Risk of colorectal cancer for people with a mutation in both a *MUTYH* and a DNA mismatch repair gene

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ABSTRACT

The base excision repair protein, MUTYH, functionally interacts with the DNA mismatch repair (MMR) system. As genetic testing moves from testing one gene at a time, to gene panel and whole exome next generation sequencing approaches, understanding the risk associated with having germline mutations in these two genes will be important for clinical interpretation and management. From the Colon Cancer Family Registry, we identified 10 carriers who had both a *MUTYH* mutation (6 with c.1187G>A p.(Gly396Asp), 3 with c.821G>A p.(Arg274Gln), and 1 with c.536A>G p.(Tyr179Cys)) and a MMR gene mutation (3 in *MLH1,* 6 in *MSH2,* and 1 in *PMS2*), 375 carriers of a single (monoallelic) *MUTYH* mutation alone, and 469 carriers of a MMR gene mutation alone. Of the 10 carriers of both gene mutations, 8 were diagnosed with colorectal cancer. Using a weighted cohort analysis, we estimated that risk of colorectal cancer for carriers of both a *MUTYH* and a MMR gene mutation was substantially higher than that for carriers of a *MUTYH* mutation alone (hazard ratio [HR] 21.5, 95% confidence interval [CI] 9.19–50.1; p<0.001), but not different from that for carriers of a MMR gene mutation alone (HR 1.94, 95% CI 0.63–5.99; p=0.25). Within the limited power of this study, there was no evidence that a monoallelic *MUTYH* gene mutation confers additional risk of colorectal cancer to carriers of a MMR gene mutation alone. Our finding suggests *MUTYH* mutation testing in MMR gene mutation carriers is not clinically warranted.

Keywords: *MUTYH,* mismatch repair, colorectal cancer, Lynch syndrome

INTRODUCTION

People with a heterozygous germline mutation in one of the DNA mismatch repair (MMR) genes *MLH1, MSH2, MSH6,* or *PMS2,* are at a substantially increased risk of colorectal cancer i.e. Lynch syndrome, previously known as hereditary non-polyposis colorectal cancer or HNPCC.[1, 2] People with a heterozygous germline mutation in the DNA base-excision repair gene *MUTYH* (monoallelic *MUTYH* mutation carriers) are at a small increased risk of colorectal cancer.[3-6] As genetic testing advances from the iterative process of testing one gene at a time, to gene panel and whole exome next generation sequencing approaches in which multiple genes are tested simultaneously, understanding of cancer risks associated with the co-existence of mutations within these genes is becoming increasingly important for appropriate clinical interpretation and management.

Several studies have investigated potential genetic modifiers of cancer risks for MMR gene mutation carriers by examining the role of mutations in other genes and intergenic regions on their risk of colorectal cancer.[7-15] A protein-protein interaction between MSH6 and MUTYH in the oxidative DNA damage repair pathway has been reported by Gu *et al.*[16] Some studies have observed that a mutation in *MSH6* is more likely to be present in individuals with a *MUTYH* mutation who have colorectal cancer.[17, 18] Other studies, however, have observed no such associations.[19-23] In this study, we aimed to estimate the risk of colorectal cancer for carriers of both a *MUTYH* mutation and a MMR gene mutation compared with carriers of monoallelic *MUTYH* mutation alone and carriers of a MMR gene mutation alone.

MATERIALS AND METHODS

Study Sample

The study comprised of carriers of a mutation in one or both of *MUTYH* and a MMR gene, from both population- and clinic-based families of the Colon Cancer Family Registry that is described in detail elsewhere[24] and at http://coloncfr.org. Between 1997 and 2012, the Colon Cancer Family Registry recruited families via: population-based probands who were recently diagnosed colorectal cancer cases from state or regional population cancer registries in the USA (Washington, California, Arizona, Minnesota, Colorado, New Hampshire, North Carolina, and Hawaii), Australia (Victoria) and Canada (Ontario); and clinic-based probands who were enrolled from multiple-case families referred to family cancer clinics in the USA (Mayo Clinic, Rochester, Minnesota, and Cleveland Clinic, Cleveland, Ohio), Canada (Ontario), Australia (Melbourne, Adelaide, Perth, Brisbane, Sydney) and New Zealand (Auckland). Probands were asked for permission to contact their relatives to seek their enrolment in the Cancer Family Registry. For population-based families, first-degree relatives of probands were recruited and recruitment was extended to more distant relatives by some registries. For clinic-based families, recruitment was based on availability but attempts were made to recruit up to second-degree relatives of affected individuals (detailed in Newcomb *et al.*[24]). Informed consent was obtained from all study participants, and the study protocol was approved by the institutional research ethics review board at each centre of the Colon Cancer Family Registry.

Data Collection

Information on demographics, personal characteristics, personal and family history of cancer, cancer-screening history, history of polyps, polypectomy, and other surgeries was

obtained by questionnaires from all probands and participating relatives. Participants were followed approximately every 5 years after baseline to update this information. The present study was based on all available baseline and follow-up data. Reported cancer diagnoses and age at diagnosis were confirmed using pathology reports, medical records, cancer registry reports, and death certificates, where possible. The tumor anatomic location and histology were coded and stored using the International Classification of Diseases for Oncology.[25] Blood samples from all participants and tumor tissue samples from all colorectal cancer-affected participants were obtained.

MMR gene mutation testing

Testing for germline mutations in *MLH1*, *MSH2*, *MSH6*, and *PMS2* was performed for all population-based probands with a MMR-deficient colorectal tumor as evidenced by either high levels of tumor microsatellite instability (MSI-high) and/or loss of expression of one or more of the four MMR proteins by immunohistochemistry (IHC). Testing was undertaken for the youngest-onset colorectal cancer participant from each clinic-based family regardless of tumor microsatellite instability or MMR protein expression status. Testing for *MLH1*, *MSH2* and *MSH6* mutations was performed using Sanger sequencing or denaturing high performance liquid chromatography, followed by confirmatory DNA sequencing (reference sequences and exon numbering: for *MLH1*, NG_007109.2 and NM_000249.3; for *MSH2*, NG_007110.2 and NM_000251.2; and for *MSH6*, NG_007111.1 and NM_000179.2). Large duplication and deletions including those involving *EPCAM*, which lead to *MSH2* methylation, were detected by Multiplex Ligation Dependent Probe Amplification according to the manufacturer's instructions (MRC Holland, Amsterdam, The Netherlands).[24, 26, 27] *PMS2* mutations were identified using a modified protocol from Senter *et al.*[28] where exons 1-5, 9 and 11-15 were amplified in three long-range polymerase chain reactions

(PCRs), followed by nested exon-specific PCR and sequencing. The remaining exons (6, 7, 8 and 10) were amplified and sequenced directly from genomic DNA. Large-scale deletions in *PMS2* were detected using the P008-A1 MLPA kit according to manufacturer's specifications (MRC Holland, Amsterdam, The Netherlands) (reference sequence for *PMS2*, NG_008466.1 and NM_000535.5). The relatives of probands with a pathogenic MMR germline mutation, who provided a blood sample, underwent testing for the specific mutation identified in the proband.

MUTYH mutation testing

All probands were tested for *MUTYH* mutations. Genomic DNA extracted from each participant was tested at a central testing facility (Analytic Genetics Technology Center, Toronto, Canada) as previously described by Cleary *et al*.[6] DNA was screened for 12 previously identified *MUTYH* mutations: c.536A>G p.(Tyr179Cys), c.1187G>A p.(Gly396Asp), c.312 C>A p.(Tyr104Ter), c.821G>A p.(Arg274Gln), c.1438G>T p.(Glu480Ter), c.1171C>T p.(Gln391Ter), c.1147delC p.(Ala385ProfsTer23), c.933+3A>C, c.1437_1439delGGA, c.721C>T, p.(Arg241Trp), c.1227_1228dup, c.1187-2A>G using the MassArray MALDI-TOF Mass Spectrometry (MS) system (Sequenom, San Diego, CA) (*MUTYH* reference sequence and exon numbering based on NC_000001.10 and NM_001128425.1). To confirm the *MUTYH* mutations and identify additional mutations, screening of the entire *MUTYH* coding region, promoter, and splice site regions was performed on all samples exhibiting MS mobility shifts using denaturing high-performance liquid chromatography (Transgenomic Wave 3500HT System; Transgenomic, Omaha, NE). All MS-detected mutations and WAVE mobility shifts were submitted for sequencing for mutation confirmation (ABI PRISM 3130XL Genetic Analyser). That is, if a monoallelic *MUTYH* mutation was identified, then the *MUTYH* gene was screened for any additional

mutations not captured by the sequenom genotyping screen to ensure all potential compound heterozygous carriers were identified. The relatives of probands with a pathogenic *MUTYH* germline mutation, underwent testing for the specific mutation identified in the proband.

Statistical Analysis

As a proportion of mutation carriers from this study were ascertained from multiple-case cancer families, and colorectal cancer cases were preferentially tested for a *MUTYH* or a MMR gene mutation, subject selection for testing genetic mutation was not random with respect to disease status. We adjusted for this non-random ascertainment by applying probability weights to carriers based on the approach described by Antoniou *et al.*[29] and created a synthetic cohort representative of carriers in the general population. A simulation study of the weighted cohort approach applied to Cox regression demonstrated that applying rates from an external reference population to adjust for the non-random ascertainment removed bias when the external rates were correctly specified and reduced bias even if the external rates were not completely accurate.[29]

Age-specific incidences of colorectal cancer for carriers were calculated by multiplying the age-specific population incidence by the hazard ratio (HR) of colorectal cancer for carriers of *MUTYH*[4] or MMR[30] gene mutations. Data on age-, sex- and country-specific population-based incidences of colorectal cancer in 1998 to 2002 for 5-year intervals was obtained for Australia, Canada, and USA.[31] These age-specific incidences of colorectal cancer for carriers were used to calculate sampling fractions to weigh the proportion of colorectal cancer-affected carriers and unaffected carriers in each age stratum so that the proportion of affected carriers in each age group was equal to the proportion in the general population.

Cox proportional hazard regressions with age as the time metric were used to estimate the risk of colorectal cancer for carriers of both a *MUTYH* and a MMR gene mutation compared with carriers of a monoallelic *MUTYH* mutation alone and carriers of a MMR gene mutation alone. The time at risk for each carrier started at birth, and ended at: age at first diagnosis of colorectal cancer for carriers diagnosed with colorectal cancer; and age at first diagnosis of extracolonic cancer or last contact or death, whichever occurred the earliest, for carriers without colorectal cancer. Multivariable models were fitted after adjusting for sex and country of recruitment. HRs and robust estimates of corresponding 95% confidence intervals (CIs) were calculated by taking into account clustering by family membership to allow for correlation of risk between relatives from the same family.[32, 33] Student's *t*-tests were used to compare estimated mean age at diagnosis of colorectal cancer between carriers of a mutation in single gene and carriers of a mutation in both genes. Fisher's exact tests were used to compare the frequency of carriers. All statistical tests were two-sided. All statistical analyses were performed using Stata 13.0.[34]

RESULTS

In the Colon Cancer Family Registry, we identified a total of 854 individuals (593 probands and 261 relatives) who were known to carry a mutation in one or both of *MUTYH* and a MMR gene. Of these, there were 469 carriers of a MMR gene mutation alone, 375 carriers of a monoallelic *MUTYH* mutation alone, and 10 carriers of both a monoallelic *MUTYH* mutation and a MMR gene mutation. Of the 375 carriers of a monoallelic *MUTYH* mutation alone, 76 were genotyped and confirmed as non-carriers of MMR gene mutation, and the remainders were assumed to be non-carriers of a MMR gene mutation (details in Figure 1).

Of the 10 carriers of both a monoallelic *MUTYH* mutation (6 with c.1187G>A p.(Gly396Asp), 3 with c.821G>A p.(Arg274Gln), and 1 with c.536A>G p.(Tyr179Cys)) and a MMR gene mutation (3 in *MLH1,* 6 in *MSH2,* and 1 in *PMS2*), 8 were diagnosed with colorectal cancer, of which 4 (50%) were located in the sigmoid colon or rectum. For affected carriers of a monoallelic *MUTYH* mutation alone, 31% of their tumors were in the proximal colon, 62% in the distal colon or rectum, and 7% in a non-specific site of the colon. For affected carriers of a MMR gene mutation alone, 58% of their tumors were in the proximal colon, 34% in the distal colon or rectum, and 8% in a non-specific site of the colon (Table 2). The mean age at diagnosis of colorectal cancer for carriers of both a *MUTYH* and a MMR gene mutation was 46.6 (standard deviation [SD] 14.0) years, which was not different from carriers of a monoallelic *MUTYH* mutation alone (mean 53.5, SD 12.1; p=0.12) or carriers of a MMR gene mutation alone (mean 43.4, SD 10.6; p=0.39). The majority of carriers of both a *MUTYH* and a MMR gene mutation (7 of 10) also developed extracolonic cancers in the small bowel, uterus, biliary tract and skin (Table 1).

In the 385 monoallelic *MUTYH* mutation carriers (including the 10 who also carried a MMR gene mutation), the frequency of MMR gene mutations was higher in those with colorectal cancer (8/159, 5.0%) compared with those without colorectal cancer (2/226, 0.9%; p=0.02). In the 479 MMR gene mutation carriers (including the 10 who also carried a *MUTYH* mutation), there was no evidence for a difference in the frequency of monoallelic *MUTYH* mutations between those with colorectal cancer (8/411, 2.0%) and those without colorectal cancer (2/68, 2.9%; p=0.64) (Table 2).

Based on the weighted cohort analyses, the risk of colorectal cancer for carriers of both a *MUTYH* and a MMR gene mutation was 21.5-times (95% CI 9.19–50.1; p<0.001) higher than for carriers of a monoallelic *MUTYH* mutation alone. A sensitivity analysis

restricted to individuals with genotype data on both *MUTYH* and MMR gene mutations (n=86) showed a very similar result to the main analysis (HR 14.5, 95% CI 3.30–63.7; p<0.001). There was no statistical evidence of a difference in the risk of colorectal cancer between carriers of both a *MUTYH* and a MMR gene mutation and carriers of a MMR gene mutation alone (HR 1.94, 95% CI 0.63–5.99; p=0.25).

DISCUSSION

As the MMR system functionally interacts with the base excision repair protein MUTYH,[16] we hypothesized that the combined effect of having a mutation in both a *MUTYH* gene and a MMR gene may increase colorectal cancer risk compared with only carrying a single gene mutation. From identifying the 10 individuals with a germline mutation in both a *MUTYH* gene and a MMR gene, we found no evidence that a monoallelic *MUTYH* mutation confers additional risk of colorectal cancer to carriers of a MMR gene mutation.

We found that, amongst MMR gene mutation carriers, there was no difference in the frequency of monoallelic *MUTYH* mutations between those with colorectal cancer and those without colorectal cancer. This finding is consistent with other studies: Steinke *et al.*[19] found that the frequency of monoallelic *MUTYH* mutations in *MSH6* mutation carriers with colorectal cancer (2/64, 3.1%) was not significantly higher than that of healthy controls (9/577, 1.6%, p=0.30); Ashton *et al.*[22] found that the frequency of *MUTYH* mutations in *MLH1* and *MSH2* mutation carriers (2/209, 1%) was not higher than that of healthy controls (4/296, 1.35%; p=0.69); van Puijenbroek *et al.*[21] found no evidence of the combined effect of heterozygous *MSH6* and *MUTYH* mutations, except perhaps for urothelial tumors; and Gorgens *et al.*[23] found only one monoallelic *MUTYH* mutation of c.1187G>A p.(Gly396Asp) in 50 individuals with suspected HNPCC compared with no *MUTYH*

mutation in 116 healthy controls. Further, Stormorken *et al.*[20] reported that frequency of monoallelic *MUTYH* mutations in individuals with HNPCC, HNPCC-like or dominantly inherited late-onset colorectal cancer (2/96, 2%) was not more than expected by chance, and concluded that monoallelic *MUTYH* mutations may not increase risk of cancer in these individuals.

In contrast with our finding, Niessen *et al.*[18] found an over-representation of monoallelic *MUTYH* mutations in colorectal or endometrial cancer cases with a *MSH6* missense mutation (4/20, 20%) compared with colorectal cancer cases without a MMR mutation (1/134, 0.7%; p=0.002) and healthy controls (1.5%, p=0.001). This finding, however, might be attributable to indiscriminate MMR testing of all individuals despite the appearance of normal tumor IHC staining for MSH6 and low frequency MSI (MSI-low) whereas our study only performed MMR genetic testing on population-based probands with tumor tissue that was MSI-high or showed loss of MMR protein expression by IHC testing. It is possible, due to our testing regimen, that our findings are confided to MMR gene mutations that are at the higher end of pathogenicity, compared to the study of Niessen *et al.*[18]

We found that the risk of colorectal cancer for carriers of both a *MUTYH* and a MMR gene mutation was significantly higher than for those with a monoallelic *MUTYH* mutation alone. This finding is likely to be a reflection of the substantially increased risk of colorectal cancer due to a MMR gene mutation[35], and a small increased risk due to a monoallelic *MUTYH* mutation[4]. That is, a monoallelic *MUTYH* mutation might not modify the risk of colorectal cancer for carriers of a MMR gene mutation. In this study, we found that the frequency of MMR gene mutations was higher in monoallelic *MUTYH* mutation carriers with colorectal cancer than those without colorectal cancer. This is consistent with the finding by Giráldez *et al.*[17] that *MSH6* mutations were more frequent in *MUTYH* mutation carriers

than non-carriers (3/26 = 11.5% vs. 0/50 = 0%, p=0.04). However, we did not observe any individuals with a *MSH6* mutation in the monoallelic *MUTYH* mutation carriers in this study.

Previous studies have reported that MMR gene mutation-associated colorectal cancers[36] and those attributed to biallelic mutations in *MUTYH*[6, 37] are more likely to be diagnosed in the proximal colon. We found an apparently different distribution of tumor location for carriers of both a *MUTYH* and a MMR gene mutation given that about half of colorectal cancers in these mutation carriers were located in the sigmoid colon or rectum. However, we cannot make a conclusion on colorectal tumor location distribution as well as extracolonic cancer distribution for carriers of both a *MUTYH* and a MMR gene mutation because of the small number of cases. Individuals who carried both biallelic *MUTYH* mutations and a MMR gene mutation were not observed in our study, consistent with previous reports.[19-23, 38]

In this study, risk estimates were corrected for possible selection bias by application of appropriate weights to minimise bias when participants are selected on the basis of phenotype.[29] Additionally standardized epidemiologic assessments and uniformly high-quality testing for gene mutations were used across the Colon Cancer Family Registry centers.[24] A major limitation of the study is that we were not able to sequence the MMR genes for all *MUTYH* mutation carriers regardless of tumor phenotype (tumor mismatch repair status) in the Colon Cancer Family Registry. However, a sensitivity analysis on individuals with genotype data on both *MUTYH* and MMR gene mutations showed a very similar result to the main analysis. Another limitation is that there is a possibility of survival bias since cases with poor survival were unable to participate in the study as they would not have been able to provide a blood sample for genetic testing. Since we preferentially included individuals with a pathogenic MMR gene mutation that are likely to cause colorectal cancer

especially colorectal cancer with tumor MMR deficiency, then our findings are relevant to individuals with a MMR gene mutation likely to cause colorectal cancer, which we would argue, are more likely relevant to family cancer clinics.

Within the limited power of this study, we observed we observed no differences in the risk of colorectal cancer for MMR gene mutation carriers with additional carrying a *MUTYH* mutation. In contrast, there is a substantially high risk of colorectal cancer for *MUTYH* mutation carriers with additional carrying a MMR gene mutation. Our data suggest that for MMR gene mutation carriers, there is no advantage to the carrier, with respect to their screening recommendations, for having additional testing for *MUTYH*. However, for monoallelic *MUTYH* mutation carriers, additional testing for MMR germline mutations (if their tumor shows MMR deficiency) is warranted as co-existance of a pathogenic MMR gene mutation would result in recommendations for increased screening.

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DISCLAIMER

The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the CFRs, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government or the CFR. Authors had full responsibility for the design of the study, the collection of the data, the analysis and interpretation of the data, the decision to submit the manuscript for publication, and the writing of the manuscript.

DISCLOSURE

The authors have no conflict of interest to declare with respect to this manuscript.

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Titles and legends to figures

Figure 1. Carriers of a mutation in *MUTYH* and/or a DNA mismatch repair gene and their colorectal cancer affected status

Table 1. Genotype and phenotype characteristics of carriers of both a *MUTYH* mutation and a mismatch repair gene mutation

Person	MMR	*MUTYH*	Sex	Colorectal cancer						Other Cancer sites (age)	Last known age
				Site (age)	Histology	Grade	TNM stage	IHC	MSI		
1	*MSH2* c.136_164del, p.His46GlyfsTer26	c.821G>A p.(Arg274Gln)	F	Rectum (35) Caecum (51) Splenic flexure (53) Transverse colon adenoma (53)	NA Mucinous NA NA	NA Moderate NA NA	NA T3N0Mx NA -	MSH2 absent	MSI-H	Uterus (32) Skin (50, 51) Duodenum (54)	55 (deceased)
2	*MLH1* c.793C>T, p.His264LeufsTer2	c.1187G>A p.(Gly396Asp)	F	Caecum (72)	Adenocarcinoma	Moderate	T2N0Mx	MLH1 absent	MSI-H	Uterus (59)	81 (deceased)
3	*MLH1* c.1975C>T, p.[Glu633_Glu663del, Arg659Ter]	c.1187G>A p.(Gly396Asp)	M	Ascending colon (39)	Mucinous	Moderate	T3N1Mx	MLH1 absent	MSI-H	No	45
4	*MLH1*. c.588delA p.Lys196AsnfsTer6	c.1187G>A p.(Gly396Asp)	M	Colon (34) Rectum (58)	NA Adenocarcinoma	NA Poor	NA T3N1Mx	MLH1 & PMS2 absent	NA	Prostate (68)	68
5	*MSH2*. c.863delA p.Gln288fsArgfsTer4	c.536A>G p.(Tyr179Cys)	F	Rectum (61)	NA	NA	NA	MSH2 & MSH6 absent	MSI-H	Small bowel (54) Duodenum (58,62)	68
6	*MSH2* c.792+1G>T r.spl? p.?	c.1187G>A p.(Gly396Asp)	M	Caecum (53) Transverse colon(53)	Signet ring NA	Well NA	TxN0Mx NA	MSH2 & MSH6 absent	NA	Biliary tract (23)	60
7	*MSH2* c.1660A>G, p.Gly504AlafsX3	c.1187G>A p.(Gly396Asp)	F	Rectum (37)	Adenocarcinoma	Well	T1NxMx	MSH2 absent	NA	Skin (44)	48
8	*PMS2*. c.1939A>T p.Lys647Ter	c.1187G>A p.(Gly396Asp)	M	Sigmoid colon (43)	Mucinous	Well	T3N0M0	PMS2 absent	MSI-H	No	53
9	*MSH2* c.136_164del, p.His46GlyfsTer26	c.821G>A p.(Arg274Gln)	M	No						Skin (50, 51)	57
10	*MSH2* c.136_164del, p.His46GlyfsTer26	c.821G>A p.(Arg274Gln)	M	No						No	35

M, male; F, female; MMR, mismatch repair; NA, not available; MSI, microsatellite instability; MSI-H, high microsatellite instability; IHC, immunohistochemistry

Table 2. Characteristics of carriers of monoallelic *MUTYH* mutation alone, carriers of a mismatch repair (MMR) gene mutation alone, and carriers of both a *MUTYH* and a MMR gene mutation.

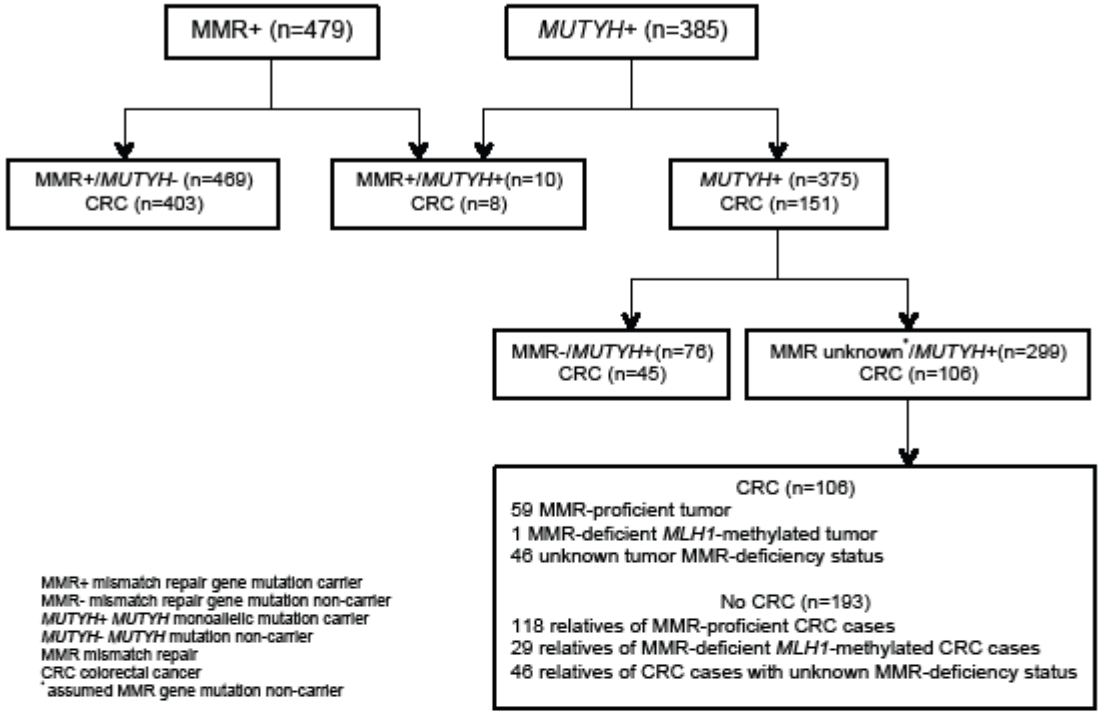
	Carriers of monoallelic *MUTYH* mutation alone[¶] (n=375)		Carriers of a MMR gene mutation alone (n=469)		Carriers of both a *MUTYH* and a MMR gene mutation (n=10)	
	No colorectal cancer (n=224)	Colorectal cancer (n=151)	No colorectal cancer (n=66)	Colorectal cancer (n=403)	No colorectal cancer (n=2)	Colorectal cancer (n=8)
MUTYH mutation						
c.1187G>A p.(Gly396Asp)	152 (67.9)	98 (64.9)			0 (0)	6 (75.0)
c.536A>G p.(Tyr179Cys)	55 (24.5)	39 (25.8)			0 (0)	1 (12.5)
c.821G>A p.(Arg274Gln)	9 (4.0)	9 (6.0)			2 (100)	1 (12.5)
Other mutations	8 (3.6)	5 (3.3)			0 (0)	0 (0)
MMR gene mutation						
MLH1			28 (42.4)	166 (41.2)	0 (0)	3 (37.5)
MSH2			33 (50.0)	162 (40.2)	2 (100)	4 (50.0)
MHS6			3 (4.6)	39 (9.7)	0 (0)	0 (0)
PMS2			2 (3.0)	36 (8.9)	0 (0)	1 (12.5)
Country						
USA	93 (41.5)	86 (57.0)	13 (19.7)	153 (38.0)	0 (0)	3 (37.5)
Canada	80 (35.7)	41 (27.1)	44 (67.7)	79 (19.6)	2 (100)	3 (37.5)
Australia	51 (22.8)	24 (15.9)	9 (13.6)	171 (42.4)	0 (0)	2 (25.0)
Sex						
Male	97 (43.3)	77 (51.0)	27 (40.9)	208 (51.6)	2 (100)	4 (50.0)
Female	127 (56.7)	74 (49.0)	39 (59.1)	195 (48.4)	0 (0)	4 (50.0)
Proband status						
Proband	49 (21.9)	143 (94.7)	4 (6.1)	389 (96.5)	0 (0)	8 (100)
Non-proband	175 (78.1)	8 (5.3)	62 (93.9)	14 (3.5)	2 (100)	0 (0)
Age[*] (SD),years	58.2 (14.6)	53.5 (12.1)	48.6 (11.8)	43.4 (10.6)	42.5 (10.6)	46.6 (14.0)
Tumor location						
Proximal colon[**]		47 (31.1)		236 (58.6)		3 (37.5)
Distal colon[^]		48 (31.8)		70 (17.4)		1 (12.5)
Rectosigmoid junction		20 (13.3)		25 (6.2)		0 (0)
Rectum		26 (17.2)		41 (10.1)		3 (37.5)
Non-specific site of colon		10 (6.6)		31 (7.7)		1 (12.5)

[*]Age at first diagnosis of colorectal cancer for carriers diagnosed with colorectal cancer; age at first diagnosis of extracolonic cancer or last contact or death, whichever occurred the earliest, for carriers without colorectal cancer.

[**] caecum, ascending colon, hepatic flexure and transverse colon

[^] splenic flexure, descending colon and sigmoid colon

[¶] Of 375 individuals, 76 were confirmed non-carriers of a MMR gene and the remainders were not tested for a MMR gene mutation (See details in Figure 1)



MMR+ mismatch repair gene mutation carrier
MMR- mismatch repair gene mutation non-carrier
MUTYH+ *MUTYH* monoallelic mutation carrier
MUTYH- *MUTYH* mutation non-carrier
MMR mismatch repair
CRC colorectal cancer
* assumed MMR gene mutation non-carrier