In addition, we observed in single-marker polytomous logistic regression analyses that higher fiber intake was associated with a decreased risk for MSS/MSI-low, CIMP-negative, *BRAF*-wildtype, and *KRAS*-wildtype molecular subtypes compared with controls. The association in the combined-marker analysis also showed a trend toward a negative association. These negative associations were stronger than they were for MSI-high, CIMP-positive, *BRAF*-mutated, or *KRAS*-mutated tumors, but the differences were not statistically significant. Increased fiber intake was differentially associated with a

Table 2. ORs and 95% CIs for the association of fruits, vegetables, and fiber intake with the risk of molecular subtypes of colorectal cancer in case-only analysis.

	Quartile				
	Lowest (Q1)	Second (Q2)	Third (Q3)	Highest (Q4)	P_{trend}
Fruits (servings/day)					
MSI-high vs. MSS/MSI-low					
High (*n*)/Stable/low cases (*n*)	354/1,844	383/2,076	322/1,701	238/1,338	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.12 (0.94–1.33)	0.99 (0.84–1.17)	0.88 (0.73–1.07)	0.14
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.14 (0.96–1.35)	1.02 (0.86–1.21)	0.91 (0.75–1.10)	0.27
CIMP-positive vs. CIMP-negative					
High (*n*)/Low cases (*n*)	274/1,293	334/1,559	274/1,278	232/1,066	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.09 (0.90–1.33)	0.97 (0.80–1.19)	0.95 (0.77–1.18)	0.43
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.10 (0.91–1.34)	1.00 (0.82–1.23)	0.99 (0.80–1.23)	0.73
BRAF-mutated vs. *BRAF*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	284/1,780	277/2,161	254/1,691	169/1,336	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.91 (0.75–1.11)	0.87 (0.72–1.06)	0.71 (0.57–0.88)	2.9e-03
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.93 (0.76–1.13)	0.91 (0.75–1.10)	0.75 (0.60–0.94)	0.02
KRAS-mutated vs. *KRAS*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	502/1,093	703/1,410	544/1,070	430/853	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.16 (1.00–1.36)	1.12 (0.96–1.30)	1.06 (0.90–1.25)	0.55
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.16 (0.99–1.35)	1.11 (0.95–1.29)	1.04 (0.88–1.22)	0.75
Vegetables (servings/day)					
MSI-high vs. MSS/MSI-low					
High (*n*)/Stable/low cases (*n*)	267/1,438	470/2,535	377/1,976	204/1,101	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.02 (0.86–1.21)	1.03 (0.86–1.23)	0.97 (0.79–1.20)	0.87
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.03 (0.87–1.22)	1.05 (0.87–1.25)	0.98 (0.80–1.21)	0.99
CIMP-positive vs. CIMP-negative					
High (*n*)/Low cases (*n*)	261/1,074	351/1,715	294/1,529	214/921	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.99 (0.82–1.21)	1.02 (0.83–1.25)	1.08 (0.86–1.35)	0.47
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.00 (0.82–1.22)	1.05 (0.86–1.29)	1.10 (0.88–1.38)	0.33
BRAF-mutated vs. *BRAF*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	203/1,442	362/2,545	276/1,982	150/1,104	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.12 (0.92–1.36)	1.08 (0.88–1.33)	0.99 (0.78–1.26)	0.89
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.13 (0.93–1.38)	1.12 (0.91–1.38)	1.01 (0.79–1.29)	0.90
KRAS-mutated vs. *KRAS*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	444/974	785/1,531	598/1,196	378/756	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.12 (0.97–1.30)	1.07 (0.92–1.25)	1.06 (0.89–1.26)	0.68
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.12 (0.97–1.30)	1.06 (0.91–1.24)	1.06 (0.89–1.26)	0.75
Fiber (g/day)					
MSI-high vs. MSS/MSI-low					
High (*n*)/Stable/low cases (*n*)	199/1,092	202/1,094	191/1,095	235/1,101	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.98 (0.78–1.21)	0.91 (0.72–1.14)	1.11 (0.87–1.41)	0.51
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.99 (0.79–1.23)	0.94 (0.74–1.18)	1.15 (0.90–1.49)	0.35
CIMP-positive vs. CIMP-negative					
High (*n*)/Low cases (*n*)	195/822	231/849	218/826	235/863	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.21 (0.96–1.52)	1.15 (0.90–1.46)	1.18 (0.92–1.52)	0.30
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.23 (0.98–1.54)	1.18 (0.93–1.50)	1.22 (0.94–1.57)	0.20
BRAF-mutated vs. *BRAF*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	166/1,070	171/1,056	175/1,049	183/1,072	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.98 (0.77–1.24)	0.98 (0.77–1.26)	0.99 (0.76–1.29)	0.97
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.99 (0.78–1.26)	1.02 (0.80–1.31)	1.05 (0.80–1.37)	0.69
KRAS-mutated vs. *KRAS*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	375/735	375/743	383/723	402/735	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.98 (0.82–1.17)	1.04 (0.86–1.25)	1.09 (0.89–1.32)	0.34
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.97 (0.81–1.17)	1.02 (0.84–1.23)	1.04 (0.85–1.27)	0.60

[a]Minimally adjusted OR was adjusted for age, sex, study, and total energy (continuous).
[b]In addition to minimally adjusted OR, multivariate-adjusted OR was further adjusted for tobacco smoking (never, past, and current smoker <25, 25–<50, 50–<75, ≥75 pack-years), red meat intake (study- and sex-specific quartiles as continuous), and processed meat intake (study- and sex-specific quartiles as continuous).

decreased risk of CIMP-negative compared with CIMP-positive tumors in cohort studies, but not in case–control studies. We did not identify any differences in vegetable intake with colorectal cancer risk in subtype analyses examining MSI, CIMP, and *KRAS* and *BRAF* mutations separately or in combination.

Colorectal cancer development is caused by different etiological pathways underlying different genetic and epigenetic aberrations, which have been defined by specific molecular subtypes associated with distinct development trajectories. If dietary risk factors for colorectal cancer, such as fruit, vegetable, and fiber intake, affect

Table 3. ORs and 95% CIs for the association of fruits, vegetables, and fiber intake with the risk of molecular subtypes of colorectal cancer in case–control analysis.

	Quartile					
	Lowest (Q1)	Second (Q2)	Third (Q3)	Highest (Q4)	P_{trend}	P_{diff}[a]
Fruits (servings/day)						
MSI-high vs. Controls						
Cases (*n*)/Controls (*n*)	275/1,938	404/1,736	297/1,682	199/1,430		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.27 (1.06–1.52)	1.01 (0.84–1.22)	0.85 (0.69–1.04)	0.047	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.32 (1.10–1.58)	1.09 (0.91–1.32)	0.95 (0.77–1.16)	0.40	
MSS/MSI-low vs. Controls						
Cases (*n*)/Controls (*n*)	1,447/1,938	2,199/1,736	1,556/1,682	1,128/1,430		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.22 (1.10–1.35)	1.02 (0.92–1.13)	0.98 (0.87–1.09)	0.24	0.19
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.25 (1.13–1.39)	1.07 (0.96–1.19)	1.06 (0.94–1.18)	0.83	0.37
CIMP-positive vs. Controls						
Cases (*n*)/Controls (*n*)	242/1,938	360/1,736	268/1,682	205/1,430		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.18 (0.97–1.42)	0.95 (0.78–1.15)	0.91 (0.73–1.12)	0.14	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.21 (1.00–1.47)	1.02 (0.84–1.25)	1.02 (0.82–1.26)	0.77	
CIMP-negative vs. Controls						
Cases (*n*)/Controls (*n*)	1,124/1,938	1,656/1,736	1,250/1,682	933/1,430		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.26 (1.13–1.41)	1.06 (0.95–1.19)	0.94 (0.83–1.06)	0.11	0.59
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.30 (1.16–1.46)	1.12 (0.99–1.25)	1.02 (0.90–1.16)	0.82	0.87
BRAF-mutated vs. Controls						
Cases (*n*)/Controls (*n*)	233/1,938	321/1,736	235/1,682	145/1,430		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.09 (0.89–1.32)	0.86 (0.70–1.06)	0.72 (0.57–0.91)	1.7e-03	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.13 (0.93–1.38)	0.94 (0.77–1.16)	0.82 (0.65–1.04)	0.06	
BRAF-wildtype vs. Controls						
Cases (*n*)/Controls (*n*)	1,407/1,938	2,298/1,736	1,577/1,682	1,117/1,430		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.27 (1.14–1.41)	1.05 (0.94–1.17)	1.01 (0.90–1.13)	0.43	6.5e-03
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.31 (1.18–1.45)	1.10 (0.99–1.23)	1.09 (0.97–1.22)	0.54	0.03
KRAS-mutated vs. Controls						
Cases (*n*)/Controls (*n*)	438/1,938	755/1,736	548/1,682	369/1,430		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.39 (1.20–1.61)	1.16 (0.99–1.34)	1.04 (0.88–1.22)	0.93	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.43 (1.23–1.66)	1.21 (1.04–1.41)	1.12 (0.95–1.33)	0.38	
KRAS-wildtype vs. Controls						
Cases (*n*)/Controls (*n*)	883/1,938	1,577/1,736	1,070/1,682	738/1,430		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.31 (1.17–1.47)	1.06 (0.94–1.20)	1.05 (0.93–1.20)	0.84	0.94
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.36 (1.21–1.53)	1.13 (1.00–1.28)	1.16 (1.02–1.32)	0.18	0.63
Vegetables (servings/day)						
MSI-high vs. Controls						
Cases (*n*)/Controls (*n*)	255/1,772	441/1,987	319/1,828	178/1,238		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.08 (0.90–1.29)	0.98 (0.81–1.19)	0.87 (0.70–1.09)	0.17	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.10 (0.92–1.31)	1.03 (0.85–1.25)	0.91 (0.73–1.13)	0.35	
MSS/MSI-low vs. Controls						
Cases (*n*)/Controls (*n*)	1,397/1,772	2,301/1,987	1,779/1,828	929/1,238		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.03 (0.93–1.14)	0.97 (0.87–1.08)	0.89 (0.79–1.00)	0.046	0.81
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.03 (0.93–1.15)	0.99 (0.89–1.11)	0.91 (0.81–1.03)	0.13	0.93
CIMP-positive vs. Controls						
Cases (*n*)/Controls (*n*)	261/1,772	380/1,987	246/1,828	191/1,238		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.15 (0.95–1.38)	1.00 (0.82–1.22)	1.05 (0.84–1.31)	0.96	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.17 (0.97–1.41)	1.05 (0.86–1.28)	1.09 (0.88–1.36)	0.67	
CIMP-negative vs. Controls						
Cases (*n*)/Controls (*n*)	1,060/1,772	1,711/1,987	1,451/1,828	784/1,238		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.05 (0.94–1.18)	0.98 (0.87–1.11)	0.90 (0.79–1.03)	0.09	0.35
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.06 (0.95–1.19)	1.00 (0.89–1.13)	0.93 (0.81–1.06)	0.22	0.26
BRAF-mutated vs. Controls						
Cases (*n*)/Controls (*n*)	207/1,772	370/1,987	228/1,828	135/1,238		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.17 (0.96–1.42)	0.99 (0.80–1.22)	0.90 (0.71–1.15)	0.23	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.19 (0.98–1.45)	1.04 (0.84–1.28)	0.94 (0.74–1.20)	0.45	
BRAF-wildtype vs. Controls						
Cases (*n*)/Controls (*n*)	1,408/1,772	2,374/1,987	1,793/1,828	914/1,238		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.01 (0.91–1.12)	0.93 (0.84–1.05)	0.87 (0.77–0.98)	0.01	0.94
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.02 (0.92–1.13)	0.96 (0.86–1.07)	0.89 (0.79–1.01)	0.04	0.77

(Continued on the following page)

Table 3. ORs and 95% CIs for the association of fruits, vegetables, and fiber intake with the risk of molecular subtypes of colorectal cancer in case–control analysis. (Cont'd)

	Quartile					
	Lowest (Q1)	Second (Q2)	Third (Q3)	Highest (Q4)	P_{trend}	P_{diff}[a]
KRAS-mutated vs. Controls						
Cases (*n*)/Controls (*n*)	448/1,772	812/1,987	561/1,828	315/1,238		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.10 (0.95–1.27)	1.01 (0.87–1.18)	0.95 (0.80–1.13)	0.40	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.11 (0.96–1.29)	1.04 (0.89–1.21)	0.98 (0.82–1.16)	0.65	
KRAS-wildtype vs. Controls						
Cases (*n*)/Controls (*n*)	968/1,772	1,616/1,987	1,104/1,828	611/1,238		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.00 (0.89–1.13)	0.96 (0.85–1.09)	0.90 (0.79–1.04)	0.12	0.71
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.02 (0.90–1.14)	0.99 (0.88–1.12)	0.93 (0.81–1.07)	0.33	0.76
Fiber (g/day)						
MSI-high vs. Controls						
Cases (*n*)/Controls (*n*)	214/1,606	200/1,621	184/1,624	230/1,634		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	0.88 (0.71–1.09)	0.74 (0.59–0.92)	0.86 (0.68–1.08)	0.11	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	0.91 (0.73–1.12)	0.79 (0.63–0.99)	0.95 (0.75–1.20)	0.47	
MSS/MSI-low vs. Controls						
Cases (*n*)/Controls (*n*)	1,161/1,606	1,079/1,621	1,050/1,624	1,092/1,634		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	0.88 (0.79–0.99)	0.81 (0.72–0.91)	0.78 (0.69–0.88)	4.3e-05	0.56
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	0.90 (0.80–1.01)	0.84 (0.74–0.94)	0.83 (0.73–0.95)	3.4e-03	0.40
CIMP-positive vs. Controls						
Cases (*n*)/Controls (*n*)	212/1,606	226/1,621	209/1,624	233/1,634		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	1.09 (0.88–1.34)	0.93 (0.75–1.16)	1.01 (0.80–1.27)	0.69	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	1.12 (0.91–1.38)	0.99 (0.80–1.24)	1.11 (0.88–1.41)	0.63	
CIMP-negative vs. Controls						
Cases (*n*)/Controls (*n*)	899/1,606	836/1,621	786/1,624	840/1,634		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	0.90 (0.80–1.02)	0.79 (0.69–0.90)	0.79 (0.69–0.91)	1.8e-04	0.08
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	0.92 (0.81–1.04)	0.82 (0.72–0.94)	0.85 (0.74–0.98)	9.0e-03	0.049
BRAF-mutated vs. Controls						
Cases (*n*)/Controls (*n*)	178/1,606	170/1,621	167/1,624	181/1,634		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	0.89 (0.71–1.12)	0.79 (0.62–1.00)	0.80 (0.62–1.03)	0.053	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	0.92 (0.73–1.16)	0.86 (0.67–1.09)	0.90 (0.70–1.16)	0.36	
BRAF-wildtype vs. Controls						
Cases (*n*)/Controls (*n*)	1,139/1,606	1,038/1,621	1,012/1,624	1,059/1,634		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	0.87 (0.78–0.97)	0.80 (0.71–0.90)	0.78 (0.68–0.88)	4.0e-05	0.88
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	0.88 (0.79–0.99)	0.83 (0.74–0.94)	0.83 (0.73–0.95)	3.5e-03	0.58
KRAS-mutated vs. Controls						
Cases (*n*)/Controls (*n*)	403/1,606	370/1,621	372/1,624	391/1,634		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	0.89 (0.76–1.05)	0.84 (0.71–0.99)	0.84 (0.71–1.01)	0.047	
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	0.90 (0.77–1.06)	0.87 (0.73–1.03)	0.89 (0.74–1.07)	0.18	
KRAS-wildtype vs. Controls						
Cases (*n*)/Controls (*n*)	791/1,606	733/1,621	674/1,624	739/1,634		
Minimally adjusted OR (95% CI)[b]	1.00 (Reference)	0.91 (0.80–1.03)	0.78 (0.68–0.89)	0.80 (0.70–0.93)	4.7e-04	0.44
Multivariate-adjusted OR (95% CI)[c]	1.00 (Reference)	0.93 (0.82–1.06)	0.82 (0.72–0.94)	0.88 (0.76–1.02)	0.03	0.72

[a]P_{diff} tests the difference of the dietary risk factor and colorectal cancer association for the two cancer subtypes, such as MSI-high vs. MSS/MSI-low. This is based on the case-only analysis testing the difference in the trend across the 4 quartiles.
[b]Minimally adjusted OR was adjusted for age, sex, study, and total energy (continuous).
[c]In addition to minimally adjusted OR, multivariate-adjusted OR was further adjusted for tobacco smoking (never, past, and current smoker <25, 25–<50, 50–<75, ≥75 pack-years), red meat intake (study- and sex-specific quartiles as continuous), and processed meat intake (study- and sex-specific quartiles as continuous).

specific etiologic pathways, then we can expect that the specific dietary factors are differentially associated with these molecular subtypes. For specific molecular subtypes, the mutation in the *KRAS* oncogene has been widely known as an acting driver of colorectal cancer development (28). In addition, mutation of the *BRAF* oncogene induces proliferation and inhibits normal apoptosis of colonic epithelial cells (29). Both *KRAS* and *BRAF* are key players of the MAPK pathway (24). Another driving force of colorectal cancer development is CIMP. Widespread methylation of numerous promoter CpG island loci is responsible for inactivation of tumor-suppressor genes and other tumor-related genes (28). CIMP is also strongly related to the serrated pathway. Silencing of tumor-suppressor genes such as *p16INK4a* and *IGFBP7* via the synergistic effects of mutation of *BRAF* and CIMP resulting from hypermethylation could facilitate progression to serrated colorectal polyps (24). MSI is recognized by high frequency of genetic alterations in repeated microsatellite sequences of DNA resulting from a DNA mismatch repair deficiency (30).

Fruit intake was associated in *BRAF*-mutated tumors, which is of interest given that *BRAF*-mutated tumors have particularly poor survival (31). Differences in findings for fruit intake by study design may be due to differences in sample size, given that approximately two thirds of cases were from case–control studies. To evaluate study-specific differences among case–control studies, we explored consistency of the case–control study findings. The meta-analysis OR from

Table 4. ORs and 95% CIs for the association of fruits, vegetables, and fiber intake with the risk of molecular subtypes of colorectal cancer in case-only analysis, stratified by study design.

	Quartile				
	Lowest (Q1)	**Second (Q2)**	**Third (Q3)**	**Highest (Q4)**	P_{trend}
Cohort studies					
Fruits (servings/day)					
MSI-high vs. MSS/MSI-low					
High (*n*)/Stable/low cases (*n*)	114/591	94/500	103/505	94/502	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.92 (0.67–1.25)	0.93 (0.69–1.26)	0.83 (0.60–1.15)	0.31
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.92 (0.68–1.26)	0.94 (0.69–1.27)	0.83 (0.60–1.15)	0.31
CIMP-positive vs. CIMP-negative					
High (*n*)/Low cases (*n*)	126/575	129/479	112/512	119/495	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.15 (0.86–1.55)	0.90 (0.66–1.22)	1.04 (0.76–1.43)	0.81
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.15 (0.85–1.54)	0.90 (0.66–1.22)	1.03 (0.75–1.41)	0.74
BRAF-mutated vs. *BRAF*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	109/586	86/508	100/510	90/501	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.88 (0.65–1.22)	0.99 (0.73–1.35)	0.96 (0.69–1.34)	0.97
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.88 (0.64–1.22)	0.99 (0.72–1.35)	0.94 (0.67–1.32)	0.89
KRAS-mutated vs. *KRAS*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	205/457	219/349	214/366	194/375	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.32 (1.03–1.68)	1.24 (0.97–1.58)	1.05 (0.81–1.35)	0.79
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.31 (1.03–1.66)	1.22 (0.96–1.56)	1.02 (0.79–1.32)	0.93
Vegetables (servings/day)					
MSI-high vs. MSS/MSI-low					
High (*n*)/Stable/low cases (*n*)	123/603	98/516	91/538	93/440	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.88 (0.65–1.19)	0.78 (0.57–1.07)	0.95 (0.69–1.32)	0.55
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.90 (0.66–1.22)	0.80 (0.59–1.09)	0.96 (0.70–1.33)	0.59
CIMP-positive vs. CIMP-negative					
High (*n*)/Low cases (*n*)	146/573	126/505	105/528	109/454	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.13 (0.84–1.51)	0.93 (0.68–1.26)	1.12 (0.82–1.54)	0.79
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.16 (0.86–1.55)	0.94 (0.69–1.28)	1.14 (0.83–1.57)	0.75
BRAF-mutated vs. *BRAF*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	114/611	106/501	86/539	79/454	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.24 (0.91–1.68)	0.94 (0.68–1.29)	1.00 (0.71–1.41)	0.65
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.27 (0.94–1.73)	0.96 (0.69–1.32)	1.01 (0.71–1.42)	0.67
KRAS-mutated vs. *KRAS*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	215/484	227/348	219/369	171/345	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.33 (1.05–1.69)	1.23 (0.97–1.57)	1.01 (0.78–1.31)	0.94
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.34 (1.05–1.70)	1.23 (0.97–1.57)	1.00 (0.77–1.30)	0.99
Fiber (g/day)					
MSI-high vs. MSS/MSI-low					
High (*n*)/Stable/low cases (*n*)	95/533	95/542	106/531	125/528	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.91 (0.66–1.25)	1.01 (0.73–1.38)	1.15 (0.84–1.58)	0.28
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.92 (0.67–1.27)	1.05 (0.76–1.44)	1.19 (0.86–1.65)	0.20
CIMP-positive vs. CIMP-negative					
High (*n*)/Low cases (*n*)	112/516	125/525	137/507	153/519	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.12 (0.83–1.52)	1.29 (0.95–1.76)	1.35 (0.99–1.85)	0.04
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.13 (0.83–1.53)	1.33 (0.98–1.81)	1.35 (0.98–1.85)	0.04
BRAF-mutated vs. *BRAF*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	95/544	99/535	105/527	109/536	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.06 (0.77–1.46)	1.15 (0.83–1.58)	1.17 (0.84–1.62)	0.31
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.05 (0.77–1.45)	1.17 (0.85–1.61)	1.15 (0.82–1.61)	0.34
KRAS-mutated vs. *KRAS*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	200/409	206/399	200/395	228/404	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.03 (0.81–1.32)	1.00 (0.78–1.29)	1.14 (0.89–1.47)	0.35
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.02 (0.80–1.30)	0.98 (0.76–1.26)	1.08 (0.84–1.40)	0.64
Case–control studies					
Fruits (servings/day)					
MSI-high vs. MSS/MSI-low					
High (*n*)/Stable/low cases (*n*)	240/1,253	289/1,576	219/1,196	144/836	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.12 (0.94–1.33)	0.99 (0.84–1.17)	0.88 (0.73–1.07)	0.58
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.24 (1.01–1.54)	1.05 (0.85–1.28)	0.93 (0.73–1.17)	0.42

(Continued on the following page)

Table 4. ORs and 95% CIs for the association of fruits, vegetables, and fiber intake with the risk of molecular subtypes of colorectal cancer in case-only analysis, stratified by study design. (Cont'd)

	Quartile				
	Lowest (Q1)	**Second (Q2)**	**Third (Q3)**	**Highest (Q4)**	P_{trend}
CIMP-positive vs. CIMP-negative					
High (*n*)/Low cases (*n*)	148/718	205/1,080	162/766	113/571	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.06 (0.81–1.39)	1.03 (0.80–1.34)	0.87 (0.65–1.17)	0.41
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.08 (0.83–1.42)	1.10 (0.84–1.43)	0.96 (0.71–1.29)	0.89
BRAF-mutated vs. *BRAF*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	175/1,194	191/1,653	154/1,181	79/835	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.92 (0.71–1.19)	0.83 (0.65–1.06)	0.58 (0.43–0.77)	3.5e-04
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.94 (0.73–1.22)	0.88 (0.69–1.12)	0.64 (0.47–0.86)	4.9e-04
KRAS-mutated vs. *KRAS*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	297/636	484/1,061	330/704	236/478	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.06 (0.87–1.29)	1.04 (0.85–1.26)	1.07 (0.87–1.33)	0.58
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.05 (0.86–1.28)	1.02 (0.84–1.24)	1.05 (0.84–1.30)	0.76
Vegetables (servings/day)					
MSI-high vs. MSS/MSI-low					
High (*n*)/Stable/low cases (*n*)	144/835	372/2,019	286/1,438	111/661	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.12 (0.90–1.39)	1.17 (0.93–1.46)	0.97 (0.74–1.28)	0.87
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.12 (0.91–1.39)	1.19 (0.95–1.50)	0.98 (0.75–1.30)	0.75
CIMP-positive vs. CIMP-negative					
High (*n*)/Low cases (*n*)	115/501	225/1,210	189/1,001	105/467	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.92 (0.70–1.20)	1.07 (0.81–1.41)	1.04 (0.75–1.42)	0.46
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.91 (0.70–1.19)	1.11 (0.84–1.46)	1.06 (0.77–1.46)	0.31
BRAF-mutated vs. *BRAF*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	89/831	256/2,044	190/1,443	71/650	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.07 (0.82–1.40)	1.16 (0.88–1.54)	0.98 (0.69–1.38)	0.77
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.07 (0.82–1.40)	1.21 (0.91–1.60)	1.00 (0.71–1.41)	0.58
KRAS-mutated vs. *KRAS*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	229/490	558/1,183	379/827	207/411	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.01 (0.83–1.22)	0.96 (0.78–1.18)	1.10 (0.87–1.39)	0.64
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.00 (0.83–1.21)	0.95 (0.78–1.17)	1.09 (0.86–1.38)	0.70
Fiber (g/day)					
MSI-high vs. MSS/MSI-low					
High (*n*)/Stable/low cases (*n*)	104/559	107/552	85/564	110/573	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.01 (0.75–1.38)	0.77 (0.55–1.10)	0.99 (0.68–1.45)	0.65
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.03 (0.75–1.39)	0.80 (0.56–1.13)	1.02 (0.70–1.51)	0.79
CIMP-positive vs. CIMP-negative					
High (*n*)/Low cases (*n*)	83/306	106/324	81/319	82/344	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	1.30 (0.92–1.85)	0.90 (0.61–1.34)	0.87 (0.55–1.37)	0.28
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	1.32 (0.93–1.88)	0.95 (0.64–1.42)	0.94 (0.60–1.50)	0.50
BRAF-mutated vs. *BRAF*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	71/526	72/521	70/522	74/536	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.85 (0.59–1.23)	0.73 (0.49–1.10)	0.70 (0.45–1.11)	0.11
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.88 (0.61–1.27)	0.80 (0.53–1.21)	0.80 (0.50–1.28)	0.33
KRAS-mutated vs. *KRAS*-wildtype					
Mutated (*n*)/Wildtype cases (*n*)	175/326	169/344	183/328	174/331	
Minimally adjusted OR (95% CI)[a]	1.00 (Reference)	0.93 (0.71–1.21)	1.08 (0.81–1.45)	1.03 (0.74–1.44)	0.66
Multivariate-adjusted OR (95% CI)[b]	1.00 (Reference)	0.92 (0.70–1.21)	1.07 (0.79–1.43)	1.00 (0.71–1.41)	0.79

[a]Minimally adjusted OR was adjusted for age, sex, study, and total energy (continuous).

[b]In addition to minimally adjusted OR, multivariate-adjusted OR was further adjusted for tobacco smoking (never, past, and current smoker <25, 25–<50, 50–<75, ≥75 pack-years), red meat intake (study- and sex-specific quartiles as continuous), and processed meat intake (study- and sex-specific quartiles as continuous).

case–controls studies was similar to the pooled estimate from all studies, and we did not observe substantial heterogeneity among the case–control studies [$OR_{case-control\ meta\text{-}analysis} = 0.85$ (95% CI, 0.78–0.93), P heterogeneity $= 0.52$]. However, selection and recall biases, which are more likely to occur in case–control studies, may also explain observed differences due to study design. Selection bias could occur when colorectal cancer cases and/or controls are not representative of the target population. Although it could be possible that controls with a healthy diet are more willing to participate in studies, this is unlikely to explain the observed differential effect of fruit intake specifically on *BRAF*-mutated versus *BRAF*-wildtype tumors in the case-only analysis. For this case-only finding to be explained by selection bias, the participation of *BRAF*-mutated versus *BRAF*-wildtype colorectal cancer cases would need to differ by fruit intake. This may be possible if survival after colorectal cancer diagnosis affects participation and differs by *BRAF* mutational status and by fruit intake. However, we recently showed in a pooled analysis that *BRAF*-mutational status is not associated with survival after colorectal cancer diagnosis (32), and previous studies do not show strong evidence for the impact of fruit intake on survival (33, 34). For recall bias to explain the case-only

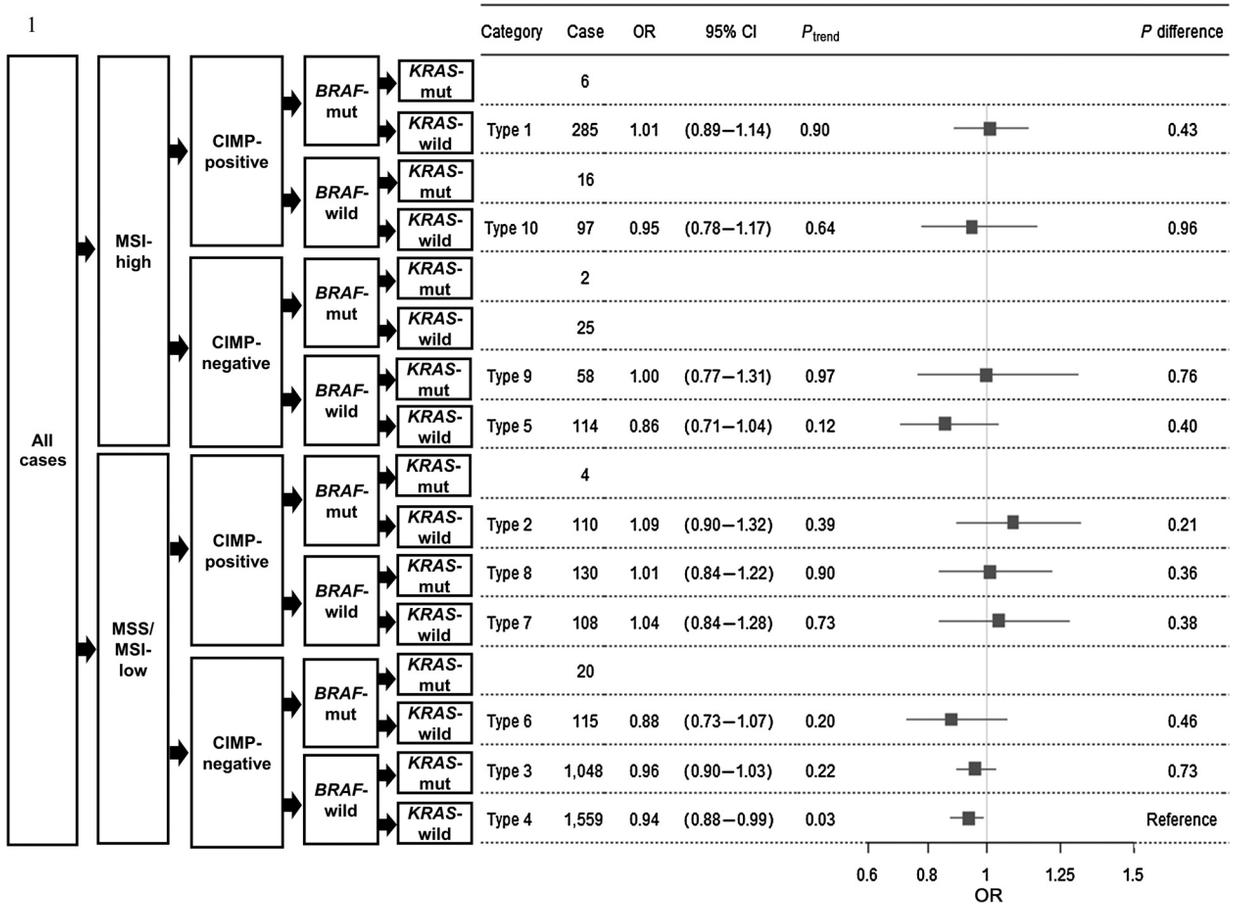

Figure 1.

Forest plot of the association between fiber intake and colorectal cancer risk by combined molecular subtypes using polytomous logistic regression analysis. ORs and 95% CIs for multivariate-adjusted models are presented for each increasing quartile of fiber intake and each combined molecular subtypes of colorectal cancer risk. A total of 3,697 cases and 6,485 controls are included. Gray boxes are centered at multivariate-adjusted ORs, and lines depict their 95% CIs. Subtype classifications with less than 50 cases were excluded from analyses. The number of cases per molecular subtype is listed under "Case." P_{trend} was calculated by assigning ordinal values for quartile categories of fiber intake and modeling that variable continuously. *P* difference is the degree of difference in *P* value of multivariate-adjusted OR between Type 4 and each of the other types.

finding, cases with and without *BRAF* mutation would need to recall their fruit intake differently. If treatment with *BRAF* inhibitors could have an impact is unknown; however, the majority of colorectal cancer cases included in this study were diagnosed before treatment with *BRAF* inhibitors were introduced (35). As *BRAF* mutation is measured by standard methods, it is probably also less likely that measurement error could explain the observed difference in the case-only analysis for *BRAF*-mutated versus wildtype tumors.

To our knowledge, only one study has published findings on fruit intake and colorectal cancer risk by *BRAF* mutation status (15). This cohort study of 186 cases found no clear differences in risk. Several meta-analyses of studies not accounting for tumor markers have been published. A meta-analysis published in 2003 reported statistically significant inverse associations between fruit intake and colorectal cancer risk in case–control studies, but the relative risk was null when restricted to cohort studies (36). In addition, a previous pooled analysis of 14 cohort studies published in 2007 also showed no overall association (37). However, a more recent meta-analysis of 19 cohort studies published in 2018 reported statistically significant inverse associations between fruit intake and colorectal cancer risk, and reported nonlinear relationships between fruit intake and colorectal cancer risk (2). It is possible as sample sizes of meta-analyses increase that the power becomes sufficient to capture a significant association in a subset of the cancers (in this case *BRAF*-mutated tumors). Additional studies ideally conducting in cohorts are needed to replicate our finding.

Fruit intake may plausibly play a role in inhibiting events of *BRAF*-mutated tumors. Fruits are rich in many nutrients and biologically active compounds, such as vitamins, carotenoids, and folic acid, which may be cancer-preventive (38). Accordingly, a previous laboratory study showed that high levels of vitamin C specifically kill *BRAF*-mutated colorectal cancer cells, but not *BRAF*-wildtype cells (39). This effect is due to uptake of dehydroascorbate, which is the oxidized form of vitamin C. Increased intracellular dehydroascorbate uptake accumulates cellular reactive oxygen species and inactivates GAPDH. Inhibition of GAPDH in *BRAF*-mutated colorectal cancer cells leads to an energetic crisis and cell death through inhibiting glycolysis and depleting ATP (39). However, two randomized, double-blind,

placebo-controlled trials with a mean follow-up period ranging from 8.0 to 9.4 years did not find that 500 mg of vitamin C supplementation daily was associated with a decreased colorectal cancer incidence in both men and women (40, 41). Fruit intake may also affect *BRAF*-mutated colorectal cancer development through affecting the MAPK pathway. It has been previously reported that *Lycium barbarum* fruit, also known as Chinese Wolfberry, inhibits cancer cell growth by cell-cycle arrest and apoptosis through regulating the activation of MAPK-signaling pathway (42). Furthermore, previous studies have shown that fisetin, a dietary flavonoid found in apples, strawberries, kiwi, and other fruits, inhibits cell invasion by targeting MAPK signaling pathway (43) and reduces the anti-invasive and antimetastatic effects of *BRAF*-mutated cells (44). Accordingly, fruit intake may reduce *BRAF*-mutated colorectal cancer through multiple nutrients and biologically active compounds.

With regard to colorectal carcinogenic pathways, we investigated three different pathways: (i) a serrated pathway (Types 1 and 2), (ii) an alternate pathway (Type 3), and (iii) a traditional pathway (Types 4 and 5; refs. 23, 24). Because this colorectal carcinogenic hypothesis only shows the predominant pathways, there is overlap between them (24). We observed that higher intake of fiber was associated with a decreased risk of combined colorectal cancer subtypes that were MSS/MSI-low, CIMP-negative, *BRAF*-wildtype, and *KRAS*-wildtype (Types 4 and 5: traditional pathway). Moreover, higher fiber intake was associated with a decreased risk for CIMP-negative tumors, but not for CIMP-positive tumors in cohort studies. Higher dietary fiber intake has "probable strong evidence" for decreasing the risk of colorectal cancer as defined by WCRF/AICR (4), which is in line with our findings given that the traditional pathway is the predominate pathway for colorectal cancer development. A previous meta-analysis of seven observational studies investigating how lifestyle exposures may increase or decrease the risk of serrated colorectal polyps presented a suggestive trend toward an inverse association between fiber intake and risk of serrated colorectal polyps, but findings were not statistically significant (45), which is not consistent with our finding. Currently functional data are missing that could explain why the association with fiber intake is restricted to a subset of molecularly-defined colorectal cancer tumors.

A strength of our pooled analysis from 9 observational studies with up to 9,592 colorectal cancer cases and 7,869 controls is that we were well powered to detect associations of fruit, vegetables, and fiber intake with risk of major colorectal cancer molecular subtypes. Due to this sizable dataset, we could further assess the association of colorectal cancer molecular subtype markers in combination. Furthermore, we used a consistent approach to harmonize all dietary variables across the studies to enable a pooled analysis of individual level data. In addition, we have robust data on other factors including age at diagnosis, total energy consumption, and sex as covariates.

Our study has some limitations. First, it is difficult to exclude potential selection bias. Although molecular subtypes are ideally available for all cases, there may be tissue retrieval biases with tissue availability potentially associated with tumor size and stage (46). However, some of the included studies previously have shown that there were no differences in age, diet, or other lifestyle characteristics between cases with and without available tumor tissue (47, 48). Second, fruit, vegetables, and fiber intake are likely measured with error because they were assessed via in-person interviews or self-administered questionnaires, resulting in exposure misclassification. To best account for differences between dietary assessment methods, we harmonized data as study- and sex-specific quartiles rather than the continuous intake value, as it is commonly done for pooled dietary analyses (37). As exposure misclassification would likely be nondifferential, it would result in an underestimation of the association of these dietary variables with colorectal cancer risk. Third, we analyzed the colorectal cancer risk only using the lifestyle information measured at a single point in time, whereas lifestyle habits of study participants might change during the relevant etiologic time window. However, as such changes are not differential between cases and controls, this would have led to an attenuation of the associations. Fourth, some studies had a sizable number of missing covariate data; however, the missing covariate data were imputed by sex-specific mean for each study to ensure no samples were missed in the analysis due to missing confounders.

In summary, we found in our large pooled analysis for well-defined molecular subtypes that higher fruit intake was associated with a decreased risk of *BRAF*-mutated colorectal cancer but not with *BRAF*-wildtype colorectal cancer. In additionally, higher fiber intake may be associated with a stronger decreased risk of colorectal cancer developing by the traditional adenoma-colorectal cancer pathway that is MSS/MSI-low, CIMP-negative, *BRAF*-wildtype, and *KRAS*-wildtype tumors. These results potentially explain in part the inconsistent findings between fruit or fiber intake and overall colorectal cancer risk that have previously been reported.

Disclosure of Potential Conflicts of Interest

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Authors' Contributions

A. Hidaka: Conceptualization, data curation, formal analysis, validation, investigation, visualization, methodology, writing-original draft, writing-review and editing. **T.A. Harrison:** Conceptualization, formal analysis, supervision, investigation, methodology, writing-original draft, project administration, writing-review and editing. **Y. Cao:** Writing-review and editing. **L.C. Sakoda:** Resources, writing-review and editing. **R. Barfield:** Methodology, writing-review and editing. **M. Giannakis:** Writing-review and editing. **M. Song:** Writing-review and editing. **A.I. Phipps:** Resources, writing-review and editing. **J.C. Figueiredo:** Writing-review and editing. **S.H. Zaidi:** Writing-review and editing. **A.E. Toland:** Writing-review and editing. **E.L. Amitay:** Writing-review and editing. **S.I. Berndt:** Resources, writing-review and editing. **I. Borozan:** Writing-review and editing. **A.T. Chan:** Resources, writing-review and editing. **S. Gallinger:** Resources, writing-review and editing. **M.J. Gunter:** Resources, writing-review and editing. **M.A. Guinter:** Writing-review and editing. **S. Harlid:** Writing-review and editing. **H. Hampel:** Resources,

writing-review and editing. **M.A. Jenkins:** Resources, writing-review and editing. **Y. Lin:** Data curation, software, writing-review and editing. **V. Moreno:** Resources, writing-review and editing. **P.A. Newcomb:** Resources, writing-review and editing. **R. Nishihara:** Writing-review and editing. **S. Ogino:** Resources, writing-review and editing. **M. Obón-Santacana:** Writing-review and editing. **P.S. Parfrey:** Resources, writing-review and editing. **J.D. Potter:** Resources, writing- and editing. **M.L. Slattery:** Resources, writing-review and editing. **R.S. Steinfelder:** Data curation, writing-review and editing. **C.Y. Um:** Writing-review and editing. **X. Wang:** Writing-review and editing. **M.O. Woods:** Resources, writing-review and editing. **B. Van Guelpen:** Resources, writing-review and editing. **S.N. Thibodeau:** Resources, writing-review and editing. **M. Hoffmeister:** Resources, writing-review and editing. **W. Sun:** Formal analysis, writing-review and editing. **L. Hsu:** Formal analysis, methodology, writing-review and editing. **D.D. Buchanan:** Resources, writing-review and editing. **P.T. Campbell:** Resources, writing-review and editing. **U. Peters:** Conceptualization, supervision, funding acquisition, methodology, project administration, writing-review and editing.

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