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Other Health-related Behaviours and Cancer

Multivitamin, calcium and folic acid supplements and the risk of colorectal cancer in Lynch syndrome

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Abstract

Background: People with a DNA mismatch repair (MMR) gene mutation have a substantially elevated risk of colorectal cancer (CRC) but the modifiers of this risk are not well established. We investigated the association between dietary supplement intake and CRC risk for carriers.

Methods: This study included 1966 (56% female) carriers of an MMR gene mutation (719 *MLH1*, 931 *MSH2*, 211 *MSH6* and 105 *PMS2*) who were recruited from the USA, Canada, Australia and New Zealand into the Colon Cancer Family Registry between 1997 and 2012. Information on lifestyle factors including supplement intake was collected at the time of recruitment. Using Cox proportional hazards regression weighted to correct for ascertainment bias, we estimated hazard ratios (HRs) and 95% confidence intervals (CIs) for associations between self-reported multivitamin, calcium and folic acid supplement intake and CRC risk.

Results: Of 744 carriers with CRC, 18%, 6% and 5% reported intake of multivitamin, calcium and folic acid supplements for at least 1 month, respectively, compared with 27%, 11% and 10% of 1222 carriers without CRC. After adjusting for identified confounding variables, a decreased CRC risk was associated with multivitam inintake for at least 3 years (HR 0.47, 95% CI 0.32–0.69) and calcium intake for at least 3 years(HR 0.42, 95% CI 0.23–0.74), compared with never users. There was no evidence of an association between folic acid supplement intake and CRC risk ($P = 0.82$).

Conclusion: Intake of multivitamin and calcium supplements might be associated with a decreased risk of CRC for MMR gene mutation carriers.

Key words: Colorectal cancer, DNA mismatch repair, Lynch syndrome, multivitamin, calcium, folic acid

Key Messages

- People with a DNA mismatch repair gene mutation have a substantially elevated risk of colorectal cancer and several other cancers.
- Almost nothing is known about lifestyle factors influencing the risk of colorectal cancer in these mutation carriers.
- Regular intake of multivitamin and calcium supplements for at least 3 years was found to be associated with an approximately 50% reduced risk of colorectal cancer for people with mismatch repair gene mutations.

Introduction

Colorectal cancer (CRC) is the third most common cancer in both men and women and the third leading cause of cancer-related deaths in the USA.[1] The lifetime risk of CRC is 5% in men and 4.7% in women.[1] Approximately 2–4% of all colorectal cancers,[2–9] but 10–15% of colorectal cancers diagnosed before age 50 years,[2,3,10] are attributable to Lynch syndrome. Lynch syndrome is an autosomal dominant disorder of cancer predisposition caused by a germline mutation in one of the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6* and *PMS2*.[11] Approximately 1 in 3100 people in the general population carry a mutation in an MMR gene.[12] MMR gene mutation carriers have a substantially increased risk of colorectal cancer (CRC) to age 70 years, estimated to be between 20% and 70% depending on sex and the mutated MMR gene.[13–16]

Personal and lifestyle factors may modify the risk of CRC for MMR gene mutation carriers. Identifying modifiers of disease risk is important for understanding carcinogenesis, as it may indicate potential initiators or promoters of the disease. In addition, identifying potentially protective factors, or conversely, harmful and avoidable risk factors might provide opportunities for mutation carriers to reduce their risk of cancer. Previous studies have reported that increased body mass index (BMI),[17–19] alcohol consumption[20] and cigarette smoking[18,20–23] are associated with an increased risk of CRC for mutation carriers, whereas aspirin intake,[24,25] and fruit and dietary fibre intake[18] are associated with a decreased risk.

For the general population, intake of multivitamin,[26] calcium[27] and dietary folate or folic acid supplements[28] have been reported to be associated with a decreased risk of CRC, although individual study findings have been inconsistent. In the current study, we report associations between intake of multivitamin, calcium and folic acid supplements and CRC risk for people with Lynch syndrome.

Materials and Methods

Study sample

The study sample comprised carriers of a germline pathogenic mutation in an MMR gene, who had been recruited by the Colon Cancer Family Registry. Detailed description of the study design and recruitment strategies have been previously published.[29] Between 1997 and 2012, families were recruited either via population-based probands who were recently diagnosed with CRC and identified from state or regional population cancer registries in the USA (Washington, California, Arizona, Minnesota, Colorado, New Hampshire, North Carolina and Hawaii), Australia (Victoria) and Canada (Ontario), or via probands who were members of, multiple-case cancer families referred to family cancer clinics in the USA (Mayo Clinic, Rochester, Minnesota and Cleveland Clinic, Cleveland, Ohio), Canada (Ontario), Australia (Melbourne, Adelaide, Perth, Brisbane, Sydney) and New Zealand (Auckland). Probands were asked for permission to contact their relatives to seek their enrolment in the Colon Cancer Family Registry. Informed consent was obtained from all study participants and the study protocol was approved by the institutional research ethics review board at each recruitment centre.

Data Collection

Information on demographics, personal characteristics, personal and family history of cancer, CRC screening, colorectal polyps and polypectomy and other surgical procedures, as well as lifestyle factors including intake of multivitamin, calcium and folic acid supplements, were obtained from all probands and participating relatives, at the time of recruitment. The information was collected using standardized questionnaires via personal interviews, telephone interviews or mails. The questionnaires are available at: [http://coloncfr.org/questionnaires]. We attempted to request and obtain blood samples from all participants and tumour tissues from all CRC-affected participants.

MMR gene mutation testing

Testing for germline mutations in *MLH1, MSH2, MSH6* and *PMS2* was performed for all population-based probands who had a colorectal tumour displaying evidence of MMR-deficiency as evidenced by either tumour microsatellite instability (MSI) and/or loss of MMR-protein expression by immunohistochemistry, and for the youngest-onset CRC case from each clinic-based family regardless of tumour MMR-deficiency status. Details of germline testing methods have been described elsewhere.[30] Relatives of the probands with a pathogenic MMR germline mutation, who provided a blood sample, underwent testing for the specific mutation identified in the proband. All probands and relatives with a pathogenic MMR gene mutation were eligible for the current study.

Exposure definitions

Intakes of multivitamin, calcium and folic acid supplements were the three main exposure variables. Ever users of supplements were defined as those who answered 'yes' to 'Have you ever taken [supplement] at least twice a week for 1 month or longer?' Never users were defined as those who answered 'no' to 'Have you ever taken [supplement] at least twice a week for 1 month or longer?' Duration of supplement intake was defined as self-reported total number of years of supplement intake before age at CRC diagnosis or censored age. Given that the age at first intake of these supplements was not reported, we estimated the age at first intake by subtracting the reported duration of intake from the age at the baseline questionnaire, assuming that the duration was continuous and recent. Frequency of supplement intake was defined as self-reported weekly frequency of supplement intake.

Statistical analysis

Proportional Cox regression, with age as the time scale, was used to estimate hazard ratios (HRs) for associations between multivitamin, calcium and folic acid supplement intake and CRC risk for MMR gene mutation carriers. Time at risk started at birth and ended at: age at diagnosis of CRC ($n = 744$); or age at diagnosis of non-colorectal cancer ($n = 343$); or first polypectomy ($n = 317$); or interview ($n = 562$), whichever occurred first. We censored at the age of first polypectomy to avoid potential bias, because polypectomy reduces CRC risk. We censored at age of diagnosis of a non-colorectal cancer because intake of supplements and CRC risk may be changed by diagnosis and treatment of a non-colorectal cancer.[31,32]

As some carriers from this study were ascertained because they had CRC, and diagnosed at a young age, the subject selection for testing for genetic mutations was not random with respect to the CRC status. To reduce bias of estimates of hazards, we adjusted for this non-random

ascertainment by applying probability weights based on the approach described by Antoniou *et al.*[33] Weights were calculated for the carriers with CRC and without CRC for each age-stratum, so the proportion of carriers with CRC at each age-stratum equalled that expected based on the age-specific incidences of CRC for carriers.[34]

The proportional hazards (PH) assumption was tested using the Schoenfeld and scaled Schoenfeld residuals.[35] Univariable and multivariable models were fitted separately for intake of each supplement (multivitamin, calcium and folic acid) as well as intake of two or more of the supplements. The variables that we considered as potential confounders are listed in Table 1. The variables that did not meet the PH assumption were included in the model as time-dependent covariates. Interactions were assessed by a change in the log-likelihood ratio after the addition of a cross-product term between the exposure and potential effect modifiers identified a priori (country of recruitment, ascertainment method, mutated gene, sex and site of colorectal cancer). The overall model fit was assessed using Cox–Snell residuals as the time variable and plotting them against the Nelson–Aalen cumulative hazard function.[36]

For multivariable models, missing data were handled using both complete case analysis and multiple imputations. Numbers of missing values for all the variables are shown in Table 1. Missing data were imputed using chained interactions, assuming that missingness was at random.[37] Outcome status, age at the time of colorectal cancer diagnosis or censored age, year of birth, country, mutated gene, ascertainment method and whether the carrier was a proband were included in the imputation model; 50 imputed datasets were generated.

The following additional analyses were conducted: (i) analysis restricted to carriers who were diagnosed with CRC or censored within 5 years before interview, to reduce survival bias; and (ii) analysis in which outcome was defined as colorectal neoplasia, i.e. either CRC or colorectal polyps.

The 95% confidence intervals (CIs) of HRs were calculated accounting for clustering by family membership to allow for correlation of risk between relatives from the same family, using the Huber–White robust variance correction.[38,39] All statistical tests were two-sided. All analyses were performed using Stata 13.0.[40]

Results

From the Colon Cancer Family Registry, we identified a total of 2003 carriers of a pathogenic mutation in an MMR gene. Of these carriers, we excluded 11 (0.5%) who were censored before 18 years of age and 26 (1.3%) with missing data for all main exposure variables. A total of 1966 (56% female) carriers contributed to the analysis; 719 carried a germline mutation in *MLH1*, 931 in *MSH2*, 211 in *MSH6* and 105 in *PMS2*. Carriers excluded due to missing data did not differ in main characteristics from those who were not excluded. Characteristics of the carriers included in the study are summarized in Table 1. For carriers who were diagnosed with CRC or censored at an age younger than age at interview, the mean time interval between diagnosis or censored age and age at interview was 9.6 [standard deviation (SD) 9.8] years for carriers with CRC and 10.0 (SD 9.2) years for carriers without CRC ($P = 0.40$).

A total of 744 (38%) MMR mutation carriers were reported to be diagnosed with CRC at a mean age of 42.4 (SD 10.5) years. Of these, 681 (91%) were confirmed by pathology reports or medical records or cancer registry linkage data. Overall, 18%, 6% and 5% of carriers affected with CRC reported intake of multivitamin, calcium and folic acid supplements, respectively, at least twice a week for at least 1 month, compared with 27%, 11% and 10% of unaffected carriers. Intakes of multivitamin and calcium supplements were higher in women than men and in carriers from the USA than those from Canada, Australia or New Zealand, and increased with age (Table 2).

Overall, we found that intake of multivitamin and calcium supplements was associated with a decreased risk of CRC compared with never users (HR 0.55, 95% CI 0.40–0.75; and HR 0.46, 95% CI 0.30–0.71, respectively). There was a decreased risk of CRC associated with an increased duration of multivitamin intake ($\geq$ 3 years: HR 0.47, 95% CI 0.32–0.69; per year: HR 0.95, 95% CI 0.91–0.98), and calcium intake ($\geq$ 3 years: HR 0.42, 95% CI 0.23–0.74; per year: HR 0.90, 95% CI 0.83–0.98). There was no evidence of an association between folic acid supplement intake and CRC risk ($P = 0.82$). The results from the multiple imputation analysis were similar to the complete case analysis (Table 3). When we additionally adjusted for recent BMI, red meat intake and fruit and vegetable intake in the multiple imputation analysis, the results were similar to the main analysis (Supplementary Table 1, available as Supplementary data at *IJE* online). There was no evidence of interactions between each supplement intake and country of recruitment, ascertainment method, mutated gene, sex or site of colorectal cancer (Supplementary Table 2, available as Supplementary data at *IJE* online).

There was an inverse association between multivitamin supplement intake and CRC risk for both males and females (HR 0.45, 95% CI 0.28–0.72, and HR 0.64, 95% CI 0.43–0.95, respectively). There was an inverse association with calcium supplement intake in women (HR 0.63,

Table 1. Characteristics of carriers of germline mutations in DNA mismatch repair genes

Exposure variable	No colorectal cancer (n = 1222 (62.2%))	Colorectal cancer (n = 744 (37.8%))	Total (n = 1966)
Sex, *n* (%)			
Female	767 (62.8)	341 (45.8)	1,108 (56.4)
Male	455 (37.2)	403 (54.2)	858 (43.6)
Country of recruitment, *n* (%)			
Australia or New Zealand	707 (57.9)	349 (46.9)	1,056 (53.7)
USA	349 (28.6)	278 (37.4)	627 (31.9)
Canada	166 (13.6)	117 (15.7)	283 (14.4)
Race, *n* (%)			
Caucasian	1160 (94.9)	675 (90.7)	1,835 (93.3)
Other	43 (3.5)	58 (7.8)	101 (5.1)
Missing	19 (1.6)	11 (1.5)	30 (1.5)
Age (years)[a]			
mean (SD)	41.5 (13.1)	42.4 (10.5)	41.8 (12.2)
median [range]	41 [18 - 85]	42 [19 - 75]	42 [18 - 85]
Year of birth, *n* (%)			
1914–43	250 (20.5)	210 (28.2)	460 (23.4)
1944–54	265 (21.7)	214 (28.8)	479 (24.4)
1955–65	304 (24.9)	212 (28.5)	516 (26.3)
1966–90	403 (33.0)	108 (14.5)	511 (26.0)
Ascertainment			
Population	970 (79.4)	491 (66.0)	1461 (74.3)
Cancer clinic	252 (20.6)	253 (34.0)	505 (25.7)
Education level, *n* (%)			
Primary or less	11 (0.9)	17 (2.3)	28 (1.4)
Some high school	229 (18.7)	158 (21.2)	387 (19.7)
Completed high school / some tertiary	426 (34.9)	245 (32.9)	671 (34.1)
Vocational/technical school	222 (18.2)	121 (16.3)	343 (17.5)
University degree	324 (26.5)	192 (25.8)	516 (26.3)
Missing	10 (0.8)	11 (1.9)	21 (1.1)
MMR gene mutated, *n* (%)			
MLH1	401 (32.8)	318 (42.7)	719 (36.6)
MSH2	613 (50.2)	318 (42.7)	931 (47.4)
MSH6	149 (12.2)	62 (8.3)	211 (10.7)
PMS2	59 (4.8)	46 (6.2)	105 (5.3)
Number of screening colonoscopies			
0	402 (32.9)	184 (24.7)	586 (29.8)
1	372 (30.4)	378 (50.8)	750 (38.2)
2	138 (11.3)	26 (3.5)	164 (8.3)
$\geq$ 3	193 (15.8)	61 (8.2)	254 (12.9)
Missing	117 (9.6)	95 (12.8)	212 (10.8)
Diabetes, *n* (%)			
No	1,183 (96.8)	705 (94.8)	1,888 (96.0)
Yes	33 (2.7)	32 (4.3)	65 (3.3)
Missing	6 (0.5)	7 (0.9)	13 (0.7)
Regular physical activity,[b] *n* (%)			
<3 months	159 (13.0)	86 (11.6)	245 (12.5)
$\geq$3 months	1,063 (87.0)	658 (88.4)	1,721 (87.5)
Aspirin and/or ibuprofen intake,[c] *n* (%)			
<1 month	953 (78.0)	614 (82.5)	1,567 (79.7)
$\geq$1 month	195 (16.0)	90 (12.1)	285 (14.5)
Missing	74 (6.1)	40 (5.4)	114 (5.8)

(Continued)

Table 1. Continued

Exposure variable	No colorectal cancer (n = 1222 (62.2%))	Colorectal cancer (n = 744 (37.8%))	Total (n = 1966)
Cigarette smoking, *n* (%)			
Never smoker	664 (54.3)	337 (45.3)	1,001 (50.9)
Former smoker[d]	276 (22.6)	156 (21.0)	432 (22.0)
Current smoker[e]	277 (22.7)	250 (33.6)	527 (26.8)
Missing	5 (0.4)	1 (0.1)	6 (0.3)
Average number of alcoholic beverages consumed per day[f]			
0	309 (25.3)	186 (25.0)	495 (15.2)
≤ 0.5	197 (16.1)	128 (17.2)	325 (16.5)
0.5–1	199 (16.3)	100 (13.4)	299 (15.2)
1–2	169 (13.8)	101 (13.6)	270 (13.7)
2+	174 (14.2)	136 (18.3)	310 (15.8)
Missing	174 (14.2)	93 (12.5)	267 (13.6)
BMI[g] at age 20 years, *n* (%)			
Normal	804 (65.8)	470 (63.2)	1,274 (64.8)
Overweight	195 (16.0)	139 (18.7)	334 (17.0)
Obese	48 (3.9)	41 (5.5)	89 (4.5)
Underweight	106 (8.7)	61 (8.2)	167 (8.5)
Missing	69 (5.7)	33 (4.4)	102 (5.2)
BMI[g] 2 years before diagnosed / censored,[h] *n* (%)			
Normal	356 (29.1)	92 (12.4)	448 (22.8)
Overweight	236 (19.3)	104 (14.0)	340 (17.3)
Obese	117 (9.6)	58 (7.8)	175 (8.9)
Underweight	24 (2.0)	9 (1.2)	33 (1.7)
Missing	489 (40.0)	481 (64.7)	970 (49.3)
Daily fruit and vegetable intake[i] 2 years before interview,[h] *n* (%)			
0–2	197 (16.1)	109 (14.7)	306 (15.6)
2–3	141 (11.5)	44 (5.9)	185 (9.4)
3–4	139 (11.3)	46 (6.2)	184 (9.4)
4+	274 (22.4)	63 (8.5)	337 (17.1)
Missing	472 (38.6)	482 (64.8)	954 (48.5)
Daily red meat intake[i] 2 years before interview,[h] *n* (%)			
0–0.3	220 (18.0)	83 (11.2)	303 (15.4)
0.3–0.6	261 (21.4)	82 (11.0)	343 (17.5)
0.6–0.9	106 (8.7)	43 (5.8)	149 (7.6)
0.9+	161 (13.2)	55 (7.4)	216 (11.0)
Missing	474 (38.8)	481 (64.7)	955 (48.6)
Number of live births,[j] *n* (%)			
None	222 (28.9)	60 (17.6)	282 (25.5)
1	80 (10.4)	49 (14.4)	129 (11.6)
2	211 (27.5)	94 (27.6)	305 (27.5)
≥ 3	235 (30.6)	125 (36.7)	360 (32.5)
Missing	19 (2.5)	13 (3.8)	32 (2.9)
Age at menarche,[j] *n* (%)			
≤ 12 years	204 (26.6)	109 (32.0)	313 (28.3)
13 years	225 (29.3)	90 (26.4)	315 (28.4)
≥ 14 years	319 (41.6)	134 (39.3)	453 (40.9)
Missing	19 (2.5)	8 (2.4)	27 (2.4)
Age at menopause,[j] *n* (%)			
Pre-menopause	521 (67.9)	228 (66.9)	749 (67.6)
< 50 years	162 (21.1)	88 (25.8)	250 (22.6)
≥ 50 years	50 (6.5)	16 (4.7)	66 (6.0)
Missing	34 (4.4)	9 (2.6)	43 (3.9)

(Continued)

Table 1. Continued

Exposure variable	No colorectal cancer (n = 1222 (62.2%))	Colorectal cancer (n = 744 (37.8%))	Total (n = 1966)
Hormonal contraception,[j] *n* (%)			
< 1 year	200 (26.1)	94 (27.6)	294 (26.5)
≥ 1 year	558 (72.8)	237 (69.5)	795 (71.8)
Missing	9 (1.2)	10 (2.9)	19 (1.7)
HRT used,[j] *n* (%)			
< 6 months	641 (83.6)	293 (85.9)	934 (84.3)
≥ 6 months, estrogen only	61 (8.0)	29 (8.5)	90 (8.1)
≥ 6 months, estrogen and progestin	44 (5.7)	12 (3.5)	56 (5.1)
Missing	21 (2.7)	7 (2.1)	28 (2.5)
Multivitamin supplement intake,[c], *n* (%)			
Never	830 (67.9)	573 (77.0)	1,403 (71.4)
1 month to 11.9 months	81 (6.6)	25 (3.4)	106 (5.4)
1 year to 2.9 years	82 (6.7)	40 (5.4)	122 (6.2)
≥ 3 years	165 (13.5)	72 (9.7)	237 (12.1)
Missing	64 (5.2)	34 (4.6)	98 (5.0)
Calcium supplement intake,[c] *n* (%)			
Never	1,045 (85.5)	685 (92.1)	1,730 (88.0)
1 month to 11.9 months	31 (2.5)	4 (0.5)	35 (1.8)
1 year to 2.9 years	41 (3.4)	19 (2.6)	60 (3.1)
≥ 3 years	58 (4.7)	25 (3.4)	83 (4.2)
Missing	47 (3.9)	11 (1.5)	58 (3.0)
Folic acid supplement intake,[c] *n* (%)			
Never	1,073 (87.8)	693 (93.1)	1,766 (89.8)
1 month to 11.9 months	57 (4.7)	11 (1.5)	68 (3.5)
1 year to 2.9 years	38 (3.1)	18 (2.4)	56 (2.9)
≥ 3 years	22 (1.8)	8 (1.1)	30 (1.5)
Missing	32 (2.6)	14 (1.9)	46 (2.3)

MMR, mismatch repair; BMI, body mass index; HRT, hormone replacement therapy.

[a]Age at first diagnosis of colorectal cancer or age at first polypectomy or diagnosis of another cancer or age at interview, whichever occurred first.

[b]Regular physical activity defined as any physical activity for at least 30 min per week for at least 3 months.

[c]At least twice a week.

[d]Former smokers defined as carriers who had smoked at least 1 cigarette per day for at least 3 months and had quit more than 2 years before age at colorectal cancer or censored age.

[e]Current smokers defined as carriers who had smoked at least 1 cigarette per day for at least 3 months and continued within 2 years of age at colorectal cancer or censored age.

[f]4-oz. glasses of wine, or 12-oz. cans or bottles of beer or hard cider, or 1-oz. servings of sake or liquor (spirits).

[g]Underweight (< 18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), obese (≥ 30 kg/m^2).

[h]Carriers who were diagnosed with colorectal cancer or censored more than 2 years before interview had 'missing' for these variables.

[i]Number of servings.

[j]Limited to female carriers.

95% CI 0.38–1.04) but there was no evidence of such an association in men ($P = 0.29$) (Table 4).

In the analysis restricted to carriers who were diagnosed with CRC or censored within 5 years before interview, the results were similar to the main analysis (Supplementary Table 3, available as Supplementary data at *IJE* online). In the analysis where the outcome was defined as colorectal neoplasia, the results were similar to the main analysis (Supplementary Table 4, available as Supplementary data at *IJE* online). There was no evidence that main exposure variables violated the PH assumption in any of the final models.

Discussion

We found regular intake of multivitamin and/or calcium supplements for at least 3 yearsto be associated with an approximate halving of CRC risk for MMR gene mutation carriers, independently of other factors including aspirin intake and colonoscopy screening. In our study sample, multivitamin and calcium supplement intakes were higher in women and in older people, similar to the pattern observed by the US National Health and Nutrition Examination Survey.[41]

Only one previous study has reported associations between supplement intake and the risk of colorectal

Table 2. Prevalence of multivitamin, calcium and folic acid supplement intake at least twice a week for at least 1 month in carriers of germline mutation in DNA mismatch repair gene by country, sex and age group

Supplement intake for at least 1 month	Country / age group	Male *n* (% of total)	Female *n* (% of total)	Overall *n* (% of total)
Multivitamin	USA			
	≤ 35	17 (20.2)	26 (31.3)	43 (25.8)
	36–45	20 (24.1)	41 (41.41)	61 (33.5)
	46–55	33 (47.1)	34 (52.3)	67 (49.6)
	55+	19 (43.2)	26 (61.9)	45 (52.3)
	All	89 (31.7)	127 (43.9)	216 (37.9)
	Australia / New Zealand			
	≤ 35	39 (23.1)	51 (24.5)	90 (23.9)
	36–45	16 (12.9)	35 (19.9)	51 (17.0)
	46–55	11 (11.0)	20 (14.7)	31 (13.1)
	55+	6 (10.5)	10 (13.5)	16 (12.2)
	All	72 (16.0)	116 (19.5)	188 (18.0)
	Canada			
	≤ 35	6 (21.4)	8 (19.5)	14 (20.3)
	36–45	9 (25.7)	12 (22.2)	21 (23.6)
	46–55	4 (13.8)	7 (17.5)	11 (15.9)
	55+	5 (41.7)	10 (66.7)	15 (55.6)
	All	24 (23.1)	37 (24.7)	61 (24.0)
	Overall	185 (22.2)	280 (27.1)	465 (24.9)
Calcium	USA			
	≤ 35	1 (1.1)	9 (10.7)	10 (5.8)
	36–45	4 (4.7)	19 (17.3)	23 (11.8)
	46–55	8 (11.0)	20 (29.9)	28 (20.0)
	55+	5 (11.1)	23 (50.0)	28 (30.8)
	All	18 (6.2)	71 (23.1)	89 (14.9)
	Australia / New Zealand			
	≤ 35	5 (3.0)	11 (5.3)	16 (4.2)
	36–45	2 (1.6)	10 (5.7)	12 (4.0)
	46–55	2 (2.0)	15 (11.0)	17 (7.2)
	55+	2 (3.5)	18 (25.0)	20 (15.5)
	All	11 (2.4)	54 (9.1)	65 (6.2)
	Canada			
	≤35	1 (3.3)	6 (13.3)	7 (9.3)
	36–45	0 (0)	3 (5.8)	3 (3.3)
	46–55	1 (3.5)	7 (17.1)	8 (11.4)
	55+	1 (8.3)	5 (33.3)	6 (22.2)
	All	3 (2.7)	21 (13.7)	24 (9.1)
	Overall	32 (3.8)	146 (13.8)	178 (9.3)
Folic acid	USA			
	≤ 35	0 (0)	9 (10.2)	9 (5.2)
	36–45	0 (0)	10 (8.9)	10 (5.1)
	46–55	2 (2.7)	6 (8.1)	8 (5.4)
	55+	2 (4.4)	4 (8.9)	6 (6.6)
	All	4 (1.4)	29 (9.1)	33 (5.4)
	Australia /New Zealand			
	≤ 35	1 (0.6)	44 (21.3)	45 (12.0)
	36–45	1 (0.8)	34 (19.3)	35 (11.6)
	46–55	1 (1.0)	12 (8.8)	13 (5.5)
	55+	3 (5.3)	6 (8.1)	9 (6.9)
	All	6 (1.3)	96 (16.2)	102 (9.8)
	Canada			
	≤ 35	0 (0)	5 (11.9)	5 (6.9)

(Continued)

Table 2. Continued

Supplement intake for at least 1 month	Country / age group	Male *n* (% of total)	Female *n* (% of total)	Overall *n* (% of total)
	36–45	0 (0)	7 (13.0)	7 (7.5)
	46–55	0 (0)	4 (8.9)	4 (5.7)
	55+	1 (8.3)	2 (11.1)	3 (10.0)
	All	1 (0.9)	18 (11.3)	19 (7.1)
	Overall	11 (1.3)	143 (13.3)	154 (8.0)

adenoma in Lynch syndrome.[42] The prospective study of 470 MMR gene mutation carriers reported no evidence of an association between colorectal adenoma risk and multivitamin intake (HR 1.15, 95% CI 0.72–1.84) or calcium supplement intake (HR 0.69, 95% CI 0.25–1.92). Although the results of this study are not overall contrary to our results (overlapping CIs, especially for calcium supplement intake), different definitions of supplement intake used in the studies (during the past month vs at least twice a week for at least 1 month), study sample (470 carriers and 122 colorectal adenoma cases vs 1966 carriers and 744 CRC cases) and outcome definition (colorectal adenoma vs CRC) may explain the stronger inverse association observed in our study.

Similar to our finding of an inverse association between multivitamin supplement intake and CRC risk for MMR gene mutation carriers, a prospective study previously reported that multivitamin supplement intake was associated with a lowering of the excess risk of colon cancer for a person having a first-degree relative affected by CRC (a cohort more likely to carry CRC-predisposing gene mutations than the general population).[43] For the general population, multivitamin supplement intake has also been shown to have an inverse association with CRC risk. A large randomized controlled trial showed a small protective effect of multivitamin supplements on the risk of cancers, including colorectal cancer for men.[44] A pooled analysis of prospective cohort studies reported that ever use of multivitamin supplements was associated with a 12% lower risk of colon cancer compared with never use [relative risk (RR) 0.88, 95% CI 0.81–0.96].[26]

We also found an inverse association between calcium supplement intake and CRC risk for MMR gene mutation carriers. Two case-control studies have investigated the association between dietary calcium intake and CRC with and without MSI, a feature that indicates loss of MMR function due to either somatic methylation or inherited MMR gene mutations.[45,46] Similar to our results, both of these studies reported inverse associations between dietary calcium intake and MSI-high CRC. A meta-analysis of randomized controlled trials[47] showed a protective effect of calcium supplementation against recurrent colorectal adenoma (RR 0.80, 95% CI 0.68–0.93) although a recent trial[48] showed no such association. A meta-analysis of 24 prospective cohort studies also reported an inverse association between calcium supplement intake and CRC risk for the general population (RR 0.86, 95% CI 0.79–0.95).[27]

Given that our estimates for the strengths of associations between multivitamin and calcium supplement intakes and CRC risk for MMR gene mutation carriers were stronger than those for the general population ($P < 0.05$), it could be suggested that, if the association is causal, the possibly protective effect of multivitamin and calcium on CRC is likely to be stronger for those with MMR gene mutations. However, we were only able to report the overall effect of multivitamins rather than individual vitamins and minerals in the current study. Further, data on dosage and composition of multivitamins were not available in our database. The underlying mechanism of a potential beneficial role of multivitamins in the prevention of CRC is not clear, but it has been hypothesized to be due to potential chemopreventive roles of several single vitamins and minerals included in the multivitamin supplement.

The relationship between folic acid supplements or dietary folate intake and CRC is complex. A large-scale meta-analysis showed that intake of folic acid supplements or dietary folate had a small inverse association with CRC for the general population, although the findings were inconsistent depending on dose, duration and source in studies that were conducted mainly before widespread fortification of the food supply with folic acid.[28] The role of supplemental folic acid, the non-natural folate form currently used in all supplements, on cancer risk in the post-fortification period is controversial.[49–56] Two prospective cohort studies reported no evidence for an association between total folate intake, from both diet and supplements, and the risk of MSI-high CRCs (although the MSI-high CRCs in these studies were predominantly sporadic).[57,58] Similarly, in the current study, where the majority of carriers were queried about folic acid supplement intake after 1996 when widespread voluntary fortification began in the USA, Canada and Australia, no evidence of an association between folic acid supplement intake and CRC risk for a high-risk post-fortification population was observed. However, the lack of

Table 3. Univariable and multivariable hazard ratios for associations between intake of multivitamin, calcium and folic acid supplements and the risk of colorectal cancer for carriers of germline mutations in DNA mismatch repair genes

	Univariable model					Multivariable model[b]			
						Complete case analysis		Multiple imputation	
	No. of carriers with colorectal cancer	Total no. of carriers	Person-years	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Multivitamin supplement intake									
Never[a]	573	1403	58122	1 (ref)	–	1 (ref)	–	1 (ref)	–
≥ 1 month	137	465	19924	0.49 (0.37–0.64)	< 0.001	0.52 (0.36–0.75)	< 0.001	0.55 (0.40–0.75)	< 0.001
1 month to 11.9 months	25	106	3777	0.58 (0.35–0.98)	0.04	0.46 (0.25–0.85)	0.01	0.55 (0.30–0.99)	0.05
1 year to 2.9 years	40	122	5132	0.70 (0.43–1.14)	0.15	0.67 (0.37–1.22)	0.19	0.69 (0.41–1.17)	0.17
≥ 3 years	72	237	11015	0.38 (0.28–0.53)	< 0.001	0.45 (0.29–0.69)	< 0.001	0.47 (0.32–0.69)	< 0.001
Multivitamin supplement intake (per year)	710	1868	78046	0.93 (0.90 – 0.96)	< 0.001	0.93 (0.89–0.97)	0.001	0.95 (0.91–0.98)	0.003
Calcium supplement intake									
Never[a]	685	1730	71025	1 (ref)	–	1 (ref)	–	1 (ref)	–
≥ 1 month	48	178	8635	0.29 (0.20–0.41)	< 0.001	0.44 (0.27–0.71)	0.001	0.46 (0.30–0.71)	< 0.001
1 month to 11.9 months	4	35	1347	0.40 (0.05–0.51)	0.002	0.28 (0.07–1.12)	0.07	0.32 (0.09–1.11)	0.07
1 year to 2.9 years	19	60	2738	0.40 (0.23–0.68)	0.001	0.60 (0.30–1.20)	0.15	0.60 (0.32–1.13)	0.11
≥ 3 years	25	83	4550	0.27 (0.17–0.43)	< 0.001	0.40 (0.21–0.76)	0.01	0.42 (0.23–0.74)	0.003
Calcium supplement intake (per year)	733	1908	79660	0.84 (0.76 – 0.93)	0.001	0.87 (0.79–0.99)	0.002	0.90 (0.83–0.98)	0.02
Acid folic supplement intake									
Never[a]	693	1766	74195	1 (ref)	–	1 (ref)	–	1 (ref)	–
≥1 month	37	154	6225	0.51 (0.32–0.79)	0.003	1.05 (0.64–1.75)	0.84	0.95 (0.58–1.53)	0.82
1 month to 11.9 months	11	68	2627	0.31 (0.14–0.70)	0.004	0.64 (0.25–1.59)	0.33	0.60 (0.25–1.41)	0.24
1 year to 2.9 years	18	56	2258	0.84 (0.44–1.60)	0.60	1.50 (0.76–2.97)	0.24	1/39 (0.72–2.69)	0.33
≥ 3 years	8	30	1340	0.40 (0.18–0.89)	0.02	1.24 (0.52–2.98)	0.63	0.87 (0.36–2.08)	0.76
Acid folic supplement intake (per year)	730	1920	80420	0.86 (0.74–1.00)	0.05	0.97 (0.92–1.02)	0.22	0.96 (0.90–1.01)	0.14
Any supplement intake									
None of three[a]	541	1252	51604	1 (ref)	–	1 (ref)	–	1 (ref)	–
Multivitamin only	86	281	11694	0.50 (0.36–0.70)	< 0.001	0.46 (0.29–0.72)	< 0.001	0.51 (0.35–0.74)	< 0.001
Calcium only	6	40	1949	0.11 (0.05–0.26)	< 0.001	0.23 (0.08–0.69)	0.01	0.22 (0.08–0.60)	0.003
Folic acid only	10	52	2007	0.31 (0.13–0.75)	0.009	0.70 (0.27–1.84)	0.47	0.63 (0.25–1.56)	0.32
Multivitamin and calcium	26	87	4305	0.27 (0.17–0.44)	< 0.001	0.28 (0.14–0.57)	< 0.001	0.36 (0.20–0.64)	< 0.001
Multivitamin and folic acid	12	49	1841	0.59 (0.27–1.28)	0.18	0.93 (0.38–2.24)	0.87	0.92 (0.41–2.03)	0.83
Calcium and folic acid	4	16	750	0.23 (0.07–0.76)	0.02	0.40 (0.12–1.42)	0.16	0.39 (0.11–1.37)	0.14
All of three	10	29	1294	0.53 (0.25–1.10)	0.09	1.54 (0.79–3.00)	0.21	1.08 (0.48–2.46)	0.84

HR, hazard ratio; CI, confidence interval.

[a]Never user defined as carriers who answered 'No' to 'Have you ever taken [supplement] at least twice a week for at least 1 month?'

[b]Adjusted for ascertainment (binary), education (categorical), country (categorical), sex, number of screening colonoscopies (categorical), regular physical activity (binary), cigarette smoking (categorical) and intake of aspirin and/or ibuprofen (binary).

Table 4. Sex-specific multivariable hazard ratios for associations between intake of multivitamin, calcium and folic acid supplements and the risk of colorectal cancer for carriers of germline mutations in DNA mismatch repair genes

	Male[b]					Female[c]				
				Multivariable model Multiple imputation					Multivariable model Multiple imputation	
	No. of carriers with colorectal cancer	Total no. of carriers	Person-years	HR (95% CI)	*P*-value	No. of carriers with colorectal cancer	Total no. of carriers	Person-years	HR (95% CI)	*P*-value
Multivitamin										
Never[a]	325	650	27109	1 (ref)		248	753	31013	1 (ref)	
≥ 1 month	68	185	7893	0.45 (0.28–0.72)	0.001	69	280	12031	0.64 (0.43–0.95)	0.03
1 month to 11.9 months	12	46	1603	0.44 (0.19–1.03)	0.06	13	60	2174	0.70 (0.31–1.56)	0.38
1 year to 2.9 years	19	47	1973	0.52 (0.24–1.13)	0.10	21	75	3159	0.73 (0.39–1.36)	0.32
≥ 3 years	37	92	4317	0.41 (0.23–0.75)	0.004	35	145	6698	0.55 (0.33–0.93)	0.02
Multivitamin supplement intake (per year)	393	835	35002	0.93 (0.88–1.00)	0.04	317	1033	43033	0.96 (0.92–1.01)	0.10
Calcium supplement intake										
Never[a]	388	821	34227	1 (ref)		297	909	36798	1 (ref)	
≥ 1 month	14	32	1495	0.61 (0.25–1.51)	0.29	34	146	7140	0.63 (0.38–1.04)	0.07
1 month to 11.9 months	2	9	322	0.72 (0.14–3.64)	0.69	2	26	1025	0.35 (0.07–1.65)	0.18
1 year to 2.9 years	3	7	294	0.82 (0.16–4.09)	0.81	16	53	2444	0.84 (0.39–1.79)	0.65
≥ 3 years	9	16	879	0.55 (0.17–1.74)	0.31	16	67	3671	0.53 (0.27–1.04)	0.07
Calcium supplement intake (per year)	402	853	35722	0.89 (0.80–0.99)	0.03	331	1055	43938	0.95 (0.89–1.02)	0.18
Folic acid supplement intake										
Never*	392	837	34950	1 (ref)	–	301	929	39245	1 (ref)	
≥ 1 month	7	11	583	2.06 (0.58–7.33)	0.26	30	143	5642	0.83 (0.49–1.40)	0.49
1 month to 11.9 months	2	3	126	–	–	9	65	2501	0.39 (0.17–0.87)	0.02
1 year to 2.9 years	2	3	169	–	–	16	53	2089	1.47 (0.71–3.03)	0.30
≥ 3 years	3	5	288	–	–	5	25	1052	0.76 (0.29–2.00)	0.57
Folic acid supplement intake (per year)	399	848	35533	0.94 (0.87–1.01)	0.09	331	1072	44887	0.99 (0.90–1.08)	0.76

[a]Never user defined as carriers who answered 'No' to 'Have you ever taken [supplement] at least twice a week for at least 1 month?'

[b]Adjusted for education (categorical), ascertainment (binary), number of screening colonoscopies (categorical), regular physical activity (binary), country (categorical), cigarette smoking status (categorical), intake of aspirin and/or ibuprofen (binary), and average lifetime alcohol intake (continuous).

[c]Adjusted for education (categorical), ascertainment (binary), number of screening colonoscopies (categorical), regular physical activity (binary), country (categorical), cigarette smoking status (categorical), intake of aspirin and/or ibuprofen (binary), hormonal contraceptive intake (binary), live births (binary) and age at menopause (continuous).

an observed association between folate intake and CRC risk in this study might be attributed to poor statistical power.

Our study is, to the best of our knowledge, the largest study to date investigating associations between multivitamin, calcium and folic acid supplement intake and CRC risk for MMR gene mutation carriers. To overcome bias in retrospective studies when subjects are selected on the basis of phenotype, we used a weighted cohort approach.[33] The data for this study came from the Colon Cancer Family Registry where we have used standardized and uniform materials for collection of epidemiological and cancer data as well as genetic testing.[29]

The current study findings may have been affected by recall or response bias, given that data of the intake of multivitamin, calcium and folic acid supplements (and the other variables) were collected at the time of recruitment after the diagnoses of CRC had occurred. There may be residual confounding in the current study due to lack of high-quality data of dietary intake, total energy intake, physical activity and recent BMI. As carriers with poor survival were less likely to be included in the analysis, because they were unable to provide a blood sample for genetic testing and complete a questionnaire, there is a possibility of survival and selection bias if age at onset and survival of cases were related to exposure variables and/or mutation status. To determine whether survival bias influenced the observed associations, we conducted a sensitivity analysis restricted to carriers who were diagnosed with CRC or censored within 5 years before interview; we observed findings similar to the main analysis. Further, we were not able to investigate the association with vitamin D in combination with calcium intake. We were also not able to report the dose-response relationships with CRC risk, as information on the specific doses of supplements intake was not available. Our definition of duration of supplements intake was based on the assumption that the intake was continuous and recent before the interview. However, participants might have used supplements intermittently, and we were unable to account for this variation in our analysis. In addition, immortal time bias is a potential limitation of our analysis because we did not have data on the carriers' age at first intake of supplements and were not able to use a time-dependent approach. Our analytical approach might have overestimated the association between intake of supplements and the risk of CRC. Another limitation is that we were only able to report the overall effect of multivitamins rather than individual vitamins and minerals.

In conclusion, we found intake of multivitamin and calcium supplements to be associated with a decreased risk of CRC for MMR gene mutation carriers. Further prospective studies and clinical trials are required to confirm this finding, as it might provide evidence on important options to reduce CRC risk for these high-risk people.

Supplementary Data

Supplementary data are available at *IJE* online.

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