Germline MutY Human Homologue Mutations and Colorectal Cancer: A Multisite Case-Control Study

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See related article, Chiu H–M et al, on page 463 in *CGH*.

Background & Aims: **The *MutY* human homologue (*MYH*) gene is a member of the base-excision repair pathway involved in the repair of oxidative DNA damage. The objective of this study was to determine colorectal cancer (CRC) risk associated with mutations in the MYH gene.** ***Methods:*** **A total of 3811 CRC cases and 2802 controls collected from a multisite CRC registry were screened for 9 germline *MYH* mutations; subjects with any mutation underwent screening of the entire *MYH* gene. Logistic regression was used to estimate age- and sex-adjusted odds ratios (AOR). Clinicopathologic and epidemiologic data were reviewed to describe the phenotype associated with *MYH* mutation status and assess for potential confounding and effect modification.** ***Results:*** **Twenty-seven cases and 1 control subject carried homozygous or compound heterozygous *MYH* mutations (AOR, 18.1; 95% confidence interval, 2.5–132.7). CRC cases with homozygous/compound heterozygous mutations were younger at diagnosis ($P = .01$), had a higher proportion of right-sided ($P = .01$), synchronous cancers ($P < .01$), and personal history of adenomatous polyps ($P = .003$). Heterozygous *MYH* mutations were identified in 87 CRC cases and 43 controls; carriers were at increased risk of CRC (AOR, 1.48; 95% confidence interval, 1.02–2.16). There was a higher prevalence of low-frequency microsatellite instability (MSI) in tumors from heterozygous and homozygous/compound heterozygous *MYH* mutation carriers ($P = .02$); MSI status modified the CRC risk associated with heterozygous *MYH* mutations (P interaction $< .001$).** ***Conclusions:*** **Homozygous/compound heterozygous MYH mutations account for less than 1% of CRC cases. Heterozygous carriers are at increased risk of CRC. Further studies are needed to understand the possible interaction between the base excision repair and low-frequency MSI pathways.**

Colorectal cancer (CRC) is the third most common malignancy in North America[1] and up to one third of cases have a family history of the disease, suggesting a hereditary component in many cases.[2–4] Recent reports have explored the role of germline mutations of the *MutY* human homologue (*MYH*) gene in predisposition to CRC.[5] MYH is a member of the base-excision repair pathway that detects and protects against oxidative DNA damage. Several recent clinic-based studies in North America and Europe[6–14] have shown an attenuated polyposis phenotype inherited in an autosomal-recessive pattern in individuals with germline mutations in both MYH alleles, carrying either homozygous (2 identical) or compound heterozygous (2 different) mutations. However, these studies focused on small numbers of highly selected clinic-based patients recruited based on a specific polyposis phenotype and may not accurately describe the contribution of MYH mutations to CRC risk in the general population or the full spectrum of MYH-associated phenotypes.

We[15] and others[16–19] have conducted population-based case-control studies to characterize the population frequency of homozygous and compound heterozygous MYH mutations and to determine the CRC risk, if any, associated with heterozygous mutations. These studies showed homozygous or compound heterozygous MYH mutations in 0.4%–1% of population-based CRC cases; the risk of CRC

Abbreviations used in this paper: AOR, adjusted odds ratio; C-CFR, Colorectal Cancer Family Registry; CI, confidence interval; CRC, colorectal cancer; MS, mass spectrometry; MSI, microsatellite instability; MSI-L, low-frequency microsatellite instability; MYH, mutY human homologue; OR, odds ratio.

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associated with heterozygous MYH mutations is the subject of ongoing debate, with several studies[15,17,18,20–22] showing a nonsignificant increased odds of CRC, whereas others[16,19] have failed to find this association. Furthermore, several population-based and clinic-based series have only screened subjects for the 2 common MYH mutations, Y165C and G382D, and as a result the true prevalence of MYH mutations in CRC and the contribution of less common pathogenic variants is unclear.

To more thoroughly address the role of MYH mutations in CRC risk, we performed a large multisite case-control study in Canada, the United States, and Australia.

Methods

CRC patients and controls were recruited through the resources of the Colorectal Cancer Family Registry (C-CFR), a National Cancer Institute–supported consortium dedicated to the study of genetic and epidemiologic factors in CRC. Recruitment of cases and controls was undertaken at 6 international study sites and included the collection of family history, epidemiologic and pathologic data, and the collection of pathologic specimens and blood samples for genetic testing. Recruitment also included the collection of epidemiologic and family history information, and blood from age- and sex- frequency-matched controls with no prior personal history of CRC at the time of recruitment.

In the current study, population-derived case and control subjects were obtained from 3 C-CFR sites (Ontario, Canada; Melbourne, Australia; and Seattle, WA, USA) as well as the Newfoundland Familial Colorectal Cancer Registry; details of the study design and recruitment at each site have been published previously.[23–25] Briefly, the Australasian Colorectal Cancer Family Registry recruited CRC cases aged 18–59 years identified through the Victoria Cancer Registry; controls were selected though electoral rolls. The Seattle Familial Colorectal Cancer Registry identified incident population-based CRC cases aged 20–74 in 3 Washington state counties through the Puget Sound SEER program; controls were recruited through drivers license lists (age, <65 y) and through health care finance files (age, 65–74 y). The Ontario Familial Colon Cancer Registry identified incident CRC cases in the province of Ontario, Canada, through the Ontario Cancer Registry as described by Cotterchio et al.[24] Briefly, CRC cases were stratified into high risk (Amsterdam criteria), intermediate risk (based on demographic or pathologic factors such as young age/multiple polyps), or low risk/nonfamilial (absence of familial risk factors). After stratification, all high- and intermediate-risk probands and a 25% random sample of nonfamilial cases were recruited into the Ontario Familial Colon Cancer Registry. Controls were recruited through residential telephone lists as well as the Ontario Ministry of Finance property-assessment file for the year 2000.

The fourth site, the Newfoundland Familial Colorectal Cancer Registry recruited CRC cases (age, <75 y) from 1999 to 2003 in the province of Newfoundland and Labrador, Canada, through the provincial cancer registry; controls were recruited using random digit dialing and a list of residential telephone numbers.[25]

CRC cases with a diagnosis of invasive CRC during the phase 1 recruitment period (1997–2002), and controls who provided a blood specimen were submitted for genetic analysis. Known cases of familial adenomatous polyposis, nonincident cases, and those diagnosed with in situ malignancies were excluded.

All protocols described earlier were approved by local institutional research ethics review boards.

MYH Mutation Testing

Genomic DNA from each subject was sent to a central testing facility (Analytic Genetics Technology Centre, Toronto, Canada) for analysis. Samples were aliquoted on 96-well plates with 5% repeat samples on each plate as internal controls. Cases and controls were screened for 12 known *MYH* mutations: G382D, Y165C, 1103delC, 891+3A→C, E466X, 1395delGGA, Q377X, R260Q, Y90X, R227W 1186_7insGG, IVS12-2A→G using the MassArray MALDI-TOF Mass Spectrometry (MS) system (Sequenom, San Diego, CA). Screening for R227W, 1186_7insGG, and IVS12-2A→G was discontinued when testing of 6000 samples failed to identify any mutation carriers. Samples with incomplete *MYH* mutation testing for all 9 mutations were excluded from subsequent analyses. The functional impact of the mutations in this series have been shown by our group[26] and others.[5,27]

All samples with MS mobility shifts underwent screening of the entire *MYH* coding region, promoter, and splice sites regions by denaturing high-performance liquid chromatography (Transgenomic Wave 3500HT System; Transgenomic, Omaha, NE), to confirm the mutation and to identify additional mutations. All MS-detected variants and WAVE mobility shifts were submitted for sequencing for mutation confirmation (ABI PRISM 3130XL Genetic Analyzer). All cases and controls were not retested for novel mutations detected on screening of the MYH gene.

Tumor microsatellite instability (MSI) status was determined for cases as described by Lindor et al[28] using a 10-microsatellite marker panel. If at least 4 markers amplified, the proportion of unstable markers was calculated and tumors were classified as microsatellite stable (0% markers unstable), low-frequency MSI (MSI-L; 1%–29% markers unstable), and high-frequency MSI (>30% markers unstable). If fewer than 4 markers amplified, then immunohistochemistry results for MLH1, MSH2, MSH6, and PMS2 were examined; the tumor was scored as high-frequency MSI if at least 1 mismatch repair protein was deficient.

Statistical Analysis

The association between CRC risk and compound heterozygous, homozygous, and heterozygous *MYH* mutation status was assessed using unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs). All variables had less than 10% missing values unless otherwise stated. ORs were adjusted for age and sex using a multivariate model. Adjustment for study site and familial risk catogory[24] in the multivariate model did not alter the results of the analysis, and thus were not included in the final models. To evaluate epidemiologic variables (CFR site, family history, nonsteroidal anti-inflammatory drug use, calcium supplementation, smoking status, inflammatory bowel disease, and red meat and vegetable intake) as potential confounders, we compared the multivariate adjusted OR (AOR) for heterozygous *MYH* mutation status in models with and without the epidemiologic variable of interest. Variables were considered confounders if their inclusion altered the AOR for *MYH* mutations by greater than 20%.[29] Potential modification (by MSI status) of the association between heterozygous MYH mutations and CRC risk was assessed. A *P* value for the interaction was obtained using the log rank test, comparing the multivariate models with and without the multiplicative interaction (MYH*MSI) term for heterozygous MYH mutation status and the effect modifier. Age- and sex-adjusted ORs for heterozygous MYH mutations stratified by MSI status were calculated using polytomous logistic regression.

All statistical analyses were conducted using SAS 9.1.3 software (SAS Institute, Cary, NC); all tests of significance were 2-sided.

Results

A total of 6769 subjects were genotyped for 9 *MYH* mutations. Interpretable MS results were obtained for 98%–99% of subjects for each mutation. A total of 132 subjects who did not have complete screening results for all 9 mutations and 24 cases with germline mutations in mismatch repair genes (MLH1, MSH2, and MSH6) were excluded from the analysis. Three hundred and fifty samples were submitted randomly for repeat MS testing and there was 100% concordance among duplicate genotyping results. The 6613 subjects with complete *MYH* genotyping results included 3811 cases and 2802 controls (Table 1). Results for 1238 cases and 1255 controls from Ontario have been reported previously, although a more limited mutation screening algorithm was used (prescreening for only Y165C and G382D)[15]; these cases were rescreened using the more extensive algorithm and the results are reported here. Table 1 shows associations of CRC risk with known risk factors, namely higher risk with ulcerative colitis, smoking, and red-meat consumption, and lower risk with nonsteroidal anti-inflammatory drug use and calcium supplementation.

We identified 132 heterozygous, 11 compound heterozygous, and 17 homozygous germline *MYH* mutation carriers (Table 2). Denaturing high-performance liquid chromatography screening of the promoter, splice sites, and entire coding region of the *MYH* gene in heterozygous mutation carriers identified 2 additional novel *MYH* mutations, Y114X and R231H. Fifteen *MYH* mutation carriers carried pathogenic variants other than the 2 common mutations, Y165C and G382D. We did not identify any carriers of the E466X, R227W, 1186_7insGG, and IVS12-2A→G mutations, likely owing to ethnicity of the study subjects because greater than 85% of probands in the C-CFR are Caucasian and the majority are of Northern European descent.[23]

Table 1. Description of Cases and Controls—Distribution of Demographic and Subject Characteristics by Case-Control Status[†]

	Cases N (%)	Controls N (%)	OR (95% CI)
N	3811	2802	
CFR site			
Ontario	1289 (34)	1295 (46)	
Australia	598 (16)	251 (9)	
Seattle	1406 (37)	735 (26)	
Newfoundland	518 (14)	521 (19)	
Age group, y			
20–49	782 (21)	468 (17)	1.0 (ref)
50–59	1238 (32)	812 (29)	0.91 (0.79–1.05)
60–69	1260 (33)	960 (34)	0.78 (0.68–0.90)
70–80	531 (14)	562 (20)	0.56 (0.47–0.66)
Sex			
Female	1818 (49)	1351 (48)	1.0
Male	1913 (51)	1439 (52)	0.97 (0.88–1.07)
Crohn's disease			
No	3590 (99)	2748 (99)	1.0
Yes	33 (1)	15 (0.5)	1.69 (0.92–3.12)
Ulcerative colitis			
No	3454 (97)	2716 (98)	1.0
Yes	101 (3)	40 (1)	1.99 (1.38–2.89)
NSAID use[a]			
No	2077 (55)	1218 (43)	1.0
Yes	1734 (45)	1584 (57)	0.69 (0.62–0.76)
Calcium[b]			
No	2625 (74)	1857 (67)	1.0
Yes	934 (26)	907 (33)	0.73 (0.65–0.81)
Cigarette smoking			
No	1381 (39)	1207 (44)	1.0
Yes	2157 (61)	1560 (56)	1.21 (1.09–1.34)
Vegetable servings/wk			
<7	606 (17)	408 (15)	1.0
7–10	1136 (32)	874 (32)	0.88 (0.75–1.02)
10–17	907 (26)	750 (27)	0.82 (0.70–0.96)
>17	868 (25)	708 (26)	0.83 (0.71–0.97)
Red meat servings/wk			
<2	473 (14)	480 (18)	1.0
2–2.9	622 (18)	528 (20)	1.19 (1.00–1.42)
3–5	1248 (36)	953 (36)	1.33 (1.14–1.54)
>5	1078 (31)	661 (25)	1.65 (1.41–1.94)

NSAID, nonsteroidal anti-inflammatory drug.
[a]Taken aspirin or ibuprofen-based medications at least twice a week for >1 month.
[b]Taken calcium pills or tablets at least twice a week for >1 month.
[†]Chi-square test.

Table 2. The Distribution of MYH Genotypes by Colorectal Cancer Cases and Controls, and OR Estimates

Genotype	Cases N = 3811 (%)	Controls N = 2802 (%)	Unadjusted OR (95% CI)	AOR[a] (95% CI)
Heterozygous	87 (2.3)	43 (1.5)	1.54 (1.06–2.3)	1.48 (1.02–2.16)
G382D	63	32	1.60 (1.05–2.43)	1.6 (1.05–2.44)
Y165C	15	10	1.69 (0.80–3.59)	1.64 (0.77–3.47)
R260Q	4	1		
1103delC	2	0		
891+3A>C	2	0		
Y90X	1	0		
E466X	0	0		
1395delGGA	0	0		
Q377X	0	0		
Homozygous/compound Heterozygous	27 (0.7)	1 (0.04)	20.7 (2.8–153)	18.1 (2.5–132.7)
G382D/G382D	11	0		
Y165C/Y165C	5	1		
G382D/Y165C	5	0		
G382D/Y114X	1	0		
Y165C/R231H	1	0		
Y165C/891+3A>C	1	0		
G382D/891+3A>C	1	0		
Y165C/Y90X	1	0		
1395delGGA/Q377X	1	0		

[a]OR adjusted by age and sex.

Among heterozygous *MYH* mutation carriers, there were 87 (2.3%) CRC cases and 43 (1.5%) controls. Heterozygous *MYH* mutation status showed a statistically significant association with CRC risk with an OR of 1.54 (95% CI, 1.06–2.3); this remained statistically significant after adjusting for age and sex (AOR, 1.48; 95% CI, 1.02–2.16). Heterozygous G382D carriers were at increased risk of CRC (AOR, 1.6; 95% CI, 1.05–2.44) whereas all other mutations were more common in cases than controls. The prevalence of *MYH* mutations at each study site is presented in Table 3; adjustment for study site in multivariate models did not alter the results of the analysis.

We identified 27 CRC cases (0.7%) with germline *MYH* mutations affecting both alleles, 16 homozygotes and 11 compound heterozygotes. One compound heterozygous carrier did not harbor either of the common Y165C or G382D mutations. Surprisingly, a homozygous Y165C mutation was detected in a control subject with no prior personal history of CRC but who had 1 affected parent and 3 affected siblings. This individual had undergone screening colonoscopy 3 years before MYH testing, at age 64, with 2 adenomas detected at that time. The individual was referred for genetic counseling and repeat colonoscopy was performed that indentified multiple colonic polyps. The subject subsequently underwent a total proctocolectomy at age 67 and pathologic examination revealed approximately 300 adenomas; all were less than 1 cm in diameter and many had focal high-grade dysplasia. The highest polyp density (4 per cm^2) was in the cecum.

The phenotypic characteristics of CRC cases according to MYH mutation status are shown in Table 4. Homozygous and compound heterozygous mutation carriers were significantly younger at diagnosis (mean age ± SD, 51.7 ± 9.5 y) than either heterozygous carriers (mean age ± SD, 58.2 ± 10.7 y) or those without MYH mutations (mean age ± SD, 58.3 ± 10.2 y) ($P = .006$). Homozygous and compound heterozygous mutation carriers had a higher frequency of right-sided cancers than noncarriers ($P = .012$) and also had a significantly higher prevalence of adenomas adjacent to CRC ($P = .035$) and synchronous cancers ($P < .001$). Although exact polyp counts were not available for most MYH wild-type and heterozygous CRC cases, homozygous and compound heterozygous carriers had a higher prevalence of self-reported personal history of polyps ($P < .001$). A detailed review of surgical pathology and colonoscopy reports of 26 homozygous and compound heterozygous carriers (summarized in Table 5) showed an absence of polyps in 9 (35%) cases at the time of cancer diagnosis.

The high-frequency MSI phenotype was observed in the tumors of 9 heterozygous carriers (5 MLH1-deficient, 2 MSH2-deficient, 1 PMS2-deficient, and 1 MLH1/PMS2-deficient) and 1 Y165C homozygous carrier (MLH1-deficient). We observed a high prevalence of MSI-L tumors among both homozygous/compound heterozygous carriers (23.5%) and heterozygous carriers (18.8%) compared with CRC cases without an MYH mutation (9%). To examine the potential association between MYH mutation status and MSI tumor status we performed a stratified polytomous logistic regression. Because low numbers of homozygous/compound heterozygous cases and controls created an unstable multivariate model, the potential effect modification of heterozygous MYH mutation status by MSI was examined by this method. As shown in Table 6, there was significant heterogeneity in the association between heterozygous mutations and CRC risk when stratified by MSI status, indicating that CRC risk associated with heterozygous MYH mutations is modi-

Table 3. Genotype of *MYH* Mutations by Study Site

	Ontario, cases/controls	Australia, case/controls	Seattle, cases/controls	Newfoundland, cases/controls
G382D				
No mutation	1257/1283	589/247	1379/723	505/517
Heterozygous[a]	28/12	6/4	25/12	11/4
Homozygous	4/0	3/0	2/0	2/0
Allele frequency	0.014/0.005	0.01/0.008	0.01/0.008	0.014/0.004
Y165C				
No mutation	1274/1292	596/250	1399/730	514/519
Heterozygous[a]	12/2	2/1	7/5	2/2
Homozygous	3/1	0/0	0/0	2/0
Allele frequency	0.007/0.002	0.002/0.002	0.002/0.003	0.006/0.002
1103delC				
No mutation	1289/1295	598/251	1405/735	517/521
Heterozygous[a]	0/0	0/0	1/0	1/0
Homozygous	0/0	0/0	0/0	0/0
Allele frequency	0/0	0/0	0.0004/0	0.0009/0
891+3 A>C				
No mutation	1286/1295	597/251	1406/735	518/521
Heterozygous[a]	3/0	1/0	0/0	0/0
Homozygous	0/0	0/0	0/0	0/0
Allele frequency	0.0012/0	0.0008/0	0/0	0/0
1395delGGA				
No mutation	1288/1295	598/251	1406/735	518/521
Heterozygous[a]	1/0	0/0	0/0	0/0
Homozygous	0/0	0/0	0/0	0/0
Allele frequency	0.0004/0	0/0	0/0	0/0
Q377X				
No mutation	1288/1295	598/251	1406/735	518/521
Heterozygous[a]	1/0	0/0	0/0	0/0
Homozygous	0/0	0/0	0/0	0/0
Allele frequency	0.0004/0	0/0	0/0	0/0
R260Q				
No mutation	1287/1295	597/251	1406/734	517/521
Heterozygous[a]	2/0	1/0	0/1	1/0
Homozygous	0/0	0/0	0/0	0/0
Allele frequency	0.008/0	0.0008/0	0/0.0007	0.0009/0
Y90X				
No mutation	1287/1295	598/251	1406/735	518/521
Heterozygous[a]	2/0	0/0	0/0	0/0
Homozygous	0/0	0/0	0/0	0/0
Allele frequency	0.008/0	0/0	0/0	0/0

[a]Individuals who have compound heterozygous *MYH* mutations are recorded as heterozygous at 2 mutation loci.

fied by MSI status. Immunohistochemical staining results for mismatch repair proteins was available for 11 of 12 and 3 of 4 cases that were MSI-L and heterozygous and homozygous/compound heterozygous MYH-mutation positive, respectively. All 14 cases underwent testing for MLH1 and MSH2, 11 cases were tested for MSH6, and 8 cases were tested for PMS2. No mismatch repair protein deficiency was detected in any of the tumors tested among MSI-L MYH mutation carriers.

Discussion

The *mutYh*, or MYH, gene is a member of the base-excision repair pathway involved in the detection and repair of oxidative DNA damage.[30] Al-Tassan et al[5] described 2 mutations in the MYH gene, Y165C and G382D, in a Welsh family with CRC and multiple adenomatous polyps. These 2 mutations appear to account for almost 90% of MYH mutations in Caucasian patients of Northern European ancestry. Additional mutations have been described in Caucasians[13,31,32] and individuals of South Asian descent.[7]

The present study represents a large analysis of the association between germline MYH mutations and CRC risk. We characterized MYH mutations in 3811 population-derived CRC cases and 2802 controls from 3 sites of the C-CFR, in 3 countries: Canada, the United States, and Australia; and a fourth site including subjects from Newfoundland, Canada. As a population-derived series, the cases are more likely to represent the full spectrum of disease present in the general population and allow more thorough characterization of the phenotype associated with these mutations. More than 90% of cases and controls completed a detailed epidemiologic questionnaire that provided data for an analysis of potential modifiers

Table 4. Phenotype Characteristics of CRC Cases by MYH Mutation Status

Variable	No mutation N (%)	Heterozygous mutation carrier N (%)	Homozygous and compound heterozygous mutation carrier N (%)	*P*
Total	3697	87	27	
CFR site				
Ontario	1240 (96)	35 (3)	14 (1)	
Australia	585 (98)	9 (2)	4 (0.8)	
Seattle	1374 (98)	27 (2)	5 (0.5)	
Newfoundland	498 (96)	16 (3)	4 (1)	.11
Sex				
Male	1854 (51)	47 (57)	12 (46)	.47
Female	1769 (49)	35 (43)	14 (54)	
Age at diagnosis, y				.01
20–50	749 (20)	20 (23)	13 (48)	
50–60	1201 (32)	28 (32)	9 (33)	
60–70	1228 (33)	27 (31)	5 (19)	
70–80	519 (14)	12 (14)	0	
Location of primary				
Right colon	936 (27)	19 (25)	12 (50)	
Left colon	528 (15)	10 (12)	2 (8)	
Rectosigmoid	2066 (58)	49 (62)	10 (42)	.12
Primary histology				
Adenocarcinoma	3217 (88)	75 (91)	24 (92)	
Mucinous adenocarcinoma	388 (11)	7 (9)	2 (8)	
Signet ring cancer	32 (1)	0	0	
Adenocarcinoid	1 (0.03)	0	0	.94
Tumor stage				
Stage 1	1584 (43)	35 (40)	11 (41)	
Stage 2	1105 (30)	33 (38)	8 (30)	
Stage 3	823 (22)	17 (20)	7 (26)	
Stage 4	208 (6)	3 (3)	1 (4)	.75
Lymph node status				
Negative	1348 (36)	28 (32)	9 (33)	
Positive	945 (25)	17 (19)	7 (26)	
(Unknown)	1427 (38)	43 (49)	11 (41)	.87
Depth of invasion (T-stage)				
T1	351 (10)	8 (9)	7 (26)	
T2	525 (14)	7 (8)	1 (4)	
T3	1645 (44)	42 (48)	13 (48)	
T4	164 (5)	5 (6)	1 (4)	
(Unknown)	1014 (27)	26 (30)	5 (19)	.07
Tumor grade				
Well differentiated	290 (8)	8 (9)	2 (7)	
Moderately differentiated	2184 (59)	50 (58)	15 (56)	
Poorly differentiated	474 (13)	8 (9)	4 (13)	.87
(Unknown)	751 (20)	21 (24)	6 (22)	
Tumor microsatellite instability status				
MS-stable	2098 (56)	43 (49)	12 (44)	
MSI-L	245 (7)	12 (14)	4 (15)	.02
MSI-high	347 (10)	8 (10)	1 (5)	
(Unknown)	1009 (27)	24 (27)	10 (37)	
Synchronous colorectal cancers				
Yes	74 (3)	1 (2)	5 (28)	
No	2483 (97)	54 (98)	13 (72)	<.001
Adjacent adenoma				
Yes	521 (21)	16 (29)	7 (41)	
No	2027 (80)	39 (71)	10 (59)	.03
Reported history of polyps[a]				
Yes	1679 (48)	42 (51)	21 (81)	
No	1836 (52)	40 (49)	5 (19)	.003

NOTE. Numbers may not add to totals due to missing data.

[†]Chi-square test for right-sided tumors in homozygous/compound heterozygous vs controls ($P = .012$).

[a]Self-reported lifetime history of colonic polyps. As a result, polyps may have been detected before or after CRC diagnosis.

Table 5. Mutation and Phenotypic Details of Homozygous and Compound Heterozygous MYH Mutation Carriers

Subject	Site	MYH mutations	Polyps number, type, source	Cancer age, location, stage	MSI status
1	NFLD	Y165C/Y165C	No polyps, colonoscopy	T3N1 rectum	MSS
2	NFLD	Y165C/Y165C	Multiple tubular/tubulovillous adenomas, total proctocolectomy specimen	T3N0 right colon	MSS
3	NFLD	G382D/G382D	3 tubular adenomas, multiple hyperplastic polyps, total proctocolectomy specimen	T2N0 rectum T1N0 cecum	
4	NFLD	G382D/G382D	12 adenomas, 11 hyperplastic polyps, subtotal colectomy specimen	T3N0 sigmoid	MSS
5	ONT	G382D/G382D	Multiple adenomatous polyps, colonoscopy	56 T3N0 transverse 67 T2N0 sigmoid	
6	ONT	1395delGGA/Q377X	10 adenomas, subtotal colectomy	33 T3N1 rectosigmoid	
7	ONT	Y165C/ 891+3A>C	Several adenomas, segmental colectomy	45 T3N0 right colon	MSI-L
8	ONT	Y165C/G382D	4 adenomas, colonoscopy	66 T1N0 right colon	MSS
9	ONT	Y165C/G382D	No polyps, colonoscopy	52 T3N0 sigmoid	
10	ONT	Y165C/Y165C	Multiple tubular/tubulovillous adenomas, subtotal colectomy	47 T3N0 cecum 48 T2N1 left colon 48 T2N0 left colon	MSS
11	ONT	G382D/G382D	No polyps, colonoscopy	47 T1N0 rectum	MSI-L
12	ONT	G382D/G382D	No polyps, colonoscopy	67 T1N0 sigmoid	
13	ONT	Y165C/Y90X	6 adenomas, colonoscopy	35 T3N0 cecum	MSS
14	ONT	G382D/G382D	No polyps, colonoscopy	48 T3N0 left colon	MSS
15	ONT	Y165C/G382D	No polyps,[a] colonoscopy	58 T3N0 sigmoid	MSS
16	ONT	Y165C/Y165C	10 adenomas, segmental resection	50 T3N1 right colon 50 T1N1 right colon	MSI-H
17	ONT	Y165C/ 891+3A>C	3 adenomas, colonoscopy	41 T3N0 rectum	MSI-L
18	ONT	Y165C/Y165C	Single adenoma, segmental resection	58 T3N0 sigmoid	
19	AUS	Y165C/R321H	4 adenomas, segmental resection	39 T3N1 right colon 39 T3N1 right colon	MSI-L
20	AUS	G382D/G382D	No polyps, segmental resection	T1N0 right colon	
21	AUS	G382D/G382D	Single adenomas, segmental resection	T3N1 rectum	MSS
22	AUS	G382D/G382D	2 adenomas, colonoscopy	T1N0 right colon	MSS
23	SEA	G382D/G382D	Multiple adenomas	67 T1N0 sigmoid	MSS
24	SEA	G382D/Y114X	No polyps, segmental resection	T1N0 transverse colon	MSS
25	SEA	Y165C/G382D	Multiple (100) adenomas, total proctocolectomy	54 T3N1 right colon 54 T3N1 right colon 54 T1 right colon	
26	SEA	Y165C/G382D	No polyps,[a] segmental resection	61 T2N0 right colon	

AUS, Australia; MSI-H, high-frequency MSI; MSS, microsatellite stable; NFLD, Newfoundland; ONT, Ontario; SEA, Seattle.
[a]Subject was diagnosed with solitary polyps in follow-up endoscopy after CRC diagnosis and surgery.

of MYH mutations, although a definitive study of gene-environment interactions will require an even larger sample size. Unlike many previous population-based studies[15–19,22,33] that screened for only the 2 common mutations found in Caucasians, Y165C and G382D, we screened subjects for a larger panel of mutations. By using this 9-mutation panel, we identified an additional 10 heterozygous carriers and 1 additional compound heterozygous carrier who harbored confirmed pathogenic MYH mutations but did not carry either of the 2 common mutations.

Cases and controls at each study site were selected from population-based sampling frames providing comparable and representative groups for comparison. In addition, we observed an increased cancer risk for known CRC risk factors,[34] suggesting that the study groups were repre-

Table 6. OR for Heterozygous MYH Mutation Status Stratified by MSI Status

MYH status	Controls N (%)	MSS N (%)	MSS AOR[a] (95% CI)	MSI-L N (%)	MSI-L AOR[a] (95% CI)	MSI-H N (%)	MSI-H AOR[†] (95% CI)	*P* interaction[b]
No mutation	2757 (98)	2098 (98)	1.0	245 (95)	1.0	347 (98)	1.0	$P < .001$
Heterozygous mutation carrier	44 (2)	43 (2)	1.36 (0.9–2.1)	12 (5)	3.7 (2–6.9)	8 (2)	1.59 (0.8–3.3)	

MSI-H, high-frequency MSI; MSS, microsatellite stable.
[a]Age and sex AORs were calculated using polytomous logistic regression.
[b]Test for heterogeneity of AOR for heterozygous MYH mutation between MSI-stable, MSI-L, and high-frequency MSI: *P*-interaction was calculated using the log-rank test of multivariate model with and without a multiplicative interaction term.

sentative of the general population.[35] As with most case-control studies, there were several limitations. First, survival bias was possible because deceased cases were not recruited and response rates are lower for cases with later-stage disease. It is unlikely that this would affect our findings because MYH status does not appear to be associated with stage of disease. Response bias is possible when high response rates are not achieved.[23,24] Although all 4 sites undertook population-based collection of CRC cases, the recruitment strategies of each site differed. The stratified recruitment method at the Ontario site[24] may have lead to selection bias because MYH mutation carriers may not have a strong family history of CRC but may have certain pathologic characteristics that increased their likelihood of recruitment. However, adjustment for risk-strata and study site did not alter the findings of this study. Clinically obvious cases of florid polyposis were excluded from the C-CFR, which may have rendered some homozygous and compound heterozygous carriers ineligible for recruitment, leading to an underrepresentation of these mutations in this series.

We identified homozygous and compound heterozygous MYH mutations in 0.7% of the CRC cases in this series with a range of 0.4%–1% of cases from each site, indicating that these mutations likely contribute to a minority of CRC. Phenotypic characterization of CRC cases with homozygous and compound heterozygous MYH mutations showed that carriers had a younger age of diagnosis and a higher prevalence of polyps, right-sided and synchronous cancers. Although data on demographic and incident tumor characteristics were abstracted from original pathology reports, detailed polyp counts and colonoscopy reports were not available for all heterozygous and wild-type MYH cases; information for these subjects was based on synchronous polyps in the colectomy specimen or self-reported history of polyps or polypectomy. This combined with the exclusions of polyposis cases as well as the increased recruitment of multiple polyp CRC cases in Ontario limit the detailed interpretation of the phenotypic data for wild-type and heterozygous MYH carriers in this series. These findings do support the observation that the majority of carriers with mutations affecting both MYH alleles develop a mild polyposis syndrome as reported in clinic-based studies of MYH.[6–14] However, as with other series[15,18] we observed homozygous and compound heterozygous mutation carriers who developed CRC without multiple polyps. Homozygous and compound heterozygous carriers of the Y165C and G382D mutations developed CRC with and without polyps, indicating that neither the type nor location of the germline mutation affects the polyposis phenotype.

There is considerable debate whether an association exists between heterozygous MYH mutations and increased risk of CRC. Several studies have suggested that heterozygous MYH mutation may confer increased risk[15,18,20–22] by showing a higher rate of heterozygous carriers among cases compared with controls, but each study alone lacked statistical power to definitively establish this relationship. Farrington et al[18] reported an increased odds of CRC among heterozygous cases older than age 55, whereas Tenesa et al[22] and Jenkins et al[36] showed a statistically significant association by combining the results of several population-based series. Other studies[19,33,37] have challenged this potential association by failing to find a relationship between heterozygous MYH mutations and CRC. The current study found a statistically significant association between heterozygous MYH mutation and CRC (AOR, 1.45; 95% CI, 1.01–2.10) and showed an increased risk for the G382D mutation (AOR, 1.6; 95% CI, 1.05–2.44). This risk associated with single MYH mutations is supported by the fact that mutations in other DNA repair pathways are inherited in a dominant fashion, loss of heterozygosity of 1p is a common somatic event in CRC,[38,39] and higher rates of 1p loss of heterozygosity have been documented in heterozygous MYH carriers by our group[15] and others.[37] Our ability to detect this association was aided by a greater sample size as well as the expanded mutation screening panel. If screening in this series had been limited to only Y165C and G382D, we would have detected heterozygous mutations in 78 cases and 43 controls, yielding an AOR of 1.45 (95% CI, 0.95–2.3). Furthermore, less common variants were detected in 15 cases (9 heterozygotes and 6 compound heterozygotes) and in only 1 control subject, suggesting that these rare variants may be more penetrant than the more common Y165C and G382D mutations. We have found that although most mutations abolish glycosylase and DNA binding activities of MYH, certain variants such as R260Q may retain reduced enzymatic function,[26] raising the possibility that the functional characteristics of each MYH mutation may result in different risks or clinical manifestations.

We observed MSI-L tumors in 9% of MYH–wild-type cases, a frequency that is consistent with other large studies.[40–42] MSI-L was detected in 18.8% and 23.5% of tumors from heterozygous and homozygous/compound heterozygous MYH-mutation carriers, respectively. We showed heterogeneity of the OR associated with heterozygous mutation status when stratified by MSI, indicating that the CRC risk associated with MYH variants differs by tumor MSI status. Although we did not show this finding for homozygous and compound heterozygous mutations owing to low numbers and instability of multivariate models, this effect modification also may hold for the risk associated with mutations affecting both alleles. Although the association between MYH and MSI was suggested by Kambara et al,[37] it is difficult to draw comparisons with that study because the MYH V22M variant was considered pathogenic and comprised 60% of the variants detected in CRC cases in that series; in vitro data from our group[26] have shown this variant is

a polymorphism with no impact on glycosylase or DNA binding function. The nature and significance of MSI-L in CRC is unclear[43]; however, there is an emerging consensus that although MSI-L cancers do not differ histologically from microsatellite stable cancers, they do have higher rates of mutations in K-ras and CpG-island methylation.[44] Lipton et al[45] showed the presence of G:C→T:A transversions in K-ras in 63% of MYH-associated cancers. We have shown that cancers associated with MYH mutations do not differ histologically from sporadic adenocarcinomas, which are associated commonly with chromosomal instability and are microsatellite stable.[44,46] Furthermore, MYH has been shown to interact with the MSH2/MSH6 heterodimer and the function of MYH is enhanced by this interaction.[47]

The mechanism(s) by which germline MYH mutations would predispose to the development of MSI-L tumors is not clear; however, we offer a number of hypotheses. Because tumors with deficiencies in DNA repair may accumulate mutations in other DNA-damage signaling and repair pathways,[48] cancers with disabled base-excision repair pathways caused by MYH mutations may develop somatic alterations in MMR genes resulting in a MSI-L phenotype. Our observation of intact mismatch repair proteins in MSI-L MYH mutation–positive cases would not appear to support this hypothesis. Alternatively, neoplastic progression along the MSI-L pathway may lead to mutations in MYH providing a second hit in heterozygous mutation carriers,[49] thus accelerating tumorigenesis. Finally, because the MutSα complex is involved in both base excision and mismatch recognition and repair, it is possible that high levels of G:C→T:A transversions caused by deficiencies in MYH may overload the MutSα complex and lead to a MSI-L phenotype.[45,47]

In conclusion, we have shown homozygous and compound heterozygous mutations in a minority (<1%) of CRC cases and a single control (out of 2802) obtained from a large multisite population-derived registry. As with previous studies, not all biallelic carriers had an attenuated polyposis phenotype. Although biallelic carriers have an increased tendency to develop multiple polyps and right-sided CRC at a younger age than nonmutation carriers, none of these pathologic features are specific for homozygous and/or compound heterozygous MYH mutations. The absence of specific pathologic features and the lack of family history of CRC in these individuals indicates that identification of cases of MYH-associated CRC may be difficult. To date, a robust immunohistochemical assay to detect absent MYH protein in tumors from homozygous/compound heterozygous carriers is not available.[46] The current study also conclusively shows an increased risk of CRC associated with heterozygous MYH mutations. Future studies are required to delineate the molecular pathways and genetic characteristics of MYH-associated CRC, to examine possible interaction between the base-excision repair and MMR pathways, and to determine optimal screening strategies for homozygous/compound heterozygous and heterozygous carriers.

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Conflict of interest

The authors disclose no conflicts.

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