Ethnicity and Risk for Colorectal Cancers Showing Somatic BRAF V600E Mutation or CpG Island Methylator Phenotype

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Abstract

Colorectal cancers arising from serrated polyps are characterized by the CpG island methylator phenotype (CIMP) and somatic mutation (V600E) in the BRAF proto-oncogene. Few epidemiologic studies have investigated risk factors for these tumors. We conducted a cohort study of 41,328 residents of Melbourne, Australia that included 9,939 participants of southern European origin and 31,389 of Anglo-Celtic origin. Colorectal cancers arising from serrated polyps and their malignant counterparts (11-17). In addition, BRAF mutation has been identified in aberrant crypt foci, which show evidence of epithelial serration (18), suggesting that these changes are established early in colorectal carcinogenesis and induce commitment to the serrated neoplasia pathway (19).

Different molecular subsets of colorectal cancer may have different environmental, genetic, and lifestyle risk factors, and investigation of possible risk factors separately for different molecular subtypes may lead to a better understanding of how to prevent disease. For example, in Australia, men have higher incidence of colorectal cancer than women (20), but CIMP and BRAF-related tumors are more common in women (21). Smoking was associated with risk of CIMP and BRAF-related tumors in a case-control study but not with other colorectal cancers (22), although there were no clear differences in dietary risk factors between the subtypes (23).

The Melbourne Collaborative Cohort Study consists mostly of people of British or Irish descent (usually referred to in Australia as of “Anglo-Celtic” descent)
migrants to Australia from Greece or Italy (southern Europeans). The migrants were included to increase the heterogeneity of the cohort with respect to lifestyle (especially diet), genes, and rates of disease outcomes. When southern European migrants arrive in Australia, they initially have substantially lower mortality from colorectal cancer, but the differences diminish over time (24-26), suggesting that environmental factors after migration might be important risk factors.

Within the Melbourne Collaborative Cohort Study, we determined CIMP and \textit{BRAF} mutation status for colorectal cancers diagnosed during follow-up with a view to identifying those arising from the serrated pathway and determining whether their risk factors differed from the more common cancers that arise from adenomas. Here, we report on the incidence of colorectal cancer with CIMP and \textit{BRAF} mutation in relation to ethnic origin and gender.

\textbf{Materials and Methods}

\textbf{Subjects.} The Melbourne Collaborative Cohort Study is a prospective cohort study of 41,528 participants, ages 27 to 75 years at recruitment from 1990 to 1994 (almost all were ages 40-69 years), and includes 5,425 migrants from Italy and 4,535 from Greece. For this analysis, 200 participants who had a colorectal cancer diagnosed before baseline were excluded, leaving 41,328.

Subjects were recruited via the electoral rolls (registration to vote is compulsory for adults in Australia), advertisements, and community announcements in local media (e.g., television, radio, and newspapers). Comprehensive lists of Italian and Greek surnames were also used to target southern European migrants listed in the phone books and on electoral rolls. The study protocol was approved by the Cancer Council Victoria’s Human Research Ethics Committee. Participants gave written consent for participation and for the investigators to obtain their medical records.

\textbf{Baseline Data Collection.} A structured interview schedule was used to obtain information on potential risk factors including education, country of birth, alcohol consumption, smoking habits, current physical activity during leisure time, education, and, for women, reproductive history, menopausal status including age at menopause, and use of hormone replacement therapy. Information on current diet was obtained from a dietary questionnaire that contained a 121-item food frequency questionnaire that was developed for this study (27). Height, weight, and waist and hip circumference were measured.

\textbf{Cohort Follow-up and Case Ascertainment.} Cases were participants who had a first diagnosis of invasive cancer of the colon or rectum during follow-up to December 31, 2004 and were identified by linkage to population-based cancer registries in all Australian states. Addresses and vital status of the subjects were determined by record linkage to electoral rolls, the National Death Index, Victorian death records, from electronic phone books, and from responses to mailed questionnaires and newsletters.

\textbf{Molecular Pathology.} Archival tumor tissue was sought for all primary, histopathologically confirmed adenocarcinomas diagnosed in Victoria. Diagnosis was verified and pathology was reviewed by an experienced histopathologist (J.R.J.). Immunohistochemistry for DNA mismatch repair genes \textit{MLH1}, \textit{MSH2}, \textit{MSH6}, and \textit{PMS2} was done as reported by Lindor et al. (28). Microsatellite instability (MSI) was examined using 10 microsatellite markers (28). \textit{BRAF} V600E mutation analysis was done by a real-time PCR-based allelic discrimination method (29). CIMP status and \textit{MLH1} methylation was determined by MethyLight analysis of five markers (\textit{RNUX3}, \textit{CACNA1G}, \textit{SOCS1}, \textit{NEUROG1}, and \textit{IGF2}), and \textit{MLH1}, respectively (21). Exon 1 of \textit{K Ras} was analyzed by direct sequencing (21). Positive methylation measurements were determined by the presence of amplification product for the three CIMP markers and \textit{MLH1}. Samples were considered negative for methylation if no marker-specific probe amplification was seen together with the Alu reference probe displaying amplification with a Ct value <25, indicating the initial amount of bisulfite converted template was significant enough to allow the amplification and detection of the methylation markers. Tumors were classified as CIMP positive when at least three markers were positive for methylation. For participants who had both colon and rectal tumors, results for colon tumors are reported here.

\textbf{Statistical Analysis.} Ethnicity was defined based on country of birth and was classified into two groups: southern European (born in Italy or Greece) and Anglo-Celtic (born in Australia, New Zealand, United Kingdom, or Ireland).

Cox regression, with age as the time scale, was used to estimate hazard ratios (HR), 95% confidence intervals (95% CI), and \( P \) values (30). To estimate HR separately for molecular subtypes and to test their difference, Cox models based on competing risks were fitted using a data duplication method (31). Calculation of person-time began at baseline and ended at the earliest of the date of diagnosis of colorectal cancer, date of diagnosis of cancer of unknown primary site, date of death, date last known to be in Australia, or December 31, 2004. Tests based on Schoenfeld residuals and graphical methods using Kaplan-Meier curves (32) showed no evidence that proportional hazard assumptions were violated for any analyses.

Level of education, smoking status (never, former, current), alcohol intake (g/d), waist circumference, physical activity, frequency of red meat consumption, intakes of cereal fiber, calcium, dietary folate and energy, multivitamin use, calcium supplements, and, for women, hormone replacement therapy use were all considered as potential confounders. Only waist circumference and alcohol intake changed the HR for sex or ethnic origin by >10% and were retained in the final models.

Differences between characteristics of tumors for which molecular measurements were done and those for which no measurements were made were tested using \( \chi^2 \) tests for categorical variables and the Mann-Whitney rank sum test for continuous variables. All statistical tests are two sided, with \( P < 0.05 \) considered statistically significant. All statistical analyses were done in Stata version 9.2 (StataCorp).

\textbf{Results}

Baseline characteristics of the cohort by ethnic origin are shown in Table 1. Southern Europeans had larger mean...
waist circumference than Anglo-Celts and were more likely to be lifetime abstainers from alcohol, to have lower educational attainment, less likely to engage in recreational physical activity, were more likely to eat red meat frequently, had lower intakes of cereal fiber, calcium, and folate, and were less likely to use multivitamins, calcium supplements, or hormone replacement therapy. Most of the migrants had lived in Australia for >30 years (median, 33 years; interquartile range, 28-37 years).

During an average of 11 years of follow-up per person to December 31, 2004, 55 participants were known to have left Australia and 718 were diagnosed with colorectal cancer, including 449 with colon tumors (236 proximal and 173 distal), 269 with rectal tumors (three subjects had both colon and rectal tumors), and 40 with colorectal cancers of unspecified site.

Archival tissue was obtained for 656 cases (92%); of the remaining cases, 9 were diagnosed in states other than Victoria, and for 53, the material could not be found or was not provided by the pathology laboratory. Molecular analysis of archival tumor tissue was done for 585 cases for which CIMP status was determined for 579 and BRAF V600E mutation status for 582. Of those patients for whom no molecular analysis was attempted, 10 did not have a histopathologic diagnosis, and on review of the diagnostic slides, 25 were found to have metastatic disease, 11 were classified as having adenomas only, and 25 did not have tissue suitable for molecular analysis.

A slightly lower proportion of tumors from men than from women had no molecular measurements (79% versus 84%; \( P = 0.05 \)), but there was little difference in the proportions with molecular measurements by ethnicity (\( P = 0.8 \)), age at diagnosis (\( P = 0.4 \)), waist circumference (\( P = 0.1 \)), smoking status (\( P = 0.3 \)), alcohol consumption (\( P = 0.6 \)), education (\( P = 0.4 \)), physical activity (\( P = 0.3 \)), red meat consumption (\( P = 0.8 \)), or for the site (rectum, proximal, or distal colon; \( P = 0.7 \)).

CIMP and BRAF mutation status were strongly associated; 54 (74%) of 73 CIMP-positive tumors had BRAF mutations compared with 37 (7%) of 506 CIMP-negative tumors, giving an odds ratio of 36

Table 1. Baseline demographic, dietary, and lifestyle characteristics by ethnic origin

<table>
<thead>
<tr>
<th>Ethnic origin</th>
<th>Anglo-Celtic</th>
<th>Southern European</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>31,389</td>
<td>9,939</td>
</tr>
<tr>
<td>Age (y)*</td>
<td>55.1 (8.9)</td>
<td>55.9 (7.8)</td>
</tr>
<tr>
<td>Waist circumference (cm)*</td>
<td>84 (13)</td>
<td>90 (12)</td>
</tr>
<tr>
<td>Females</td>
<td>18,923 (60)</td>
<td>5,459 (55)</td>
</tr>
<tr>
<td>Smoking status † (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>17,878 (57)</td>
<td>5,855 (59)</td>
</tr>
<tr>
<td>Former</td>
<td>10,198 (32)</td>
<td>2,709 (27)</td>
</tr>
<tr>
<td>Current</td>
<td>3,310 (11)</td>
<td>1,368 (14)</td>
</tr>
<tr>
<td>Alcohol intake †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lifetime abstainer</td>
<td>7,694 (25)</td>
<td>4,138 (42)</td>
</tr>
<tr>
<td>Former drinker</td>
<td>1,280 (4)</td>
<td>369 (4)</td>
</tr>
<tr>
<td>1-19 g/d</td>
<td>15,612 (50)</td>
<td>3,351 (34)</td>
</tr>
<tr>
<td>20-39 g/d</td>
<td>4,326 (14)</td>
<td>1,210 (12)</td>
</tr>
<tr>
<td>40+ g/d †</td>
<td>2,452 (9)</td>
<td>857 (9)</td>
</tr>
<tr>
<td>Education †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>1,369 (4)</td>
<td>6,653 (67)</td>
</tr>
<tr>
<td>Some secondary school</td>
<td>13,936 (44)</td>
<td>1,847 (19)</td>
</tr>
<tr>
<td>Completed secondary school</td>
<td>7,437 (24)</td>
<td>1,093 (11)</td>
</tr>
<tr>
<td>Degree/diploma</td>
<td>8,644 (28)</td>
<td>340 (3)</td>
</tr>
<tr>
<td>Recreational physical activity †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5,638 (18)</td>
<td>3,551 (36)</td>
</tr>
<tr>
<td>Low</td>
<td>6,079 (19)</td>
<td>2,206 (22)</td>
</tr>
<tr>
<td>Medium</td>
<td>11,461 (37)</td>
<td>3,238 (33)</td>
</tr>
<tr>
<td>High</td>
<td>8,211 (26)</td>
<td>940 (9)</td>
</tr>
<tr>
<td>Multivitamin use †</td>
<td>6,052 (19)</td>
<td>652 (7)</td>
</tr>
<tr>
<td>Calcium supplement use †</td>
<td>3,888 (12)</td>
<td>497 (5)</td>
</tr>
<tr>
<td>Hormone replacement therapy use † (women only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>13,353 (71)</td>
<td>4,667 (87)</td>
</tr>
<tr>
<td>Former</td>
<td>1,762 (9)</td>
<td>329 (6)</td>
</tr>
<tr>
<td>Current</td>
<td>5,717 (20)</td>
<td>341 (6)</td>
</tr>
<tr>
<td>Red meat consumption † (times a week)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>7,642 (24)</td>
<td>2,334 (24)</td>
</tr>
<tr>
<td>5-6</td>
<td>7,363 (24)</td>
<td>2,125 (21)</td>
</tr>
<tr>
<td>7-9</td>
<td>8,679 (28)</td>
<td>2,357 (24)</td>
</tr>
<tr>
<td>10+</td>
<td>7,678 (24)</td>
<td>3,104 (31)</td>
</tr>
<tr>
<td>Energy intake (kJ/d) †</td>
<td>8,899 [7,090-11,101]</td>
<td>8,092 [6,287-10,414]</td>
</tr>
<tr>
<td>Cereal fiber intake (g/d) †</td>
<td>10.5 [7.5-14.5]</td>
<td>8.1 [5.5-11.5]</td>
</tr>
<tr>
<td>Calcium intake (mg/d) †</td>
<td>826 [633-1,064]</td>
<td>680 [509-908]</td>
</tr>
<tr>
<td>Folate intake (mg/d) †</td>
<td>319 [249-405]</td>
<td>253 [187-344]</td>
</tr>
</tbody>
</table>

* Mean (SD).
† Numbers do not always add up to total numbers due to missing data.
‡ Median [interquartile range].

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(95% CI, 19-71; \( P < 0.001 \)). Both features were more common in tumors from females than from males (\( P < 0.005 \)) and in tumors of the right colon (\( P < 0.005 \)), were positively associated with MSI-high tumor status (\( P < 0.001 \) and MLH1 methylation (\( P < 0.001 \)), and were inversely associated with \( KRAS \) mutations (\( P < 0.001; \) Table 2).

Table 3 shows HR by sex and ethnic origin. Overall, women had a slightly lower incidence rate of colorectal cancer than did men (HR, 0.88; 95% CI, 0.74-1.05) and also had slightly lower incidence of tumors that were CIMP negative (HR, 0.84; 95% CI, 0.69-1.02) or that did not have \( BRAF \) mutations (HR, 0.83; 95% CI, 0.68-1.01) but had higher incidence rates for tumors that were CIMP positive or that had \( BRAF \) mutations (CIMP positive: HR, 1.83; 95% CI, 1.11-3.01; \( BRAF \) mutation: HR, 1.67; 95% CI, 1.08-2.58). For both molecular features, the differences between the HR for the two subtypes were significant (Table 3).

The HR for ethnicity in relation to tumors of the right colon were similar to those for all sites, although the 95% CI were wider (data not shown); \( P \) values for the differences between HR for the two subtypes were 0.07 for CIMP status and 0.008 for \( BRAF \) mutation status.

**Discussion**

Overall, people of southern European origin had ~20% lower incidence rate of colorectal cancer than did people of Anglo-Celtic origin. This was largely due to their much lower incidence rate of tumors with \( BRAF \) mutations or the CIMP, because both ethnic groups had similar incidence rates of other colorectal cancer. Like others, we also found that \( BRAF \) mutation and CIMP were strongly associated with each other and were more common in tumors from females and the proximal colon (10, 21). In addition, also as reported previously, \( BRAF \) mutation was positively associated with MSI-high status and MLH1 methylation and inversely associated with the presence of \( KRAS \) in a tumor (33).

Our study has several strengths and limitations. We had almost complete ascertainment of colorectal cancer and little loss to follow-up but were unable to obtain archival tissue from the primary lesion for 19% of cases. However, this is unlikely to have biased the observed associations because the proportions of cases with no
suitable tissue varied little by ethnicity or sex. Our measurement of CIMP used a panel of recently reported markers with high sensitivity and specificity, and the associations between CIMP and BRAF mutation status, MSI status, MLH1 methylation, KRAS mutations, and subsite are supported by earlier studies, indicating that our measurements of CIMP and BRAF mutation status are in general agreement with those of other investigators (21).

Despite residing in Australia for many years, the migrants maintained substantial differences in dietary patterns from those born in Australia (34) and also differed from the Australian-born participants in their smoking habits, alcohol intake, waist circumference, and education. Nevertheless, control for these risk factors had little effect on the results. It is unlikely that family history, which was not recorded, could account for a strong inverse association between southern European descent and risk of tumors with CIMP or BRAF mutation status but little or no association with risk of tumors not displaying these features. We cannot rule out chance as an explanation for the ethnic differences in risk of BRAF-related colorectal cancer.

The HR for all colorectal cancer for the southern European migrants (0.76) is consistent with descriptive studies of mortality. Compared with Australian-born residents, migrants who had lived in Australia for at least 30 years had mortality rate ratios between 0.7 (25) and 0.8 (95% CI, 0.7-1.0; ref. 26). This suggests that although migrants’ risk for the more common form of colorectal cancer converges to that of the Australian born with increasing duration of residence, the same is not true for tumors with BRAF mutation or the CIMP. Risk factors that act primarily before migration, or genetic factors (35, 36), could explain the lack of convergence for these tumor types.

Evidence for a genetic predisposition for tumors bearing BRAF mutations comes from a case-control study of colorectal cancer, where microsatellite-stable tumors with BRAF mutation were over four times more likely than tumors without BRAF mutation to be associated with a family history of colorectal cancer (37). In addition, familial syndromes associated with BRAF mutation-bearing tumors have been described from Australia and Sweden (29, 38). There is also indirect evidence regarding an association between ethnicity and the risk of colorectal cancer with CIMP and BRAF mutation from a study of hyperplastic polyposis syndrome, which is a rare condition characterized by multiple serrated polyps and tumors with CIMP and BRAF mutation. In a case series from a gastroenterology clinic in New Zealand, all 24 cases were people of European origin, whereas only 46% of patients attending the clinic were of European descent (39). Thus, the possibility exists that a sequence variant in the Anglo-Celtic population may underlie the development of CIMP-related colorectal cancer either directly or through modulation of an environmental influence; however, at present, none has been definitively identified.

Two studies have examined associations between common genetic variants and CIMP status for patients with colorectal cancer, including genes in the folate pathway. van Rijnsoever et al. found that overall there was no association between a variant in the 5,10-methylenetetrahydrofolate reductase gene (C677T; rs1801133) and type of colorectal cancer, but that females had a higher proportion of CIMP-positive tumors (40). CIMP was defined as methylation of two of three genes (CDKN2A, MINT2, and MLH1) present, none has been definitively identified.

<table>
<thead>
<tr>
<th>Type of colorectal cancer</th>
<th>Male</th>
<th>Female</th>
<th>P*</th>
<th>Anglo-Celtic</th>
<th>Southern European</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person-years</td>
<td>187,654</td>
<td>277,942</td>
<td></td>
<td>346,149</td>
<td>119,447</td>
<td></td>
</tr>
<tr>
<td>All colorectal cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>371</td>
<td>347</td>
<td></td>
<td>545</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>Partially adjusted</td>
<td>1</td>
<td>0.65 (0.57-0.80)</td>
<td>1</td>
<td>0.84 (0.70-0.99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fully adjusted</td>
<td>1</td>
<td>0.88 (0.74-1.05)</td>
<td></td>
<td>0.78 (0.65-0.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIMP status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>24</td>
<td>49</td>
<td></td>
<td>65</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Partially adjusted</td>
<td>1</td>
<td>1.37 (0.84-2.24)</td>
<td>1</td>
<td>0.35 (0.17-0.72)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fully adjusted</td>
<td>1</td>
<td>1.83 (1.11-3.01)</td>
<td></td>
<td>0.32 (0.16-0.67)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>266</td>
<td>240</td>
<td></td>
<td>374</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Partially adjusted</td>
<td>1</td>
<td>0.63 (0.53-0.75)</td>
<td>1</td>
<td>0.92 (0.76-1.12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fully adjusted</td>
<td>1</td>
<td>0.84 (0.69-1.02)</td>
<td>0.003</td>
<td>0.86 (0.70-1.05)</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>BRAF mutation status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>33</td>
<td>62</td>
<td></td>
<td>85</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Partially adjusted</td>
<td>1</td>
<td>1.26 (0.83-1.93)</td>
<td>1</td>
<td>0.33 (0.17-0.62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fully adjusted</td>
<td>1</td>
<td>1.67 (1.08-2.58)</td>
<td></td>
<td>0.30 (0.16-0.58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>257</td>
<td>230</td>
<td></td>
<td>356</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>Partially adjusted</td>
<td>1</td>
<td>0.63 (0.52-0.75)</td>
<td>1</td>
<td>0.96 (0.79-1.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fully adjusted</td>
<td>1</td>
<td>0.83 (0.68-1.01)</td>
<td>0.003</td>
<td>0.90 (0.74-1.11)</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

* P value for test that fully adjusted HR for two tumor subtypes are equal.
† HR (95% CI) adjusted for both variables in the table and with age as the time scale.
‡ HR (95% CI) adjusted for both variables in the table plus waist circumference and alcohol intake and with age as the time scale.
was higher for colorectal cancer patients with a poly-
morphism in the gene coding 5-methyltetrahydro-
olate-homocysteine methyltransferase, also known as 
methionine synthase reductase (41). Other genes in-
volved in the folate pathway, those encoding including 
5-methyltetrahydrofolate-homocysteine methyltrans-
ferase and thymidylate synthetase, have been shown to 
be associated with colorectal cancer in general (42).

Colorectal cancers with CIMP and BRAF mutation 
ascend in a subset of serrated polyps called sessile 
serrated adenomas (3). Given our findings, it is likely that sessile 
serrated adenomas, lesions with high levels of BRAF 
mutation (10), may also be more common in people of 
Anglo-Celtic descent than in people of southern 
European descent. The prevalence of these lesions in 
patients undergoing colonoscopy ranges from 4% to 9% 
(43, 44) and is associated with increased polyp burden 
(44). CIMP tumors are frequently located in the proximal 
colon (40), and the detection of their sessile precursor 
lesions may prove difficult (45). CIMP-related colorectal 
tumors have been shown to account for almost all 
nonfamilial MSI-high colorectal cancers (11) and are 
associated with improved survival for this subgroup (46).

In contrast, tumors that show somatic BRAF mutation in 
the absence of a MSI-high phenotype may have a 
relatively poor outcome (37).

In summary, we have confirmed the findings of 
Weisenberger et al. (21) that their five-marker panel 
effectively identifies CIMP colorectal cancer and its 
association with BRAF mutation, female sex, proximal 
location in the colon, MSI-high tumor status, and MLH1 
methylation. Further, we report the novel finding that 
individuals with Anglo-Celtic ancestry were more likely 
to develop CIMP-related colorectal cancer than were 
individuals of Italian or Greek origin, and this difference 
was unlikely to be due to environmental factors 
commonly associated with colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Section 1734 solely to indicate this fact.

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