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Prevalence and Spectrum of Germline Cancer Susceptibility Gene Mutations Among Patients With Early-Onset Colorectal Cancer

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Abstract

IMPORTANCE—Hereditary cancer syndromes infer high cancer risks and require intensive cancer surveillance, yet the prevalence and spectrum of these conditions among unselected patients with early-onset colorectal cancer (CRC) is largely undetermined.

OBJECTIVE—To determine the frequency and spectrum of cancer susceptibility gene mutations among patients with early-onset CRC.

DESIGN, SETTING, AND PARTICIPANTS—Overall, 450 patients diagnosed with colorectal cancer younger than 50 years were prospectively accrued from 51 hospitals into the Ohio Colorectal Cancer Prevention Initiative from January 1, 2013, to June 20, 2016. Mismatch repair (MMR) deficiency was determined by microsatellite instability and/or immunohistochemistry. Germline DNA was tested for mutations in 25 cancer susceptibility genes using next-generation sequencing.

MAIN OUTCOMES AND MEASURES—Mutation prevalence and spectrum in patients with early-onset CRC was determined. Clinical characteristics were assessed by mutation status.

RESULTS—In total 450 patients younger than 50 years were included in the study, and 75 gene mutations were found in 72 patients (16%). Forty-eight patients (10.7%) had MMR-deficient tumors, and 40 patients (83.3%) had at least 1 gene mutation: 37 had Lynch syndrome (13, *MLH1* [including one with constitutional *MLH1* methylation]; 16, *MSH2;* 1, MSH2/monoallelic *MUTYH;* 2, *MSH6;* 5, *PMS2*); 1 patient had the *APC* c.3920T>A, p.I1307K mutation and a *PMS2* variant; 9 patients (18.8%) had double somatic MMR mutations (including 2 with germline biallelic *MUTYH* mutations); and 1 patient had somatic *MLH1* methylation. Four hundred two patients (89.3%) had MMR-proficient tumors, and 32 patients (8%) had at least 1 gene mutation: 9 had mutations in high-penetrance CRC genes (5, *APC;* 1, *APC/PMS2;* 2, biallelic *MUTYH;* 1, *SMAD4*); 13 patients had mutations in high- or moderate-penetrance genes not traditionally associated with CRC (3, *ATM*; 1, *ATM/CHEK2;* 2, *BRCA1;* 4, *BRCA2;* 1, *CDKN2A;* 2, *PALB2*); 10 patients had mutations in low-penetrance CRC genes (3, *APC* c.3920T>A, p.I1307K; 7, monoallelic *MUTYH*). Importantly, 24 of 72 patients (33.3%) who were mutation positive did not meet established genetic testing criteria for the gene(s) in which they had a mutation.

CONCLUSIONS AND RELEVANCE—Of 450 patients with early-onset CRC, 72 (16%) had gene mutations. Given the high frequency and wide spectrum of mutations, genetic counseling and testing with a multigene panel could be considered for all patients with early-onset CRC.

Colorectal cancer (CRC) is the third most common cancer diagnosed in the United States, excluding nonmelanoma skin cancers.[1] The median age of CRC diagnosis is 69 years in males and 73 years in females; 10% of patients with CRC are diagnosed when they are younger than 50 years.[1] Early-onset cancer is a hallmark of inherited cancer predisposition. Identification of hereditary cancer syndromes has significant implications for patients and families, as it facilitates risk assessment, directs clinical management, and can guide treatment options.

Lynch syndrome, caused by germline mutations in the mismatch repair (MMR) genes *MLH1, MSH2, MSH6* and *PMS2*, or *EPCAM*, is the most common known cause of hereditary CRC and accounts for 4% to 13.5% of patients with early-onset CRC.[2–6] Patients with tumors exhibiting characteristics of MMR deficiency are more likely to have Lynch syndrome; therefore, professional guidelines recommend all patients with CRC receive tumor screening for Lynch syndrome, with referral to genetic counseling for those with MMR deficiency.[7,8] The National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology for Genetic/Familial High-Risk Assessment: Colorectal (NCCN Guidelines) suggests that all patients with CRC diagnosed younger than 50 years consider genetic testing for Lynch syndrome.[9]

The prevalence of other hereditary cancer syndromes among patients with early-onset CRC is largely unknown because previous studies are limited and have been confined to selected (high-risk) patient populations.[5,6] With the advent of next-generation sequencing (NGS), genetic testing for hereditary CRC has shifted from phenotype-specific single gene assessment to broad panels providing simultaneous assessment of multiple genes implicated in various hereditary cancer syndromes. Previous studies have shown that multigene panel testing for hereditary CRC is feasible, timely, and more cost-effective than single gene

testing.[10] However, the clinical utility of multigene panel testing among patients with early-onset CRC is not known.

Using multigene panel testing, we determined the prevalence and spectrum of germline mutations in 25 genes associated with various hereditary cancer syndromes in 450 patients with CRC diagnosed at younger than 50 years, unselected for family history or MMR status of the tumor.

Methods

Patients

As of June 20, 2016, 2785 patients who had surgical resection in Ohio for newly diagnosed invasive colorectal adenocarcinoma between the ages of 17 and 96 years, on or after January 1, 2013, were prospectively enrolled into the ongoing Ohio Colorectal Cancer Prevention Initiative (OCCPI). The OCCPI was created to decrease CRC incidence in Ohio by identifying patients with hereditary predisposition (statewide universal tumor screening for newly diagnosed patients with CRC), increasing colonoscopy compliance for first-degree relatives of patients with CRC and encouraging future research through the creation of a biorepository. The 51 Ohio hospitals participating in the OCCPI (eTable 1 in the Supplement) were selected to represent a cross-section of clinical centers in the state based on high reported volume of patients with CRC, affiliation with a high volume hospital, or interest in participation. Institutional review board approval was obtained by the individual hospitals, Community Oncology Programs, or by ceding review to the Ohio State University (OSU) institutional review board. Written informed consent was obtained.

Of the total patients enrolled, 594 were diagnosed at younger than 50 years, and 450 of those ostensibly unrelated patients had all of their testing completed in time for inclusion in this analysis (75.8% of the total patients enrolled younger than 50 years). Using tumor registry numbers from 2013, there were an estimated 1207 patients diagnosed with CRC at younger than 50 years in the state of Ohio between January 2013 and June 2016.[11] Therefore, this analysis includes 37.3% (450 of 1207) of eligible patients.

Samples

Blood and a paraffin-embedded tumor block or unstained slides were submitted for each patient. Study pathologists confirmed the tumor histology and marked areas with at least 30% tumor and normal adjacent tissue. Blood and tissue (tumor and normal) underwent DNA extraction using standard methods.[12]

Tumor Screening for Lynch Syndrome

All tumors were screened for MMR deficiency by microsatellite instability (MSI) testing and/or immunohistochemical (IHC) analysis. Tumor screening was performed centrally at OSU, if not already completed in a Clinical Laboratory Improvement Amendments–approved laboratory for clinical care. Microsatellite instability testing was completed using tumor and normal DNA to detect a size change in microsatellites using the Promega MSI Analysis System version 1.2 (Promega Corporation). This included fluorescently labeled

primers for coamplification of 7 repeat markers (BAT-25, BAT-26, NR-21, NR-24, MONO-27, Penta C, Penta D). Tumors showing MSI at 0 markers were classified as microsatellite stable (MSS). Tumors showing MSI at 1 marker were classified as microsatellite low. Tumors showing MSI at 2 or more markers were classified as microsatellite high (MSI-H). Immunohistochemistry of the MMR proteins was performed using the 2-stain method as previously described.[13] Staining for all 4 MMR proteins was attempted if MSI could not be performed. Antibodies included MLH-1 Clone: Leica ES05 (Mouse: NCL-L-MLH1), MSH-2 Clone: Calbiochem FE11 (Mouse: NA27), MSH-6 Clone: Epitomics EP49 (Rabbit: AC-0047), PMS-2 Clone: BD Pharmingen A16-4 (Mouse: 556415). Proteins with convincing stain in more than 1% of cells were considered present. Methylation of the *MLH1* promoter was assessed at 4 CpG sites between −209 and −188 using pyrosequencing[14] when tumors were MSI-H and/or absent MLH1 and PMS2 proteins on IHC. The average percent of methylation detected at the 4 CpG sites was used to classify tumors as methylated (≥10% methylation) or not (<10% methylation).

Germline Genetic Testing

The testing strategy is detailed in the Figure. All patients underwent germline testing for 25 cancer susceptibility genes: *APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11, TP53*. Two clinical laboratories were used for germline testing based on the MMR status of the tumor. Genetic testing for patients with MMR deficient tumors (MSI-H and/or abnormal IHC without *MLH1* methylation) was performed at the University of Washington (UW). Genomic regions were captured using biotinylated RNA oliognucleotides (SureSelect; Agilent Technologies) and sequenced on an Illumina HiSeq2000 instrument (Illumina Inc).[15] Large rearrangements were detected.[16] Genetic testing for patients with MMR-proficient tumors (MSS and/or normal IHC) or *MLH1* methylation was performed at Myriad Genetics Inc, and ultra-deep targeted sequencing was performed using the RainDance Thunder-Storm platform (RainDance Technologies) for DNA amplification and Illumina MiSeq and HiSeq2000 instruments.[17] Large rearrangements were detected. The Clinical Laboratory Improvement Amendments–approved laboratories adjudicated the pathogenicity of all mutations using criteria established by the American College of Medical Genetics and International Agency for Research on Cancer guidelines.[18,19]

Tumor Genetic Testing

Select patients underwent additional testing of the MMR genes in tumor DNA at UW using methods previously described.[20] For patients with unexplained MMR deficiency (MMR-deficient tumor, no germline MMR mutation or *MLH1* methylation), tumors were assessed for 2 MMR mutations or 1 MMR mutation with loss of heterozygosity of the opposite allele (double somatic MMR mutations), which has been shown to cause sporadic MMR deficiency.[20] For patients with MMR-deficient tumors and a germline MMR variant of uncertain significance, tumors were assessed for additional MMR mutations or loss of heterozygosity to attempt to clarify the pathogenicity of the variant. Variants were reclassified as likely pathogenic when tumor screening results supported pathogenicity and 1

additional pathogenic mutation was identified in the tumor using methods previously described.[20]

Statistical Analysis

Descriptive statistics were provided. Wilson score intervals with continuity correction were used to compute confidence intervals. Pearson χ^2 tests with continuity correction and Fisher exact test were used to estimate *P* values; all tests were 2-sided, and level of significance was set at .05.

Results

Patient characteristics are shown in Table 1. Men accounted for 52.2% (n = 235) of patients; the mean age at CRC diagnosis was 42.5 years. Over 85% of patients self-reported their race as white (n = 385) and 9.1% of patients (n = 41) self-reported their race as black. Among the patients, 8.2% (n = 37) had an additional malignancy. Overall, patients reported a family history of at least 1 first-degree relative with cancer(s) of the colon (n = 86 [19.1%]), endometrium (n = 19 [4.2%]), breast (n = 42 [9.3%]), ovary (n = 11 [2.4%]), and/or pancreas (n = 10 [2.2%]).

Tumor Screening for Lynch syndrome

Among the patients, 10.7% (n = 48) had MMR-deficient tumors (n = 8 [16.7%], stage I; n = 13 [27.1%], stage II; n = 23 [47.9%], stage III; n = 4 [8.3%], stage IV). Three patients with MMR-deficient tumors had *MLH1* methylation. All tumor screening results (MSI and IHC) were concordant for each patient, except for 1 (MSI-H, normal IHC). In total, 402 patients (89.3%) had MMR-proficient tumors (n = 41 [10.2%], stage I; n = 80 [19.9%] stage II; n = 174 [43.3%], stage III; n = 104 [25.9%], stage IV; n = 3 [0.7%], stage unavailable).

Overall Germline Genetic Testing

Among 450 patients with early-onset CRC, 75 pathogenic or likely pathogenic cancer susceptibility gene mutations were found in 72 patients (16%; 95% CI, 12.8%–19.8%). The spectrum of mutations is shown in Table 2. Thirty-six patients (8%) had Lynch syndrome only; 2 patients (0.4%) had Lynch syndrome and another hereditary cancer syndrome; 34 patients (7.6%) had a different hereditary cancer syndrome (including a third patient with 2 syndromes). Sixty-one patients (13.6%) had mutations in high- or moderate-penetrance genes, and 11 patients (2.4%) had mutations in low-penetrance genes.

Genotype and phenotype data (including family history) for patients with pathogenic mutations are provided in eTable 3 in the Supplement. Patients with pathogenic mutations were more likely to report a family history of colon cancer (45.8% vs 14%; $P < .001$) and endometrial cancer (11.1% vs 2.9%; $P = .005$)compared with patients without mutations. Nine patients came from unique families with a known mutation in a cancer susceptibility gene but had not undergone predictive testing for the familial mutation prior to their CRC diagnosis. Patients with Lynch syndrome (19 of 37) were diagnosed at earlier stages (I, II) compared with patients with another hereditary cancer syndrome (9 of 35) (51.4% vs 25.7%; $P = .047$).

Germline Results for Patients With MMR-Deficient Tumors

Forty (83.3%) of the 48 patients with MMR-deficient tumors had at least 1 mutation in a cancer susceptibility gene. Thirty-seven patients had Lynch syndrome (13, *MLH1;* 16, *MSH2;* 1, MSH2/monoallelic *MUTYH;* 2, *MSH6;* 5, *PMS2*), 2 had biallelic *MUTYH* mutations, and 1 had the common low-penetrance *APC* c.3920T>A, p.I1307K mutation[21–23] and a variant of uncertain significance in *PMS2* (c.322G>T, p.G108W). Nine patients' MMR-deficient tumors were explained by double somatic MMR mutations (including 2 patients with germline biallelic *MUTYH* mutations) (eTable 4 in the Supplement). Of the 3 patients with *MLH1* methylation, 1 was found to have a *MSH2* mutation (the tumor showed absence of all 4 MMR proteins on IHC), 1 was found to have constitutional *MLH1* methylation by testing blood, and the third patient did not have any germline mutations and constitutional methylation was ruled out.

Germline Results for Patients With MMR-Proficient Tumors

Thirty-two (8%) of 402 patients with MMR-proficient tumors had at least 1 mutation in a cancer susceptibility gene. Nine patients had mutations in high-penetrance genes with established CRC risk (5, APC; 1, *APC/PMS2;* 2, biallelic *MUTYH;* 1, *SMAD4*), 13 had mutations in high- or moderate-penetrance genes not traditionally associated with CRC risk (3, ATM; 1, *ATM/CHEK2;* 2, *BRCA1;* 4, *BRCA2;* 1, *CDKN2A;* 2, *PALB2*), and 10 had mutations in low-penetrance CRC genes (3, *APC* c.3920T>A, p.I1307K; 7, monoallelic *MUTYH*).

Variants of Uncertain Significance

One hundred seventy-eight variants of uncertain significance were found in 145 patients (32.2%) (eTable 2 in the Supplement). The genes most likely to have a variant discovered included *ATM* (n = 30 [16.9%]), *APC* (n = 19 [10.7%]), and *CHEK2* (n = 18 [10.1%]). Five variants were upgraded to pathogenic (or likely pathogenic) after additional testing. One patient with double somatic MMR mutations had a germline pathogenic *MUTYH* mutation and *MUTYH* variant c.698G>A, p.G233D, that was reclassified to likely pathogenic based on segregation analysis and clinical history. Tumor sequencing clarified that *PMS2* c.215G>A, p.G72E and *MSH2* c.1832T>A, p.V611E were likely pathogenic owing to the presence of the germline variant plus 1 additional pathogenic somatic mutation, in addition to supportive tumor screening results. RNA studies showing altered MLH1 splicing (exon 2 skipping) in a majority of transcripts and cosegregation with disease proved that *MLH1* c.207 + 5G>C was pathogenic. Cosegregation with disease proved that *MSH6* c.1109T>C, p.L370S was pathogenic.

Discussion

This prospective, statewide study indicates that 1 of every 6 patients with CRC diagnosed younger than 50 years has at least 1 pathogenic cancer susceptibility gene mutation (16%). While the prevalence of Lynch syndrome reported herein (8.4%) is consistent with previous publications,[2,3] this is the first study to our knowledge to determine the prevalence and spectrum of other hereditary cancer syndromes (8%) found in an unselected series of patients with early-onset CRC. All patients found to have pathogenic mutations received

genetic counseling and current evidence-based guidelines for intensive cancer surveillance based on their mutation status.[9] For some patients, the identification of MMR tumor status and/or gene mutation(s) provided actionable therapeutic targets for their current CRC (eg, PARP [poly adenosine diphosphate–ribose polymerase] inhibitors and anti-PD1 [programmed cell death protein 1] immunotherapy).[24,25] At-risk family members benefited from genetic counseling and cascade testing to determine the necessity of potentially life-saving cancer surveillance and prevention options.

Multigene panel testing facilitated identification of hereditary cancer syndromes in patients who may have otherwise been missed. Importantly, 24 of 72 patients (33.3%) with pathogenic mutations did not meet NCCN Guidelines for at least 1 of the gene(s) in which they were found to have a mutation.[9] Forty-four of the 72 patients (61.1%) with pathogenic mutations did meet NCCN Guidelines owing to having MMR-deficient tumors and/or the presence of more than 10 adenomatous polyps; however, they likely would have only received phenotype-specific genetic testing for Lynch syndrome or polyposis had testing been done outside of this study.[9] In that scenario, 4 patients (5.6%) would have had at least 1 of their mutations missed without the use of a broad multigene panel. Three patients with MMR-deficient tumors were found to have additional mutations in genes that would not have been assessed: 1 had biallelic *MUTYH* mutations (without polyposis), 1 had a monoallelic *MUTYH* mutation, and 1 had the *APC* c.3920T>A, p.I1307K mutation. One patient with polyposis (due to a known *APC* mutation) and an MMR-proficient tumor was unexpectedly found to also have Lynch syndrome caused by a pathogenic *PMS2* mutation.

While many of the detected mutations were in genes with established CRC risk, 13 of 72 patients (18.1%) had mutations in genes not traditionally associated with CRC: 3, ATM;1, *ATM/CHEK2;* 2, *BRCA1;* 4, *BRCA2;* 1, *CDKN2A;* and 2, *PALB2*. Notably, 6 patients had mutations in *BRCA1/2* (known to cause hereditary breast-ovarian cancer syndrome [HBOC]). Four patients with *BRCA1/2* mutations met NCCN genetic testing criteria for HBOC, and 2 patients with *BRCA1/2* mutations did not have personal or family history of breast or ovarian cancer and did not meet NCCN genetic testing criteria for HBOC. Previous studies have reported early-onset CRC in women with *BRCA1* mutations[26] and *BRCA2* mutations in families with familial colorectal cancer type X.[27] It is possible that these 13 mutations are incidental findings; however, the cancer spectrum and penetrance for many well-established and newly discovered genes will likely be redefined now that multigene panel testing is becoming more routine.

Another novel aspect of this study was the use of tumor sequencing to elucidate the etiology of patients with early-onset CRC with unexplained MMR deficiency and clarify the pathogenicity of MMR variants of uncertain significance. It was previously reported that 68% of patients with CRC and endometrial cancer with unexplained MMR deficiency do not have Lynch syndrome, and their MMR-deficient tumors were the result of double somatic MMR mutations.[20] In our study, 100% (9/9) of patients with early-onset CRC and unexplained MMR deficiency were found to have double somatic MMR mutations (including 2 patients with germline biallelic *MUTYH* mutations). This finding has significant clinical implications and underscores the importance of somatic testing for patients with early-onset CRC and unexplained MMR deficiency. Traditionally, patients with

CRC (particularly those diagnosed at a young age) with unexplained MMR deficiency would be medically managed as if they had Lynch syndrome with an unidentifiable germline MMR mutation. Proving that their MMR deficiency was the result of double somatic mutations likely means that they do not have Lynch syndrome and may not need to follow intensive Lynch syndrome surveillance guidelines. It is possible that these patients have something undiscovered that predisposes them to the development of somatic MMR mutations, but most likely, their CRC was sporadic and it would be appropriate for their family members to follow screening guidelines based on the family history of early-onset CRC. In addition, this is the first time that we know of that somatic testing was used specifically to aid in MMR variant reclassification, assisting in the reclassification of 2 variants among patients with MMR-deficient tumors.

Limitations

While the recruiting personnel were strongly discouraged from preferentially enrolling patients with a strong family history of cancer or young age of diagnosis, it is possible that this occurred. Patient's self-reported family history is a limitation; medical record review would have ensured accuracy but was not feasible. We had 3-generation pedigrees for patients who tested positive and received genetic counseling. However, we only had first-degree relative cancer history for patients who tested negative, so we were unable to provide complete risk assessment or determine who met NCCN genetic testing criteria among patients who did not have a mutation.

The 16% prevalence of hereditary cancer syndromes among patients with early-onset CRC reported herein is likely an underestimate for several reasons. There are likely other CRC susceptibility genes, some of which have not yet been discovered, for which our patients may not have been tested. Additionally, some of the variants of uncertain significance may eventually be found to be pathogenic. For example, at the time of publication there were 2 mutations (*CHEK2* c.470T>C, p.I157T and *MLH1* c.1897-2A>G) with discrepant pathogenicity classifications from various clinical laboratories (ranging from variant of uncertain significance to pathogenic). For this study, they were considered variants of uncertain significance.

Conclusions

Overall, 75 pathogenic cancer susceptibility gene mutations were found in 72 patients of 450 diagnosed with CRC younger than 50 years (16%). While it is important to continue MMR tumor screening for all patients with CRC for treatment purposes (ie, checkpoint inhibitors, if initial findings are validated in subsequent trials), genetic counseling and testing with a broad multigene panel should be considered for all patients with early-onset CRC due to their high prevalence of hereditary cancer syndromes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

Question

What is the frequency and spectrum of cancer susceptibility gene mutations among patients with colorectal cancer diagnosed at younger than 50 years?

Findings

In this cohort study of 450 patients with early-onset colorectal cancer, 72 (16%) had a pathogenic mutation. Panel testing identified mutations in patients that may have otherwise been missed; specifically, 24 of 72 patients (33.3%) who were mutation positive did not meet testing criteria for the gene(s) in which they had a mutation.

Meaning

Multigene panel testing should be considered for all patients with early-onset colorectal cancer.

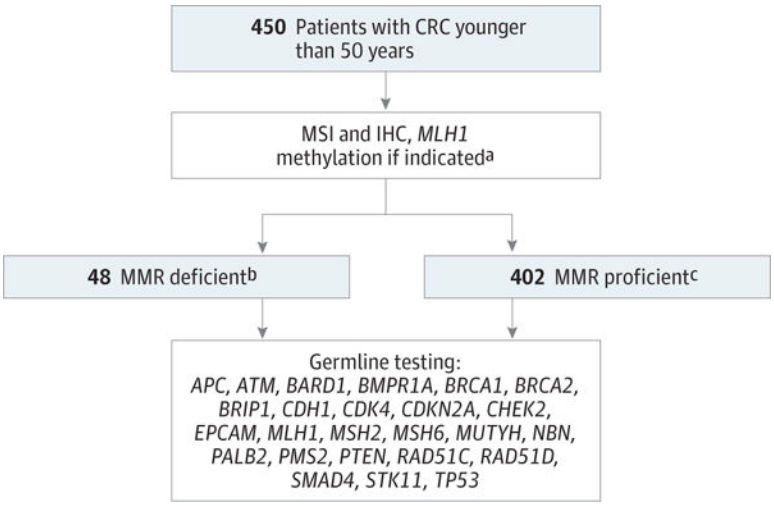

Figure. Testing Strategy

CRC indicates colorectal cancer; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability.

[a]*MLH1* methylation indicated if tumor MSI-high and/or absent MLH1/PMS2 proteins on IHC.

[b]MMR-deficient tumor indicates MSI-high and/or abnormal IHC.

[c]MMR-proficient tumor indicates microsatellite stability and/or normal IHC.

Table 1

Clinicopathologic Characteristics by Mutation Status

Characteristic	Mutations					Study Population (n = 450)
	All (n = 72)	MMR Only (n = 37)[a,b]	Other CRC (n = 22)[c,d]	Non-CRC (n = 13)[e]	No Mutation or VUS (n = 378)	
Average age at CRC diagnosis, y	41.1	41.7	38.9	43.2	42.8	42.5
Diagnosed, No. (%)						
Younger than 20 y	0	0	0	0	1 (0.3)	1 (0.2)
Between 20–29 y	4 (5.6)	2 (5.4)	2 (9.0)	0	14 (3.7)	18 (4.0)
Between 30–39 y	21 (29.2)	9 (24.3)	10 (45.5)	2 (15.4)	77 (20.4)	98 (21.8)
Between 40–49 y	47 (65.3)	26 (70.3)	10 (45.5)	11 (84.6)	286 (75.7)	333 (74.0)
CRC site, No. (%)						
Right colon	25 (34.7)	19 (51.4)	4 (18.2)	2 (15.4)	76 (20.1)	101 (22.4)
Left colon	22 (35.6)	9 (24.3)	9 (40.9)	4 (30.8)	178 (47.1)	200 (44.4)
Transverse	5 (6.9)	4 (10.8)	0	1 (7.7)	11 (2.9)	16 (3.6)
Rectum	20 (27.8)	5 (13.5)	9 (40.9)	6 (46.2)	105 (27.8)	125 (27.8)
Not specified[f]	0	0	0	0	8 (2.1)	8 (1.8)
Stage, No. (%)						
I	12 (16.7)	8 (21.6)	3 (13.6)	1 (7.7)	37 (9.8)	49 (10.9)
II	16 (22.2)	11 (29.7)	3 (13.6)	2 (15.3)	77 (20.4)	93 (20.7)
III	29 (40.3)	15 (40.5)	9 (40.9)	5 (38.5)	168 (44.4)	197 (43.8)
IV	15 (20.8)	3 (8.1)	7 (31.8)	5 (38.5)	93 (24.6)	108 (24.0)
Unavailable	0	0	0	0	3 (0.8)	3 (0.6)
Sex, No. (%)						
Male	37 (51.4)	20 (54.1)	12 (54.5)	5 (38.5)	198 (52.4)	235 (52.2)
Female	25 (48.6)	17 (45.9)	10 (45.5)	8 (61.5)	180 (47.6)	215 (47.8)
Self-reported race, No. (%)						
White	62 (86.1)	31 (83.8)	19 (86.4)	12 (92.3)	323 (85.5)	385 (85.6)
African American/black	8 (11.1)	6 (16.2)	1 (4.5)	1 (7.7)	33 (8.7)	41 (9.1)
Asian	0	0	0	0	8 (2.1)	8 (1.8)

Characteristic	Mutations					
	All (n = 72)	MMR Only (n = 37)[a,b]	Other CRC (n = 22)[c,d]	Non-CRC (n = 13)[e]	No Mutation or VUS (n = 378)	Study Population (n = 450)
Other	2 (2.8)	0	2 (9.0)	0	8 (2.1)	10 (2.2)
Not reported	0	0	0	0	6 (1.6)	6 (1.3)
Hispanic, No. (%)						
Yes	2 (2.8)	2 (5.4)	0	0	3 (0.8)	5 (1.1)
No	70 (97.2)	35 (94.6)	22 (100)	13 (100)	375 (99.2)	445 (98.9)
Other self-reported malignancy, No. (%)						
Cancer						
Synchronous (colon)	6 (8.3)	5 (13.5)	1 (4.5)	0	7 (1.9)	13 (2.9)
Metachronous (colon)	2 (2.8)	2 (5.4)	0	0	1 (0.3)	3 (0.7)
Endometrial	1 (1.4)	1 (2.7)	0	0	1 (0.3)	2 (0.4)
Breast	1 (1.4)	0	0	1 (7.7)	1 (0.3)	2 (0.4)
Ovarian	0	0	0	0	0	0
Pancreatic	0	0	0	0	0	0
Other	4 (5.6)	2 (5.4)	2 (9.0)	0	14 (3.7)	18 (4.0)
None	59 (81.9)	28 (75.7)	19 (86.4)	12 (92.3)	354 (93.7)	413 (91.8)
Self-reported family cancer history, No. (%)[g]						
Cancer						
Colon	33 (45.8)	27 (73.0)	5 (22.7)	1 (7.7)	53 (14)	86 (19.1)
Endometrial	8 (11.1)	8 (21.6)	0	0	11 (2.9)	19 (4.2)
Breast	8 (11.1)	3 (8.1)	2 (9.1)	3 (23.1)	34 (9.0)	42 (9.3)
Ovarian	2 (2.8)	1 (2.7)	0	1 (7.7)	9 (2.4)	11 (2.4)
Pancreatic	2 (2.8)	1 (2.7)	1 (4.5)	0	8 (2.1)	10 (2.2)

Abbreviations: CRC, colorectal cancer; MMR, mismatch repair; VUS, variant of uncertain significance.

[a] MMR genes include *MLH1, MSH2, MSH6, PMS2*.

[b] Includes 1 patient with both *MSH2/MUTYH* mutations.

[c] Other CRC genes include APC, *APC* p.I1307K, biallelic *MUTYH*, monoallelic *MUTYH*, and *SMAD4*.

[d] Includes 1 patient with both *APC/PMS2* mutations (tumor was microsatellite stable) and a separate patient with both *APC* p.I1307K/PMS2 VUS (tumor was microsatellite unstable).

[e] Non-CRC genes include *ATM, BRCA1, BRCA2, CDKN2A, CHEK2, PALB2*.

[f] Stage IV or metastatic biopsy.

[g] First-degree relatives.

Table 2

Germline Mutations Identified and Associated Syndromes

Gene	Associated Syndrome or Cancer(s)	Overall Penetrance	Patients With Mutation, No. (%)	(95% CI)
Any pathogenic or likely pathogenic mutation			72 (16)	(12.8–19.8)
Genes associated with colon cancer			59 (13.1)	(10.2–16.7)
MLH1	Lynch syndrome	High	13 (2.9)	(1.6–5.0)
MSH2	Lynch syndrome	High	16 (3.6)	(2.1–5.8)
MSH2/monoallelic *MUTYH*	Lynch syndrome/colon cancer	High/low	1 (0.2)	(0.01–1.4)
MSH6	Lynch syndrome	Moderate	2 (0.4)	(0.08–1.8)
PMS2	Lynch syndrome	Moderate	5 (1.1)	(0.4–2.7)
APC	Familial adenomatous polyposis (FAP)	High	5 (1.1)	(0.4–2.7)
APC p.I1307K	Colon cancer	Low	4 (0.9)	(0.3–2.4)
MUTYH				
Biallelic	MUTYH-associated polyposis (MAP)	High	4 (0.9)	(0.3–2.4)
Monoallelic	Colon cancer	Low	7 (1.6)	(0.7–3.3)
SMAD4	Juvenile polyposis syndrome	High	1 (0.2)	(0.01–1.4)
APC/PMS2	FAP/Lynch syndrome	High/moderate	1 (0.2)	(0.01–1.4)
Genes not traditionally associated with colon cancer			13 (2.9)	(1.6–5.0)
BRCA1	Hereditary breast-ovarian cancer syndrome	High	2 (0.4)	(0.08–1.8)
BRCA2	Hereditary breast-ovarian cancer syndrome	High	4 (0.9)	(0.3–2.4)
ATM	Breast cancer, pancreatic cancer	Moderate	3 (0.7)	(0.2–2.1)
ATM/CHEK2	Breast cancer, pancreatic cancer	Moderate	1 (0.7)	(0.01–1.4)
PALB2	Breast cancer, pancreatic cancer	Moderate	2 (0.4)	(0.08–1.8)
CDKN2A	Melanoma, pancreatic cancer	High	1 (0.2)	(0.01–1.4)