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Aspirin and the Risk of Colorectal Cancer in Relation to the Expression of 15-Hydroxyprostaglandin Dehydrogenase (*15-PGDH, HPGD*)

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Abstract

Aspirin use reduces the risk of colorectal neoplasia, at least in part, through inhibition of prostaglandin-endoperoxide synthase 2 (PTGS2, cyclooxygenase 2)-related pathways. Hydroxyprostaglandin dehydrogenase 15-(NAD) (15-PGDH, HPGD) is downregulated in colorectal cancers and functions as a metabolic antagonist of PTGS2. We hypothesized that the effect of aspirin may be antagonized by low *15-PGDH* expression in the normal colon. In the Nurses' Health Study and the Health Professionals Follow-up Study, we collected data on aspirin use and other risk factors every two years and followed up participants for diagnoses of colorectal cancer. Duplication-method Cox proportional, multivariable-adjusted, cause-specific hazards regression for competing risks data was used to compute hazard ratios (HRs) for incident colorectal cancer according to *15-PGDH* mRNA expression level measured in normal mucosa from colorectal cancer resections. Among 127,865 participants, we documented 270 colorectal cancer cases that developed during 3,166,880 person-years of follow-up and from which we could assess *15-PGDH* expression. Compared with nonuse, regular aspirin use was associated with lower risk of colorectal cancer that developed within a background of colonic mucosa with high *15-PGDH* expression (multivariable HR=0.49; 95% CI, 0.34–0.71), but not with low *15-PGDH* expression (multivariable HR=0.90; 95% CI, 0.63–1.27) (*P* for heterogeneity=0.018). Regular aspirin use was associated with lower incidence of colorectal cancers arising in association with high *15-PGDH* expression, but not with low *15-PGDH* expression in normal colon mucosa. This suggests that *15-PGDH* expression level in normal colon mucosa may serve as a biomarker which may predict stronger benefit from aspirin chemoprevention.

Keywords

colon cancer; rectal cancer; adenocarcinoma; epigenomics; epigenetics

INTRODUCTION

Although substantial evidence from randomized controlled trials and observational studies demonstrates that aspirin reduces the risk of colorectal cancer, susceptibility to aspirin likely varies between individuals (1–10). Understanding of mechanisms underlying the anticancer effects of aspirin is important to establish effective chemopreventive strategies which target those individuals most likely to benefit from long-term use of the drug.

Aspirin, at least at high doses, may exert its effect through inhibition of prostaglandin-endoperoxide synthase 2 (PTGS2, cyclooxygenase 2), which catalyzes metabolic conversion of arachidonic acid to prostaglandins (11, 12). Constitutive up-regulation of PTGS2 activity is a key event in colorectal tumorigenesis (11–13). We and others have shown that hydroxyprostaglandin dehydrogenase 15-(NAD) (15-PGDH, EC 1.1.1.141) (encoded by the *HPGD* gene) plays an important role as an antagonist of PTGS2 during oncogenesis (14–20). 15-PGDH catalyzes prostaglandin degradation and is ubiquitously down-regulated in colorectal cancer (14–19). In a mouse model, knock out of *HPGD* increased colonic PGE2, markedly increased colon tumor numbers, and conferred resistance to the anti-colon tumor

effect of the PTGS2 inhibitor celecoxib (17). Similarly, in a pilot analysis of a clinical trial, low *15-PGDH* mRNA expression in normal colon mucosa was associated with lack of response to celecoxib for the prevention of recurrent adenomatous colon polyps (17).

Based on these findings, we hypothesized that susceptibility to aspirin might differ according to *15-PGDH* expression level in the colon. Within 2 nationwide cohorts, we examined whether regular aspirin use was associated with a lower risk of colorectal cancer arising in settings of high *15-PGDH* mRNA expression in normal colonic mucosa, but not in cancers arising with low normal colon *15-PGDH* expression.

RESULTS

Characteristics of the participants

Among 127,865 participants (82,095 women and 45,770 men), we documented 270 colorectal cancer cases [165 cases in the Nurses' Health Study (NHS) and 105 cases in the Health Professionals Follow-up Study (HPFS)] with available data on *15-PGDH* expression level in normal colonic mucosa and aspirin use status, which developed during 3,166,880 person-years of follow-up (2,272,266 person-years in the NHS and 894,613 person-years in the HPFS). We classified aspirin use status based on the cumulative mean for the number of aspirin tablets consumed per week up to each biennial follow-up cycle. Aspirin use status and other covariates were modeled as time-varying variables to take into account potential changes in exposures over follow-up time. At baseline, compared with nonusers, regular aspirin users were more likely to be older, have a higher BMI, smoke, undergo endoscopy, use postmenopausal hormone (women only), consume higher amounts of alcohol and folate, use multivitamins, and engage in less physical activity (Table 1).

Correlation of regular aspirin use and incident colorectal cancer with colon *15-PGDH* expression

15-PGDH mRNA expression was quantified in matched normal mucosa from 270 colorectal cancer cases, enabling these cases to be divided into those arising in colons with high (greater than median) *15-PGDH* expression versus those arising in colons with low (lower than median) *15-PGDH* expression. These two groups were respectively designated normal colon high *15-PGDH* versus normal colon low *15-PGDH* type colon cancers. Both types of cancer were then modeled simultaneously in a competing risk model. Demographic, clinical, and pathological characteristics at diagnosis, including status of *BRAF* mutation, *PIK3CA* mutation, and PTGS2 expression which have been previously noted to differ by aspirin use, were not significantly different according to *15-PGDH* expression level (Table S1). In the combined cohort, the benefit of regular aspirin use appeared to be confined to decreasing the risk only of normal colon high *15-PGDH* colorectal cancers (*P* for heterogeneity=0.018) (Table 2). Compared with nonuse, the age-adjusted HR of regular aspirin use for developing normal colorectal high *15-PGDH* type cancers was 0.51 (95% CI, 0.35–0.73). This inverse association remained significant after adjustment for lifestyle and other risk factors (multivariable HR=0.49; 95% CI, 0.34–0.71). In contrast, regular aspirin use was not associated with a lower risk of developing normal colon low *15-PGDH* cancers, with a multivariable HR of 0.90 (95% CI, 0.63–1.27). This finding was independently observed

within both study cohorts, with regular aspirin use associated with reduced multivariable HRs for normal colon high *15-PGDH* colorectal cancers to 0.58 (95% CI, 0.35–0.94) in the NHS and to 0.44 (95% CI, 0.25–0.76) in the HPFS. In contrast, regular aspirin use was not associated with reduced HR for developing normal colon low *15-PGDH* type cancers in either the NHS or HPFS. Consistent results were observed in the analyses of aspirin according to dosage and duration (Table S2 and Table S3).

We further conducted a secondary analysis that additionally adjusted for use of non-steroidal anti-inflammatory drugs (NSAIDs) (Table S4). Inclusion of data on NSAID use did not materially alter the differential association we observed, in which regular aspirin use was associated with reduced risk of only normal colon high *15-PGDH* type cancers. After adjustment for NSAIDs, regular aspirin use was again associated with a lower risk of normal colon high *15-PGDH* colorectal cancers (multivariable and NSAID-adjusted HR=0.47; 95% CI, 0.32–0.68) but not of normal colon low *15-PGDH* type cancers (multivariable and NSAID-adjusted HR=0.85; 95% CI, 0.60–1.21).

DISCUSSION

In two prospective cohorts, we found that regular aspirin use was associated with a lower incidence of colorectal cancers arising in association with high *15-PGDH* expression in the normal colon mucosa, whereas regular aspirin use showed no association with reduced incidence of colorectal cancers that arose in colon mucosa with low normal *15-PGDH* expression. Consistent results were observed in the analyses of aspirin according to dosage and duration. These findings support the hypothesis that the anticancer activity of aspirin in colonic mucosa is at least in part dependent on high *15-PGDH* expression and that low expression of *15-PGDH* may impart potential resistance to aspirin's tumor preventive effects.

15-PGDH is a physiologic antagonist to the PTGS2 oncogene that mediates oncogenesis through inflammatory responses (14–17). In a mouse model, we have previously shown that knockout of the *HPGD* gene confers resistance to anti-tumor effect of the PTGS2-selective inhibitor celecoxib, resulting in continued colon tumor development in celecoxib-treated knockout but not in celecoxib-treated wild-type mice (17). In parallel, the ability of celecoxib to lower colonic PGE2 was also attenuated in knockout mice (17). Moreover, in a pilot analysis of patients enrolled in a clinical trial of celecoxib for chemoprevention of recurrent adenoma, low *15-PGDH* expression in normal colon mucosa was associated with celecoxib resistance and with continued adenoma development during celecoxib treatment (17). Taken together with previous findings that *15-PGDH* expression is consistently abolished within colorectal cancers (14–16), our results further confirm a model in which 15-PGDH functions as a key tumor suppressor of colon neoplasms, and suggest that a critical level of 15-PGDH activity is required for aspirin to exert its anti-neoplastic effects. Conversely, reduction of *15-PGDH* expression and its attendant tumor suppressive activity within normal mucosa may abrogate the chemopreventive benefits of aspirin.

Despite extensive experimental studies on both the anti-cancer properties of aspirin and the central role of 15-PGDH in colorectal neoplasia (21–27), there are no data available

regarding whether the effectiveness of aspirin varies among individuals of differing *15-PGDH* expression levels (14–20). We have previously shown that certain molecular features are associated with a subclass of colon cancers that are resistant to aspirin prevention, including low expression of PTGS2 and mutation of *BRAF*, which is a key regulator of PTGS2 activity in RAF-MAPK signaling (11, 13). Our present results substantially extend upon these findings by demonstrating that constitutional differences in individuals' normal colons, namely in *15-PGDH* expression, may also be associated with resistance to chemoprevention with aspirin. Both aspirin and 15-PGDH act to lower prostaglandin levels, aspirin through inhibition of PTGS and 15-PGDH through catalyzing prostaglandin degradation. Our data suggest that both effective PTGS inhibition and robust prostaglandin degradation are required to lower risk of colorectal cancer. Although aspirin is a highly effective chemopreventive agent, widespread use of the drug is not currently recommended due to uncertainty about its risk-benefit profile (28). Our findings suggest the possibility that *15-PGDH* levels in normal colonic mucosa could be exploited to identify individuals who will have greatest likelihood of benefit from regular use of aspirin for chemoprevention.

Our study had several strengths. First, we used prospectively collected data on aspirin use in relation to colorectal cancer, minimizing potential recall bias. Second, our follow-up of over 28 years allowed us to evaluate the long-term association between regular aspirin use and colorectal cancer incidence. Third, repeated measures and updated assessments of aspirin use in each 2-year questionnaire enabled us to consider potential changes over time. Finally, comprehensive collection of data on other lifestyle risk factors for colorectal cancer allowed us to adjust for potential confounding variables.

Our study also had limitations. First, we did not have information on expression of *15-PGDH* in colon mucosa before the diagnosis of cancer. Second, although normal colon blocks are in most cases prepared from the surgical margins at the edge of a colon resection, the location of the normal colon samples tested would have differed among the cases studied, and we lacked precise information on this point. Mitigating these two limitations, we have recently shown that *15-PGDH* expression is stable along the length of the colon, and is also stable over time (29). Additionally, we have also shown that aspirin administration does not significantly alter colonic *15-PGDH* expression (29). Moreover, in our previous study we found that levels of *15-PGDH* in biopsies taken prior to treatment with celecoxib predicted likelihood of the development of new adenomas (17). Nonetheless, future studies will be needed in which *15-PGDH* levels are assessed prospectively, both before and during aspirin treatment, in individuals monitored for development of colonic neoplasia. A third limitation is that we relied on self-reported aspirin use, but this has previously been shown to be highly reproducible within our cohorts, because the participants were all health professionals (11). Fourth, the potential for confounding by unmeasured or unknown factors could not be fully eliminated, although we adjusted for several major lifestyle and risk factors in the analysis. Fifth, we did not have measurements of *15-PGDH* expression in normal mucosa for all cases of colorectal cancer that developed within our cohort. Last, our study population was predominantly non-Hispanic white, and our results might not be generalizable to other ethnic groups.

In summary, we show that regular aspirin use is associated with a lower risk of colorectal cancer that arises in association with high, but not low, expression of *15-PGDH* in the normal colon mucosa. Our data provide evidence that the anticancer effect of aspirin requires cooperation with an active 15-PGDH tumor suppressor pathway. Further studies are needed to determine the potential utility of using *15-PGDH* expression in colonic mucosa as a biomarker to risk-stratify individuals for aspirin-based chemoprevention.

MATERIALS AND METHODS

Study design

This study aimed to examine whether susceptibility to aspirin might differ according to *15-PGDH* expression in the colon. The hypothesis was developed on the basis of our previously reported observations in a mouse model and pilot analysis of a clinical trial (17). Analysis of *15-PGDH* mRNA expression was performed by validated real-time PCR assay in a blinded fashion. The *15-PGDH* mRNA expression was determined as the average levels obtained from three independent real-time PCR reactions. Information on aspirin use was collected every 2 years prospectively. To assess our hypothesis, duplication-method, multivariable-adjusted, cause-specific Cox proportional hazards regression for competing risks data was used to compute HRs for incident colorectal cancer according to *15-PGDH* mRNA expression.

Study population

The Nurses' Health Study (NHS) was established in 1976, enrolling 121,701 US registered female nurses aged 30 to 55 years. The Health Professionals Follow-up Study (HPFS) was initiated in 1986, including 51,529 US male health professionals aged 40 to 75 years. Every two years, questionnaires were mailed to participants, and information on lifestyle factors and medical history has been collected. We obtained informed consent from all participants. This study was approved by Human Subjects Committees at Harvard School of Public Health and Brigham and Women's Hospital.

Identification of colorectal cancer cases

In both cohorts, cancer and other disease outcomes were reported by participants on the biennial questionnaires. Deaths were reported by family members or the postal system, or identified through a search of the National Death Index. After obtaining consent from the participants or their next-of-kin, medical records and pathological reports were obtained and reviewed by study physicians to abstract information on tumor location, stage, and histologic type of the cancer. We retrieved formalin-fixed paraffin-embedded tissue blocks from hospitals throughout the U.S. where participants with colorectal cancer had undergone surgical resection (30). We collected diagnostic biopsy specimens for rectal cancer patients who received preoperative treatment, to minimize biases associated with preoperative radiation treatment. Hematoxylin and eosin stained tissue sections, including normal and tumor sections from all colorectal cancer cases, were reviewed by a pathologist (S.O.).

Assessment of aspirin use

Detailed description of aspirin assessment in the cohorts has been reported previously (11, 13). In the NHS, we collected the information on aspirin usage and the number of tablets taken per week, beginning in 1980 and every 2 years thereafter except in 1986. In the HPFS, we inquired about aspirin usage, beginning in 1986 and every 2 years thereafter. The number of tablets taken per week was first collected in 1992 for the HPFS. In both cohorts, participants were asked about standard dose (325 mg) tablets. Participants were also requested to convert intake of 4 baby aspirin tablets (81 mg) to 1 standard aspirin tablet in the questionnaires, beginning in 1992. To minimize within individual variation and to estimate long-term influence, we calculated the cumulative mean for the number of aspirin tablets consumed per week, which was the mean of all available data on tablets per week up to each biennial follow-up cycle (11, 13).

In the NHS, we defined regular aspirin users as women who consumed 2 or more standard-dose (325 mg) aspirin tablets per week and non-users as those who reported intake of fewer aspirin per week. In the HPFS, men who reported consumption of standard-dose (325 mg) aspirin at least 2 times per week were defined as regular users, and men who reported less frequent aspirin consumption were defined as nonusers. To better reflect potential changes of aspirin use over follow-up time, status of regular aspirin use was updated every 2 years in our analysis.

Analysis of *15-PGDH* (*HPGD*) expression

In most cases, normal colon was less than 20 cm away from the tumor. Four unstained sections of 5 μm thickness were cut from the block of normal colon tissue, and the mucosal layer was retrieved by scraping. RNA was extracted with a High Pure RNA FFPE RNA Micro kit (Roche). Real-time PCR was performed as described previously (17) to quantitate the expression level of *15-PGDH* mRNA, following the MIQE guidelines (31) and the software programs (32–34), as fully detailed in Supplementary methods.

Statistical analysis

We included participants who provided baseline aspirin data in 1980 for the NHS and in 1986 for the HPFS. We excluded participants with a history of cancer (except for nonmelanoma skin cancer), inflammatory bowel disease, or familial polyposis at baseline. Participants were followed from the date of baseline questionnaire return through July 1, 2008, date of colorectal cancer diagnosis, or date of death, whichever came first.

We used a Cox proportional cause-specific hazards regression model with a duplication method for competing risks data and computed hazard ratio (HR) and 95% confidence interval (CI), to examine whether the association between aspirin use and colorectal cancer incidence differed according to *15-PGDH* expression in normal colonic mucosa. A heterogeneity test was conducted using a likelihood ratio test, comparing a model that allowed for different associations of aspirin use by *15-PGDH* expression with a model that assumed a common association. We used SAS software version 9.3 (SAS Institute Inc.) to stratify all analyses by age (in months), sex (in the analysis using combined cohorts), and year of questionnaire return. We further conducted multivariable analyses, including body

mass index (<25, 25–29.9 or ≥ 30 kg/m^2), smoking status (never, former, or current), family history of colorectal cancer in any first-degree relative (yes or no), previous lower gastrointestinal endoscopy (no endoscopy, confirmed history of adenomatous polyps, or endoscopy without detection of neoplasia), postmenopausal hormone use (for women only, never or ever), physical activity [quintiles of metabolic-equivalent task (MET) hours per week], total red meat intake (quintiles of servings per day), alcohol consumption (0 or quartile of grams per day), total caloric intake (quintiles of kcal per day), folate intake (quintiles of μg per day), calcium intake (quintiles of mg per day), and current multivitamin use (yes or no). MET scores were calculated as previously described (35, 36), and values for individual activities were summed to give a total MET hours per week. A secondary analysis, which adjusted for the regular use of NSAIDs, was conducted using the data from 1990 for the NHS and from 1986 for the HPFS, the time points at which information on NSAIDs use began to be collected routinely in these cohorts.

For all analyses, we used the most updated data prior to each follow-up cycle, and modeled them as time-varying variables to take into account potential changes over time. Cumulative mean was used for the continuous variables, including body mass index, physical activity, total red meat intake, alcohol consumption, and intake of calories, folate and calcium. Before pooling the two cohorts, we examined the possible heterogeneity between cohorts, using the Q statistics for the association between aspirin use and colorectal cancer incidence. We did not observe significant heterogeneity between cohorts (P=0.52 for cancer with low *15-PGDH; P*=0.46 for cancer with high *15-PGDH*). We tested the proportional hazards assumption based on the interaction terms between aspirin use and follow-up time and observed no evidence of violation (P=0.56 for cancer with low *15-PGDH; P*=0.88 for cancer with high *15-PGDH*). All analyses were two sided, and P<0.05 was considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

15-PGDH	hydroxyprostaglandin dehydrogenase 15-(NAD)
BMI	body mass index
CI	confidence interval
HPFS	Health Professionals Follow-up Study
HR	hazard ratio
MAPK	mitogen-activated protein kinase
MET	metabolic equivalent task
NHS	Nurses' Health Study
NSAID	non-steroidal anti-inflammatory drug
PTGS2	prostaglandin-endoperoxide synthase 2
SD	standard deviation

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Table 1

Age-adjusted demographic characteristics according to regular aspirin use status at baseline

	All (combined cohorts)		Men (Health Professionals Follow-up Study)		Women (Nurses' Health Study)	
	Nonusers (N=85,725)	Regular users (N=42,140)	Nonusers (N=32,398)	Regular users (N=13,372)	Nonusers (N=53,327)	Regular users (N=28,768)
Age[a], year; mean (SD)	49.1 (8.9)	49.8 (9.1)	53.4 (9.7)	56.1 (9.8)	46.5 (7.2)	46.8 (7.1)
BMI, kg/m^2; mean (SD)	24.5 (3.9)	24.7 (4.1)	25.4 (3.2)	25.7 (3.3)	23.9 (4.1)	24.3 (4.4)
Smoking status, %						
Never	46	42	49	42	44	42
Former	32	34	41	47	27	28
Current	21	24	10	10	28	30
Family history of colorectal cancer in any first-degree relative, %	8	8	8	8	8	8
Lower gastrointestinal endoscopy status, %						
No endoscopy	83	82	71	69	90	89
Endoscopy[b]	17	18	28	31	10	11
Postmenopausal hormone use (ever), %	-	-	-	-	41	46
Physical activity, METs hours/week[c]; mean (SD)	17.3 (25.4)	16.0 (23.0)	21.0 (29.1)	20.9 (29.5)	14.3 (21.3)	13.5 (18.4)
Total red meat intake, servings/day; mean (SD)	1.3 (0.8)	1.3 (0.8)	1.1 (0.8)	1.1 (0.8)	1.4 (0.8)	1.4 (0.8)
Alcohol consumption, g/day; mean (SD)	7.9 (12.5)	8.6 (13.0)	10.8 (15.0)	12.5 (16.1)	6.1 (10.4)	6.8 (10.8)
Total calorie intake, Kcal/day; mean (SD)	1712 (585)	1723 (576)	1974 (618)	2021 (626)	1549 (499)	1594 (502)
Folate intake, µg/day; mean (SD)	403 (285)	411 (273)	472 (274)	498 (280)	360 (284)	371 (260)
Calcium intake, mg/day; mean (SD)	794 (370)	785 (360)	890 (423)	917 (429)	736 (320)	725 (304)
Multivitamin use, %	34	42	39	50	31	38

BMI, body mass index; MET, metabolic equivalent task; SD, standard deviation.

Values are standardized to the age distribution of the study population.

In the NHS, regular aspirin use was defined as the consumption of at least two 325 mg tablets per week, and nonuse was defined as consumption of fewer than two tablets per week. In the HPFS, regular aspirin use was defined as the consumption of aspirin at least two times per week, and nonuse use was defined as the consumption of aspirin fewer than two times per week.

[a] Age (year) is not age adjusted.

[b] Endoscopy includes sigmoidoscopy or colonoscopy.

[c] MET calculated according to the frequency of a range of physical activities in 1986 for both women and men.

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Table 2

Regular aspirin use and incident colorectal cancer grouped by *HPGD* (*15-PGDH*) mRNA expression in normal mucosa

		Nonusers	Regular users	*P* value	*P* for heterogeneity[c]
All (combined cohorts)					
Low expression of *15-PGDH* mRNA	Person-years	1,849,337	1,317,543		
	Incidence (n)	72	62		
	Age-adjusted HR (95% CI)[a]	1 [referent]	0.91 (0.64–1.28)	0.58	
	Multivariable HR (95% CI)[b]	1 [referent]	0.90 (0.63–1.27)	0.53	0.018
High expression of *15-PGDH* mRNA	Incidence (n)	93	43		
	Age-adjusted HR (95% CI)[a]	1 [referent]	0.51 (0.35–0.73)	0.0003	
	Multivariable HR (95% CI)[b]	1 [referent]	0.49 (0.34–0.71)	0.0002	
Women (Nurses' Health Study)					
Low expression of *15-PGDH* mRNA	Person-years	1,364,478	907,788		
	Incidence (n)	51	37		
	Age-adjusted HR (95% CI)[a]	1 [referent]	0.84 (0.55–1.28)	0.41	
	Multivariable HR (95% CI)[b]	1 [referent]	0.82 (0.53–1.26)	0.36	0.28
High expression of *15-PGDH* mRNA	Incidence (n)	53	24		
	Age-adjusted HR (95% CI)[a]	1 [referent]	0.58 (0.36–0.94)	0.028	
	Multivariable HR (95% CI)[b]	1 [referent]	0.58 (0.35–0.94)	0.027	
Men (Health Professionals Follow-up Study)					
Low expression of *15-PGDH* mRNA	Person-years	484,858	409,755		
	Incidence (n)	21	25		
	Age-adjusted HR (95% CI)[a]	1 [referent]	1.06 (0.59–1.93)	0.84	
	Multivariable HR (95% CI)[b]	1 [referent]	1.05 (0.57–1.91)	0.88	0.034
High expression of *15-PGDH* mRNA	Incidence (n)	40	19		
	Age-adjusted HR (95% CI)[a]	1 [referent]	0.43 (0.25–0.74)	0.003	
	Multivariable HR (95% CI)[b]	1 [referent]	0.44 (0.25–0.76)	0.004	

CI, confidence interval; HR, hazard ratio.

In the NHS, regular aspirin use was defined as the consumption of at least two 325 mg tablets per week and nonuse was defined as consumption of fewer than two tablets per week. In the HPFS, regular aspirin use was defined as the consumption of aspirin at least two times per week and nonuse was defined as the consumption of aspirin fewer than two times per week.

[a] All analyses were stratified by age (in month), year of questionnaire return, and sex in the analysis of combined cohorts.

[b] Multivariable HR was further adjusted for body mass index (<25 vs. 25–29.9 vs. ≥ 30 kg/m^2), smoking status (never vs. former vs. current), family history of colorectal cancer in any first-degree relative, endoscopy status (no endoscopy vs. history of adenomatous polyps vs. negative endoscopy), physical activity level [quintiles of mean metabolic equivalent (MET) task hours per week], red meat intake (quintiles of servings/day), total calorie intake (quintiles of kcal/day), alcohol consumption (0 or quartiles of g/day), folate intake (quintiles of μg/day), calcium intake (quintiles of mg/day), and current multivitamin use. Models were adjusted for postmenopausal hormone use in the analysis of women.

[c] *P* for the heterogeneity of the association of regular aspirin use with colorectal cancer, grouped by *HPGD* (*15-PGDH*) mRNA expression in normal mucosa.