DOI:10.1093/jnci/djs482
Advance Access publication November 21, 2012

ARTICLE

Prospective Study of Family History and Colorectal Cancer Risk by Tumor LINE-1 Methylation Level

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Manuscript received August 15, 2012; revised September 27, 2012; accepted October 18, 2012.

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Background Beyond known familial colorectal cancer (CRC) syndromes, the mechanisms underlying the elevated CRC risk associated with CRC family history remain largely unknown. A recent retrospective study suggests familial clustering of CRC with hypomethylation in long interspersed nucleotide element 1 (LINE-1). We tested the hypothesis that CRC family history might confer a higher risk of LINE-1 methylation-low CRC.

Methods Using the Nurses' Health Study and the Health Professionals Follow-up Study, we prospectively examined the association between CRC family history and the risk of rectal and colon cancer (N = 1224) according to tumor LINE-1 methylation level by duplication method Cox proportional hazards regression. We examined microsatellite instability (MSI) status to exclude the influence of Lynch syndrome. All statistical tests were two-sided.

Results The association between CRC family history and non-MSI CRC risk differed statistically significantly by LINE-1 methylation level ($P_{heterogeneity}$ = .02). CRC family history was associated with a statistically significantly higher risk of LINE-1 methylation-low non-MSI cancer (multivariable hazard ratio [HR] = 1.68, 95% confidence interval [CI] = 1.19 to 2.38 for 1 vs 0 first-degree relatives with CRC; multivariable HR = 3.48, 95% CI = 1.59 to 7.6 for ≥2 vs 0 first-degree relatives with CRC; P_{trend} < .001). In contrast, CRC family history was not statistically significantly associated with LINE-1 methylation-high non-MSI cancer (P_{trend} = .35).

Conclusions This molecular pathological epidemiology study shows that CRC family history is associated with a higher risk of LINE-1 methylation-low CRC, suggesting previously unrecognized heritable predisposition to epigenetic alterations. Additional studies are needed to evaluate tumor LINE-1 methylation as a molecular biomarker for familial cancer risk assessment.

J Natl Cancer Inst 2013;105:130–140

Epidemiological evidence indicates that a family history of colorectal cancer (CRC) is associated with higher personal CRC risk (1–5). Beyond known familial CRC syndromes (including Lynch and polyposis syndromes), which together constitute less than 5% of CRC cases (6), the mechanisms underlying familial clustering of CRC remain largely unknown (7–9). Recent data by Goel et al. (10) suggest that one potential mechanism of familial clustering of CRC may be heritable predisposition to epigenomic instability and the development of tumors with hypomethylation in long interspersed nucleotide element 1 [LINE-1, which comprises approximately 17% of the human genome (11)]. Thus, we hypothesized that a family history of CRC might be associated with higher risk of CRC with low-level LINE-1 methylation.

Because LINE-1 methylation-low CRC has been associated with aggressive tumor behavior (12–14), it is imperative to develop effective prevention strategies tailored to those who are susceptible to the development of this unfavorable cancer subtype.

To test our hypothesis of possible familial clustering of LINE-1 methylation-low CRC, we utilized two US nationwide prospective cohort studies. We prospectively examined the relationship between a history of CRC in a first-degree relative and subsequent risk of developing CRC with varying degrees of LINE-1 methylation. We also examined the status of tumor microsatellite instability (MSI) and CpG island methylator phenotype (CIMP), both of which have been inversely associated with LINE-1 methylation level (15,16). In particular, we utilized tumor MSI status in our attempt to exclude the potential influence of Lynch syndrome (ie, heritable susceptibility to mismatch repair-deficient cancer, most likely to be MSI-high cancer). We aimed to support not only a possible link between the heritability of CRC and tumor epigenetic instability but also the possible presence of a previously unrecognized familial cancer trait. Akin to current MSI testing in CRC, tumor LINE-1 methylation level may potentially serve as a tumor biomarker for familial cancer risk assessment.

Methods

Study Population

Details on our study population are described in the Supplementary Materials (available online). We utilized the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) (5,17,18). Eligible participants included 86 172 women (NHS) and 47 907 men (HPFS). The Harvard School of Public Health and Brigham and Women's Hospital Institutional Review Boards approved this study. All subjects provided informed consent.

Family History Data Collection

We utilized family history data prospectively collected from questionnaires (before a participant developed CRC if it occurred) to avoid recall bias, which is a major problem in assessing family history of cancer (19–22). A history of CRC in first-degree relatives was collected in 1982, 1988, 1992, 1996, 2000, and 2004 in the NHS and in 1986, 1990, 1992, and 1996 in the HPFS.

Assessment of Incident CRC Cases

On each biennial follow-up questionnaire, participants were asked whether they had a diagnosis of CRC. For nonresponders, we searched the National Death Index to discover deaths and ascertain any diagnoses of CRC. We collected paraffin-embedded CRC tissue blocks from hospitals where participants with CRC had undergone tumor resection (18). For rectal cancer, we collected diagnostic biopsy and resection specimens to avoid any effect of preoperative treatment on tumor tissue analyses. Based on the colorectal continuum model (23,24), we used both colon and rectal cancers as outcomes. Based on the availability of tumor tissue data, 1224 CRC cases diagnosed up to 2008 were included as outcome data. A pathologist (S. Ogino) reviewed the histopathology of all 1224 CRC cases. Distributions of age, sex, tumor subsite location, disease stage, and pathologic features of our CRC cases have been previously described (23) and are generally consistent with cancer registry data in the United States. Furthermore, patient characteristics did not appreciably differ between cases with and without available tissue (18), so no analyses by major racial/ethnic group were done.

Tumor LINE-1 Methylation Analysis

DNA was extracted from archival tumor tissue. We employed validated bisulfite DNA treatment (25), polymerase chain reaction, and pyrosequencing assay to measure LINE-1 methylation level (26). Precision of the LINE-1 methylation assay was high, with a coefficient of variation of approximately 3% to 4% (26). Moreover, DNA from a whole tissue tumor section yielded LINE-1 methylation values comparable with DNA from pure tumor cells collected by laser capture microdissection (26). We classified LINE-1 methylation level into low (<55%), intermediate (55%–64.9%), and high (≥65%) designations, which reflected methylation levels relative to the overall distribution of the 1224 tumors. The cut points of 55% and 65% LINE-1 methylation levels were chosen to subclassify tumors, as previously described (27), to keep consistency in classification. An alternative strategy would be tertile or quartile classification. Although distribution of LINE-1 methylation level in our dataset resembled a normal distribution, the lower tail had many more cases than expected by a normal distribution (28). Neither tertile nor quartile cut points worked well to capture the nature of methylation-low cases because their lowest cut points were greater than 55%. Quintile cut points would have made too many categories and yielded less robust effect estimates.

Analysis for CIMP

Using a validated real-time polymerase chain reaction assay (MethyLight) on bisulfite-treated DNA (25), we quantified DNA methylation in eight CIMP-specific promoters (*CACNA1G*, *CDKN2A* (p16), *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3*, and *SOCS1*) (29–31). CIMP-high was defined as the presence of six or more of eight methylated promoters, and CIMP-low/negative (CIMP-low/0) was defined as zero of eight to five of eight methylated promoters, as per established criteria (31,32).

MSI Analysis

MSI analysis was performed utilizing 10 microsatellite markers (31). MSI-high cancer was defined as instability in 30% or more of the markers, and microsatellite stable (MSS) cancer was defined as instability in 0% to 29% of the markers (31).

Statistical Analysis

Detailed statistical analysis methods are described in the Supplementary Materials (available online). All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Our primary study hypothesis was that CRC family history was associated with an increased risk of LINE-1 methylation-low MSS cancer (after controlling for the effect of Lynch syndrome) but not with the risk of LINE-1 methylation-high MSS cancer. We used Cox proportional hazards regression model to estimate a hazard ratio (HR) with a 95% confidence interval (CI) of developing a specific CRC subtype by CRC family history, adjusted for multiple potential confounders. Nonetheless, we cautiously interpreted statistical significance in subset analyses that resulted from our molecular pathological epidemiology design (33,34). All statistical tests were two-sided. A P value less than .05 was considered statistically significant.

Results

Family History and CRC Risk According to Molecular Subtypes

We followed a baseline population of 86 172 women and 47 907 men in the NHS and the HPFS, respectively. Characteristics of the population during the follow-up period are summarized in Table 1. There was no substantial difference in characteristics according to CRC family history status, except for age. During 3 184 415 person-years of follow-up in both cohorts, we documented 1224 incident CRC cases with available tumor molecular data. Distributions of LINE-1 methylation levels are shown in Figure 1 (overall CRCs) and Figure 2 (in relation to family history status).

In the NHS (women), compared with individuals without CRC family history, those with CRC family history experienced a statistically significantly higher overall CRC risk and higher risks for all

Table 1. Age-adjusted characteristics of participants (during follow-up), according to family history of colorectal cancer (CRC) in first-degree relatives*

No. of first-degree relatives with CRC†	Women (Nurses' Health Study)			Men (Health Professionals Follow-up Study)			Total		
	0	1	≥2	0	1	≥2	0	1	≥2
No. of study participants in each family history category as final status‡	70 770 (82.1%)	13 831 (16.1%)	1571 (1.8%)	41 336 (86.3%)	6244 (13.0%)	327 (0.7%)	112 106 (83.6%)	20 075 (15.0%)	1898 (1.4%)
Total person-years	1 967 983	262 761	22 250	819 660	107 327	4433	2 787 643	370 088	26 684
Age, y	58.6 (10.5)	62.2 (10.1)	66.5 (9.4)	62.7 (10.9)	65.2 (10.6)	69.6 (9.9)	59.8 (10.8)	63.1 (10.3)	67.0 (9.6)
Regular aspirin use, %	42	42	42	51	50	51	44	44	44
Multivitamin use, %	49	49	48	54	55	55	51	51	49
Physical activity, MET score/week	16.7 (22.0)	17.1 (21.9)	17.0 (21.5)	34.2 (40.0)	34.7 (38.9)	33.0 (38.3)	21.8 (29.6)	21.8 (28.5)	19.2 (25.0)
Body mass index, BMI, kg/m^2	24.3 (4.7)	24.3 (4.8)	24.1 (4.5)	25.5 (3.3)	25.5 (3.3)	25.2 (3.5)	24.6 (4.4)	24.6 (4.5)	24.3 (4.4)
Folate intake, μg/day §	365 (276)	366 (263)	357 (245)	481 (277)	485 (278)	504 (315)	400 (282)	399 (273)	378 (262)
Methionine intake, g/day §	1.9 (0.5)	1.9 (0.5)	1.9 (0.5)	2.2 (0.5)	2.2 (0.5)	2.2 (0.4)	2.0 (0.5)	2.0 (0.5)	1.9 (0.5)
Calcium intake, mg §	908 (350)	914 (343)	905 (343)	924 (375)	921 (377)	869 (346)	912 (357)	917 (353)	905 (346)
Red meat intake, serving/week §	2.1 (1.5)	2.1 (1.4)	2.0 (1.3)	1.7 (1.4)	1.7 (1.4)	1.7 (1.4)	2.0 (1.5)	2.0 (1.4)	2.0 (1.3)
Alcohol intake, g/day	6.3 (10.3)	6.4 (10.5)	6.1 (10.2)	11.1 (15.0)	11.1 (14.8)	11.9 (17.3)	7.7 (12.1)	7.7 (12.0)	6.9 (11.7)
Smoking, current/former, %	56	55	56	55	54	50	55	55	56

* Updated information of exposures from biennial questionnaires was averaged using person-years in each category of family history status up to censoring (including death from other causes) or immediately before personal CRC diagnosis if it occurred. In the rows from "Age" to "Smoking," each value represents a mean (standard deviation) or percentage number and is standardized to the age distribution of the study population (except for age). BMI = body mass index; MET = metabolic equivalent task.

† Not including offspring.

‡ Final status was derived from updated family history status at censoring (including death from other causes) or immediately before personal CRC diagnosis if it occurred (to avoid recall bias).

§ Energy-adjusted intake.

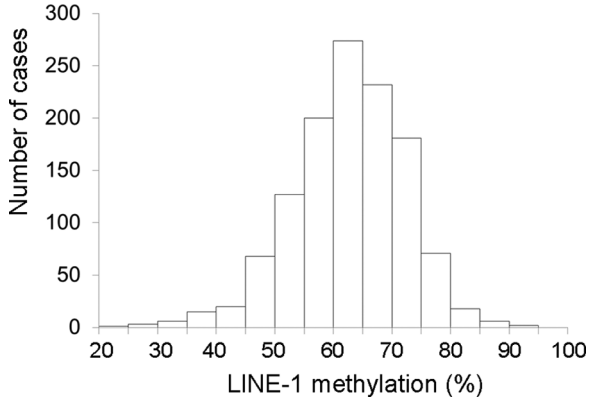

Figure 1. Distribution of long interspersed nucleotide element 1 (LINE-1) methylation levels in 1224 colorectal cancer cases in the Nurses' Health Study and the Health Professionals Follow-up Study. LINE-1 methylation level was determined by polymerase chain reaction on bisulfite-modified genomic DNA from archival paraffin-embedded tumor tissue, followed by pyrosequencing, as described in the Methods.

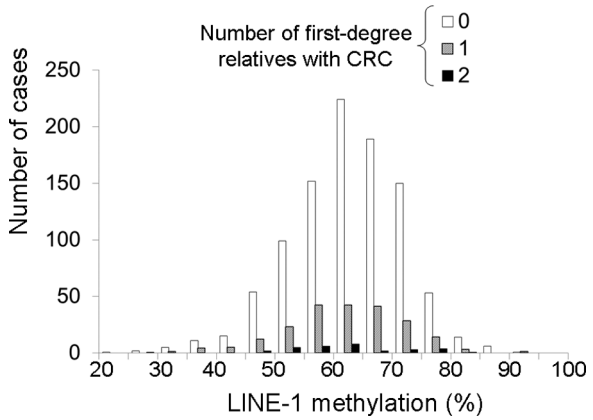

Figure 2. Distribution of long interspersed nucleotide element 1 (LINE-1) methylation levels in 1224 colorectal cancer cases according to the number of first-degree relatives with colorectal cancer (CRC) in the Nurses' Health Study and the Health Professionals Follow-up Study. Information on the number of first-degree relatives with CRC was obtained from cohort participants before CRC diagnosis. LINE-1 methylation level was determined by polymerase chain reaction on bisulfite-modified genomic DNA, followed by pyrosequencing, as described in the Methods.

tumor subtypes examined (Table 2). The cancer risk associated with family history appeared to be higher for the LINE-1 methylation-low tumor subtype than the LINE-1 methylation-high subtype and higher for the MSI-high subtype than the MSS subtype, although the differences were not statistically significant ($P_{heterogeneity} > .10$).

In the HPFS (men), compared with individuals without CRC family history, those with CRC family history experienced a statistically significantly higher overall CRC risk and higher risks for all tumor subtypes examined except for the LINE-1 methylation-high subtype (Table 3). The cancer risk associated with family history appeared to be higher for the LINE-1 methylation-low subtype than the LINE-1 methylation-high subtype and higher for the MSI-high subtype than the MSS subtype, although the differences were not statistically significant ($P_{heterogeneity} > .09$).

There was no statistically significant heterogeneity between the two cohorts (ie, women and men) in the relationships of CRC family history with overall CRC risk or with the risk of each tumor subtype (Q statistics $P \geq .30$). To increase statistical power, we combined the two cohorts for further analyses.

In the combined cohorts, the association between the number of first-degree relatives with colorectal cancer (0, 1, ≥2; an ordinal scale) and CRC risk appeared to differ by LINE-1 methylation status (an ordinal scale of 3 levels), although the difference was not statistically significant ($P_{heterogeneity} = .06$) (Table 4). Compared with individuals without CRC family history, those with CRC family history experienced a substantially higher risk of LINE-1 methylation-low CRC (multivariable HR = 1.63, 95% CI = 1.16 to 2.28 for 1 vs 0 first-degree relatives with CRC; multivariable HR = 4.32, 95% CI = 2.15 to 8.67 for ≥2 vs 0 first-degree relatives with CRC; $P_{trend} < .001$) (Table 4). To a lesser degree, CRC family history was associated with higher risks of LINE-1 methylation-intermediate CRC ($P_{trend} < .001$) and LINE-1 methylation-high CRC ($P_{trend} = .007$) (Table 4).

In the combined cohorts, the association between the number of first-degree relatives with CRC (0, 1, ≥2; an ordinal scale) and CRC risk statistically significantly differed by MSI status ($P_{heterogeneity} = .03$) (Table 4). In contrast, CRC risk associated with family history did not differ statistically significantly by CIMP status ($P_{heterogeneity} = .40$).

CRC Family History and MSS Cancer Risk According to LINE-1 Methylation Level

We aimed to examine the relationship between CRC family history and CRC risk according to LINE-1 methylation level while attempting to exclude influence of Lynch syndrome (ie, hereditary susceptibility to mismatch repair-deficient cancer, most likely to be MSI-high cancer). For this purpose, we examined the risk of MSS (non-MSI-high) CRC according to LINE-1 methylation level (censoring MSI-high cancer incidence) (Table 5). The association between the number of first-degree relatives with CRC (0, 1, ≥2; an ordinal scale) and MSS CRC risk statistically significantly differed by LINE-1 methylation status (ordinal 3 levels) ($P_{heterogeneity} = .02$). CRC family history was associated with a statistically significant increase in LINE-1 methylation-low MSS cancer (multivariable HR = 1.68, 95% CI = 1.19 to 2.38 for 1 vs 0 first-degree relatives with CRC; multivariable HR = 3.48, 95% CI = 1.59 to 7.63 for ≥2 vs 0 first-degree relatives with CRC; $P_{trend} < .001$). To a lesser degree, CRC family history was associated with a higher risk of LINE-1 methylation-intermediate MSS cancer (multivariable HR = 1.50, 95% CI = 1.17 to 1.93 for 1 vs 0 first-degree relatives with CRC; multivariable HR = 1.42, 95% CI = 0.64 to 3.16 for ≥2 vs 0 first-degree relatives with CRC; $P_{trend} = .002$) (Table 5). In contrast, CRC family history was not statistically significantly associated with LINE-1 methylation-high MSS cancer ($P_{trend} = .35$). Our data implied that the association between CRC family history and MSS cancer risk was strongest for the LINE-1 methylation-low tumor subtype, followed by the LINE-1 methylation-intermediate subtype, and weakest or null for the LINE-1 methylation-high tumor subtype.

Discussion

In this large, prospective study, we showed that a family history of CRC was associated with a high risk of CRC with low-level LINE-1 methylation. After attempting to exclude the potential influence of

Table 2. Family history of colorectal cancer (CRC) and subsequent risk of developing CRC according to molecular subtypes in the Nurses' Health Study*

CRC molecular subtype	No. of first-degree relatives with CRC†			P_{trend}	$P_{heterogeneity}$‡
	0	1	≥2		
Person-years	1 967 983	262 761	22 250		
All CRCs					
No. of cancers	517	119	23		
Age-adjusted incidence rate§	28.3	43.6	96.3		
Age-adjusted HR (95% CI)	1 (referent)	1.41 (1.16 to 1.73)	2.66 (1.74 to 4.06)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.39 (1.13 to 1.70)	2.60 (1.70 to 3.97)	<.001	
LINE-1 methylation					.25
Methylation-low, <55% (n = 115, 17%)					
No. of cancers	86	24	5		
Age-adjusted incidence rate§	4.6	9.9	28.9		
Age-adjusted HR (95% CI)	1 (referent)	1.79 (1.12 to 2.86)	3.98 (1.57 to 10.1)	.001	
Multivariable HR (95% CI)‖	1 (referent)	1.77 (1.11 to 2.82)	3.96 (1.58 to 9.94)	.001	
Methylation-intermediate 55%–64.9% (n = 270, 41%)					
No. of cancers	216	44	10		
Age-adjusted incidence rate§	12.4	16.2	38.7		
Age-adjusted HR (95% CI)	1 (referent)	1.28 (0.92 to 1.77)	2.76 (1.45 to 5.25)	.006	
Multivariable HR (95% CI)‖	1 (referent)	1.25 (0.90 to 1.74)	2.69 (1.42 to 5.12)	.009	
Methylation-high, ≥65% (n = 274, 42%)					
No. of cancers	215	51	8		
Age-adjusted incidence rate§	11.4	17.6	28.4		
Age-adjusted HR (95% CI)	1 (referent)	1.40 (1.03 to 1.91)	2.12 (1.04 to 4.32)	.005	
Multivariable HR (95% CI)‖	1 (referent)	1.37 (1.01 to 1.87)	2.06 (1.01 to 4.20)	.008	
MSI status					.11
MSS (n = 515, 80%)					
No. of cancers	411	93	11		
Age-adjusted incidence rate§	23.0	36.3	47.4		
Age-adjusted HR (95% CI)	1 (referent)	1.41 (1.12 to 1.77)	1.68 (0.92 to 3.07)	.001	
Multivariable HR (95% CI)‖	1 (referent)	1.39 (1.10 to 1.75)	1.66 (0.91 to 3.02)	.002	
MSI-high (n = 126, 20%)					
No. of cancers	93	23	10		
Age-adjusted incidence rate‡	5.2	6.1	38.7		
Age-adjusted HR (95% CI)	1 (referent)	1.40 (0.89 to 2.18)	5.23 (2.66 to 10.3)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.37 (0.87 to 2.14)	4.98 (2.54 to 9.76)	<.001	
CIMP status					.87
CIMP-low/negative (n = 516, 79%)					
No. of cancers	409	92	15		
Age-adjusted incidence rate§	22.9	36.0	63.7		
Age-adjusted HR (95% CI)	1 (referent)	1.42 (1.13 to 1.79)	2.37 (1.41 to 4.00)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.40 (1.11 to 1.76)	2.34 (1.40 to 3.94)	<.001	
CIMP-high (n = 140, 21%)					
No. of cancers	107	26	7		
Age-adjusted incidence rate§	5.4	7.3	23.6		
Age-adjusted HR (95% CI)	1 (referent)	1.34 (0.88 to 2.04)	3.01 (1.37 to 6.57)	.01	
Multivariable HR (95% CI)‖	1 (referent)	1.31 (0.86 to 2.00)	2.87 (1.31 to 6.27)	.02	

* CI = confidence interval; CIMP = CpG island methylator phenotype; HR = hazard ratio; LINE-1 = long interspersed nucleotide element 1; MSI = microsatellite instability; MSS = microsatellite stable.

† Not including offspring.

‡ *P* for heterogeneity for trends (0 vs 1 vs ≥2 affected first-degree relatives) between tumor molecular subtypes. A test for LINE-1 methylation subtypes assessed an ordinal linear trend for exposure (0 vs 1 vs ≥2 affected first-degree relatives) and for LINE-1 methylation-low to methylation-intermediate to methylation-high subtype.

§ Age-adjusted incidence rates (per 100 000) were standardized to the age distribution of the population.

‖ Adjusted for body mass index, cumulative mean physical activity, alcohol, folate, methionine, calcium, red meat intake, current smoking status, current multivitamin use, and regular aspirin use.

Lynch syndrome (ie, familial predisposition to MSI-positive cancer), the relationship between CRC family history and the risk of LINE-1 methylation-low MSS cancer persisted. The relationship between CRC family history and subsequent MSS cancer risk differed statistically significantly by tumor LINE-1 methylation level. Our data suggest a possible link between the heritability of CRC and tumoral epigenetic changes. It is possible that at least some LINE-1 methylation-low MSS cancer may represent a previously unrecognized familial CRC trait.

Genetic predisposition to CRC may underlie specific molecular alterations in neoplastic cells (35–39). Familial clustering of CRCs has been commonly associated with a specific molecular

Table 3. Family history of colorectal cancer (CRC) and subsequent risk of developing CRC according to molecular subtypes in Health Professionals Follow-up Study*

CRC molecular subtype	No. of first-degree relatives with CRC†			P_{trend}	$P_{heterogeneity}$‡
	0	1	≥2		
Person-years	819660	107327	4433		
All CRCs					
No. of cancers	459	97	9		
Age-adjusted incidence rate§	59.4	85.1	123.3		
Age-adjusted HR (95% CI)	1 (referent)	1.41 (1.13 to 1.76)	2.80 (1.43 to 5.50)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.41 (1.12 to 1.75)	2.88 (1.46 to 5.67)	<.001	
LINE-1 methylation					.12
Methylation-low, <55% (n = 125, 22%)					
No. of cancers	101	21	3		
Age-adjusted incidence rate§	13.5	19.3	38.1		
Age-adjusted HR (95% CI)	1 (referent)	1.54 (0.95 to 2.49)	4.75 (1.61 to 14.0)	.009	
Multivariable HR (95% CI)‖	1 (referent)	1.50 (0.93 to 2.44)	5.18 (1.81 to 14.8)	.01	
Methylation-intermediate, 55%–64.9% (n = 204, 36%)					
No. of cancers	160	40	4		
Age-adjusted incidence rate§	12.4	16.2	38.7		
Age-adjusted HR (95% CI)	1 (referent)	1.63 (1.15 to 2.31)	3.22 (1.23 to 8.43)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.66 (1.17 to 2.35)	3.22 (1.22 to 8.54)	<.001	
Methylation-high, ≥65% (n = 236, 42%)					
No. of cancers	198	36	2		
Age-adjusted incidence rate§	25.6	32.1	25.5		
Age-adjusted HR (95% CI)	1 (referent)	1.17 (0.82 to 1.68)	1.51 (0.37 to 6.09)	.31	
Multivariable HR (95% CI)‖	1 (referent)	1.16 (0.81 to 1.66)	1.55 (0.38 to 6.36)	.33	
MSI status					.10
MSS (n = 487, 89%)					
No. of cancers	399	82	6		
Age-adjusted incidence rate§	51.2	71.0	76.2		
Age-adjusted HR (95% CI)	1 (referent)	1.38 (1.08 to 1.76)	2.05 (0.95 to 4.45)	.002	
Multivariable HR (95% CI)‖	1 (referent)	1.38 (1.08 to 1.75)	2.13 (0.99 to 4.59)	.002	
MSI-high (n = 62, 11%)					
No. of cancers	46	13	3		
Age-adjusted incidence rate§	6.3	12.4	47.2		
Age-adjusted HR (95% CI)	1 (referent)	1.72 (0.93 to 3.18)	13.8 (4.18 to 45.7)	.002	
Multivariable HR (95% CI)‖	1 (referent)	1.69 (0.92 to 3.10)	13.2 (3.87 to 44.8)	.003	
CIMP status					.17
CIMP-low/negative (n = 447, 88%)					
No. of cancers	367	75	5		
Age-adjusted incidence rate§	47.3	66.5	63.6		
Age-adjusted HR (95% CI)	1 (referent)	1.37 (1.07 to 1.76)	1.99 (0.86 to 4.59)	.004	
Multivariable HR (95% CI)‖	1 (referent)	1.37 (1.07 to 1.76)	2.07 (0.90 to 4.77)	.004	
CIMP-high (n = 61, 12%)					
No. of cancers	45	14	2		
Age-adjusted incidence rate§	5.5	12.0	34.3		
Age-adjusted HR (95% CI)	1 (referent)	1.95 (1.05 to 3.60)	6.22 (1.33 to 29.2)	.005	
Multivariable HR (95% CI)‖	1 (referent)	1.91 (1.03 to 3.54)	5.89 (1.22 to 28.5)	.007	

* CI = confidence interval; CIMP = CpG island methylator phenotype; HR = hazard ratio; LINE-1 = long interspersed nucleotide element 1; MSI = microsatellite instability; MSS = microsatellite stable.

† Not including offspring.

‡ *P* for heterogeneity for trends (0 vs 1 vs ≥2 affected first-degree relatives) between tumor molecular subtypes. A test for LINE-1 methylation subtypes assessed an ordinal linear trend for exposure (0 vs 1 vs ≥2 affected first-degree relatives) and for LINE-1 methylation-low to methylation-intermediate to methylation-high subtype.

§ Age-adjusted incidence rates (per 100000) were standardized to the age distribution of the population.

‖ Adjusted for body mass index, cumulative mean physical activity, alcohol, folate, methionine, calcium, red meat intake, current smoking status, current multivitamin use, and regular aspirin use.

subtype, namely MSI-high tumors in the setting of Lynch syndrome (36,40,41). Nonetheless, it has also been shown that familial clustering suggestive of Lynch syndrome is not always associated with hereditary mismatch repair defects or MSI-high tumors (10,40,42). In a recent study by Goel et al. (10), non-MSI-high tumors occurring in a familial pattern suggestive of Lynch syndrome frequently exhibited LINE-1 hypomethylation, which is consistent with our current data. Besides intense familial cancer clustering, a common family history of colorectal cancer in a first-degree relative (observed in approximately 20% of all colorectal cancer cases) may help to identify novel risk alleles by genome-wide linkage analysis (7). A recent study highlights the novel opportunities of using family history data

Table 4. Family history of colorectal cancer (CRC) and subsequent risk of developing CRC according to molecular subtypes in the combined cohorts*

CRC molecular subtype	No. of first-degree relatives with CRC†			P_{trend}	$P_{heterogeneity}$‡
	0	1	≥2		
Person-years	2 787 643	370 088	26 684		
All CRCs					
No. of cancers	976	216	32		
Age-adjusted incidence rate§	37.2	54.6	100.4		
Age-adjusted HR (95% CI)	1 (referent)	1.41 (1.22 to 1.64)	2.70 (1.88 to 3.86)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.40 (1.20 to 1.62)	2.67 (1.86 to 3.82)	<.001	
LINE-1 methylation					.06
Methylation-low, <55% (n = 240, 20%)					
No. of cancers	187	45	8		
Age-adjusted incidence rate§	7.0	12.4	29.1		
Age-adjusted HR (95% CI)	1 (referent)	1.66 (1.19 to 2.32)	4.21 (2.08 to 8.56)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.63 (1.16 to 2.28)	4.32 (2.15 to 8.67)	<.001	
Methylation-intermediate, 55%–64.9% (n = 474, 39%)					
No. of cancers	376	84	14		
Age-adjusted incidence rate§	14.7	21.3	42.6		
Age-adjusted HR (95% CI)	1 (referent)	1.42 (1.12 to 1.81)	2.90 (1.70 to 4.95)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.42 (1.12 to 1.80)	2.83 (1.66 to 4.83)	<.001	
Methylation-high, ≥65% (n = 510, 42%)					
No. of cancers	413	87	10		
Age-adjusted incidence rate§	15.7	21.4	28.3		
Age-adjusted HR (95% CI)	1 (referent)	1.30 (1.03 to 1.64)	1.94 (1.03 to 3.66)	.005	
Multivariable HR (95% CI)‖	1 (referent)	1.28 (1.01 to 1.61)	1.92 (1.02 to 3.63)	.007	
MSI status					.03
MSS (n = 1002, 84%)					
No. of cancers	810	175	17		
Age-adjusted incidence rate§	30.9	45.0	49.7		
Age-adjusted HR (95% CI)	1 (referent)	1.40 (1.18 to 1.65)	1.79 (1.11 to 2.89)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.38 (1.17 to 1.63)	1.79 (1.12 to 2.89)	<.001	
MSI-high (n = 188, 16%)					
No. of cancers	139	36	13		
Age-adjusted incidence rate§	5.6	8.1	41.1		
Age-adjusted HR (95% CI)	1 (referent)	1.50 (1.04 to 2.15)	6.20 (3.43 to 11.2)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.47 (1.02 to 2.10)	5.86 (3.25 to 10.6)	<.001	
CIMP status					.40
CIMP-low/negative (n = 963, 83%)					
No. of cancers	776	167	20		
Age-adjusted incidence rate§	30.1	44.1	62.6		
Age-adjusted HR (95% CI)	1 (referent)	1.40 (1.18 to 1.66)	2.26 (1.45 to 3.52)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.39 (1.17 to 1.64)	2.27 (1.46 to 3.52)	<.001	
CIMP-high (n = 201, 17%)					
No. of cancers	152	40	9		
Age-adjusted incidence rate§	5.6	8.8	26.7		
Age-adjusted HR (95% CI)	1 (referent)	1.51 (1.07 to 2.13)	3.45 (1.72 to 6.92)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.48 (1.04 to 2.09)	3.29 (1.64 to 6.59)	<.001	

* CI = confidence interval; CIMP = CpG island methylator phenotype; HR = hazard ratio; LINE-1 = long interspersed nucleotide element 1; MSI = microsatellite instability; MSS = microsatellite stable.

† Not including offspring.

‡ *P* for heterogeneity for trends (0 vs 1 vs ≥2 affected first-degree relatives) between tumor molecular subtypes. A test for LINE-1 methylation subtypes assessed an ordinal linear trend for exposure (0 vs 1 vs ≥2 affected first-degree relatives) and for LINE-1 methylation-low to methylation-intermediate to methylation-high subtype.

§ Age-adjusted incidence rates (per 100 000) were standardized to the age distribution of the population.

‖ Adjusted for body mass index, cumulative mean physical activity, alcohol, folate, methionine, calcium, red meat intake, current smoking status, current multivitamin use, and regular aspirin use.

in genome-wide association study datasets to gain new evidence for disease risk alleles (43). Family history of cancer remains pivotal in our attempts to decipher genetic etiologies (beyond genome-wide association studies) and their interactions with environment.

LINE-1 represents a major repetitive element and occupies approximately 17% of the human genome (11). Thus, methylation level in LINE-1 has been shown to correlate with global DNA methylation level in tumor cells (44). Genomic hypomethylation has been linked to genomic and chromosomal instability leading to carcinogenesis (45–49). In addition to its role as a surrogate of genomic hypomethylation, LINE-1 hypomethylation may, in itself, have carcinogenic effects through deregulation of gene

Table 5. Family history of colorectal cancer (CRC) and subsequent risk of developing microsatellite stable (MSS) CRC by long interspersed nucleotide element 1 (LINE-1) methylation level (censoring microsatellite instability-high cancers) in the combined cohorts*

MSS CRC molecular subtype	No. of first-degree relatives with CRC†			P_{trend}	$P_{heterogeneity}$‡
	0	1	≥2		
Person-years	2 787 643	370 088	26 684		
LINE-1 subtyping in MSS tumors					.02
Methylation-low, <55%					
No. of cancers	172	43	6		
Age-adjusted incidence rate§	6.8	11.8	17.4		
Age-adjusted HR (95% CI)	1 (referent)	1.71 (1.21 to 2.42)	3.37 (1.52 to 7.50)	<.001	
Multivariable HR (95% CI)‖	1 (referent)	1.68 (1.19 to 2.38)	3.48 (1.59 to 7.63)	<.001	
Methylation-intermediate, 55%–64.9%					
No. of cancers	330	77	6		
Age-adjusted incidence rate§	12.5	19.7	20.0		
Age-adjusted HR (95% CI)	1 (referent)	1.50 (1.17 to 1.93)	1.42 (0.64 to 3.16)	.002	
Multivariable HR (95% CI)‖	1 (referent)	1.50 (1.17 to 1.93)	1.42 (0.64 to 3.14)	.002	
Methylation-high, ≥65%					
No. of cancers	308	55	5		
Age-adjusted incidence rate§	11.6	14.1	12.4		
Age-adjusted HR (95% CI)	1 (referent)	1.12 (0.84 to 1.50)	1.43 (0.59 to 3.48)	.31	
Multivariable HR (95% CI)‖	1 (referent)	1.11 (0.83 to 1.48)	1.43 (0.59 to 3.49)	.35	

* CI = confidence interval; HR = hazard ratio.

† Not including offspring.

‡ A heterogeneity test for LINE-1 methylation subtypes assessed an ordinal linear trend for exposure (0 vs 1 vs ≥2 affected first-degree relatives) as well as for LINE-1 methylation-high to methylation-intermediate to methylation-low subtype.

§ Age-adjusted incidence rates (per 100 000) were standardized to the age distribution of the population.

‖ Adjusted for body mass index, cumulative mean physical activity, alcohol, folate, methionine, calcium, and red meat intake, current smoking status, current multivitamin use, and regular aspirin use.

transcription and activation of retrotransposons (50–52). Tumor LINE-1 hypomethylation has been associated with inferior prognosis in not only colon cancer (12–14) but also many different human cancer types (53). We previously found that the degree of colon cancer LINE-1 hypomethylation was linearly associated with aggressive tumor behavior and observed an approximately fivefold increase in cancer-specific mortality associated with tumors at the low end of the methylation spectrum, compared with those at the high end (12). In addition, LINE-1 hypomethylated CRC has been associated with young age of onset (28) and CRC familial clustering (10), but not with polymorphisms in one-carbon metabolizing enzyme genes (54). Taking these findings together with our current data, LINE-1 methylation level may serve as a potential tumor biomarker for prognostication as well as familial cancer risk assessment.

Interestingly, we showed that CRC family history was associated with elevated risk of almost all molecular subtypes that we examined. One reason for this phenomenon may be that many common CRC susceptibility variants may increase the risks of many different molecular subtypes. As indicated by the unique tumor principle (55,56), each tumor confers its own differential familial risk given the uniqueness of each human genome. Nonetheless, we use molecular classification (such as MSI-high vs MSS) to better predict familial risk, natural history, and response to treatment (55,56). A second reason is mutual confounding of molecular subtypes (55). For example, MSI-high cancers are strongly associated with CRC family history, and are present in multiple molecular subtypes, including LINE-1 methylation-high, CIMP-low, and CIMP-negative subtypes, which is why we needed to perform our primary hypothesis testing within MSS cancers.

In CRC, CIMP has been associated with sporadic MSI-high cancers due to epigenetic silencing of *MLH1* (57,58). One retrospective case–case study (N = 47) (59) reported a statistically significant association between CRC family history and CIMP, whereas our much larger current study and retrospective case–case study (N = 547) (60) failed to confirm this association, indicating overall lack of evidence for a strong link between CRC family history and CIMP.

Our study possesses several key strengths. First, collection of family history information and data on numerous dietary and lifestyle covariables relevant to cancer risk was performed repeatedly over decades, with high rates of follow-up in both cohorts. Second, our study participants were distributed throughout the United States, and thus our CRC cases were more representative of cases in the US white population than CRC cases from several referral hospitals. Third, data collection was prospective, eliminating differential recall bias, which is a major concern in analyses involving family history of cancer (19,20). In addition, our robust and extensive molecular characterization of CRC enabled a molecular pathological epidemiology study design (33,34). Molecular pathological epidemiology studies have yielded several interesting observations relevant to carcinogenesis and personalized patient management strategies (18,33,34,61–66).

One limitation of our study is the use of questionnaire-based data collection for family history, which relies on knowledge and compliance of study participants. Nonetheless, a recent study (67) has validated the predictive accuracy of self-reporting of CRC in first-degree relatives (positive predictive value = 0.86, 95% CI = 0.64 to 0.95; negative predictive value = 0.98, 95% CI = 0.94 to 0.99), in keeping with an existing report (68). Furthermore, all of our study participants were health professionals, which increased accuracy

of family history information. In addition, we ascertained family history at multiple time points, further increasing accuracy. A second limitation is that we could not obtain tumor tissue specimens from all incident CRCs. Nonetheless, the demographic and clinical characteristics did not statistically significantly differ between cases with and without available tumor tissue, and the pathologic and molecular features of our CRC cases were generally compatible with data from the US cancer registry and published literature (23). Additionally, any biomarker research should be interpreted with caution, and study findings must be carefully evaluated (69).

Clinical implications of our findings warrant further discussion. As shown in Table 4, the multivariable hazard ratio for MSI-high cancer by the presence of two or more affected first-degree relatives was 5.86 (95% CI = 3.25 to 10.6), and the multivariable hazard ratio for LINE-1 methylation-low cancer was 4.32 (95% CI = 2.15 to 8.67). Thus, the presence of these tumor molecular features imply further elevated familial CRC risk, compared with the approximately twofold increase in CRC risk associated with CRC family history (without considering tumor molecular features). As the effect estimates imply, the ability of MSI testing to predict familial risk is higher than that of LINE-1 testing. Nonetheless, considering the importance of MSI testing in clinical and pathology practice, additional studies are necessary to assess implications of LINE-1 methylation status in the estimation of familial CRC risk.

In summary, our current study suggests that a family history of CRC is associated with a high risk of CRC with low-level LINE-1 methylation and that genetic predisposition likely underlies somatic epigenetic changes. It is possible that at least a subset of LINE-1 methylation-low CRC may constitute a previously unrecognized familial cancer trait. Previous studies have shown that LINE-1 methylation-low colon cancer is an aggressive cancer subtype (12–14), which demands effective prevention and surveillance strategies. Therefore, our findings may have considerable clinical and public health implications. Additional studies are needed to assess utility of tumor LINE-1 methylation testing in clinical settings before it can be implemented as a tumor molecular biomarker for prognostication in the proband and for cancer risk assessment in family members, akin to current MSI testing in CRC. We also anticipate that future studies will further elucidate the mechanisms that underlie the association between the heritability of CRC and somatic epigenetic alterations.

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Funding

This work was supported by the National Institute of Health (P01 CA87969 [to S. E. Hankinson], P01 CA55075 [to W. C. Willett], P50 CA127003 [to CSF], R01 CA151993 [to SO], and R01 CA137178 [to ATC]; the Bennett Family Fund for Targeted Therapies Research; and the National Colorectal Cancer Research Alliance. PL is a Scottish Government Clinical Academic Fellow and is supported by a Harvard University Knox Memorial Fellowship. ATC is a Damon Runyon Clinical Investigator.

Notes

S. Ogino, R. Nishihara, and P. Lochhead contributed equally to this study. A. T. Chan, E. Giovannucci, and C. S. Fuchs contributed equally to this study.

A.T. Chan was a consultant of Bayer Healthcare, Millennium Pharmaceuticals, and Pfizer Inc. This study was not funded by Bayer Healthcare, Millennium Pharmaceuticals, or Pfizer Inc. No other authors report any conflicts of interest.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Funding agencies did not have any role in the design of the study; the collection, analysis, or interpretation of the data; the decision to submit the manuscript for publication; or the writing of the manuscript.

Recently, Antelo et al. (70) reported that LINE-1 hypomethylated colorectal cancer was associated with early onset disease, consistent with our previous data (28), and possible genetic susceptibility that may also lead to synchronous LINE-1 hypomethylated colorectal cancers (71).

We deeply thank the Nurses' Health Study and Health Professionals Follow-up Study cohort participants who have agreed to provide us with information through questionnaires and biological specimens and hospitals and pathology departments throughout the United States for generously providing us with tissue specimens. In addition, we thank the participants and staff of the Nurses' Health Study and the Health Professionals Follow-Up Study for their valuable contributions and the US state cancer registries for their help.

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