Patient and Tumor Characteristics of Colon Cancers with Microsatellite Instability: A Population-based Study[1]

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Abstract

Molecular screening for microsatellite instability (MSI) in colon cancers has been proposed to identify individuals with hereditary nonpolyposis colorectal cancer. To date, most reports of MSI in colorectal cancer have been based on studies of clinical case series or high-risk families. We examined the proportion of incident colon cancers in the general population that exhibit MSI by patient and tumor characteristics. We interviewed 201 colon cancer cases ascertained by the New Mexico Tumor Registry in the metropolitan Albuquerque area for demographic information, lifestyle factors, medical history, and family cancer history. Paired normal and tumor tissue specimens were obtained for each case. Three microsatellite markers were used; instability was defined as observed alteration at two or more loci. Overall, 37 of 201 (18%) colon cancers exhibited instability. MSI was more common among cases >70 years (26%) and most common among cases >80 years (38%). MSI was significantly associated with tumors in the proximal colon and with later stage and poor differentiation among cases >70 years. MSI was not associated with a history of polyps. Family history of colorectal cancer was associated with MSI only among cases <50 years. When all factors were analyzed jointly in a regression model, proximal subsite and poor differentiation remained significantly associated with MSI. One patient, whose tumor exhibited MSI, fulfilled the Amsterdam Criteria for hereditary nonpolyposis colorectal cancer. Our study provides a population-based estimate of MSI in colon tumors and a representative estimate of the proportion of colorectal cancer patients in the general population who consent to be interviewed for family cancer history and to have biological samples analyzed.

Introduction

Colorectal cancer is the third most commonly diagnosed cancer and the third leading cancer cause of death in the United States (1). Since 1993, an enormous amount of knowledge has emerged about the genetic basis of HNPCC,[3] the most common form of familial colon cancer (2, 3). Several laboratories showed that the majority of HNPCC tumors exhibited a mutator phenotype, MSI, characterized by small deletions or expansions in the length of microsatellites—short, tandemly repeated DNA sequences that occur randomly throughout the genome (4–7). MSI in HNPCC was subsequently found to arise from germline mutations in at least four human MMR genes (8–11).

HNPCC tumors usually occur in patients in their mid-forties, show predominance in the proximal colon, are of mucinous histology and poor differentiation, and are more likely to be synchronous and metachronous in the colon and extracolonic sites (12–14). Prior to the identification of MSI and MMR gene mutations, HNPCC (also known as Lynch Syndromes I and II) was defined clinically by family history of colorectal and other cancers. The Amsterdam Criteria were developed in 1991 to standardize the definition of HNPCC (15), although they have been deemed overly restrictive, and other criteria have been developed, including the modified Amsterdam Criteria and the Bethesda Guidelines (16).

MSI also has been observed in sporadic, or nonfamilial, colorectal cancers, with prevalence estimates ranging from 9 to 20% (13–14, 17–22). MSI in sporadic colorectal cancers can arise from mutations in MMR (or other) genes or hypermethylation of the promotor region of the *hMLH1* gene (23–28). Sporadic MSI tumors are generally larger in size, are of mucinous histology and poor differentiation, predominantly occur in the proximal colon, and are less likely to arise from adenomas or show aneuploidy and p53 mutations (13, 29–33).

Most reports of MSI in colon cancers to date have been based on studies of clinical case series, high-risk families, or referral patient populations. Population-based estimates of MSI in colorectal cancers are needed to apply knowledge on the genetic basis of colorectal cancers to the general population for primary and secondary prevention. Screening for MSI has been proposed for all early onset colorectal cancers and for cases

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[3] The abbreviations used are: MSI, microsatellite instability; HNPCC, hereditary nonpolyposis colorectal cancer; OR, odds ratio; CI, confidence interval; MMR, mismatch repair.

exhibiting a family history of colorectal or extracolonic cancers, to identify those with germline mutations in MMR genes (22, 34). Such screening for family cancer history and MSI would necessitate patient consent to interview and biological sample collection and testing. We report here a cancer registry-based study of the proportion of incident colon cancer cases in the general population that exhibit MSI, among persons who consented to be interviewed for family cancer history and to have biological samples tested.

Patients and Methods

Study Subjects. Colon cancer cases were diagnosed from January 1, 1996, to December 31, 1997, among residents of the two-county metropolitan Albuquerque area. Cases were ascertained by the New Mexico Tumor Registry, a population-based cancer registry established in 1969 and a member of the National Cancer Institute Surveillance, Epidemiology, and End Results program since 1973. The study included histologically confirmed carcinomas of all histology defined by primary site codes C18.0 to C18.9 according to the International Classification of Diseases for Oncology (35). Eligible cases included English-speaking subjects of all racial/ethnic groups and all ages residing in the two-county area, subjects ages 50 years and younger living throughout the state of New Mexico, and subjects diagnosed in one of the participating study hospitals in Albuquerque. Six Hispanic patients were excluded because of the English language requirement. Proximal colon included subsites from the cecum to the splenic flexure; cases with unspecified primary subsite code were excluded from subsite-specific analyses. Rectal cases with primary site codes C19.9 and C20.9 were excluded from analyses.

A total of 365 eligible colon cancer cases were ascertained during the study period in the metropolitan Albuquerque area and were invited to participate in the study after their physicians were notified of the study. Upon informed consent, patients were interviewed by trained interviewers to obtain information on demographics, lifestyle factors [including physical activity and diet (food frequency method)], medical history, and family cancer history among first- and second-degree relatives using a structured questionnaire. Interviewers also were trained by a certified genetic counselor to construct a family tree, or pedigree, with each patient. Because testing for MSI was done in a research rather than diagnostic setting, test results were not divulged to subjects. This study was approved by the Institutional Review Board of all five major hospitals serving the Albuquerque community and by the Human Research Review Committee of the University of New Mexico.

Of the 365 cases, 228 (63%) were successfully interviewed, 40 (11%) were deceased, 45 (12%) declined to be interviewed, and 52 (14%) either had moved and could not be contacted, were hospitalized at the time of contact, or were not recommended to be interviewed by their physician. Of the 228 interviewed cases, 201 could be tested for MSI. Of the 27 cases that could not be tested, 13 cases did not have a tissue sample available for retrieval, 10 cases had insufficient tumor or normal tissue, and 4 cases had failed PCR amplification. Table 1 presents the distribution of cases by patient and tumor characteristics, separately among the 201 study participants who were interviewed and had a valid MSI test result and 164 nonparticipants who either were not interviewed or did not have a valid MSI test result. Study participants were significantly younger in age and diagnosed at an earlier stage than nonparticipants.

We report here completed analysis on 201 colon cancer cases, consisting of 106 men and 95 women. Racial/ethnic distribution of colon cancer cases in our study population resembles that of the population distribution of colon cancer cases reported in the state of New Mexico (69% non-Hispanic white and 31% nonwhite, including Hispanic, African American, American Indian, and Asian). Twenty % of cases in our study population were younger than 50 years, 42% of cases were 50–70 years old, and 38% of cases were 71 years old or older at diagnosis.

Table 1 Distribution of colon cancer patients by age at diagnosis, gender, and tumor characteristics, New Mexico Tumor Registry, 1996–1997

	Participants[a] $n = 201$ (%)	Nonparticipants[b] $n = 164$ (%)	P
Age at diagnosis (yr)			
<45	20 (10.0)	15 (9.2)	0.042
45–49	19 (9.5)	11 (6.7)	
50–70	85 (42.2)	53 (32.3)	
71–80	56 (27.9)	51 (31.1)	
>80	21 (10.4)	34 (20.7)	
Sex			
Male	106 (52.7)	94 (57.3)	
Female	95 (47.3)	70 (42.7)	
Race/ethnicity			
White	138 (68.7)	102 (62.2)	
Hispanic	54 (26.9)	50 (30.4)	
Other[c]	9 (4.4)	6 (3.7)	
Unknown	0	6 (3.7)	
Colon subsite			
Proximal	101 (50.2)	81 (49.4)	
Distal	87 (43.3)	63 (38.4)	
Colon, not otherwise specified	13 (6.5)	20 (12.2)	
Tumor histology			
Mucinous	26 (12.9)	14 (8.5)	
Nonmucinous	169 (84.1)	142 (86.6)	
Not specified	6 (3.0)	8 (4.9)	
Tumor grade or differentiation			
Well/moderately differentiated	147 (73.1)	101 (61.6)	
Poorly differentiated	30 (15.0)	22 (13.4)	
Not specified	24 (11.9)	41 (25.0)	
Tumor stage			
In situ	11 (5.5)	15 (9.1)	0.001
Local	64 (31.8)	38 (23.2)	
Regional	100 (49.8)	52 (31.7)	
Distant	20 (10.0)	46 (28.1)	
Unknown	6 (2.9)	13 (7.9)	

[a] Participants are colon cancer patients who were interviewed and had a MSI testing result.
[b] Nonparticipants include colon cancer patients not interviewed or who had no MSI result.
[c] Including American Indians, African Americans, and Asians.

Specimen Analysis. Paired normal and tumor remnant tissue specimens from colectomy were obtained for each case within 24 h after surgery. When fresh tissue sample was not available for a case, a paraffin-embedded block tissue specimen was obtained via the New Mexico Tumor Registry tissue acquisition system. Upon morphology review by the study pathologist (N. J.), genomic DNA was microdissected, then isolated using the QIAamp tissue kit (Qiagen, Inc., Santa Clarita, CA). PCR was performed on each pair of normal and tumor tissue, using three fluorescently labeled primers on a GeneAmp 9600 (Perkin-Elmer Corporation, Norwalk, CT). The three PCR products were then pooled and analyzed for allele sizing and quantitation using a laser-induced fluorescence capillary electrophoresis system on the ABI 310 genetic analyzer (Applied Biosystems, Inc., Foster City, CA).

Three repeat sequence microsatellite markers of single polyadenine track were used (*BAT25*, 5′-TCGCCTCCAAGAATGTAAGT-3′ and 3′-TCTGAATTTTAACTATGGC-

Table 2 Univariate and age-adjusted ORs and 95% CIs for MSI in colon cancer by patient characteristics, New Mexico Tumor Registry, 1996–1997

	Colon cancers with MSI		Total n^a	Univariate OR (95% CI)	Age-adjusted OR (95% CI)
	n	%			
MSI at 2+ loci	37	18	201		
Age at diagnosis (yr)					
<45	5	25	20	2.8 (0.8–9.6)	
45–49	3	16	19	1.6 (0.4–6.5)	
50–70	9	11	85	1.0	
71–80	12	21	56	2.3 (0.9–5.9)	
>80	8	38	21	5.2 (1.7–15.9)	
Sex					
Male	15	14	106	1.0	1.0
Female	22	23	95	1.8 (0.9–3.8)	1.9 (0.9–4.1)
Race/ethnicity					
White	29	21	138	1.0	1.0
Hispanic & other[b]	8	13	63	0.5 (0.2–1.3)	0.6 (0.2–1.4)
Personal history of polyps					
No	26	26	100	1.0	1.0
Yes	11	11	97	0.4 (0.2–0.8)	0.4 (0.2–0.8)
Family history of colorectal cancer					
No	29	17	174	1.0	1.0
Yes	8	30	27	2.1 (0.8–5.3)	1.7 (0.7–4.5)

[a] Numbers for some variables do not total 201 due to missing values.
[b] Including American Indians, African Americans, and Asians.

TC-5′; *BAT26*, 5′-TGACTACTTTTGACTTCAGCC-3′ and 3′-AACCATTCAACATTTTTAACCC-5′; and *BAT40*, 5′-ACAACCCTGCTTTTGTTCCT-3′ and 3′-GTAGAGCAAGACCACCTTG-5′) to identify high-frequency (or true) MSI tumors. This set of three markers was chosen based on their high sensitivity and consistency in detecting high-frequency MSI in our study sample and in other studies (36–40). In particular, *BAT26* has been shown to be a highly sensitive marker for MSI (36). We did not distinguish low-frequency MSI from stable tumors. The decision to use the three-marker set was based on available knowledge at the time our laboratory began specimen analysis in 1996–1997, prior to the establishment of the five-marker consensus panel by the National Cancer Institute Workshop (39). MSI was defined as expansion or reduction in tumor allelic tract length compared to paired normal DNA in at least two of three loci. This approach was validated by analysis of a subsample of 69 cases using a battery of 10 microsatellite markers (*D8S254*, *NM23*, *D18S35*, *P53-Di*, *D5S346*, *P53-Pen*, *D2S123*, *D1S2883*, *D3S1611*, and *D7S501*), with MSI defined as observed alterations in four or more markers. Results showed 100% agreement with those obtained using three markers in determining MSI status, with the three-marker set correctly identifying all high-frequency MSI tumors.

Statistical Analysis. Statistical analyses focused on comparing the proportion of colon tumors exhibiting MSI to those not exhibiting MSI, using the χ^2 or Fisher's exact test statistic (two-sided $P < 0.05$). We estimated the OR and 95% CI for MSI comparing patient and tumor characteristics. Age- and multivariate-adjusted ORs (95% CI) were estimated using logistic regression modeling with MSI as the outcome of interest. Statistical interaction was evaluated using the likelihood ratio test (at $P < 0.05$) comparing model goodness of fit with and without interaction terms between age group and each covariate (41). All statistical analyses were conducted using the software package SAS (42).

Results

Overall, 37 (18%) of the 201 colon cancer cases exhibited MSI in at least two of three loci (Table 2). Although the mean age at diagnosis of cases exhibiting MSI (66 years) was not statistically different from that of cases not exhibiting MSI (64 years), a substantial difference was observed in the proportion of cases with MSI when stratified by age group. The proportion of MSI was higher among cases less than age 45 years and among those older than age 70 years. The highest proportion of MSI was observed among cases over 80 years of age (38%), with an OR of 5.2 (95% CI, 1.7–15.9) compared to cases ages 50–70 years. MSI was inversely associated with a personal history of polyps (age-adjusted OR, 0.4; 95% CI, 0.2–0.8). Patient characteristics positively associated with MSI included being female, white, and reporting family history of colorectal cancer; these associations were not statistically significant.

Tumor characteristics associated with MSI are presented in Table 3. Tumors in the proximal colon were significantly more likely to exhibit MSI than those in the distal colon (33% compared to 4%; Fisher's exact test, $P < 0.001$), as were tumors of mucinous histology (39% compared to 15% in nonmucinous carcinomas; χ^2, $P = 0.011$) and those poorly differentiated (50% compared to 12% in tumors of well to moderate differentiation; χ^2, $P < 0.001$). Later stage of disease at diagnosis was weakly associated with MSI.

When stratified by age at diagnosis (<50 years, 50–70 years, and >70 years), the relationship between MSI and sex, family history of colorectal cancer, tumor grade, and stage appeared limited to certain age groups (Table 4). MSI was associated with family history of colorectal cancer and tumors of mucinous histology only among cases <50 years old, whereas the increased proportion of MSI in female cases was observed primarily among cases >70 years old. MSI was more common in proximal colon cancers at all ages. Poor tumor differentiation was associated with MSI in cases >50 years old; this was the only factor that showed significant interaction with age. We did not have adequate statistical power to evaluate an age interaction with other variables, such as family history of colorectal cancer and colon subsite.

When the above factors were examined simultaneously in a logistic regression model (Table 5), MSI was significantly associated with tumor being in the proximal colon and poor

Table 3 Univariate and age-adjusted ORs and 95% CIs for MSI in colon cancer by tumor characteristics, New Mexico Tumor Registry, 1996–1997

	Colon cancers with MSI		Total n^a	Univariate OR (95% CI)	Age-adjusted OR (95% CI)
	n	%			
Colon subsite					
Proximal	33	33	101	13.6 (4.0–46.2)	14.4 (4.1–50.5)
Distal	3	4	87	1.0	1.0
Tumor histology					
Mucinous	10	39	26	3.4 (1.4–8.4)	3.7 (1.5–9.4)
Nonmucinous	26	15	169	1.0	1.0
Tumor grade or differentiation					
Well/moderately differentiated	18	12	147	1.0	1.0
Poorly differentiated	15	50	30	7.2 (3.0–17.1)	6.4 (2.6–15.6)
Tumor stage					
Local	10	16	64	1.0	1.0
Regional/distant	25	21	120	1.4 (0.6–3.2)	1.6 (0.7–3.8)

[a] Numbers do not total 201 due to missing values.

Table 4 ORs and 95% CIs for MSI in colon cancer by patient and tumor characteristics, by patient age at diagnosis, New Mexico Tumor Registry, 1996–1997

	Age <50 yr (n = 39)		50–70 yrs (n = 85)		Age >70 yrs (n = 77)	
	% MSI	OR (95% CI)	% MSI	OR (95% CI)	% MSI	OR (95% CI)
Sex						
Male	23	1.0	9	1.0	17	1.0
Female	19	0.8 (0.2–4.0)	13	1.6 (0.4–6.5)	39	3.0 (1.1–8.6)
Personal history of polyps						
No	32	1.0	15	1.0	34	1.0
Yes	6	0.1 (0.02–1.3)	7	0.4 (0.1–1.8)	19	0.5 (0.2–1.3)
Family history of colorectal cancer						
No	14	1.0	11	1.0	25	1.0
Yes	40	4.2 (0.8–21.6)	0		31	1.3 (0.4–4.9)
Colon subsite						
Proximal	43	6.8 (1.1–41.0)	18	8.8 (1.0–74.8)	42	
Distal	10	1.0	2	1.0	0	1.0
Tumor histology						
Mucinous	60	8.4 (1.1–63.7)	18	2.4 (0.4–13.8)	50	3.3 (0.8–13.1)
Nonmucinous	15	1.0	9	1.0	23	1.0
Tumor grade or differentiation						
Well/moderately differentiated	19	1.0	7	1.0	15	1.0
Pooly differentiated	13	0.6 (0.1–6.1)	50	12.8 (2.0–80.7)	69	12.1 (3.3–44.3)
Tumor stage						
Local	29	1.0	4	1.0	22	1.0
Regional/distant	21	0.7 (0.1–4.2)	13	3.7 (0.4–31.4)	32	1.7 (0.6–4.9)

differentiation. Family history of colorectal cancer was no longer associated with MSI after accounting for age and tumor characteristics. Only 1 patient of 201 (0.5%) in the study sample fulfilled the strict Amsterdam Criteria for HNPCC. This 58-year-old patient was diagnosed with an invasive adenocarcinoma of the transverse colon, and the patient's tumor sample revealed instability at all three microsatellite loci.

Discussion

Our findings from a cancer registry-based study of MSI in incident colon cancers show that 18% of cases in this population-based sample exhibited MSI. Instability was more common among female cases and non-Hispanic whites and highest among cases diagnosed after age 70 years. The majority of MSI tumors in our study originated in the proximal colon and was poorly differentiated. Other than predominance in the proximal colon and lack of previous polyps in all age groups, substantial differences in the profile of cases with MSI were observed by age in our data. When patient age and tumor characteristics were accounted for, family history of colorectal cancer was no longer associated with MSI.

The most striking finding of our study was the J-shaped age distribution of colon cancers with MSI. Previous studies have in general described colon cancer cases with MSI by family history and by mean age at disease diagnosis (3, 13, 17, 29). Two European studies have reported mean age at diagnosis in MSI cases to be 68–69 years, nearly identical to that in cases without MSI (13, 22). A Japanese study found the mean age at diagnosis in cases with MSI to be 73 years, statistically different from the mean age of 61 years in cases without MSI (19). These studies did not show in which age groups MSI was more common. Similar to our data, Samowitz *et al.* (18) found highest proportions of MSI in the youngest and oldest age groups, although they excluded cases older than age 79 years, the group with the highest MSI proportion in our study. Our observation of a higher proportion of MSI in older female cases has not been reported by other studies. This association may be due, in part, to the fact that the proportion of incident proximal colon cancers among female cases increases with age in our study population (43).

The overall proportion of colon cancers with MSI in our sample falls within the 9–20% range reported previously (13,

Table 5 Univariate and multivariate-adjusted ORs and 95% CIs from logistic regression modeling of patient and tumor characteristics associated with microsatellite instability in colon cancer, New Mexico Tumor Registry, 1996–1997

	Univariate OR (95% CI)	Multivariate-adjusted OR (95% CI)[a]
Proximal colon	13.6 (4.0–46.2)	16.2 (3.3–78.6)
Poor differentiation	7.2 (3.0–17.1)	4.9 (1.7–14.2)
Mucinous histology	3.4 (1.4–8.4)	2.4 (0.8–7.4)
Age <50 yr	2.2 (0.8–6.2)	2.8 (0.7–11.4)
Age >70 yr	3.0 (1.3–7.0)	2.4 (0.8–6.8)
Family history of colorectal cancer	2.1 (0.8–5.3)	1.0 (0.3–3.1)
Regional/distant stage	1.4 (0.6–3.2)	0.8 (0.3–2.3)
Personal history of polyps	0.4 (0.2–0.8)	0.8 (0.3–2.2)

[a] Adjusted for all other variables in the table.

14, 18–22). The 18% observed in our sample of interviewed cases was somewhat higher than the 12% reported from Finland (22) but similar to the 16.4% reported from the United Kingdom (21), 16.5% from Norway (13), and 16.5% from Utah (18). Most of these studies were hospital-based and examined nonfamilial colorectal cancers. Possible explanations for the varying estimates have thus far focused on the type and number of microsatellite markers used in different studies (37, 44, 45). However, differences in study population also may account for part of the difference. Our study of colon cancers may have yielded a slightly higher proportion of MSI tumors compared to other studies that included colon and rectal cancers. The Utah study was the only published study that stated inclusion of colon cancers only (18).

Besides differences in study populations, another possible explanation for the different proportions of MSI reported is the set of case selection criteria used by each study. It has been suggested that colon cancer patients with MSI, like HNPCC patients, tend to have better prognosis and increased survival despite the fact that MSI tumors generally show characteristics associated with poor prognosis (7, 13, 21, 46–48). Thus, studies that include only interviewed cases may find a higher proportion with MSI than studies that test all available tissue samples. Indeed, previous studies that have reported higher proportions of MSI (16–20%) have in general indicated testing tissue sample from interviewed cases (13, 14, 18, 19). Studies that tested available tissue without indicating patient contact (20) and studies that obtained patient information from medical records or other sources (22, 29) tended to report lower proportions of MSI (9%–13%). The observed 18% in our sample may be higher than what would have been observed had we tested samples from all cases diagnosed during the study period regardless of interviewing status.

Our study sample under-represents American Indian cases, in whom a Navajo HNPCC kindred has been identified (49). We also have not yet determined the proportion of cases with germline mutations in MMR genes responsible for HNPCC. Based on the observation that MSI is associated with family history of colorectal cancer in the youngest cases, we postulate that some of these may represent familial cases. As other studies relying on self-reported family history of cancer, potential misclassification of this variable may partly account for the lack of association between MSI and family history among older cases. Inaccuracies in recall of family history, especially beyond first-degree relatives, may account for the observation that only one patient in our study population fulfilled the strict Amsterdam Criteria for HNPCC. However, this low proportion is consistent with the 0.3% reported in a population-based study in the United Kingdom using the Amsterdam Criteria to classify HNPCC (50).

As genetic testing for colorectal cancer susceptibility becomes integrated into clinical practice (51), screening for MSI, in combination with other patient and tumor characteristics, has been proposed for the identification of candidates to undergo testing for germline mutations in MMR genes (22, 34). Although the identification of individuals and families at high risk is important for the design and implementation of efficacious primary and secondary disease prevention strategies, the cost effectiveness and the legal and ethical implications of genetic screening, diagnosis, and counseling in clinical practice have yet to be determined in population-based samples. The 18% observed in our study, among cases consenting to be interviewed, represents a realistic estimate of what would be observed in a screening study requiring patient contact to obtain informed consent, family cancer history, and biological samples.

In summary, our study reports on patient and tumor characteristics of colon cancers that exhibit MSI from a population-based sample. The increasing proportion of MSI observed with age in our study highlights the importance of environmental as well as genetic factors in colorectal cancer carcinogenesis via the MMR pathway (52). Population-based studies with larger samples are needed to obtain stable estimates of MSI in colon cancers in the general population and to examine the interaction between the MSI phenotype, specific gene mutations or epigenetic changes, and modifiable lifestyle factors. Such studies will have important public health implications for primary disease prevention or the delay of disease onset among high-risk individuals. Unveiling the genetic basis of HNPCC has advanced knowledge not only for familial colorectal cancers but also for a better understanding of the nonfamilial cases that represent the majority of the burden of this common cancer in the general population.

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