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ARTICLE

Aspirin, Ibuprofen, and the Risk for Colorectal Cancer in Lynch Syndrome

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Abstract

Background: Inheritance of a germline mutation in one of the DNA mismatch repair (MMR) genes *MLH1, MSH2, MSH6,* and *PMS2* causes a high risk of colorectal and other cancers (Lynch Syndrome). Use of aspirin has been shown to be associated with a reduced risk of colorectal cancer for the general population as well as for MMR gene mutation carriers. The aim of this study was to determine whether use of aspirin and ibuprofen in a nontrial setting is associated with the risk of colorectal cancer risk for MMR gene mutation carriers.

Methods: We included 1858 participants in the Colon Cancer Family Registry who had been found to have a pathogenic germline mutation in a MMR gene (carriers). We used weighted Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). All statistical tests were two-sided.

Results: A total of 714 carriers (38%) were diagnosed with colorectal cancer at a mean age of 42.4 (standard deviation 10.6) years. A reduced risk of colorectal cancer was associated with aspirin use (for 1 month to 4.9 years: HR = 0.49, 95% CI = 0.27 to 0.90, P = .02; for ≥5 years: HR = 0.25, 95% CI = 0.10 to 0.62, P = .003) and ibuprofen use (for 1 month to 4.9 years: HR = 0.38, 95% CI = 0.18 to 0.79, P = .009; for ≥5 years: HR = 0.26, 95% CI = 0.10 to 0.69, P = .007), compared with less than one month of use.

Conclusion: Our results provide additional evidence that, for MMR gene mutation carriers, use of aspirin and ibuprofen might be effective in reducing their high risk of colorectal cancer.

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Lynch Syndrome, previously termed Hereditary Non-Polyposis Colorectal Cancer, is an inherited susceptibility to colorectal and other cancers. Lynch Syndrome is caused by germline mutations in the DNA mismatch repair (MMR) genes *MLH1, MSH2, MSH6,* and *PMS2,* which encode proteins that identify and excise DNA mismatches (1). The prevalence of MMR gene mutation carriers in the general population has been estimated to range from approximately one in 370 to one in 3100, depending on the population and calculation assumptions (2,3). Lynch Syndrome causes 2% to 4% of all colorectal cancers and 10% to 15% of colorectal cancers diagnosed before the age of 50 years (4,5).

The average cumulative risk of colorectal cancer for MMR gene mutation carriers is substantial: about 50% by age 70 years for *MLH1* and *MSH2* carriers (6). However, there is evidence that there is very wide variation in risk even across carriers of mutations in the same gene (6), such that the majority of carriers are either at only modestly increased risk or at very high risk, rather than being clustered around the average risk. These observations raise the possibility that risk for mutation carriers is strongly determined by other genetic and/or environmental factors and that risk might be reduced substantially if relevant modifiable factors can be identified.

The main risk-reduction strategy for mutation carriers is regular screening with colonoscopy and polypectomy, which has been shown to reduce both colorectal cancer incidence and mortality (7). Accordingly, screening guidelines recommend that MMR mutation carriers have colonoscopy every one to two years from age 25 years, or five years earlier than the youngest colorectal cancer diagnosis in the family, whichever comes first (8–12).

Another potential colorectal cancer risk reduction approach, for which evidence has been accumulating over the last two decades, is chemoprevention using aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs). Several randomized controlled trials (RCT) and large cohort studies have consistently shown that use of aspirin and other NSAIDs reduces the risk of colorectal cancer for the general population (13–16). Five RCTs have also shown a protective effect of aspirin against colorectal adenoma for individuals with a personal history of colorectal neoplasia (17,18).

For MMR gene mutation carriers, evidence of a protective effect of aspirin use was provided by the CAPP2 RCT, the first trial of aspirin chemoprevention with cancer as the primary endpoint. At the end of two years of treatment, there was no evidence for an effect of daily high-dose (600 mg) aspirin on colorectal cancer risk (19). However, an analysis after a mean follow-up of 56 months found that the mutation carriers who had been randomly assigned to aspirin had about a 40% reduction in colorectal cancer incidence compared with the control patients (20). The effect of low-dose aspirin and other NSAIDs including ibuprofen on colorectal cancer risk for carriers outside a trial setting is unknown.

We report here results from the largest observational study to date investigating the association between use of aspirin and the risk of colorectal cancer for MMR gene mutation carriers and present the first analysis of a potential association with use of ibuprofen, a widely available nonaspirin NSAID.

Methods

Study Sample

The study sample consisted of participants in the Colon Cancer Family Registry who had been genetically tested and found to be carriers of a germline pathogenic mutation in an MMR gene. A detailed description of the Colon Cancer Family Registry design and recruitment strategy has been presented elsewhere (21) and is available at http://coloncfr.org. Briefly, between 1997 and 2012, families were recruited through: 1) population-based probands with newly diagnosed colorectal cancer identified by state or regional population cancer registries in the United States (WA, CA, AZ, MN, CO, NH, NC, and HI), Australia (Victoria), and Canada (Ontario); and 2) clinic-based probands from multiple-case families referred to family cancer clinics in the United States (Mayo Clinic, Rochester, MN, and Cleveland Clinic, Cleveland, OH), Canada (Ontario), Australia (Melbourne, Adelaide, Perth, Brisbane, Sydney), and New Zealand (Auckland). We obtained permission from probands to contact their relatives to seek their enrollment in the Colon Cancer Family Registry. Informed consent was obtained from all study participants, and the study protocol was approved by the research ethics review boards of each recruitment center.

Data Collection

Epidemiologic and demographic information was collected using in-person interviews, telephone interviews, or mailed questionnaires at the time of recruitment. The questionnaires asked about participants' characteristics; personal and family history of cancer; history of cancer screening; history of polyps, polypectomy and other surgeries; and use of aspirin, ibuprofen, and other medications. The detailed questionnaires used by each Colon Cancer Family Registry center are available at: http://coloncfr.org/questionnaires. Reported cancer diagnoses and ages at diagnosis were confirmed if possible using pathology reports, medical records, cancer registry reports, and death certificates. We attempted to obtain blood samples from all study participants and tumor tissue from colorectal cancer–affected participants.

MMR Gene Mutation Testing

Screening for germline mutations in *MLH1, MSH2, MSH6,* and *PMS2* was performed for all population-based probands who had a colorectal tumor that showed evidence of impaired MMR function by either tumor microsatellite instability (MSI) or a lack of MMR protein expression by immunohistochemistry, or both. For clinic-based families, screening was performed for the youngest colorectal cancer case patient regardless of MSI or MMR protein expression status. For the *MLH1*, *MSH2,* and *MSH6* genes, mutation testing was performed by Sanger sequencing or denaturing high performance liquid chromatography (dHPLC), followed by confirmatory DNA sequencing. Large duplication and deletion mutations were detected by Multiplex Ligation Dependent Probe Amplification (MLPA) according to the manufacturer's instructions (MRC Holland, Amsterdam, the Netherlands) (21–23). For the *PMS2* gene, mutation testing involved a modified protocol from Senter et al. (24), in which exons 1–5, 9, and 11–15 were amplified using three long-range polymerase chain reactions (PCRs) followed by nested exon-specific PCR/sequencing, while the remaining exons (6, 7, 8, and 10) were amplified and sequenced directly from genomic DNA. Large-scale deletions in *PMS2* were detected using the P008-A1 MLPA kit (MRC Holland, Amsterdam, the Netherlands). Relatives of probands with a pathogenic MMR germline mutation (25) who had provided a blood sample were tested for the specific mutation identified in the proband. In this study, we included all probands and their relatives who had been found to be carriers of a germline pathogenic mutation in a MMR gene.

Exposure Definitions

Aspirin intake and ibuprofen intake were the two main exposure variables. Never use was defined as answering "no" to "Have you ever taken (aspirin/ibuprofen) at least twice a week for a month or longer?" Ever use was defined as answering "yes" to "Have you ever taken (aspirin/ibuprofen) at least twice a week for a month or longer?"

Duration of aspirin/ibuprofen use was based on the question "How long, in total, have you taken this medication for at least twice a week for a month or longer?" We calculated the age at first use of aspirin/ibuprofen (under the assumption that duration was continuous and recent) by subtracting the reported duration of use from the age at interview. Then, we calculated the duration of use as the total number of years of (aspirin/ibuprofen) use between the age at first use and the age at colorectal cancer diagnosis or censoring.

Those who answered "yes" to the question "Have you ever taken (aspirin/ibuprofen) at least twice a week for a month or longer?" and reported a duration that was shorter than the time interval between age at colorectal cancer diagnosis or censoring and age at interview, were classified as never users.

Statistical Analysis

We conducted weighted Cox proportional hazards regression analyses, with age as the time scale, to estimate the associations of use of aspirin only, ibuprofen only, and aspirin and/or ibuprofen with the risk of colorectal cancer. Time at risk started from birth and ended at age at first diagnosis of colorectal cancer (n = 714), age of diagnosis of non-colorectal cancer (n = 304), polypectomy (n = 306), or age at interview (n = 534), whichever occurred first. We chose to censor at age at any cancer diagnosis because cancer treatment could alter subsequent colorectal cancer risk. Similarly, we censored at age at first polypectomy because removal of polyps markedly reduces colorectal cancer risk (7). This prevents the potential confounding of colonoscopy screening on the associations between the exposure variables and colorectal cancer risk.

Colorectal cancer cases with a strong family history and/or early onset were preferentially tested for MMR gene mutations in the Colon Cancer Family Registry. Therefore, selection of the mutation carriers to be included in this study was not random with respect to disease status—i.e., there was an excess of colorectal cancer case patients compared with what would be expected in an unselected cohort of carriers. To adjust for this nonrandom sampling, we created a "synthetic" cohort using the weighted cohort approach described by Antoniou et al. (26), which has been previously applied in studies of disease-risk modifiers for carriers of rare genetic mutations (27–29). This method involves giving affected and unaffected carriers age-specific relative weights based on their sampling probabilities so that the age-specific incidence in the synthetic cohort is consistent with the incidence estimated for carriers in the general population (26).

In this study, as described in detail by Pande et al. (30), population-based estimates of colorectal cancer incidence for five-year intervals (31) were multiplied by the relative risks of colorectal cancer for MMR gene mutation carriers (32) to obtain age-specific incidences (see Supplementary Table 1, available online). These age-specific incidences were used to calculate sampling fractions in order to weight mutation carriers so that for each five-year interval the proportion of affected carriers in the synthetic cohort equaled the proportion of affected carriers in the general population. A simulation study of this approach using Cox regression showed that allowing for nonrandom sampling by using external age-specific incidences led to unbiased estimates of risk modifiers when the external incidences were correctly specified (26).

Aspirin-only, ibuprofen-only, and aspirin and/or ibuprofen use were fit separately as binary (ever vs never), and categorical (<1 month, 1 month, to 4.9 years, and ≥5 years) variables, and corresponding hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated for associations with colorectal cancer risk. The proportional hazards (PH) assumption was tested by examining the relationship between the scaled Schoenfeld residuals and survival time (33).

Model-building strategies were based on the "purposeful selection" method described by Hoshmer and Lemeshow (34). Selection of covariates for inclusion in the multivariable models was based on statistical significance at the 25% level in the univariate models and on clinical importance for any variables not selected with this criterion. The following factors were considered as potential confounders: 1) year of birth (1914–43, 1944–54, 1955–65, 1966–90); 2) sex; 3) country of recruitment; 4) ethnicity; 5) education; 6) smoking status (never, former, current); 7) number of alcohol drinks per day; 8) body mass index two years before interview (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]); 9) history of diabetes (yes, no); 10) multivitamin supplement use (<1 month vs ≥1 month); 11) regular physical activity (at least 30 minutes per week for at least three months); 12) acetaminophen (<1 month vs ≥1 month); and 13) laxatives(<1 month vs ≥1 month) for both sexes and 14) hormone replacement therapy use (<6 vs ≥6 months); and 15) number of live births (none, 1, 2, ≥3) for women. Variables that did not meet the PH assumption were included as time-dependent covariates in the model. Missing values of covariates were coded as an additional category to allow inclusion of as many carriers as possible in the multivariable model fits. Tests for interactions between exposures and potential effect modifiers were performed using the log-likelihood ratio test.

To identify any potential survival bias on recruitment, we further conducted an analysis restricted to carriers who were diagnosed with colorectal cancer, or censored, within five years prior to the interview. To determine whether censoring at polypectomy influenced the estimated associations, we conducted an analysis using colorectal neoplasia (i.e., either colorectal cancer or colorectal polyps) as the outcome. We also conducted an analysis restricted to carriers for whom there was complete covariate data (i.e., carriers with a missing value for any covariate were excluded).

We applied the Huber-White robust variance estimation by clustering on family membership to allow for a correlation in risk between family members (35,36). All statistical tests were two-sided, and, following convention, nominal statistical significance was determined at P values of less than .05. Analyses were performed using Stata 13 (37).

Results

From the Colon Cancer Family Registry, we identified a total of 2003 carriers of a pathogenic mutation in a MMR gene. Of these, we excluded 11 (0.5%) because they were censored before age 18 years and 134 (6.7%) because they had missing data on both main exposure variables. Excluded carriers did not differ in main characteristics from those who were entered into the analyses (details not shown).

Table 1. Characteristics of mismatch repair gene mutation carriers by colorectal cancer diagnosis status

Variables	No colorectal cancer (n = 1144, 61%)	Colorectal cancer (n = 714, 38%)	Total (n = 1858)
Study centers			
Australia or New Zealand	687 (60.0)	342 (47.9)	1029 (55.3)
USA	304 (26.5)	265 (37.1)	569 (30.6)
Canada	153 (13.3)	107 (14.9)	260 (13.9)
Ethnicity			
White	1086 (94.9)	650 (91.0)	1736 (93.4)
Other	41 (3.5)	57 (7.9)	98 (5.2)
Missing	17 (1.4)	7 (0.9)	24 (1.2)
Age, y*			
Mean (SD)	41.2 (13.0)	42.4 (10.6)	41.7 (12.2)
Median (range)	41.0 (18–85)	42.0 (19–75)	42 (18–85)
Year of birth			
1914–1943	206 (18.0)	189 (26.4)	395 (21.2)
1944–1954	262 (22.9)	218 (30.5)	480 (25.8)
1955–1965	281 (24.5)	201 (28.1)	482 (25.9)
1966–1990	395 (34.5)	106 (14.8)	501 (26.9)
Sex			
Men	430 (37.5)	390 (54.6)	820 (44.1)
Women	714 (62.4)	324 (45.3)	1038 (55.8)
Education			
Primary or less	11 (0.9)	18 (2.5)	29 (1.5)
Some high school	221 (19.3)	156 (21.8)	377 (20.2)
Completed high school/some tertiary study	389 (34.0)	229 (32.0)	618 (33.2)
Vocational/technical school	207 (18.0)	119 (16.6)	326 (17.5)
University degree	307 (26.8)	183 (25.6)	490 (26.3)
Missing	9 (0.7)	9 (1.2)	18 (0.9)
MMR gene mutated			
MLH1	373 (32.6)	308 (43.1)	681 (36.6)
MSH2	575 (50.2)	298 (41.7)	873 (46.9)
MSH6	142 (12.4)	62 (8.6)	204 (10.9)
PMS2	54 (4.7)	46 (6.4)	100 (5.3)
BMI† two years before CRC diagnosis or censoring			
Normal	334 (29.2)	98 (13.7)	432 (23.2)
Overweight	230 (20.1)	101 (14.1)	331 (17.8)
Obese	109 (9.5)	58 (8.1)	167 (8.9)
Underweight	23 (2.0)	10 (1.4)	33 (1.8)
Missing	448 (39.1)	447 (62.6)	895 (48.1)
Diabetes			
No	1110 (97.0)	679 (95.1)	1789 (96.2)
Yes	30 (2.6)	30 (4.2)	60 (3.2)
Missing	4 (0.3)	5 (0.7)	9 (0.4)
Cigarette smoking‡			
Never	623 (54.4)	325 (45.5)	948 (51.0)
Former	260 (22.7)	152 (21.2)	412 (22.1)
Current	256 (22.3)	237 (33.1)	493 (26.5)
Missing	5 (0.43)	0 (0)	5 (0.27)
Number of alcohol drinks per day			
None	289 (25.2)	175 (24.5)	464 (24.9)
1	376 (32.8)	226 (31.6)	602 (32.4)
≥2	329 (28.7)	233 (32.6)	562 (30.2)
Missing	150 (13.1)	80 (11.2)	230 (12.3)
Regular physical activity§			
<3 mos	35 (3.0)	4 (0.5)	39 (2.1)
≥3 mos	999 (87.3)	631 (88.3)	1630 (87.7)
Missing	110 (9.61)	79 (11.0)	189 (10.1)
Multivitamin intake‖			
<1 mo	788 (68.8)	547 (76.6)	1335 (71.8)
≥1 mo	306 (26.7)	134 (18.7)	440 (23.6)
Missing	50 (4.3)	33 (4.6)	83 (4.4)
Acetaminophen intake‖			
<1 mo	1033 (90.3)	656 (91.8)	1689 (90.9)
≥1 mo	91 (7.9)	40 (5.6)	131 (7.0)
Missing	20 (1.7)	18 (2.5)	38 (2.0)

Table 1. *Continued*

Variables	No colorectal cancer (n = 1144, 61%)	Colorectal cancer (n = 714, 38%)	Total (n = 1858)
Laxative intake‖			
<1 mo	1079 (94.3)	663 (92.8)	1742 (93.7)
≥1 mo	53 (4.6)	41 (5.7)	94 (5.0)
Missing	12 (1.0)	10 (1.4)	22 (1.2)
Hormone replacement therapy use¶			
<6 mos	604 (84.5)	277 (85.4)	881 (84.8)
≥6 mos	93 (13.0)	41 (12.6)	134 (12.9)
Missing	17 (2.3)	6 (1.8)	23 (2.2)
Number of live births¶			
None	209 (29.2)	56 (17.2)	265 (25.5)
1	73 (10.2)	43 (13.2)	116 (11.1)
2	199 (27.8)	94 (29.0)	293 (28.2)
≥3	215 (30.1)	119 (36.7)	334 (32.1)
Missing	18 (2.5)	12 (3.7)	30 (2.8)

* Age at first diagnosis of colorectal cancer for affected carriers; age at first polypectomy or diagnosis of non-colorectal cancer or interview for unaffected carriers (whichever came first). BMI = body mass index; CRC = colorectal cancer; MMR = mismatch repair.
† 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).
‡ Former smokers defined as carriers who had smoked at least one cigarette per day for at least three months and had quit more than two years before age at colorectal cancer or censored age; current smokers defined as carriers who had smoked at least one cigarette per day for at least three months and continued within two years of age at colorectal cancer or censored age.
§ Regular physical activity defined as any physical activity for at least 30 minutes per week for at least three months.
‖ At least twice a week.
¶ Only for female carriers.

The study therefore involved 1858 MMR gene mutation carriers from 748 families (25% population-based), of whom 1038 (55.8%) were female, who contributed a total of 77 494 person-years. Of these, 681 carried a mutation in *MLH1*, 873 in *MSH2*, 204 in *MSH6*, and 100 in *PMS2*, and 714 (38%) were diagnosed with colorectal cancer at a mean age at diagnosis of 42.4 years (SD = 10.6). A total of 653 (91%) reported cancers were confirmed by pathology, clinical, or cancer-registry records. The time interval between age at colorectal cancer diagnosis or censoring and age at interview was on average 9.2 (SD = 9.9) years for carriers with colorectal cancer and 9.9 (SD 9.4) years for carriers without colorectal cancer (P = .14).

Characteristics of participating MMR gene mutation carriers are presented in Table 1. Of all carriers, 117 (6.7%) reported having used aspirin only for at least one month, 126 (7.2%) reported having used ibuprofen only for at least one month, and 286 (14.3%) reported having used aspirin and/or ibuprofen for at least one month. The prevalence of aspirin and ibuprofen use by country, age group, and sex is shown in Table 2. The prevalence of aspirin use for carriers affected with colorectal cancer and unaffected carriers was 6.5% and 6.9%, respectively (Supplementary Table 2, available online).

Table 3 presents the results of the univariate and multivariable Cox regression models and the confounders included in each model. A reduced risk of colorectal cancer was associated with ever use of: aspirin only (HR = 0.43, 95% CI = 0.25 to 0.75, P = .003), ibuprofen only (HR = 0.35, 95% CI = 0.19 to 0.63, P = .001), and aspirin and/or ibuprofen (HR = 0.41, 95% CI = 0.28 to 0.61, P < .001). A longer duration of use was associated with a lower risk of colorectal cancer; aspirin-only use (for 1 month to 4.9 years: HR = 0.49, 95% CI = 0.27 to 0.90, P = .02; for ≥5 years: HR = 0.25, 95% CI = 0.10 to 0.62, P = .003) and ibuprofen-only use (for 1 month to 4.9 years: HR = 0.38, 95% CI = 0.18 to 0.79, P = .009; for ≥5 years: HR = 0.26, 95% CI = 0.10 to 0.69, P = .007) (Table 3). These inverse associations are illustrated in Figures 1 and 2 for aspirin and ibuprofen, respectively.

Table 4 presents the results of the multivariable analyses separately for men and women. The inverse associations with aspirin only, ibuprofen only, and aspirin and/or ibuprofen were similar to those from the combined analysis, and there was no evidence that they differed by sex (P > .7 for all interactions). Table 5 presents the results of the multivariable analyses separately for mutation carriers by the specific MMR gene mutated. There was no evidence that the strengths of association differed by gene (P > .2 for all interactions).

In the analysis restricted to carriers diagnosed with colorectal cancer or censored within five years prior to their interview (n = 1213, 65%), the results were similar to those from the main analysis (Supplementary Table 3, available online). Similar results were also observed when we used colorectal neoplasia (either polyp or colorectal cancer) as the outcome (Supplementary Table 4, available online). Finally, in the analysis restricted to carriers with complete covariate data, the results were similar to the main analysis (data not shown). All models were tested and there was no evidence that the PH assumption was violated.

Discussion

This study includes the largest sample of confirmed MMR gene mutation carriers assembled to date to investigate the association between aspirin use and colorectal cancer risk in the context of Lynch Syndrome. We estimated that, for carriers of pathogenic germline mutations in MMR genes, aspirin use for at least one month was associated with a decrease of approximately 60% in the risk of colorectal cancer. A recent meta-analysis of 10 cohort studies investigating the association between duration of aspirin use and the risk of colorectal cancer for the general population estimated 10% (95% CI = 8% to 12%) and 18% (95% CI = 14% to 22%) reductions in colorectal cancer risk for five and 10 years' use, respectively (38). In our analysis, the magnitude of the association for MMR

Table 2. Prevalence of aspirin and ibuprofen intake in mismatch repair gene mutation carriers by country, sex, and age category*

NSAID use	Country/age group, y	Men No. (%)	Women No. (%)	Overall No. (%)
Aspirin only	USA			
	≤35	4 (9.3)	1 (3.0)	5 (6.5)
	36–45	5 (10.8)	4 (7.1)	9 (8.8)
	46–55	7 (9.2)	5 (6.4)	12 (7.8)
	>55	20 (20.6)	12 (11.5)	32 (15.9)
	All	36 (13.7)	22 (8.1)	58 (10.9)
	Australia/New Zealand			
	≤35	3 (2.6)	6 (4.1)	9 (3.4)
	36–45	5 (6.0)	5 (5.2)	10 (5.5)
	46–55	4 (3.6)	3 (2.4)	7 (3.0)
	>55	7 (6.4)	16 (8.8)	23 (7.9)
	All	19 (4.5)	30 (5.5)	49 (5.1)
	Canada			
	≤35	1 (5.2)	1 (3.7)	2 (4.3)
	36–45	2 (10.0)	0 (0)	2 (4.8)
	46–55	0 (0)	1 (2.5)	1 (1.5)
	>55	2 (5.0)	3 (4.8)	5 (4.9)
	All	5 (4.8)	5 (3.3)	10 (3.9)
	Overall	60 (7.6)	57 (5.9)	117 (6.7)
Ibuprofen only	USA			
	≤35	4 (9.0)	6 (15.3)	10 (12.0)
	36–45	2 (4.7)	5 (8.3)	7 (6.8)
	46–55	7 (9.5)	7 (8.6)	14 (9.0)
	>55	3 (3.9)	3 (3.2)	6 (3.5)
	All	16 (6.8)	21 (7.7)	37 (7.3)
	Australia/New Zealand			
	≤35	6 (5.1)	11 (7.4)	17 (6.4)
	36–45	7 (8.3)	13 (12.5)	20 (10.7)
	46–55	8 (7.1)	14 (10.6)	22 (9.0)
	>55	9 (8.0)	11 (6.3)	20 (6.9)
	All	30 (7.0)	49 (8.8)	79 (8.0)
	Canada			
	≤35	0 (0)	1 (3.7)	1 (2.2)
	36–45	1 (5.0)	2 (8.0)	3 (6.6)
	46–55	2 (7.4)	2 (4.1)	4 (5.3)
	>55	0 (0)	2 (3.1)	2 (2.0)
	All	3 (3.0)	7 (4.2)	10 (3.8)
	Overall	49 (6.4)	77 (7.7)	126 (7.2)
Aspirin and/or ibuprofen	USA			
	≤35	10 (19.2)	10 (22.7)	20 (20.8)
	36–45	10 (19.2)	12 (17.9)	22 (18.4)
	46–55	16 (18.3)	16 (15.9)	32 (16.9)
	>55	27 (25.2)	22 (17.4)	49 (21.0)
	All	63 (21.1)	60 (17.7)	123 (19.3)
	Australia/New Zealand			
	≤35	10 (8.2)	19 (12.0)	29 (10.3)
	36–45	12 (13.3)	18 (16.3)	30 (15.0)
	46–55	13 (10.8)	18 (12.9)	31 (11.9)
	>55	18 (14.5)	31 (15.3)	49 (15.0)
	All	53 (11.6)	86 (14.1)	139 (13.0)
	Canada			
	≤35	1 (5.2)	2 (6.9)	3 (6.2)
	36–45	3 (13.6)	2 (7.6)	5 (10.4)
	46–55	2 (6.6)	4 (7.8)	6 (7.4)
	>55	2 (4.7)	8 (11.1)	10 (8.7)
	All	8 (7.0)	16 (8.9)	24 (8.2)
	Overall	124 (14.3)	162 (14.3)	286 (14.3)

* NSAID = nonsteroidal anti-inflammatory drugs.

gene mutation carriers was substantially stronger than that observed for the general population, particularly for aspirin use for more than five years (P = .002 for difference between estimates based on the test of interaction proposed by Altman

Table 3. Univariate and multivariable hazard ratios for associations between aspirin and ibuprofen use and the risk of colorectal cancer for mismatch repair gene mutation carriers

				Univariate model		Multivariable model	
NSAID use	n	N	Person-years	HR (95% CI)	P	HR* (95% CI)	P
Never user†	622	1572	25 743	1 (Ref)		1 (Ref)	
Aspirin-only user	48	117	2505	0.48 (0.33 to 0.71)	<.001	0.43 (0.25 to 0.75)	.003
1 month to 4.9 years	38	96	1904	0.49 (0.31 to 0.76)	.002	0.49 (0.27 to 0.90)	.02
≥5 years	10	21	601	0.45 (0.21 to 0.92)	.03	0.25 (0.10 to 0.62)	.003
Ibuprofen-only user	30	126	1373	0.33 (0.20 to 0.55)	<.001	0.35 (0.19 to 0.63)	.001
1 month to 4.9 years	22	92	1010	0.36 (0.20 to 0.66)	.001	0.38 (0.18 to 0.79)	.009
≥5 years	8	34	363	0.25 (0.11 to 0.58)	.001	0.26 (0.10 to 0.69)	.007
Aspirin and/or ibuprofen user	92	286	4548	0.40 (0.30 to 0.54)	<.001	0.41 (0.28 to 0.61)	<.001
1 month to 4.9 years	66	210	3196	0.42 (0.29 to 0.59)	<.001	0.44 (0.27 to 0.69)	<.001
≥5 years	26	76	1352	0.36 (0.22 to 0.57)	<.001	0.34 (0.19 to 0.62)	<.001

* Adjusted for year of birth (1914–43, 1944–54, 1955–65, 1966–90) and average lifetime alcohol intake (0, 1, ≥2 drinks per day) and stratified by sex, country (USA, Australia/ New Zealand, Canada), cigarette smoking status (never, former, current), regular physical activity (at least 30 minutes per week for at least three months), and multivitamin intake (<1 month, ≥1 month). All statistical tests were two-sided. CI = confidence interval; HR = hazard ratio; n = number of colorectal cancer cases; N = total number of carriers; NSAID = nonsteroidal anti-inflammatory drugs.

† Never users defined as carriers who reported not having taken either aspirin or ibuprofen or both for at least one month.

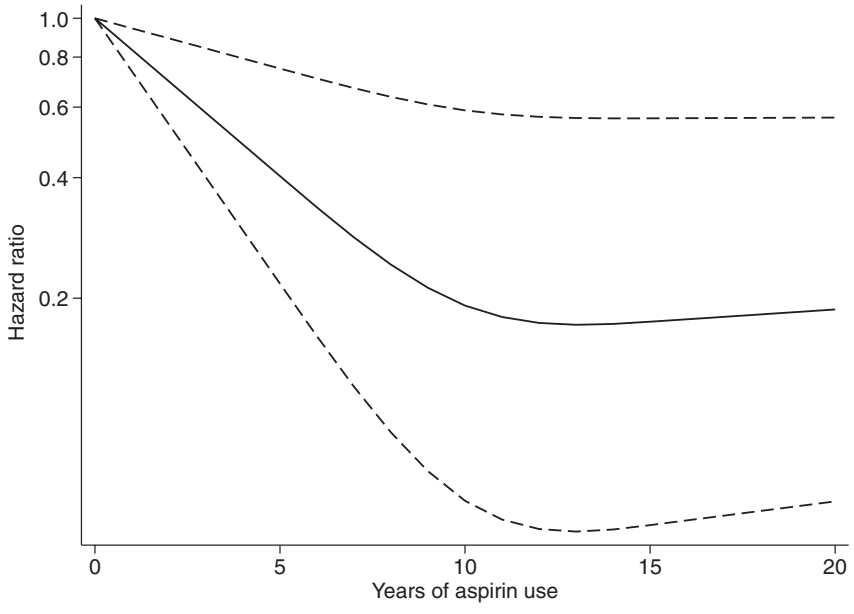

Figure 1. Cox regression analysis for association between aspirin only use and the risk of colorectal cancer over time for mismatch repair gene mutation carriers. Adjusted for year of birth (1914–43, 1944–54, 1955–65, 1966–90) and average lifetime alcohol intake (0, 1, ≥2 drinks per day) and stratified by sex, country (USA, Australia/ New Zealand, Canada), cigarette smoking status (never, former, current), regular physical activity (at least 30 minutes per week for at least three months), and multivitamin intake (<1 month, ≥1 month). **Upper and lower dashed lines** represent upper and lower limit of 95% confidence interval, respectively. **Solid line** represents the hazard ratio.

and Bland [39]). Our findings are more consistent with the risk reduction found by Burn et al. in the CAPP2 trial, in which MMR gene mutation carriers had a 58% (95% CI = 14% to 81%) reduction in colorectal cancer risk from use of 600 mg of aspirin daily for an average period of two years (this result was detected after a mean follow-up period of 4.6 years) (20). Our finding that the associations were similar when colorectal neoplasia was the outcome is consistent with use of NSAIDs delaying initiation of adenomas. This finding contrasts with the CAPP2 study, which did not find evidence for a short-term (two-year) reduction in the risk of adenoma (19). However, it is consistent with several RCTs of people with familial adenomatous polyposis (FAP), which found evidence that use of NSAIDs reduces the size and the number of colorectal polyps (40–44). It should be noted, however, that FAP and Lynch Syndrome are very different clinically and genetically.

Our analysis is the first reported investigation of a potential association between ibuprofen and the risk of colorectal cancer in the context of Lynch Syndrome. Use of ibuprofen only for at least one month was associated with a 65% decrease in the risk of colorectal cancer. This finding is consistent with previous evidence of an inverse association between nonaspirin NSAIDs, particularly those of the propionic acid derivatives class such as ibuprofen, and the risk of colorectal cancer for the general population. A case-control study by Reeves and colleagues has shown that ibuprofen might have a greater beneficial effect against colorectal cancer than aspirin (57% vs 21% risk reduction) (45).

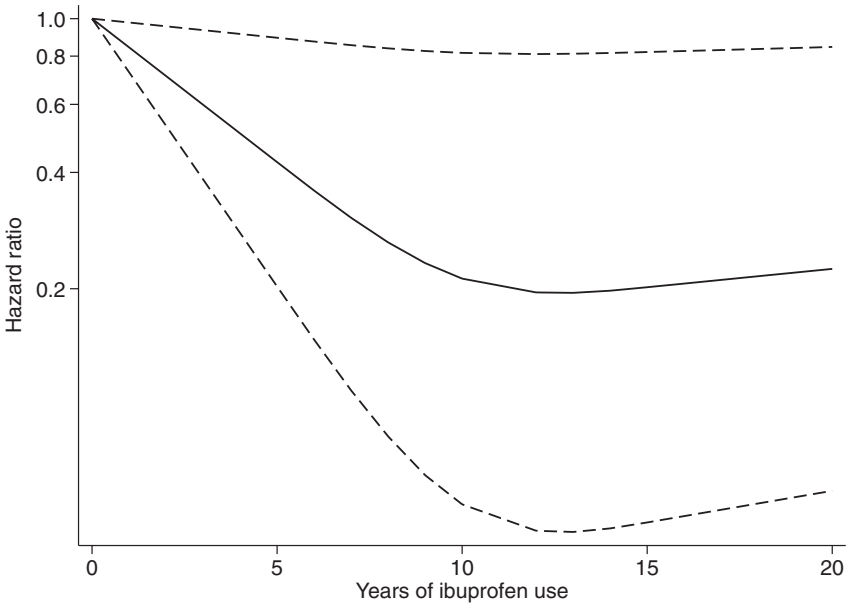

Figure 2. Cox regression analysis for association between ibuprofen only use and the risk of colorectal cancer over time for mismatch repair gene mutation carriers. Adjusted for year of birth (1914–43, 1944–54, 1955–65, 1966–90) and average lifetime alcohol intake (0, 1, ≥2 drinks per day) and stratified by sex, country (USA, Australia/ New Zealand, Canada), cigarette smoking status (never, former, current), regular physical activity (at least 30 minutes per week for at least three months), and multivitamin intake (<1 month, ≥1 month). **Upper and lower dashed lines** represent upper and lower limit of 95% confidence interval, respectively. **Solid line** represents the hazard ratio.

Table 4. Sex-specific multivariable hazard ratios for associations between aspirin and ibuprofen use and the risk of colorectal cancer for mismatch repair gene mutation carriers

	Men					Women				
NSAID use	n	N	Person-years	HR* (95% CI)	P	n	N	Person-years	HR† (95% CI)	P
Never user‡	338	696	14 137	1 (Ref)		284	876	11 606	1 (Ref)	
Aspirin-only user	29	60	1501	0.43 (0.21 to 0.86)	.02	19	57	1004	0.42 (0.17 to 1.03)	.06
1 month to 4.9 years	23	50	1128	0.45 (0.21 to 0.97)	.04	15	46	776	0.54 (0.21 to 1.41)	.21
≥5 years	6	10	373	0.32 (0.10 to 0.96)	.04	4	11	228	0.15 (0.01 to 1.32)	.09
Ibuprofen-only user	15	49	693	30 (0.14 to 0.67)	.004	15	77	680	0.35 (0.14 to 0.86)	.02
1 month to 4.9 years	11	36	517	0.38 (0.14 to 1.01)	.05	11	56	493	0.36 (0.12 to 1.09)	.07
≥5 years	4	13	176	0.21 (0.05 to 0.86)	.03	4	21	187	0.33 (0.09 to 1.15)	.08
Aspirin and/or ibuprofen user	52	124	2580	0.41 (0.24 to 0.68)	.001	40	162	1968	0.42 (0.23 to 0.78)	.006
1 month to 4.9 years	38	94	1808	0.42 (0.24 to 0.74)	.003	28	116	1388	0.39 (0.18 to 0.84)	.02
≥5 years	14	30	772	0.20 (0.06 to 0.62)	.005	12	46	580	0.52 (0.21 to 1.28)	.16

* Adjusted for year of birth (1914–43, 1944–54, 1955–65, 1966–90) and average lifetime alcohol intake (0, 1, ≥2 drinks per day) and stratified by country (USA, Australia/ New Zealand, Canada), cigarette smoking status (never, former, current), regular physical activity (at least 30 minutes per week for at least three months), and multivitamin intake (<1 month, ≥1 month). All statistical tests were two-sided. CI = confidence interval; HR = hazard ratio; n = number of colorectal cancer cases; N = total number of carriers; NSAID = nonsteroidal anti-inflammatory drugs.

† Adjusted for year of birth (1914–43, 1944–54, 1955–65, 1966–90) and number of live births (none, 1, 2, 3, or more) and stratified by country (USA, Australia/ New Zealand, Canada), cigarette smoking status (never, former, current), regular physical activity (at least 30 minutes per week for at least three months), hormone replacement therapy use (<6 months, ≥6 months), and multivitamin intake (<1 month, ≥1 month).

‡ Never users defined as carriers who reported not having taken either aspirin or ibuprofen or both for at least one month.

The potential chemopreventive effect of NSAIDs on colorectal cancer has been recognized since the late 1980s (46) and is strongly supported by experimental data (47). The mechanism of action that defines NSAIDs as a class is their ability to inhibit the cyclooxygenase (COX) activity of the enzyme prostaglandin G/H-synthase, which is expressed as two distinct isoforms, designated COX-1 and COX-2 (48). However, the exact mechanism by which aspirin reduces colorectal cancer risk is yet to be understood. Most colorectal cancers progress through the action of multiple molecular mechanisms that involve cyclooxygenases, Wnt–β-catenin, MAP kinase, cytokines, and growth factors (14). The effect of NSAIDs on colorectal neoplasia could be mediated by their ability to restore apoptosis through the inhibition of the COX pathway (49,50). In addition to the COX-inhibition pathway, several other possible mechanisms have been described (51,52).

Recently, Greenspan and colleagues investigated the effect of ibuprofen on biochemical pathways in both human

Table 5. Multivariable hazard ratios for associations between aspirin and ibuprofen use and the risk of colorectal cancer by mismatch repair gene mutated

MMR gene/NSAID use	n	N	Person-years	HR* (95% CI)	P
MLH1					
Never user†	276	600	11 043	1 (Ref)	
Aspirin-only user	13	30	609	0.34 (0.13 to 0.86)	.02
Ibuprofen-only user	13	40	603	0.91 (0.32 to 2.55)	.86
Aspirin and/or ibuprofen user	32	81	1499	0.43 (0.21 to 0.87)	.02
MSH2					
Never user†	266	751	10 975	1 (Ref)	
Aspirin-only user	18	51	887	0.52 (0.19 to 1.45)	.22
Ibuprofen-only user	8	53	344	0.27 (0.08 to 0.85)	.03
Aspirin and/or ibuprofen user	32	122	1505	0.39 (0.20 to 0.74)	.004
MSH6					
Never user†	50	148	2330	1 (Ref)	
Aspirin-only user	6	21	352	0.23 (0.04 to 1.06)	.06
Ibuprofen-only user	5	24	242	0.16 (0.03 to 0.72)	.02
Aspirin and/or ibuprofen user	12	56	643	0.27 (0.04 to 1.75)	.17
PMS2					
Never user†	30	73	1395	1 (Ref)	
Aspirin-only user	11	15	657	1.78 (0.56 to 5.67)	.33
Ibuprofen-only user	4	9	184	1.60 (0.33 to 7.59)	.55
Aspirin and/or ibuprofen user	16	27	901	0.70 (0.33 to 1.47)	.35

* Adjusted for sex, country (USA, Australia/ New Zealand, Canada), average lifetime alcohol intake (0, 1, ≥2 drinks per day), year of birth (1914–43, 1944–54, 1955–65, 1966–90), cigarette smoking status (never, former, current), regular physical activity (at least 30 minutes per week for at least three months), and multivitamin intake (<1 month, ≥1 month), where possible. All statistical tests were two-sided. CI = confidence interval; HR = hazard ratio; n = number of colorectal cancer cases; MMR = DNA mismatch repair; N = total number of carriers; NSAID = nonsteroidal anti-inflammatory drugs.
† Never users defined as carriers who reported not having taken either aspirin or ibuprofen or both for at least one month.

adenomas and colorectal cancers in vitro and showed that ibuprofen is effective in inhibiting Wnt–β-catenin nuclear translocation in both human adenomas and in colorectal cancer cells. This study confirmed that a NSAID can suppress β-catenin, a key mediator of colon tumorigenesis, in sporadic adenomas following long-term treatment in patients (53). Overall, it should be noted that it is currently unknown whether the biological processes that have been discovered so far and that potentially underlie the association between NSAIDs and sporadic colorectal cancers also apply to Lynch Syndrome–associated colorectal cancers.

One of the strengths of our study is that we were able to adjust for recognized potential confounding variables, including alcohol consumption, cigarette smoking, hormone replacement therapy, and multivitamin use. The Colon Cancer Family Registry had established standardized and uniform protocols for collection of epidemiologic data, family history, and cancer history. It had also undertaken comprehensive mutation screening (21). To minimize bias because of patients having been selected on the basis of phenotype, we used a weighted cohort approach (26).

A limitation of our study is the inability to assess the gradient in risk with specific doses of aspirin and ibuprofen, as this information was not collected by the questionnaires. Long-term follow-up of a current trial could help resolve this issue and determine optimal aspirin treatment regimen. No studies investigating the dose-response effect of ibuprofen on colorectal cancer risk have been published. There have been concerns about the potential side effects of long-term aspirin use, particularly regarding the risk of hemorrhagic strokes and gastrointestinal bleeding beyond age 70 years (54,55). NSAIDs (perhaps particularly nonaspirin NSAIDs) are also associated with side effects, including what is called diaphragm disease—a non-neoplastic overgrowth in the small intestine (56,57). More research is needed for a better understanding of the determinants of these side effects (58). Recently, a comprehensive review and synthesis of the available evidence on the benefits and harms has found a strong net benefit for the prophylactic use of aspirin to reduce the incidence of colorectal and other cancers, and mortality, in the general population (59). However, recent results from the Women's Health Study have shown that the potential benefit of aspirin use in relation to cancer outcomes might only apply after age 64 years (60). In the context of Lynch Syndrome, where absolute cancer risk is high, use of aspirin as a chemoprevention strategy for colorectal cancer is likely to present a more favorable and consistent benefit-harm profile than in the general population.

Another limitation of this study is the possibility that the findings may have been affected by recall or response bias, given that data on the intake of aspirin and ibuprofen use and other variables were collected at the time of recruitment, and therefore, for those affected, after their diagnosis of colorectal cancer. Differential recall on exposures between participants diagnosed with colorectal cancer (as well as other cancers or polypectomy) and those who were censored at the time of interview is also a potential source of bias in our study. Similarly, our definition of duration of aspirin/ibuprofen use was based on the assumption that the use was continuous and recent. However, participants might have used aspirin/ibuprofen intermittently and we were unable to account for this variation in our analysis. Further, study participants might have used NSAIDs other than aspirin and ibuprofen that were not included in the Colon Cancer Family Registry questionnaire. It is therefore possible that some participants were misclassified as nonusers in the analysis. Finally, as the carriers with poorer survival were less likely to be included in this study (as they were less likely to be alive to be recruited

and give a blood sample), there is the possibility of survival and selection bias if age at onset and survival of case patients were related to exposure variables and/or mutation status. However, our sensitivity analysis restricted to carriers who were diagnosed with colorectal cancer or censored within five years prior to the interview showed findings similar to the main analysis.

In summary, this study provides additional observational evidence that aspirin use is associated with a reduction in risk of colorectal cancer for MMR gene mutations carriers. Our results suggest that regular long-term use of aspirin or ibuprofen might be an effective way to reduce colorectal cancer risk for this group of highly susceptible people who currently rely only on frequent colonoscopies to reduce their risk.

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Notes

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The authors have no conflict of interest to declare with respect to this manuscript.

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