The CpG Island Methylator Phenotype Is Not Associated with a Personal or Family History of Cancer

Robyn Lynne Ward,1,2 Rachel Williams,1 Matthew Law,4 and Nicholas John Hawkins3

1Department of Medical Oncology, St. Vincent’s Hospital, Darlinghurst, New South Wales; and Schools of 2 Medicine and 3 Medical Sciences and 4 National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia

ABSTRACT

Colorectal cancers with widespread CpG island methylation display a number of distinct clinicopathological features, and it has been suggested that the condition has an inheritable genetic component. To address this possibility, histories of cancer were obtained from 562 individuals undergoing curative surgery for unselected colorectal cancer at one institution. Microsatellite status and methylation at p16, MINT1, 2, 12, and 31 loci were determined on fresh tumor tissue using standard methods. Fifty-five of 562 probands in this study provided a personal history of at least one other colorectal cancer, 10 reported at least one extracolonic cancer of hereditary nonpolyposis colorectal cancer type, and 84 individuals had another type of cancer. Age was strongly associated with the risk of multiple cancers, but there was no evidence that microsatellite instability or the CpG island methylator phenotype were independent risk factors for their development, either in the colorectum or elsewhere. Of the 547 individuals with knowledge of their family history, 80 (14.6%) reported a family history of colorectal cancer in a first-degree relative, and 60% of individuals reported a history of any cancer in a first-degree relative. Neither tumor CpG island methylator phenotype status nor microsatellite instability was predictive of a positive history of cancer in first- or second-degree relatives. The probability of a positive family or personal history of cancer did not increase with increasing number of methylated loci. Epigenetic silencing of multiple genes seen in some tumors is at best rarely the result of an inherited defect in the methylation apparatus. There is no justification for altering the personal or family cancer screening recommendations on the basis of tumor CpG island methylator phenotype status.

INTRODUCTION

Microsatellite unstable sporadic colorectal cancers may arise as a consequence of transcriptional silencing of the hMLH1 promoter secondary to methylation. It has been proposed that this subset of sporadic cancers overlaps with a larger group of tumors that display extensive methylation of CpG islands at MINT loci and p16 (CpG island methylator phenotype-positive; refs. 1, 2). Whereas the existence of a “CpG island methylator phenotype” pathway of tumorigenesis remains in dispute (3), it is clear that extensively methylated cancers display a number of distinguishing clinicopathological features (4). Regardless of microsatellite status, such tumors are more likely to be right-sided and to display a mucinous phenotype (5). Furthermore, we have shown that individuals with heavily methylated but microsatellite-stable tumors had a significantly worse outcome than those with microsatellite-unstable tumors or the more common nonmethylated microsatellite-stable tumors (6). Despite these findings, it is apparent the definition of CpG island methylator phenotype is imprecise and as yet there is no biochemical basis for the phenomenon (3, 7).

Given these difficulties, it is of interest that a recent study of 47 patients found that individuals with CpG island methylator phenotype-positive colorectal cancers have an increased frequency of cancer in their first-degree relatives (8). Implicit in this interesting observation is that an inheritable genetic predisposition may underlie the CpG island methylation phenotype and may lead to the development of CpG island methylator phenotype-positive tumors in multiple family members.

We have identified recently two individuals who fit clinical criteria for hereditary nonpolyposis colorectal cancer yet displayed somatic and allelic-specific hypermethylation of the hMLH1 promoter (9). These findings suggest that abnormal epigenetic silencing (epimutation) of key cancer predisposition genes may produce a phenocopy of a known genetic disease. Gazzoli et al. (10) have also identified hypermethylation of hMLH1 promoter in the peripheral blood of a mutation-negative individual with hereditary nonpolyposis colorectal cancer, and Ricciardiello et al. (11) reported that hypermethylation of hMLH1 was frequently found in individuals with first-degree relatives with colorectal cancer. Whereas these findings indicate a relationship between a germ-line epimutation in hMLH1 and tumorigenesis they do not imply that the majority or even a significant minority of CpG island methylator phenotype-positive tumors arise as a result of an inherited defect in the methylation apparatus. In this study, we have sought to study the relationship between widespread tumor methylation and a familial history of cancer, through the use of a large cohort of 603 consecutive unselected colorectal cancer patients.

MATERIALS AND METHODS

Patients. After obtaining informed consent, 603 consecutive individuals from St. Vincent’s Hospital undergoing complete surgical resection (Tumor-Node-Metastasis RO or R1; ref. 12) of colorectal cancer were entered in this prospective study. Patients presenting for resection of cancer in the setting of known familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or inflammatory bowel disease were not enrolled. This study was approved by the Hospital Ethics Committee, and enrolment was from January 1, 1994 to August 1, 2002. After collection of tumor material, 5 individuals were found to have a pathogenic germ-line mutation in the mismatch repair genes (3 in hMSH2 and 2 in hMLH1), and 1 individual was found to carry a homozygous MYH mutation. These individuals and their tumors were excluded from additional analysis. Therefore, the evaluable population consisted of 597 individuals (277 females and 320 males). The follow-up of these patients was undertaken on a 6-monthly basis for a period of 5 years or until death. At the time of diagnosis, as well as at each follow-up visit, the proband was asked to report their personal or family history of cancer. Verification of the type of other personal cancers and the type of cancer in family members was performed where possible by reference to medical records or local cancer registries. These cancers were classified as colorectal, extracolonic cancer of hereditary nonpolyposis colorectal cancer type (endometrial, ovarian, transitional cell carcinoma of the urinary tract, intestinal-type gastric cancer, or glioma), or other.

Detection of Microsatellite Instability and Methylation of p16, MINT 1, 2, 12, and 31. The full details of tumor collection, histopathological analysis, and methylation analysis have been described previously (6). Briefly, fresh representative tissue samples (500 μg) from all of the tumors and paired normal colonic mucosa were immediately frozen at −70°C. The microsatellite status of each tumor was determined as described previously using the following primer sets: Bat 25, Bat 26, Bat 40, DSS346, D2S123, and D17S250...
RESULTS

Factors Influencing Personal Cancer History of Colorectal and Other Cancers. At the time of last follow-up the study population of 277 females and 320 males had a mean age of 71.1 ± 12.1 years (range, 34 to 101 years). Thirty-five individuals were unable to provide details of family or personal history of cancer, and an additional 15 individuals had no knowledge of their family medical history. Fifty-five of 562 individuals provided a personal history of at least one other colorectal cancer (mean, 1.1; range, 1 to 4), and the probability of such a history significantly increased with advancing age (odds ratio, 1.05; 95% confidence interval, 1.03–1.08; \( P < 0.0001 \); Fig. 1). Individuals with right-sided cancers were 2.3 times more likely (95% confidence interval, 1.3–4.0; \( P = 0.004 \)) to report a personal history of colorectal cancer (30 of 206) than those with left-sided tumors (25 of 356). We also noted that a personal history of colorectal cancer was found more frequently in individuals with CpG island methylator phenotype-positive cancers (9 of 57 CpG island methylator phenotype-positive cancers) compared with CpG island methylator phenotype-negative cancers (40 of 428 CpG island methylator phenotype-negative cancers). The difference between these groups was, however, not statistically significant (odds ratio, 2.0; 95% confidence interval, 0.9–4.4; \( P = 0.08 \); Fig. 1).

Although it appeared that age, CpG island methylator phenotype-positive status, and tumor side may all predict a personal history of colorectal cancer, a multivariate analysis identified older age as the only significant predictor (odds ratio, 1.05; 95% confidence interval, 1.01–1.08; \( P = 0.003 \)).

Of the 562 individuals who were able to comment on their personal history of cancer, 10 reported at least one extracolonic cancer of hereditary nonpolyposis colorectal cancer type, and 84 individuals had another type of cancer. Given the small number of extracolonic cancers of hereditary nonpolyposis colorectal cancer type in this population, it is perhaps not surprising that the confidence intervals for age, gender, tumor side, microsatellite, and CpG island methylator phenotype status were wide and crossed unity (Fig. 1).

Older age was associated significantly with a personal history of colorectal cancer, other cancers, and all of the cancers in combination (Fig. 1). Of the 57 individuals with CpG island methylator phenotype-positive tumors, 13 reported a personal history of another cancer, two of which were extracolonic cancer of hereditary nonpolyposis colorectal cancer type. These figures did not differ significantly from the finding of 79 of 468 individuals with index CpG island methylator phenotype-negative cancers who also had another cancer (7 of which were extracolonic cancers of hereditary nonpolyposis colorectal cancer type). As expected, microsatellite instability and CpG island methylator phenotype-positive tumors were more common in older individuals (3); however, these variables were not associated with a personal history of cancer of any type (colon, extracolonic cancer of hereditary nonpolyposis colorectal cancer type, other cancer, or combined; Fig. 1). Furthermore, the total numbers of cancers in a single individual did not predict the CpG island methylator phenotype status of the index tumor (Fig. 2).

Factors Influencing a Family History of Cancer. The 547 assessable individuals with knowledge of their family history reported 332 cancers in first-degree relatives (80 colorectal, 43 extracolonic cancers of hereditary nonpolyposis colorectal cancer type, and 209 other cancers) and 200 cancers in second-degree relatives (66 colorectal, 22 extracolonic cancers of hereditary nonpolyposis colorectal cancer type, and 112 other cancers). There was no association between either tumor methylation or microsatellite status and a family history of cancer in first- (Table 1) or second-degree relatives (data not shown). These findings were the same irrespective of whether family history was analyzed using the total number of tumors in relatives or on the basis that one or more relatives with any cancer constituted a positive family history. The probability of a positive family history of cancer did not increase with increasing number of methylated loci (Fig. 2).
was defined as methylated at (extracolonic cancer of hereditary nonpolyposis colorectal cancer type), or other cancer. For each type of second cancer, OR with 95% CIs and statistical significance are shown. CIMP
MSI, microsatellite unstable; N/A, not applicable.

Inclusion and exclusion criteria applied in each study. Clearly inad-
al.

the methylation apparatus. Reconciling the conclusions of Frazier
possibility that tumors with abnormal epigenetic silencing of multiple
were 14 times more likely to have a first-degree relative with cancer
malignancy or, indeed, have a positive family history of cancer. These
elected colorectal cancers were more likely to develop a second
cancer if such an association was confirmed, it would clearly raise the
likelihood of systematic bias in the present study seems unlikely.

The clinical findings in the present study support experimental data,
which to date has failed to implicate germ-line or somatic mutations
in the DNA methyltransferase genes in tumor development. Indeed,
loss of function Dnm1 mutations in APC^{Min} mice results in a reduc-
tion in the number of colonic polyps without widespread alterations
in the methylation profile of key genes (19). Our data does not support
the concept that a germ-line defect in the methylation machinery is
responsible for the development of most tumors with multiple epimu-
tations. However, the possibility that this may occur in a few cases has
not been excluded, because population studies such as this are insen-
sitive to rare events. For instance, low-risk genotypes that determine
methyl group metabolism or shared environmental factors (such as
folate intake) may contribute to the development of CpG island
methylator phenotype-positive cancers in multiple family members.

One weakness of our study is that cancer history in first degree
relatives was self-reported and, therefore, may be underestimated (20,
21), although some studies indicate that the magnitude of this error is
quite small (22). In either case, the consequences of this type of error
can be quantified. The largest association observed in our study was
an odds ratio of 1.3 for heavy methylation associated with a first-
degree relative with colorectal cancer. For this to represent a true odds
ratio as modest as 2, assuming that misclassification rates were the
same for both subjects with and without a first-degree relative with
colorectal cancer, misclassification rates would need to have been of
the order of 11%. To put this in context, this would mean that >50 of
the 75 subjects reporting a first-degree relative with colorectal cancer
would need to misreport this event. Such extreme misclassification is
unlikely, even with self-reported data, and suggests that true associ-
tions between methylation and family history are only modest at best.

One key clinical conclusion from this study is that in the setting of
seemingly unselected colorectal cancer, there is no justification for
altering the personal or family cancer screening recommendations on
the basis of tumor methylation status. The other important conclusion

<table>
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<th>Factor</th>
<th>No. of assessable individuals</th>
<th>Number of cases (Degree relatives)</th>
<th>Number of cases (Other cancer)</th>
<th>OR (95% CI)</th>
<th>P</th>
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<td>Age</td>
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<td>150 (1.0–1.0)</td>
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<td>Microsatellite</td>
<td>91</td>
<td>54 (0.5–2.2)</td>
<td>47 (0.5–3.1)</td>
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<td>CIMP</td>
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<td>45 (0.6–2.8)</td>
<td>50 (0.3–2.3)</td>
<td>0.4</td>
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</table>

NOTE. For the 547 individuals with index colorectal cancer and assessable family history, the nature of the second cancer was characterized as colorectal cancer, HNPPC-type cancer (extracolonic cancer of hereditary nonpolyposis colorectal cancer type), or other cancer. For each type of second cancer, OR with 95% CIs and statistical significance are shown. CIMP was defined as methylated at >3 of 5 sites (MINT sites and p16). P < 0.05 was considered significant and P trend is reported for methylation status analyzed as a continuous variable.

Abbreviations: OR, odds ratio; CI, confidence interval; HNPPC, extracolonic cancer of hereditary nonpolyposis colorectal cancer type; CIMP, CpG island methylator phenotype; MSI, microsatellite unstable; N/A, not applicable.
is that the development of heavily methylated colorectal cancer does not, in the vast majority of cases, appear to involve a heritable trait.

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REFERENCES
