Cancer Susceptibility Gene Mutations in Individuals With Colorectal Cancer

Matthew B. Yurgelun, Matthew H. Kulke, Charles S. Fuchs, Brian A. Allen, Hajime Uno, Jason L. Hornick, Chinedu I. Ukaegbu, Lauren K. Brais, Philip G. McNamara, Robert J. Mayer, Deborah Schrag, Jeffrey A. Meyerhardt, Kimmie Ng, John Kidd, Nanda Singh, Anne-Renee Hartman, Richard J. Wenstrup, and Sapna Syngal

Author affiliations and support information (if applicable) appear at the end of this article.

Published at jco.org on January 30, 2017.

Corresponding author: Sapna Syngal, MD, MPH, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston, MA 02215; e-mail: ssyngal@partners.org.

© 2017 by American Society of Clinical Oncology

0732-183X/17/3510w-1086w/\$20.00

ABSTRACT

Purpose

Hereditary factors play an important role in colorectal cancer (CRC) risk, yet the prevalence of germline cancer susceptibility gene mutations in patients with CRC unselected for high-risk features (eg, early age at diagnosis, personal/family history of cancer or polyps, tumor microsatellite instability [MSI], mismatch repair [MMR] deficiency) is unknown.

Patients and Methods

We recruited 1,058 participants who received CRC care in a clinic-based setting without preselection for age at diagnosis, personal/family history, or MSI/MMR results. All participants underwent germline testing for mutations in 25 genes associated with inherited cancer risk. Each gene was categorized as high penetrance or moderate penetrance on the basis of published estimates of the lifetime cancer risks conferred by pathogenic germline mutations in that gene.

Results

One hundred five (9.9%; 95% CI, 8.2% to 11.9%) of 1,058 participants carried one or more pathogenic mutations, including 33 (3.1%) with Lynch syndrome (LS). Twenty-eight (96.6%) of 29 available LS CRCs demonstrated abnormal MSI/MMR results. Seventy-four (7.0%) of 1,058 participants carried non-LS gene mutations, including 23 (2.2%) with mutations in high-penetrance genes (five *APC*, three biallelic *MUTYH*, 11 *BRCA1/2*, two *PALB2*, one *CDKN2A*, and one *TP53*), 15 of whom lacked clinical histories suggestive of their underlying mutation. Thirty-eight (3.6%) participants had moderate-penetrance CRC risk gene mutations (19 monoallelic *MUTYH*, 17 *APC**I1307K, two *CHEK2*). Neither proband age at CRC diagnosis, family history of CRC, nor personal history of other cancers significantly predicted the presence of pathogenic mutations in non-LS genes.

Conclusion

Germline cancer susceptibility gene mutations are carried by 9.9% of patients with CRC. MSI/MMR testing reliably identifies LS probands, although 7.0% of patients with CRC carry non-LS mutations, including 1.0% with *BRCA1/2* mutations.

J Clin Oncol 35:1086-1095. © 2017 by American Society of Clinical Oncology

INTRODUCTION

Hereditary factors play a key role in colorectal cancer (CRC) risk. Studies have estimated the prevalence of hereditary CRC syndromes by performing syndrome-specific genetic testing on patients with CRC with acknowledged high-risk features. For instance, studies that used microsatellite instability (MSI) or DNA mismatch repair (MMR) protein immunohistochemistry (IHC) tumor testing in preselected patients for germline MMR gene testing have identified Lynch syndrome (LS) in 2% to 4% of patients with CRC.¹⁻⁵ Likewise, studies that

demonstrated a < 1% prevalence of familial adenomatous polyposis (FAP) and *MUTYH*-associated polyposis (MAP) included germline *APC* testing only in patients with CRC with clinical features of FAP (eg, > 100 adenomas) and only sequenced for a limited panel of *MUTYH* mutations.⁶⁻¹⁰

Because these studies focused solely on high-risk patients with CRC identified by MSI/MMR status or clinical history, the true prevalence of inherited cancer susceptibility among unselected CRC populations is unknown. Furthermore, existing estimates do not account for the contribution of germline factors linked to

ASSOCIATED CONTENT

Listen to the podcast
by Dr Ford at
ascopubs.org/jco/podcasts

Appendix
DOI: 10.1200/JCO.2016.71.0012

DOI: 10.1200/JCO.2016.71.0012

modestly increased risks of CRC, including monoallelic *MUTYH* mutations and the Ashkenazi Jewish *APC**I1307K founder allele.^{11,12} Finally, emerging data have suggested that some patients with CRC harbor mutations in breast and ovarian cancer susceptibility genes (eg, *BRCA1/2*), which raises the question about whether these mutations contribute to hereditary CRC risk.^{13,14} The primary aim of the current study was to assess the prevalence of cancer susceptibility gene mutations among patients with CRC but without preselection for high-risk features.

PATIENTS AND METHODS

All patients undergoing clinical care at the Dana-Farber Cancer Institute for GI cancer were eligible for enrollment in an institutional sample registry, which included collection of a one-time blood sample for clinical research. A cohort of 1,100 consecutively enrolled registry participants with CRC, from whom blood was collected between December 2008 to March 2014, were retrospectively identified for analysis in this study regardless of tumor MSI/MMR status, age at diagnosis, and personal/family history of cancer. Specimens were collected for research purposes only and, therefore, did not follow the Clinical Laboratory Improvement Amendments chain of custody regulations for clinical testing. Thus, genetic test results obtained in this study were considered research and were not returned to study participants or their clinicians (as was explained in the study consent form), which mirrored methods from a similar study in a cohort of women with breast cancer.¹⁵ The study was approved by the Dana-Farber/Harvard Cancer Center Institutional Review Board.

Structured medical record review and self-administered questionnaires were used to obtain information about participants' personal/family history; CRC stage; anatomic location; and molecular features, including results of MSI, MMR IHC, and *KRAS/NRAS* and *BRAF* mutation analyses. Because somatic *RAS* and *BRAF* mutations are known to be mutually exclusive in CRC, tumors with *KRAS/NRAS* mutations were presumed to be *BRAF* wild type, and those with *BRAF* mutations were presumed to be *KRAS/NRAS* wild type. *KRAS* G12C mutations were specifically analyzed because prior studies found this mutation to be overrepresented in CRCs in patients with MAP.^{16,17}

Germline DNA was extracted from frozen whole blood of each participant, polymerase chain reaction amplified with a custom amplicon library, and sequenced to detect sequence alterations and large rearrangements among 25 cancer susceptibility genes by following procedures developed in prior studies.^{13,18} Published calculations of the lifetime risks of cancer conferred by mutations in each gene were used to categorize genes as high penetrance or moderate penetrance (Table 1).^{11,12,19-27} All sequence variations and large rearrangements detected were classified for pathogenicity into the following categories, as previously described¹³: deleterious, suspected deleterious, variant of uncertain significance, favor polymorphism, and polymorphism. Variants were classified as being of uncertain significance if data were insufficient to support either a deleterious or a benign interpretation. Individuals with deleterious or suspected deleterious alterations were collectively defined as having pathogenic mutations. Individuals who carried pathogenic mutations were further assessed to determine whether they fulfilled clinical criteria for syndrome-specific germline analysis of the gene in which their mutation was found (Appendix, online only).

The primary outcome of this study was the detection of pathogenic cancer susceptibility gene mutations. Age at CRC diagnosis was analyzed as a continuous variable, and all other characteristics were analyzed as categorical variables. Bivariate association of mutation status with clinical characteristics was assessed by Fisher's exact test (categorical variables) and the two-sample *t* test (continuous variables). Clinically relevant characteristics, including those found to be significant on bivariate analysis, were examined in multivariable logistic regression models to assess for an

association with the presence of a pathogenic non-LS mutation. Stata/SE version 14.0 (StataCorp, College Station, TX) and R version 3.2.2 (R Project) software were used in these analyses. The degree of association was expressed as the odds ratio (OR) and corresponding 95% CI. *P* values were two-tailed and considered statistically significant at < .05.

RESULTS

Forty-two registry participants were excluded as a result of failed germline sequencing (*n* = 34) or a non-CRC diagnosis determined by medical record review (*n* = 8), which resulted in an evaluable population of 1,058 patients with CRC. Four hundred seventy-one (44.5%) participants were female, 939 (88.8%) were non-Hispanic white, 29 (2.7%) had a history of more than one CRC diagnosis, and 160 (15.1%) had a history of a non-CRC diagnosis (Table 2). The median age at first CRC diagnosis was 55 years (range, 21 to 92 years), and 336 (31.8%) participants received a diagnosis of CRC before age 50 years. One hundred thirty-eight (13.0%) participants had one or more first-degree relatives with CRC, and 206 (19.5%) had one or more second-degree relatives with CRC.

Five hundred seventy-two (54.1%) participants had tumors with MSI and/or MMR IHC data available, of which 70 (12.2%) were MSI-high (MSI-H) and/or MMR deficient (MMR-D). Seven hundred forty-one (70.0%) participants' tumors had available *RAS* testing results, of which 316 (42.6%) had *KRAS/NRAS* mutations, including 27 (3.6%) with *KRAS* G12C mutations. Six hundred forty-eight (61.2%) participants' tumors had *BRAF* test results available, of which 47 (7.2%) had *BRAF* mutations.

One hundred five (9.9%; 95% CI, 8.2% to 11.9%) of the 1,058 participants carried one or more pathogenic mutations, including 55 (5.2%; 95% CI, 4.0% to 6.8%) with high-penetrance mutations (four with concurrent moderate-penetrance mutations) and 50 (4.7%; 95% CI, 3.6% to 6.2%) with only moderate-penetrance mutations (Fig 1). Thirty-three (3.1%; 95% CI, 2.2% to 4.4%) of the 1,058 participants carried LS mutations (13 *MLH1*, seven *MSH2*, six *MSH6*, and seven *PMS2*; Appendix Table A1, online only) and 74 (7.0%; 95% CI, 5.6% to 8.7%) carried non-LS mutations (two both LS and non-LS mutations). Twenty-three (2.2%; 95% CI, 1.4% to 3.3%) participants carried high-penetrance non-LS mutations (five *APC*, three biallelic *MUTYH*, three *BRCA1*, eight *BRCA2*, two *PALB2*, one *CDKN2A*, and one *TP53*; Table 3). Thirty-eight (3.6%; 95% CI, 2.6% to 4.9%) participants had mutations in moderate-penetrance genes linked to CRC risk (19 monoallelic *MUTYH*, 17 *APC**I1307K, and two *CHEK2* mutations); 16 (1.5%; 95% CI, 0.9% to 2.5%) had mutations in other moderate-penetrance genes (Appendix Table A2, online only). Five participants carried more than one pathogenic mutation, including one with *MLH1/BRCA2* mutations, one with *BRCA2*/monoallelic *MUTYH* mutations, one with *MSH2/APC**I1307K mutations, and two with *BRCA1/APC**I1307K mutations.

Twenty-eight (96.6%; 95% CI, 80.4% to 99.8%) of 29 LS probands had CRCs that demonstrated MSI-H and/or MMR-D (four had missing MSI/MMR data), and 32 (97.0%; 95% CI, 82.5% to 99.8%) of 33 met clinical criteria for LS testing. Eight (72.7%) of 11 *BRCA1/2* carriers, two (40.0%) of five *APC* carriers, one (33.3%) of three biallelic *MUTYH* mutation carriers, and the *TP53*

Table 1. Cancer Predisposition Genes/Alleles Examined by a Multigene Hereditary Cancer Panel

Gene/Allele	Associated Cancer Spectrum	Estimated Lifetime Cancer Risk
High penetrance		
Lynch syndrome <i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i> , <i>PMS2</i> , <i>EPCAM</i> ¹⁹⁻²¹	Colorectal, endometrial, ovarian, gastric, urothelial, pancreaticobiliary, cutaneous sebaceous neoplasms, brain	CRC, 22%-74%; endometrial cancer, 14%-71%; ovarian cancer, 3%-22%; gastric cancer, up to 13%; urothelial cancer, up to 25%; lifetime cancer risks may be lower for <i>PMS2</i> mutation carriers
Adenomatous polyposis syndromes <i>APC</i> (FAP) ^{20,21}	Colorectal, small intestine, ampullary, gastric, desmoid, thyroid	CRC, up to 100% without colectomy
<i>MUTYH</i> (biallelic; MAP) ^{12,20,21}	Colorectal, duodenal	CRC, 43%-100% without colectomy
Hamartomatous polyposis syndromes <i>BMPR1A</i> , <i>SMAD4</i> (juvenile polyposis syndrome) ^{20,21}	Gastric, colorectal	CRC, 38%-68%; gastric cancer, up to 21%
<i>PTEN</i> (<i>PTEN</i> hamartoma tumor syndrome; Cowden syndrome) ²⁰⁻²²	Breast, endometrial, thyroid, colorectal, renal	Breast cancer, 25%-85%; endometrial cancer, up to 28%; thyroid cancer, up to 38%; CRC, 9%-16%
<i>STK11</i> (Peutz-Jeghers syndrome) ²⁰⁻²²	Breast, pancreatic, colorectal, gastric, small intestine, cervical (adenoma malignum), lung, ovarian (sex cord), testis (Sertoli cell)	Any cancer, 81% by age 80 years; breast cancer, 24%-54%; pancreatic cancer, 11%-36%; CRC, 39%; gastric cancer, 29%
Hereditary breast/ovarian cancer <i>BRCA1</i> , <i>BRCA2</i> ^{20,22}	Breast, ovarian, pancreatic, prostate, melanoma	Breast cancer, 46%-60% (<i>BRCA1</i>) and 49%-55% (<i>BRCA2</i>) by age 70 years; ovarian cancer, 12%-59% (<i>BRCA1</i>) and 6%-18% (<i>BRCA2</i>) by age 70 years
Other high-penetrance genes/syndromes		
<i>CDKN2A</i> (familial atypical multiple-mole melanoma syndrome) ^{20,25}	Melanoma, pancreatic	Melanoma, 67% by age 50 years; pancreatic cancer, 13- to 39-fold increased risk
<i>CDH1</i> (hereditary diffuse gastric cancer syndrome) ^{20,22,26}	Gastric (diffuse type), breast (lobular)	Gastric cancer, 67% (males), 83% (females); breast cancer, 39%-53%
<i>TP53</i> (Li-Fraumeni syndrome) ^{21,22,24}	Breast, sarcoma, brain (choroid plexus), adrenocortical, leukemia, colorectal, gastric, others	Any cancer, up to 73%-100%
<i>PALB2</i> ^{22,23,26}	Breast, pancreatic	Breast cancer, 35% by age 70 years and 45% by age 80 years
Moderate penetrance		
<i>ATM</i> ^{20,26}	Breast, pancreatic	Breast cancer, 27% by age 80 years; pancreatic cancer, up to threefold increased risk
<i>APC</i> *11307K ^{11,21}	Colorectal	CRC, lifetime risk not well defined
<i>BARD1</i> ²²	Breast	Breast cancer, not well defined
<i>BRIP1</i> ^{22,27}	Ovarian, possibly breast	Ovarian cancer, 5.8%
<i>CDK4</i>	Melanoma	Melanoma, lifetime risk not well defined
<i>CHEK2</i> ^{21,22,26}	Breast, colorectal, possibly others	Breast cancer, 29% by age 80 years; CRC, lifetime risk not well defined
<i>MUTYH</i> (monoallelic) ¹²	Colorectal	CRC, 10.9% (females), 13.6% (males) by age 80 years
<i>NBN</i> ²⁶	Breast, possibly prostate	Breast cancer, 23% by age 80 years
<i>RAD51C</i> ^{22,27}	Ovarian	Ovarian cancer, 5%-15%
<i>RAD51D</i> ^{22,27}	Ovarian	Ovarian cancer, 5%-15%

Abbreviations: CRC, colorectal cancer; FAP, familial adenomatous polyposis; MAP, *MUTYH*-associated polyposis.

carrier lacked personal/family histories that fulfilled criteria for germline testing for their respective syndrome. Although there are no guidelines for *PALB2* or *CDKN2A* testing, neither *PALB2* carrier had a personal/family history of breast or pancreatic cancer, and the *CDKN2A* carrier had no personal/family history of pancreatic cancer or melanoma. Thus, overall, 15 (65.2%; 95% CI, 42.8% to 82.8%) of 23 probands with high-penetrance non-LS mutations lacked clinical histories suggestive of their underlying mutation.

On bivariate analysis (Table 4), LS mutation carriers were significantly younger at CRC diagnosis and more likely to have an earlier stage of CRC, right-sided CRC, multiple CRC diagnoses, MSI-H/MMR-D CRC, a first- and/or second-degree relative with CRC, and a first-degree relative with endometrial cancer than noncarriers. On both bivariate and multivariable regression analyses (Appendix Table A3, online only), non-LS mutation carriers were significantly more likely to have had

multiple CRC diagnoses (OR, 3.70; 95% CI, 1.31 to 10.49) and CRC with a somatic *KRAS* G12C mutation (OR, 4.58; 95% CI, 1.76 to 11.92) than noncarriers. Neither age at CRC diagnosis, anatomic CRC site, personal history of non-CRC, nor having a first-degree relative with CRC was significantly associated with the presence of a non-LS mutation. Forty (54%) of 74 non-LS mutation carriers were given a diagnosis of CRC at age ≥ 50 years and lacked a family history of CRC in a first-degree relative.

Four hundred eight germline variants of uncertain significance were detected among 330 (31.2%; 95% CI, 28.4% to 34.1%) of the 1,058 participants (Appendix Fig A1, online only; Appendix Table A4, online only). Six participants with MMR-D CRC carried variants of uncertain significance in MMR genes that corresponded to their tumor's abnormal MMR protein staining, which suggests possible pathogenicity to these variants (Appendix Table A5, online only).

Table 2. Description of Cohort

Characteristic	Total Evaluable Cohort, No. (%)
No. of participants	1,058
Sex	
Male	587 (55.5)
Female	471 (44.5)
Ethnicity	
Non-Hispanic white	939 (88.8)
Non-Hispanic black	50 (4.7)
Hispanic/Latino	27 (2.6)
Asian	22 (2.1)
Native American/Alaskan	1 (0.1)
Multiple ethnicities	7 (0.7)
Missing data	12 (1.1)
Age at first CRC diagnosis, years	
Mean (SD)	55.7 (12.6)
< 30	14 (1.3)
30-39	90 (8.5)
40-49	232 (21.9)
50-59	327 (30.9)
60-69	252 (23.8)
70-79	103 (9.7)
≥ 80	40 (3.8)
Anatomic site of CRC	
Right-side colon	353 (33.4)
Left-side colon	362 (34.2)
Rectum/rectosigmoid	341 (32.2)
Unknown/not reported	2 (0.2)
Stage at initial CRC diagnosis	
0	6 (0.6)
I	123 (11.6)
II	202 (19.1)
III	404 (38.2)
IV	321 (30.3)
Unknown/not reported	2 (0.2)
MSI/MMR IHC status available (n = 572)	
MSI-H/MMR-D	70 (12.2)
MSI-L/MMR-P	13 (2.3)
MSS/MMR-P	489 (85.5)
KRAS mutation status available (n = 741)	
KRAS/NRAS wild type	425 (57.4)
KRAS/NRAS mutation (any)	316 (42.6)
KRAS G12C mutation	27 (3.6)
BRAF mutation status available (n = 648)	
BRAF wild type	602 (92.8)
BRAF mutation (any)	47 (7.2)
Personal history of more than one CRC	29 (2.7)
Personal history of other cancer (non-CRC)*	160 (15.1)
Family history	
One or more FDR with CRC	138 (13.0)
One or more SDR with CRC	206 (19.5)
One or more FDR with breast cancer	138 (13.0)
One or more FDR with endometrial cancer	25 (2.4)
One or more FDR with ovarian cancer	23 (2.2)
No known family history of cancer†	185 (17.5)
Family history data missing	3 (0.3)

Abbreviations: CRC, colorectal cancer; FDR, first-degree relatives; MSI-H, high-level microsatellite instability; MSI-L, low-level microsatellite instability; MMR-D, mismatch repair deficient; MMR-P, mismatch repair proficient; MSS, microsatellite stable; SD, standard deviation; SDR, second-degree relatives.

*Excludes cutaneous basal and squamous cell carcinomas.

†Includes adopted patients.

actionable, high-penetrance cancer susceptibility genes. Currently, patients with CRC are referred for germline testing based on the identification of high-risk phenotypic features (eg, age, family history), but beyond MSI/MMR tumor testing for LS, no systematic approach to hereditary risk assessment exists.^{20,21} Moreover, access to germline testing is inconsistent as a result of limited availability of genetic counseling and insurance coverage restrictions.²⁸ The current results demonstrate that multigene germline testing without preselection on the basis of high-risk clinical features could significantly increase opportunities for primary and secondary cancer prevention because overall, 96.2% of mutation carriers (101 of 105) had mutations in genes for which guidelines recommend specific screening and/or risk-reduction practices.¹⁹⁻²²

Consistent with prior studies that performed germline LS testing after preselection with MSI/MMR tumor testing,¹⁻⁶ 3.1% of participants in the current study had LS, virtually all of whom had abnormal MSI/MMR results and clinical phenotypes suggestive of LS. These data support the current practice of performing systematic MSI/MMR IHC for all CRCs to screen for LS^{4,5,19-21} and because MSI-H and MMR-D are important prognostic and predictive biomarkers in CRC. The large number of non-LS mutations identified in this cohort, however, demonstrates that tumor testing alone is insufficient for identifying inherited cancer risk among unselected patients with CRC.

Seven percent of patients with CRC carried mutations in non-LS genes, including 1.0% with *BRCA1/2* mutations, and nearly two thirds of probands with high-penetrance non-LS mutations lacked clinical histories suggestive of their respective syndromes. The majority of non-LS mutation carriers in this study also lacked traditional phenotypic characteristics of hereditary CRC risk (age < 50 years, CRC in a first-degree relative). Although a personal history of more than one CRC diagnosis was significantly associated with presence of non-LS mutations, a minority of carriers had multiple CRCs demonstrating that such history has limited utility in identifying mutation carriers. Somatic *KRAS* G12C mutations were significantly associated with the presence of pathogenic non-LS mutations and were found predominantly in probands with biallelic or monoallelic germline *MUTYH* mutations, consistent with prior studies.^{16,17} These findings support the notion that individuals whose CRCs harbor somatic *KRAS* G12C mutations should undergo germline evaluation.^{16,17}

One half of all FAP and MAP probands in this cohort lacked diffuse colorectal polyposis, which demonstrates that polyp number is an imperfect indicator of inherited risk.⁷ Beyond the classic hereditary CRC syndromes, mutations in other high-penetrance genes (*BRCA1/2*, *PALB2*, *CDKN2A*, and *TP53*) were identified in a notable number of CRC probands, most of whom lacked phenotypic features of these syndromes. *BRCA1/2* mutations were more common than FAP and MAP combined in this cohort, and > 70% of *BRCA1/2* probands lacked clinical features of hereditary breast and ovarian cancer, which suggests that these mutations are likely underdiagnosed among unselected patients with CRC. The finding of *BRCA1/2* mutations in 1.0% of patients with CRC (approximately 1:96) is substantially higher than the estimated 0.25% general population prevalence (1:400) and raises questions about whether such mutations predispose to CRC.^{29,30} Previously, CRC has not been included in the *BRCA1/2* tumor

DISCUSSION

In this clinic-based cohort of 1,058 patients with CRC, 9.9% carried pathogenic germline mutations, most of which were in

Fig 1. Pathogenic mutations identified with a multigene panel among 1,058 individuals with colorectal cancer. *Includes four probands with both a high- and a moderate-penetrance mutation. †One proband carried both an *MLH1* and a *BRCA2* mutation. ‡Does not include the four probands with concurrent high-penetrance mutations.

spectrum, and published guidelines do not recommend increased CRC screening for mutation carriers.^{22,31,32} However, some studies have suggested a possible modest link between CRC risk and mutations in *BRCA1/2*.^{14,33-35} Although the current data cannot establish a causal link between *BRCA1/2* mutations and probands' CRC diagnoses, these findings further support the hypothesis that *BRCA1/2* mutations predispose to a wider diversity of cancer than traditionally appreciated,¹³ potentially including CRC. Further studies with systematic somatic tumor testing (eg, *BRCA1/2* loss of heterozygosity, homologous recombination repair deficiency analyses) will be key in understanding whether such germline mutations are truly causative of these cancers.

Although the absolute risk conferred by monoallelic *MUTYH* mutations and *APC**I1307K is modest,³⁶ these moderate-penetrance mutations account for a sizeable fraction of CRC risk. The current data suggest that the identification of patients likely to harbor these moderate-penetrance mutations on the basis of phenotype alone is difficult. Evidence continues to grow in support of the role of testing for moderate-penetrance mutations to identify individuals who stand to benefit from enhanced screening,¹² and guidelines now recommend specialized colonoscopic surveillance for monoallelic *MUTYH* mutation and

*APC**I1307K carriers.²¹ Co-occurrence of these moderate-penetrance mutations with *BRCA1/2* mutations in some participants raises the possibility that CRC risk is compounded by the presence of multiple germline mutations.

Given the high prevalence of mutations seen in this cohort and the inability to clinically identify many patients with CRC with actionable, high-penetrance mutations, these data raise the provocative question of whether all patients with CRC should undergo multigene germline testing for inherited cancer susceptibility. Although precedent exists for such a test everyone approach in ovarian cancer,²² the precise clinical significance of moderate-penetrance alterations^{28,36} and even unexpected high-penetrance mutations must be more fully characterized to understand the utility of performing multigene germline testing on all patients with CRC. From a care delivery perspective, the traditional practice of using high-risk phenotypic features (age, MSI/MMR status, family history) to guide referral for germline testing inherently limits the proportion of patients with CRC who undergo hereditary cancer risk assessment. Such phenotype-driven genetic testing strategies require consistent, systematic evaluation for clinical features of underlying germline risk, which is difficult to reliably execute in real-world clinical practice^{1,37,38} and, even if performed

Table 3. Clinical Characteristics of Probands With High-Penetrance Non-Lynch Syndrome Mutations

Age, years/ sex	Tumor Site	Stage	Ethnicity	Pathogenic Germline Mutation	MSI/ MMR Status	KRAS Status	Other Personal History of Cancer (age at diagnosis, years)	Family History of Cancer (relation, age if known, years)	Germline VUS
BRCA1/2 mutation carriers 41/M	Sigmoid colon	IIIC	Non-Hispanic white	<i>BRCA1</i> c.68_69del p.E23Vfs*17 (187delAG), <i>APC</i> c.3920T>A p.I1307K	MMR-P	Wild type	None	LIV (mother, 59), BR (mat aunt, 50)	<i>CHEK2</i> c.1283C>T p.S428F
45/F	Transverse colon	I	Non-Hispanic white	<i>BRCA1</i> c.68_69del p.E23Vfs*17 (187delAG), <i>APC</i> c.3920T>A p.I1307K	MMR-P and MSS	np	MEL (46), BR (56)	CRC (father, 50; pat uncle, 50), MEL (father, 50; mat cousin, 30; mat cousin, 60), PR (pat uncle, 78), LG (mat grandmother, 50), CNS (mat grandmother, 70)	None
67/M	Sigmoid colon	IIA	More than one ethnicity	<i>BRCA1</i> c.5095C>T p.R1699W	MMR-P	Wild type	None	BR (sister, 42)	None
41/M	Cecum	IV	Non-Hispanic white	<i>BRCA2</i> c.7602del p.C2535Vfs*16	MMR-P and MSS	Mutation (G12D)	None	THY (mother), CRC (pat grandfather, 75), BR (mat grandmother)	<i>APC</i> c.835-3T>C
42/F	Rectum	I	Non-Hispanic white	<i>BRCA2</i> c.5946del p.S1982Rfs*22 (6174delT)	np	Wild type	None	MEL (brother, niece), BR (mat grandmother)	None
52/F	Ascending colon	IIIC	Non-Hispanic white	<i>BRCA2</i> c.4477G>T p.E1493X	np	Wild type	None	None	<i>STK11</i> c.1012G>A p.V338M
53/M	Descending colon	IIIB	Non-Hispanic white	<i>BRCA2</i> c.3847_3848del p.V1283Kfs*2	MSS	Wild type	CLL (53)	OV (mother, 53), CLL (father), LEU (pat grandfather)	<i>SMAD4</i> c.566G>A p.R189H
53/M	Sigmoid colon	IV	Non-Hispanic white	<i>BRCA2</i> c.8537_8538del p.E2846Gfs*22	MMR-P	Wild type	None	LG (father, 70; mat grandfather, 72), BR (pat cousin, 45)	None
62/F	Rectum	IV	Non-Hispanic white	<i>BRCA2</i> c.1796_1800del p.S599X, <i>MUTYH</i> c.494A>G Y165C	np	Wild type	BR (46)	KID (brother, 55)	None
69/M	Rectum	IV	Non-Hispanic white	<i>BRCA2</i> c.8537_8538del p.E2846Gfs*22	np	np	LG (61)	MEL (daughter, 36), THY (daughter, 36)	<i>PALB2</i> c.1987C>T p.R663C, <i>BRIP1</i> c.790C>T p.R264W
31/M	Sigmoid colon	IV	Non-Hispanic white	<i>BRCA2</i> c.3199del p.T1067Kfs*10, <i>MLH1</i> del exons 16-19	MMR-D	Mutation (G13D)	None	CRC (mat uncle, 38; mat aunt, 21; mat grandmother, 32), MEL (mother, 50)	None
Adenomatous polyposis mutation carriers									
25/M	Rectum	IV	Non-Hispanic white	<i>APC</i> c.1495C>T p.R499X	MMR-P and MSS	np	None; diffuse colorectal polyposis noted	FAP (mother), CRC (mother, 47; pat grandfather, 70), PAN (mat grandmother, 85), BR (pat grandmother, 40)	<i>BMPRI1A</i> c.1327C>T p.R443C
30/M	Splenic flexure	IIIC	Non-Hispanic white	<i>APC</i> c.3183_3187del p.Q1062X	np	Wild type	None; diffuse colorectal polyposis noted	None	None

(continued on following page)

Table 3. Clinical Characteristics of Probands With High-Penetrance Non-Lynch Syndrome Mutations (continued)

Age, years/ sex	Tumor Site	Stage	Ethnicity	Pathogenic Germline Mutation	MSI/ MMR Status	KRAS Status	Other Personal History of Cancer (age at diagnosis, years)	Family History of Cancer (relation, age if known, years)	Germline VUS
51/M	Transverse colon	IV	Non-Hispanic white	<i>APC</i> c.1213C>T p.R405X	np	Wild type	None; 80-100 polyps noted on first colonoscopy (51)	None	None
53/F	Rectum	IIA	Non-Hispanic white	<i>APC</i> c.70C>T p.R24X	np	Mutation (G12D)	None; three lifetime colorectal adenomas	PR (father, 69), EYE (pat aunt, 50), BR (mat cousin, 47; mat cousin, 48)	<i>A7M</i> c.9079dup p.S3027Kfs*36
62/F	Cecum	IV	Non-Hispanic white	<i>APC</i> c.937_938del p.E313Nfs*13	MMR-P	Mutation (Q61L)	None; 15 lifetime colorectal adenomas	UNK (great aunt)	None
37/M	Rectum and rectosigmoid (two synchronous cancers)	IV	Non-Hispanic white	<i>MUTYH</i> c.494A>G Y165C, <i>MUTYH</i> c.1145G>A G382D	MMR-P	np	None; approximately 100 colorectal polyps found (37)	OV (mother, 44), BR (mat aunt, 60), LG (mat grandmother, 55)	None
43/F	Cecum	IIIB	Non-Hispanic white	<i>MUTYH</i> c.1145G>A p.G382D (homozygous)	MMR-P and MSS	Mutation (G12C)	None; three lifetime colorectal adenomas and one sessile serrated polyp	MEL (mat grandmother, 60; pat grandmother, 70); NHL (mat grandmother, 86)	None
48/M	Hepatic flexure	IIA	Non-Hispanic white	<i>MUTYH</i> c.1145G>A G382D, <i>MUTYH</i> c.283C>T p.R95W	MMR-P and MSI-L	Mutation (G12C)	BL (38); three lifetime colorectal adenomas	None; sister with 18 colorectal adenomas known to carry biallelic <i>MUTYH</i> mutations	None
Other high-penetrance mutation carriers									
64/M	Rectum	IIIB	Non-Hispanic white	<i>PALB2</i> c.2711G>A p.W904X	np	np	LG (61)	None (adopted)	<i>NBN</i> c.1912T>C p.S638P
66/F	Rectum	IIA	Non-Hispanic white	<i>PALB2</i> c.751C>T p.Q251X	np	Mutation (G12A)	None	None	None
71/F	Cecum	IV	Non-Hispanic white	<i>CDKN2A</i> c.9_32dup p.A4_P11dup	np	Mutation (G13D)	None	BR (mother), STO (mat aunt)	None
72/M	Rectum	IV	Non-Hispanic white	<i>TP53</i> c.681del p.D228Tfs*19	np	Mutation (G12C)	MDS (76)	none	<i>TP53</i> c.394A>G p.K132D

NOTE: Relatives (e.g., aunts, uncles, grandparents) were only listed as being from the proband's maternal or paternal lineage if this was specified in the medical record. Abbreviations: BL, bladder cancer; BR, breast cancer; CLL, chronic lymphocytic leukemia; CNS, brain cancer; CRC, colorectal cancer; EYE, ocular cancer not otherwise specified; FAP, familial adenomatous polyposis; KID, kidney cancer; LEU, leukemia; LIV, liver cancer; LG, lung cancer; mat, maternal; MDS, myelodysplastic syndrome; MEL, melanoma; MMR, mismatch repair; MMR-P, mismatch repair proficient; MSS, microsatellite stable; MSI, microsatellite instability; MSI-L, low-level microsatellite instability; NHL, non-Hodgkin lymphoma; np, not performed; pat, paternal; PAN, pancreatic cancer; PR, prostate cancer; OV, ovarian cancer; STO, stomach cancer; THY, thyroid cancer; UNK, unknown cancer diagnosis; VUS, variant of uncertain significance.

Table 4. Bivariate Analysis of Mutation Carriers Versus Noncarriers

Characteristic	Noncarriers, No. (%)	Non-LS Mutation Carriers		LS Mutation Carriers	
		No. (%)	<i>P</i> *	No. (%)†	<i>P</i> *
No. of patients	953	72		33	
Sex					
Male	528 (55.4)	38 (52.8)	.71	21 (63.6)	.38
Female	425 (44.6)	34 (47.2)		12 (36.4)	
Ethnicity					
Non-Hispanic white	843 (88.5)	67 (93.1)	.14	29 (87.9)	.47
Non-Hispanic black	47 (4.9)	0 (0.0)		3 (9.1)	
Hispanic/Latino	24 (2.5)	2 (2.8)		1 (3.0)	
Other	27 (2.8)	3 (4.2)		0 (0.0)	
Mean (SD) age at first CRC diagnosis, years	56.0 (12.4)	55.8 (12.8)	.85	44.7 (13.5)	< .001
Anatomic site of CRC					
Right-side colon	308 (32.3)	25 (34.7)	.16	20 (60.6)	.004
Left-side colon	336 (35.3)	18 (25.0)		8 (24.2)	
Rectum/rectosigmoid	307 (32.2)	29 (40.3)		5 (15.2)	
Stage at initial CRC diagnosis					
0	4 (0.4)	1 (1.4)	.055	1 (3.0)	< .001
I	104 (10.9)	12 (16.6)		7 (21.2)	
II	175 (18.4)	12 (16.7)		15 (45.5)	
III	380 (39.9)	19 (26.4)		5 (15.2)	
IV	288 (30.2)	28 (38.9)		5 (15.2)	
Tumor testing results					
MSI-H/MMR-D‡	39 of 507 (7.7)	3 of 36 (8.3)	.75	28 of 29 (96.6)	< .001
<i>KRAS/NRAS</i> mutation (any)‡	282 of 668 (42.2)	24 of 54 (44.4)	.78	10 of 19 (52.6)	.48
<i>KRAS</i> G12C mutation‡	20 of 668 (3.0)	7 of 54 (13.0)	.002	0 of 19 (0.0)	1.00
<i>BRAF</i> mutation (any)‡	45 of 587 (7.7)	2 of 41 (4.9)	.76	0 of 20 (0.0)	.39
Personal history of more than one CRC	19 (2.0)	5 (6.9)	.02	5 (15.2)	< .001
Personal history of other cancer (non-CRC)§	136 (14.3)	16 (22.2)	.08	8 (24.2)	.13
Family history					
One or more FDR with CRC	116 (12.2)	10 (13.9)	.71	12 (36.4)	< .001
One or more SDR with CRC	175 (18.4)	13 (18.1)	1.00	18 (54.5)	< .001
One or more FDR with breast cancer	120 (12.6)	15 (20.8)	.07	3 (9.1)	.79
One or more FDR with endometrial cancer	22 (2.3)	0 (0.0)	.39	3 (9.1)	.048
One or more FDR with ovarian cancer	17 (1.8)	4 (5.6)	.054	2 (6.1)	.13
No known family history of cancer	165 (17.3)	14 (19.4)	.63	6 (18.2)	.82

Abbreviations: CRC, colorectal cancer; FDR, first-degree relatives; LS, Lynch syndrome; MSI-H, high-level microsatellite instability; MMR-D, mismatch repair deficient; SD, standard deviation; SDR, second-degree relatives

**P* values for comparison with noncarriers.

†One patient with both an LS and a non-LS mutation was included with LS carriers.

‡Only patients with known MSI/MMR immunohistochemistry, *KRAS/NRAS*, and *BRAF* test results were included.

§Excludes cutaneous basal and squamous cell carcinomas.

||Includes adopted patients.

correctly, will still miss a large fraction of patients with CRC with germline risk according to the current data. As germline testing becomes less expensive and additional cancer predisposition genes are identified and their associated cancer risks better clarified, it is likely that there will be a threshold at which it becomes more efficient to systematically offer multigene germline testing to patients with CRC irrespective of phenotype.³⁹

Although the identification of unexpected germline mutations in highly penetrant genes has obvious value to the healthy, at-risk family members of patients with CRC, who can pursue testing for the identified mutation and undergo appropriate risk-reducing interventions, emerging data also illustrate how treatment of probands' CRCs themselves might be influenced by the discovery of germline mutations. Immune checkpoint inhibitors, for example, have shown significant promise as treatment of patients with LS-associated CRCs and other MSI-H cancers,⁴⁰ whereas PARP inhibitors show significant activity in the treatment of a wide array of cancers (eg, breast, ovarian, pancreatic, prostate) that develop not only in *BRCA1/2* mutation carriers but also in

individuals with *ATM*, *CHEK2*, and possibly *PALB2* mutations.^{41,42}

Whether PARP inhibitors have activity in individuals with CRC who have these mutations is unknown, but the current results demonstrate that a substantial fraction of patients with CRC could potentially benefit from therapies tailored to their germline status.

This study's primary strength is its use of a large cohort of patients with CRC and detailed personal/family history and pathologic data. These data allowed for careful examination of each proband to determine the extent to which they demonstrated features of the proband's particular syndrome. Furthermore, in contrast to prior studies that attempted to estimate the prevalence of hereditary CRC by performing syndrome-specific testing only in individuals with specific high-risk features, all patients with CRC in this cohort underwent multigene germline testing regardless of age at diagnosis, personal/family history, or tumor testing results.

The study's limitations include the use of a clinic-based cohort recruited from a large academic center, which may limit generalizability to population-based patients with CRC. For instance, the median age at CRC diagnosis was 55 years, with 31.8% of

participants receiving a diagnosis before age 50 years, whereas recent SEER data have shown that the median age at CRC diagnosis is 68 years, with only 15% of patients receiving a diagnosis before age 50 years.^{43,44} By comparison, prior studies of clinic-based cohorts of patients with CRC have found a median age at CRC diagnosis of 43 to 53 years, whereas published population-based CRC cohorts have had a median age at CRC diagnosis of 58 to 71 years.²⁻⁶ Because age at CRC diagnosis was not significantly associated with the presence of a non-LS mutation in the current study, the clinic-based setting may not have significantly affected the observed mutation prevalence. We acknowledge that any attempt to categorize cancer susceptibility genes as high penetrance versus moderate penetrance is somewhat arbitrary because cancer risk is a continuum rather than a categorical or binary phenomenon. The genes categorized as high penetrance in this study are well-established to predispose to a variety of malignancies, whereas the genes considered moderate penetrance generally have less-robust data to support their associated risks (Table 1). Furthermore, the prevalence of mutations identified by multigene panel testing inherently depends on the genes included in a given panel. Several genes newly linked to CRC risk, including *POLD1*, *POLE*, and *NTHL1*, were not examined in this study, and their penetrance and associated cancer spectrum are poorly understood. With additional testing for such genes, the prevalence of cancer susceptibility gene mutations among patients with CRC ultimately may be determined as even higher than the 9.9% rate observed in this study. Finally, we had limited data on participants who were of Ashkenazi Jewish ancestry, which precludes assessment of whether the founder *BRCA1/2* and *APC**I1307K mutations identified were attributable to their background population prevalence. Even with the exclusion of Ashkenazi founder mutations, the prevalence of nonfounder *BRCA1/2* mutations (eight [0.8%] of 1,058 [approximately 1:132]; 95% CI, 0.4% to 1.6%) in this CRC cohort is higher than the 0.25% general population (1:400) prevalence,²⁹ although such population-wide prevalence estimates remain a matter of debate.³⁰

To date, clinical practice guidelines^{21,22,28} have been cautious about endorsing the use of multigene panel testing for hereditary cancer risk assessment because of a paucity of data about the clinical utility of such panels versus the established practice of phenotype-directed, syndrome-specific evaluation. Over the past decade, universal testing of all CRCs for MSI-H/MMR-D has increasingly become routine practice to systematize the recognition of LS, whereas patients with colorectal polyposis are recommended

to undergo FAP and MAP testing.^{2,4,5,19-22} The current study findings, however, clearly illustrate that genetic factors that underlie CRC extend beyond these well-recognized familial CRC syndromes, are markedly more common than previously appreciated, frequently occur in patients with CRC who lack classic high-risk features, and are found predominantly in genes for which specialized risk-reducing interventions are recommended. These data demonstrate that comprehensive germline testing in unselected patients with CRC will identify substantially more opportunities for genetically driven cancer prevention than what is found in current practice, which suggests the need to reconsider contemporary approaches to hereditary cancer risk assessment.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Matthew B. Yurgelun, Matthew H. Kulke, Charles S. Fuchs, Brian A. Allen, Robert J. Mayer, Deborah Schrag, Jeffrey A. Meyerhardt, Kimmie Ng, Anne-Renee Hartman, Richard J. Wenstrup, Sapna Syngal

Financial support: Matthew B. Yurgelun, Charles S. Fuchs, Kimmie Ng, Sapna Syngal

Administrative support: Matthew H. Kulke, Charles S. Fuchs, Philip G. McNamara, Richard J. Wenstrup, Sapna Syngal

Provision of study materials or patients: Matthew B. Yurgelun, Matthew H. Kulke, Charles S. Fuchs, Jason L. Hornick, Robert J. Mayer, Deborah Schrag, Jeffrey A. Meyerhardt, Kimmie Ng, Sapna Syngal

Collection and assembly of data: Matthew B. Yurgelun, Matthew H. Kulke, Brian A. Allen, Jason L. Hornick, Chinedu I. Ukaegbu, Lauren K. Brais, Philip G. McNamara, Anne-Renee Hartman, Richard J. Wenstrup, Sapna Syngal

Data analysis and interpretation: Matthew B. Yurgelun, Matthew H. Kulke, Charles S. Fuchs, Brian A. Allen, Hajime Uno, Jason L. Hornick, Chinedu I. Ukaegbu, Lauren K. Brais, Philip G. McNamara, Robert J. Mayer, Deborah Schrag, Jeffrey A. Meyerhardt, Kimmie Ng, John Kidd, Nanda Singh, Anne-Renee Hartman, Sapna Syngal

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

REFERENCES

1. Ward RL, Hicks S, Hawkins NJ: Population-based molecular screening for Lynch syndrome: Implications for personalized medicine. *J Clin Oncol* 31:2554-2562, 2013
2. Moreira L, Balaguer F, Lindor N, et al: Identification of Lynch syndrome among patients with colorectal cancer. *JAMA* 308:1555-1565, 2012
3. Kastrinos F, Ojha RP, Leenen C, et al: Comparison of prediction models for lynch syndrome among individuals with colorectal cancer. *J Natl Cancer Inst* 108:djv308, 2015

4. Hampel H, Frankel WL, Martin E, et al: Feasibility of screening for Lynch syndrome among patients with colorectal cancer. *J Clin Oncol* 26:5783-5788, 2008

5. Hampel H, Frankel WL, Martin E, et al: Screening for the Lynch syndrome (hereditary non-polyposis colorectal cancer). *N Engl J Med* 352:1851-1860, 2005

6. Woods MO, Younghusband HB, Parfrey PS, et al: The genetic basis of colorectal cancer in a population-based incident cohort with a high rate of familial disease. *Gut* 59:1369-1377, 2010

7. Grover S, Kastrinos F, Steyerberg EW, et al: Prevalence and phenotypes of APC and MUTYH

mutations in patients with multiple colorectal adenomas. *JAMA* 308:485-492, 2012

8. Lubbe SJ, Di Bernardo MC, Chandler IP, et al: Clinical implications of the colorectal cancer risk associated with *MUTYH* mutation. *J Clin Oncol* 27:3975-3980, 2009

9. Bertario L, Russo A, Sala P, et al: Multiple approach to the exploration of genotype-phenotype correlations in familial adenomatous polyposis. *J Clin Oncol* 21:1698-1707, 2003

10. Cleary SP, Cotterchio M, Jenkins MA, et al: Germline MutY human homologue mutations and colorectal cancer: A multisite case-control study. *Gastroenterology* 136:1251-1260, 2009

11. Boursi B, Sella T, Liberman E, et al: The APC p.11307K polymorphism is a significant risk factor for CRC in average risk Ashkenazi Jews. *Eur J Cancer* 49:3680-3685, 2013
12. Win AK, Dowty JG, Cleary SP, et al: Risk of colorectal cancer for carriers of mutations in *MUTYH*, with and without a family history of cancer. *Gastroenterology* 146:1208-1211.e5, 2014
13. Yurgelun MB, Allen B, Kaldete RR, et al: Identification of a variety of mutations in cancer predisposition genes in patients with suspected Lynch syndrome. *Gastroenterology* 149:604-613.e20, 2015
14. Phelan CM, Iqbal J, Lynch HT, et al: Incidence of colorectal cancer in *BRCA1* and *BRCA2* mutation carriers: Results from a follow-up study. *Br J Cancer* 110:530-534, 2014
15. Tung N, Lin NU, Kidd J, et al: Frequency of germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer. *J Clin Oncol* 34:1460-1468, 2016
16. Guarinos C, Juárez M, Egoavil C, et al: Prevalence and characteristics of *MUTYH*-associated polyposis in patients with multiple adenomatous and serrated polyps. *Clin Cancer Res* 20:1158-1168, 2014
17. Aimé A, Coulet F, Lefevre JH, et al: Somatic c.34G>T *KRAS* mutation: A new prescreening test for *MUTYH*-associated polyposis? *Cancer Genet* 208:390-395, 2015
18. Judkins T, Leclair B, Bowles K, et al: Development and analytical validation of a 25-gene next generation sequencing panel that includes the *BRCA1* and *BRCA2* genes to assess hereditary cancer risk. *BMC Cancer* 15:215, 2015
19. Giardiello FM, Allen JL, Axilbund JE, et al: Guidelines on genetic evaluation and management of Lynch syndrome: A consensus statement by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology* 147:502-526, 2014
20. Syngal S, Brand RE, Church JM, et al: ACG clinical guideline: Genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol* 110:223-262, 2015; quiz 263
21. NCCN Clinical Practice Guidelines in Oncology: Genetic/familial high-risk assessment: Colorectal. Version 2, 2016. http://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf
22. NCCN Clinical Practice Guidelines in Oncology: Genetic/familial high-risk assessment: Breast and ovarian. Version 1, 2017. http://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf
23. Antoniou AC, Casadei S, Heikkinen T, et al: Breast-cancer risk in families with mutations in *PALB2*. *N Engl J Med* 371:497-506, 2014
24. Malkin D: Li-Fraumeni syndrome. *Genes Cancer* 2:475-484, 2011
25. Bishop DT, Demenais F, Goldstein AM, et al: Geographical variation in the penetrance of *CDKN2A* mutations for melanoma. *J Natl Cancer Inst* 94:894-903, 2002
26. Easton DF, Pharoah PD, Antoniou AC, et al: Gene-panel sequencing and the prediction of breast-cancer risk. *N Engl J Med* 372:2243-2257, 2015
27. Ramus SJ, Song H, Dicks E, et al: Germline mutations in the *BRIP1*, *BARD1*, *PALB2*, and *NBN* genes in women with ovarian cancer. *J Natl Cancer Inst* 107:djv214, 2015
28. Robson ME, Bradbury AR, Arun B, et al: American Society of Clinical Oncology policy statement update: Genetic and genomic testing for cancer susceptibility. *J Clin Oncol* 33:3660-3667, 2015
29. McClain MR, Palomaki GE, Nathanson KL, et al: Adjusting the estimated proportion of breast cancer cases associated with *BRCA1* and *BRCA2* mutations: Public health implications. *Genet Med* 7:28-33, 2005
30. Maxwell KN, Domchek SM, Nathanson KL, et al: Population frequency of germline *BRCA1/2* mutations. *J Clin Oncol* 34:4183-4185, 2016
31. Niell BL, Rennert G, Bonner JD, et al: *BRCA1* and *BRCA2* founder mutations and the risk of colorectal cancer. *J Natl Cancer Inst* 96:15-21, 2004
32. Kirchoff T, Satagopan JM, Kauff ND, et al: Frequency of *BRCA1* and *BRCA2* mutations in unselected Ashkenazi Jewish patients with colorectal cancer. *J Natl Cancer Inst* 96:68-70, 2004
33. Kadouri L, Hubert A, Rotenberg Y, et al: Cancer risks in carriers of the *BRCA1/2* Ashkenazi founder mutations. *J Med Genet* 44:467-471, 2007
34. Suchy J, Cybulski C, Górski B, et al: *BRCA1* mutations and colorectal cancer in Poland. *Fam Cancer* 9:541-544, 2010
35. Garre P, Martín L, Sanz J, et al: *BRCA2* gene: A candidate for clinical testing in familial colorectal cancer type X. *Clin Genet* 87:582-587, 2015
36. Tung N, Domchek SM, Stadler Z, et al: Counselling framework for moderate-penetrance cancer-susceptibility mutations. *Nat Rev Clin Oncol* 13:581-588, 2016
37. Wood ME, Kadluek P, Pham TH, et al: Quality of cancer family history and referral for genetic counseling and testing among oncology practices: A pilot test of quality measures as part of the American Society of Clinical Oncology Quality Oncology Practice Initiative. *J Clin Oncol* 32:824-829, 2014
38. Grover S, Stoffel EM, Bussone L, et al: Physician assessment of family cancer history and referral for genetic evaluation in colorectal cancer patients. *Clin Gastroenterol Hepatol* 2:813-819, 2004
39. Gallego CJ, Shirts BH, Bennette CS, et al: Next-generation sequencing panels for the diagnosis of colorectal cancer and polyposis syndromes: A cost-effectiveness analysis. *J Clin Oncol* 33:2084-2091, 2015
40. Le DT, Uram JN, Wang H, et al: PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 372:2509-2520, 2015
41. Mateo J, Carreira S, Sandhu S, et al: DNA-repair defects and olaparib in metastatic prostate cancer. *N Engl J Med* 373:1697-1708, 2015
42. Kaufman B, Shapira-Frommer R, Schmutzler RK, et al: Olaparib monotherapy in patients with advanced cancer and a germline *BRCA1/2* mutation. *J Clin Oncol* 33:244-250, 2015
43. Abdelsattar ZM, Wong SL, Regenbogen SE, et al: Reply to percentage of colorectal cancer diagnoses in adults aged younger than 50 years. *Cancer* 122:1463-1464, 2016
44. Bibbins-Domingo K, Grossman DC, Curry SJ, et al: Screening for colorectal cancer: US Preventive Services Task Force recommendation statement. *JAMA* 315:2564-2575, 2016

Affiliations

Matthew B. Yurgelun, Matthew H. Kulke, Charles S. Fuchs, Hajime Uno, Chinedu I. Ukaegbu, Lauren K. Brais, Philip G. McNamara, Robert J. Mayer, Deborah Schrag, Jeffrey A. Meyerhardt, Kimmie Ng, and Sapna Syngal, Dana-Farber Cancer Institute; Matthew B. Yurgelun, Matthew H. Kulke, Charles S. Fuchs, Hajime Uno, Jason L. Hornick, Robert J. Mayer, Deborah Schrag, Jeffrey A. Meyerhardt, Kimmie Ng, and Sapna Syngal, Harvard Medical School; Matthew B. Yurgelun, Matthew H. Kulke, Charles S. Fuchs, Jason L. Hornick, Robert J. Mayer, Deborah Schrag, Jeffrey A. Meyerhardt, Kimmie Ng, and Sapna Syngal, Brigham and Women's Hospital, Boston, MA; and Brian A. Allen, John Kidd, Nanda Singh, Anne-Renee Hartman, and Richard J. Wenstrup, Myriad Genetics, Salt Lake City, UT.

Support

Supported by National Cancer Institute grants P50CA127003 (C.S.F.), K07CA148894 (K.N.), K24CA113433 (S.S.), and R01CA132829 (S.S.) and by Myriad Genetics.

Prior Presentation

Presented at the 52nd Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, June 3-7, 2016, and the 20th Annual Meeting of the Collaborative Group of the Americas on Inherited Colorectal Cancer, Seattle, WA, October 2-3, 2016.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Cancer Susceptibility Gene Mutations in Individuals With Colorectal Cancer

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/site/iffc.

Matthew B. Yurgelun

Research Funding: Myriad Genetics

Matthew H. Kulke

Consulting or Advisory Role: Lexicon Pharmaceuticals

Charles S. Fuchs

Consulting or Advisory Role: Genentech, Eli Lilly, Sanofi, Bayer AG, Celgene, Merck, Bristol-Meyers Squibb, Entrinsic Health Solutions, Five Prime Therapeutics, Agios

Brian A. Allen

Employment: Myriad Genetics

Stock or Other Ownership: Myriad Genetics

Hajime Uno

Consulting or Advisory Role: Ionis Pharmaceuticals

Jason L. Hornick

No relationship to disclose

Chinedu I. Ukaegbu

No relationship to disclose

Lauren K. Brais

No relationship to disclose

Philip G. McNamara

No relationship to disclose

Robert J. Mayer

Honoraria: Taiho Pharmaceutical

Consulting or Advisory Role: CASI Pharmaceuticals

Deborah Schrag

Consulting or Advisory Role: New Century Health, The Ohio State University

Other Relationship: *Journal of the American Medical Association*

Jeffrey A. Meyerhardt

Consulting or Advisory Role: Genentech

Kimmie Ng

Consulting or Advisory Role: Genentech, Eli Lilly, Tarrex Biopharma

Research Funding: Gilead Sciences, Trovogene, Celgene, Genentech (Inst), Pharmavite (Inst)

John Kidd

Employment: Myriad Genetics

Stock or Other Ownership: Myriad Genetics

Nanda Singh

Employment: Myriad Genetics

Stock or Other Ownership: Myriad Genetics

Anne-Renee Hartman

Employment: Myriad Genetics, Grail

Stock or Other Ownership: Myriad Genetics

Richard J. Wenstrup

Employment: Myriad Genetics

Leadership: Myriad Genetics

Stock or Other Ownership: Myriad Genetics

Sapna Syngal

Consulting or Advisory Role: Eisai

Appendix

Methods

Patients who were found to carry pathogenic germline mutations in one or more cancer susceptibility genes underwent assessment of their personal and family cancer history to determine whether they fulfilled clinical criteria for undergoing syndrome-specific genetic testing of the gene(s) in which their mutation was found.

Lynch Syndrome

Participants with colorectal cancer (CRC) were considered to fulfill guidelines for Lynch syndrome (LS) testing¹⁹⁻²¹ (Stoffel EM, et al: J Clin Oncol 33:209-217, 2015) if they had any of the following: a known LS mutation in the family; CRC diagnosed before age 50 years; personal history of more than one CRC; personal history of any other LS-associated neoplasm (endometrial cancer; gastric cancer; ovarian cancer; pancreatic cancer; small intestine cancer; urothelial cancer of the bladder, ureter, or renal pelvis; biliary cancer; brain tumor; cutaneous sebaceous adenoma/carcinoma; or keratoacanthoma); CRC with high-level microsatellite instability (MSI-H) and/or abnormal DNA mismatch repair protein immunohistochemistry; Prediction Model for *MLH1*, *MSH2*, and *MSH6* Gene Mutations score $\geq 5\%$ (<http://premm.dfci.harvard.edu>; Kastrinos F, et al: Gastroenterology 140:73-81, 2011); one or more first-degree relatives (FDR) with LS-associated neoplasm diagnosed before age 50 years; two or more FDR and/or second-degree relatives (SDR) with LS-associated neoplasms.

BRCA1 and BRCA2

Participants with CRC were considered to fulfill guidelines for *BRCA1/2* testing²² if they had any of the following: a known *BRCA1/2* mutation in the family; a personal history of ovarian cancer; a personal history of male breast cancer; a personal history of breast or pancreatic cancer and Ashkenazi Jewish ancestry; a personal history of breast cancer diagnosed at age ≤ 45 years; a personal history of multiple breast cancers, with at least one diagnosed at age ≤ 50 years; a personal history of breast cancer diagnosed at age ≤ 50 years with one or more FDR, SDR, or third-degree relatives (TDR) diagnosed with breast, pancreatic, or Gleason ≥ 7 prostate cancer at any age; a personal history of triple-negative breast cancer diagnosed at age ≤ 60 years; a personal history of breast cancer (any age) with one or more FDR, SDR, or TDR with breast cancer diagnosed at age ≤ 50 years; a personal history of breast cancer (any age) with two or more FDR, SDR, or TDR with breast cancer (any age); a personal history of breast cancer (any age) with ≥ 2 FDR, SDR, or TDR with ovarian cancer (any age); a personal history of breast cancer (any age) with two or more FDR, SDR, or TDR with pancreatic and/or Gleason ≥ 7 prostate cancer (any age); a personal history of breast cancer (any age) with one or more FDR, SDR, or TDR with male breast cancer; a personal history of Gleason ≥ 7 prostate cancer (any age) with one or more FDR, SDR, or TDR with ovarian cancer (any age); a personal history of Gleason ≥ 7 prostate cancer (any age) with one or more FDR, SDR, or TDR with breast cancer diagnosed at age ≤ 50 years; a personal history of Gleason ≥ 7 prostate cancer (any age) with two or more FDR, SDR, TDR with breast, pancreatic, or Gleason ≥ 7 prostate cancer at any age; a personal history of pancreatic cancer (any age) with one or more FDR, SDR, or TDR with ovarian cancer (any age); a personal history of pancreatic cancer (any age) with one or more FDR, SDR, or TDR with breast cancer diagnosed at age ≤ 50 years; or a personal history of pancreatic cancer (any age) with two or more FDR, SDR, or TDR with breast, pancreatic, or Gleason ≥ 7 prostate cancer at any age. Participants with CRC were also considered to fulfill criteria for *BRCA1/2* testing²² if they had a family history of an FDR or SDR who met any of these criteria or a TDR with breast and/or ovarian cancer who had two or more FDR, SDR, TDR with breast cancer (at least one who received a diagnosis at age ≤ 50 years) and/or ovarian cancer.

Adenomatous Polyposis Syndromes

Participants with CRC were considered to fulfill criteria for *APC* and *MUTYH* testing²¹ if they had any of the following: known *APC* and/or *MUTYH* mutations in the family or a personal history of ≥ 20 colorectal adenomas over their lifetime.

Li-Fraumeni Syndrome

Participants with CRC were considered to fulfill criteria for Li-Fraumeni syndrome (LFS) *TP53* genetic testing²² (Tinat J, et al: J Clin Oncol 27:e108-e109, 2009) if they had a personal/family history of any of the following: a known *TP53* mutation in the family; a personal history of breast cancer diagnosed at age < 31 years; a personal history of adrenocortical carcinoma, choroid plexus carcinoma, or rhabdomyosarcoma (any age); a personal history of any LFS-associated cancer (soft tissue sarcoma, osteosarcoma, CNS tumor, breast cancer, adrenocortical carcinoma) diagnosed at age < 46 years and one of more FDR, SDR, or TDR with any LFS-associated cancer diagnosed at age < 56 years (unless only breast cancer was present in proband/family); a personal history of any LFS-associated cancer and one or more FDR, SDR, or TDR with multiple LFS-associated cancers (any age); a personal history of two or more LFS-associated cancers (as long as at least one is a non-breast cancer) with one or more cancers diagnosed at age < 46

years; a personal history of sarcoma diagnosed at age < 45 years and an FDR with any cancer diagnosed at age < 45 years and another FDR or SDR with cancer diagnosed at age < 45 years; or a personal history of sarcoma diagnosed at age < 45 years and an FDR with any cancer diagnosed at age < 45 years and another FDR or SDR with sarcoma (any age).

Fig A1. Number of germline variants of uncertain significance (VUS), per gene, detected with a 25-gene panel in 1,058 patients with colorectal cancer.

Table A1. Clinical Characteristics of Patients With Lynch Syndrome Mutations

Age, years/ sex	Tumor Site	Stage	Ethnicity	Pathogenic Germline Mutation	MSI/MMR Status	KRAS Status	Other Personal History of Cancer (age at diagnosis, years)	Family History of Cancer (relation, age if known, years)	Germline VUS
30/F	Ascending colon	IIA	Hispanic/Latino	<i>MLH1</i> c.2070_2071insTT p.L691Lfs*ext?	MSI-H and MMR-D	Wild type	None	PAN (father, 48), CO (pat uncle, 32; pat uncle, 40)	<i>MSH2</i> 5'UTR-3G>C, <i>MSH6</i> c.3107T>G p.L1036R
33/M	Cecum	I	Non-Hispanic white	<i>MLH1</i> c.1411_1414del p.K471Dfs*19	MMR-D (MSI np)	np	None	CO (father, 50; pat grandmother, 60), KID (father, 60),	<i>NBN</i> c.511A>G p.L171V
33/M	Ascending colon	IIIC	Non-Hispanic white	del entire <i>MLH1</i> gene	MMR-D (MSI np)	Mutation (Q61H)	None	None	none
34/M	Transverse colon	IIIB	Non-Hispanic black	<i>MLH1</i> c.55A>T p.I19F	MSI-H and MMR-P	Mutation (G13D)	None	CO (mother, 55; mat aunt, 64; mat grandfather, 68), OV (mother, 58), PR (father, 75), LEU (father, 75)	<i>MSH4</i> c.3850A>G p.T1284A
35/M	Ascending colon	I	Non-Hispanic white	<i>MLH1</i> del exons 16-19	MMR-D (MSI np)	Wild type	REC (38), CO (51)	CO (sister, 26; brother, 34; mother, 61; mat uncle, 56; mat cousin, 45; aunt, CV (mother, 58), LG (father, 81)	<i>NBN</i> c.1720T>A p.L574I
37/M	Hepatic flexure	IIA	Non-Hispanic white	<i>MLH1</i> c.230G>A p.C77Y	MSI-H and MMR-D	np	None	CO (mat grandfather, 70), UNK (mat grandmother, 40), LIV (mat grandmother, 70), BR (pat grandmother, 70)	none
37/M	Ascending colon	IIA	Non-Hispanic white	<i>MLH1</i> c.1852_1854del p.K618del	MMR-D (MSI np)	np	CO (52)	CO (mother, 30; mat uncle, 30), BR (mother, 40), BL (father, 70), PR (pat uncle, 70)	<i>STK11</i> c.1211C>T p.S404F, <i>STK11</i> c.992G>A p.R331Q
45/M	Sigmoid colon	IIA	Non-Hispanic white	<i>MLH1</i> c.1667G>A p.S556N	MSI-H and MMR-P	np	CO (76)	CO (brother, 55; father, 57; pat uncle, 29; pat uncle, 50; mat uncle, 60), SM INT (brother, 52)	None
47/F	Cecum	0	Non-Hispanic white	<i>MLH1</i> c.5C>A p.S2X	MMR-D (MSI np)	np	None	CO (sister, 43; father, 42; pat grandfather, 39)	None
48/F	Transverse colon	I	Non-Hispanic white	<i>MLH1</i> c.350C>T p.T117M	MSS and MMR-D	Wild type	MEL (33), BR (47)	UT (sister, 46; sister, 50; mother, 55), CO (mat grandfather, 32; mat aunt, 75; mat cousin, 50; mat cousin, 58), CNS (mat cousin, 30), LIV (mat cousin, 30)	None
49/M	Synchronous ascending and descending colon	IV	Non-Hispanic white	<i>MLH1</i> c.678-1G>A	MSI-H and MMR-D	Mutation (G12D)	None	None	None
59/F	Cecum	IV	Non-Hispanic white	<i>MLH1</i> c.2195_2198dup p.H733Qfs*14	MMR-D (MSI np)	Wild type	SEB (60)	PER (mother), CO (mat aunt, 39)	<i>ATM</i> c.7429G>A p.G2477R, <i>BARF1</i> c.2155A>G p.T719A
31/M	Sigmoid colon	IV	Non-Hispanic white	<i>MLH1</i> del exons 16-19, <i>BRCA2</i> c.3199del p.T1067Lfs*10	MMR-D (MSI np)	Mutation (G13D)	None	CO (mat uncle, 38; mat aunt, 21; mat grandmother, 32), MEL (mother, 50)	None
33/M	Transverse colon	IIA	Non-Hispanic white	<i>MSH2</i> c.1906G>C p.A636P, <i>APC</i> c.3920T>A p.I1307K	MSI-H and MMR-D	Mutation (G13D)	None	REC (sister, 33), UT (mat aunt, 35; mat grandmother, 70)	None
36/M	Rectum	IIA	Non-Hispanic white	<i>MSH2</i> c.2074G>T p.G692W	MSI-H and MMR-D	Wild type	None	LG (mother, 42), UT (mat aunt, 30; mat grandmother, 31), URO (mat grandmother)	None
44/F	Transverse colon	IIA	Non-Hispanic white	<i>MSH2</i> del exons 9-12	MMR-D (MSI np)	np	None	None	None
46/M	Ascending colon	IIA	Non-Hispanic black	<i>MSH2</i> c.2082dup p.V695Cfs*4	MMR-D (MSI np)	np	None	None	None
50/M	Transverse colon	I	Non-Hispanic white	<i>MSH2</i> del exons 1-6	MMR-D (MSI np)	np	None	CO (mother, 43), UT (mother, 55), BR (sister, 56)	<i>MSH2</i> c.274C>G p.L92V
65/M	Transverse colon	IIIB	Non-Hispanic white	<i>MSH2</i> c.1906G>C p.A636P	np	np	SM INT (48), PAN (67)	CO (father), STO (father), URO (father), PR (father)	<i>PMS2</i> 5'UTR-7T>C

(continued on following page)

Table A1. Clinical Characteristics of Patients With Lynch Syndrome Mutations (continued)

Age, years/ sex	Tumor Site	Stage	Ethnicity	Pathogenic Germline Mutation	MSI/MMR Status	KRAS Status	Other Personal History of Cancer (age at diagnosis, years)	Family History of Cancer (relation, age if known, years)	Germline VUS
72/M	Rectum	IIA	Non-Hispanic white	<i>MSH2</i> dup exon 8	np	np	GEJ (73)	CO (daughter, brother, brother, mat uncle), OV (mother)	None
22/M	Descending colon	IIA	Non-Hispanic white	del entire <i>MSH6</i> gene	MSI-H and MMR-D	Mutation (G12D)	None	REC (cousin, 30), BR (mother, 51; aunt, 35), PR (grandfather, 78)	None
29/M	Rectum	IIA	Non-Hispanic white	<i>MSH6</i> c.3939_3957dup p.A1320Sfs*5	MSI and MMR-P	Wild type	None	None	<i>STK11</i> c.1180G>C p.G394R
34/M	Splenic flexure	IIA	Non-Hispanic white	<i>MSH6</i> c.10C>T p.Q4X	MSI-H and MMR-D	np	None	CO (mat uncle, 40), ESO (pat grandfather, 66), TES (pat cousin, 32)	None
57/F	Sigmoid colon	I	Non-Hispanic white	<i>MSH6</i> c.3939_3957dup p.A1320Sfs*5	MMR-D (MSI np)	Wild type	LG (57)	UT (sister, 53), MM (mother, 49)	None
59/M	Rectum	IIA	Non-Hispanic white	<i>MSH6</i> c.1519dup p.R507Kfs*9	MMR-D (MSI np)	Mutation (G13D)	None	UNK (sister, 27), BR (mat grandmother)	None
59/M	Cecum	IV	Non-Hispanic white	<i>MSH6</i> c.1519dup p.R507Kfs*9	MSI-H and MMR-D	Mutation (G13D)	None	CO (father, 59), UNK (aunt, 50)	None
21/F	Sigmoid colon	IIIB	Non-Hispanic white	<i>PMS2</i> c.2174+1G>A	MSI-H and MMR-D	Wild type	None	CO (pat grandmother, 75), BR (mat aunt, 35; mat aunt, 38; mat aunt, 4), CV (aunt, 38)	None
34/F	Ascending colon	I	Non-Hispanic black	<i>PMS2</i> del exon 13	MSI-H and MMR-D	np	None	PR (father, 60; pat uncle, 55), UNK (mat aunt, 55; mat uncle, 50)	<i>CDH1</i> c.2104G>A p.E702K
53/F	Ascending colon	IIA	Non-Hispanic white	<i>PMS2</i> c.2117del p.K706Sfs*19	MSI-H and MMR-D	np	None	CO (father, 43; pat grandmother, 70), UT (pat aunt, 50), UNK (pat uncle, 70), LEU (pat grandfather, 60)	None
58/F	Cecum	IIA	Non-Hispanic white	<i>PMS2</i> c.765C>G p.Y255X	MMR-D (MSI np)	Wild type	None	CO (uncle), BR (cousin, 60), NHL (cousin)	None
59/F	Descending colon	IIIB	Non-Hispanic white	<i>PMS2</i> del exons 6-15	np	Mutation (G13D)	BL (69)	TES (father, 66), PR (brother)	None
60/F	Rectum	I	Non-Hispanic white	<i>PMS2</i> c.1067del p.K356Rfs*4	np	np	THY (60), CHO (64)	HN (father, 80), CO (mat grandfather)	None
65/M	Descending colon	IIA	Non-Hispanic white	<i>PMS2</i> c.736_741delins11 p.P246Cfs*3	MMR-D (MSI np)	Mutation (G12R)	MF (65)	HL (son, 30), PR (pat grandfather), KID (mat grandmother), GB (pat grandmother)	<i>CDH1</i> c.2413G>A p.D805N

NOTE: Relatives (e.g., aunts, uncles, grandparents) were only listed as being from the proband's maternal or paternal lineage if this was specified in the medical record. Abbreviations: BL, bladder cancer; BR, breast cancer; CHO, cholangiocarcinoma; CO, colon cancer; CNS, brain cancer; CV, cervical cancer; ESO, esophageal cancer; GB, gallbladder cancer; GEJ, gastroesophageal junction cancer; HL, Hodgkin lymphoma; HN, head and neck cancer; KID, kidney cancer; LEU, leukemia; LG, lung cancer; LIV, liver cancer; mat, maternal; MEL, melanoma; MF, myelofibrosis; MM, multiple myeloma; MMR, mismatch repair; MMR-D, mismatch repair deficient; MMR-P, mismatch repair proficient; MSI, microsatellite instability; MSI-H, high-level microsatellite instability; NHL, non-Hodgkin lymphoma; np, not performed; OV, ovarian cancer; PAN, pancreatic cancer; pat, paternal; PER, perineal cancer; not otherwise specified; PR, prostate cancer; REC, rectal cancer; SEB, sebaceous carcinoma; SM INT, small intestine cancer; STO, stomach cancer; TES, testicular cancer; THY, thyroid cancer; UNK, unknown cancer diagnosis; URO, urothelial carcinoma; UT, uterine cancer; VUS, variant of uncertain significance.

Table A2. Clinical Characteristics of Patients With Moderate-Penetrance Germline Mutations

Age, years/sex	Tumor Site	Stage	Ethnicity	Pathogenic Germline Mutation	MSI/MMR Status	KRAS Status	Other Personal History of Cancer (age at diagnosis, years)	Family History of Cancer (relation, age if known, years)	Germline VUS
Genes linked to CRC risk*									
44/M	Sigmoid colon	IV	Non-Hispanic white	APC c.3920T>A p.I1307K	MMR-P	Wild type	None	CRC (mother)	None
46/M	Descending colon	IIIC	Non-Hispanic white	APC c.3920T>A p.I1307K	MMR-P	Wild type	None	BR (mother)	None
52/M	Sigmoid colon	I	Non-Hispanic white	APC c.3920T>A p.I1307K	MMR-P and MSS	np	MEL (68)	CRC (grandmother, 50)	PMS2 c.86G>C p. G29A, BRIP1 c.1735C>T p.R579C
56/F	Sigmoid colon	IIA	Non-Hispanic white	APC c.3920T>A p.I1307K	MMR-P and MSI-L	np	None	BR (mother, 73; mat cousin, 62), CRC (mat aunt, 84; mat cousin, 54), MM (mat aunt, 72)	None
58/F	Rectum	IIIC	Non-Hispanic white	APC c.3920T>A p.I1307K	np	Wild type	None	MEL (brother, 40), CRC (grandmother), BR (aunt, 40)	PMS2 c.2149G>A p.V717M
59/M†	Transverse colon (two synchronous primaries)	IIA	Non-Hispanic white	APC c.3920T>A p.I1307K	MMR-D and MSI-H (both primaries)	np	None	CRC (father, 62; pat uncle, 60), BR (mother, 85), UNK (mat grandfather)	PMS2 c.2534A>T p.H845L
61/M	Cecum and sigmoid colon (two synchronous primaries)	IV	Non-Hispanic white	APC c.3920T>A p.I1307K	np	Mutation (NOS)	PR (63)	None	None
62/F	Sigmoid colon	IV	Non-Hispanic white	APC c.3920T>A p.I1307K	np	Wild type	None	BR (mother, 73), OV (mat aunt, 74)	BMPR1A c.8A>G
63/F	Ascending colon	I	Non-Hispanic white	APC c.3920T>A p.I1307K	MMR-P	Wild type	None	CRC (pat grandmother, 90; pat uncle, 50)	PMS2 c.53T>C p.I18T
65/M	Sigmoid colon	I	Non-Hispanic white	APC c.3920T>A p.I1307K	np	np	None	KID (brother, 70), STO (pat uncle, 80)	None
68/M	Sigmoid colon	IIA	Non-Hispanic white	APC c.3920T>A p.I1307K	np	Mutation (G12C)	None	BR (sister, 50; sister, 50; pat grandmother, 60), LG (pat uncle), CNS (mat cousin), OV (mat cousin), PR (mat cousin), TES (mat cousin)	None
70/F	Rectum	IIIA	Non-Hispanic white	APC c.3920T>A p.I1307K	np	Wild type	BR (55)	CRC (father, 82), LYM (mother, 72), BR (sister, 56; pat aunt, 50; pat aunt, 74)	ATM c.6919C>T p.L2307F
71/F	Rectum	I	Non-Hispanic white	APC c.3920T>A p.I1307K	np	np	None	OV (mother, 60), BR (sister, 29)	None
74/F	Sigmoid colon	IIIB	Non-Hispanic white	APC c.3920T>A p.I1307K	np	Mutation (G12D)	None	CV (mother, 82), ESO (brother, 75)	None
43/M	Rectum	IV	Non-Hispanic white	CHEK2 c.1100del p.T367Mfs*15	np	Wild type	None	UNK (father, 68)	None
59/M	Cecum	IV	Non-Hispanic white	CHEK2 del exons 8-9	np	np	CLL (49)	None (adopted)	None
40/F	Cecum	IV	Non-Hispanic white	MUTYH c.1145G>A p.G382D	MMR-P and MSS	Mutation (G12C)	None	None (adopted)	CDKN2A c.33G>C
42/M	Rectum	IIIB	Non-Hispanic white	MUTYH c.1145G>A p.G382D	MMR-P	Wild type	None	BR (mother, 43), PR (grandfather, 70), BL (grandfather, 80), LG (grandmother, 70), UT (aunt, 61)	None
45/F	Ascending colon	IIIC	Non-Hispanic white	MUTYH c.891+3A>C	MMR-P and MSS	Mutation (G12C)	THY (38)	PR (father), HL (mat aunt), BR (mat grandmother)	PMS2 c.2012C>T p.I671M
47/M	Cecum	IV	Non-Hispanic white	MUTYH c.1145G>A p.G382D	MSS	Mutation (G12V)	None	BR (sister)	None

(continued on following page)

Table A2. Clinical Characteristics of Patients With Moderate-Penetrance Germline Mutations (continued)

Age, years/sex	Tumor Site	Stage	Ethnicity	Pathogenic Germline Mutation	MSI/MMR Status	KRAS Status	Other Personal History of Cancer (age at diagnosis, years)	Family History of Cancer (relation, age if known, years)	Germline VUS
47/F	Sigmoid colon	I	Non-Hispanic white	<i>MUTYH</i> c.494A>G p.Y165C	MMR-P and MSS	np	None	BR (mother, 64; pat grandmother, 42), HN (father, 75), LG (mat aunt, 73; mat aunt, 56), PR (pat grandfather, 95), BL (mat cousin, 46), CNS (nephew, 13)	<i>MUTYH</i> c.1234C>T p.R412C
49/F	Cecum	IIIC	Non-Hispanic white	<i>MUTYH</i> c.494A>G p.Y165C	np	Wild type	None	MCC (father, 78)	<i>CDH1</i> c.164T>G p.V55G
57/F	Rectosigmoid	IIA	Non-Hispanic white	<i>MUTYH</i> c.494A>G p.Y165C	np	Wild type	Ascending colon cancer (64), descending colon cancer (76), OV (80)	CRC (sister, 70; sister, 60), BR (sister, 60)	<i>MUTYH</i> c.1234C>T p.R412C
58/M	Rectum	IIIB	Non-Hispanic white	<i>MUTYH</i> c.1145G>A p.G382D	MMR-P	Wild type	None	LG (mother, 68)	None
58/M	Sigmoid colon	I	Non-Hispanic white	<i>MUTYH</i> c.1145G>A p.G382D	np	np	None	CRC (mat grandmother, 80), STO (mat aunt, 77)	None
60/M	Rectum	I	Non-Hispanic white	<i>MUTYH</i> c.494A>G p.Y165C	np	np	None	Missing data	None
61/F	Rectum	IIIB	Non-Hispanic black	<i>MUTYH</i> c.1282-1G>T	MMR-P and MSS	Wild type	Stage 0 transverse colon cancer (67)	CRC (sister, 57)	None
66/M	Rectum	I	Non-Hispanic white	<i>MUTYH</i> c.1145G>A p.G382D	np	Mutation (G12D)	None	PR (mat uncle, 66; mat uncle)	None
66/M	Rectum	IIIA	Non-Hispanic white	<i>MUTYH</i> c.1145G>A p.G382D	np	Mutation (G13D)	None	LEU (sister, 5), LYM (mother, 60), HN (uncle, 60)	None
71/F	Ascending colon	IV	Non-Hispanic white	<i>MUTYH</i> c.1145G>A p.G382D	MMR-P	Mutation (G13D)	None	CRC (brother, 46), LG (brother, 57)	<i>MUTYH</i> c.1234C>T p.R412C, <i>ATM</i> c.2804C>T p.T935M
71/M	Descending colon	IIA	Non-Hispanic white	<i>MUTYH</i> c.494A>G p.Y165C	np	<i>NRAS</i> Q61K mutation	PR (59), NHL (76)	OV (mother, 45), BL (father, 60)	<i>PMS2</i> c.86G>C p.G29A
80/M	Rectosigmoid	IV	Asian	<i>MUTYH</i> c.892-2A>G p.Y1544X	np	Wild type	None	None	None
80/F	Sigmoid colon	0	Non-Hispanic white	<i>MUTYH</i> c.1145G>A p.G382D	np	np	None	LIV (brother, 19), LG (brother, 60)	None
82/F	Cecum	IIIB	Non-Hispanic white	<i>MUTYH</i> c.503G>A p.R168H	MMR-D and MSI-H	Wild type	MEL (80)	CRC (mat aunt, 66; mat uncle, 78; mat uncle, 80), STO (mat aunt, 39; mat grandmother, 62; mat uncle, 80), OV (mat aunt, 44)	None
Genes not linked to CRC risk									
31/F	Rectum	IV	Hispanic	<i>ATM</i> c.8934_8935del p.E2979Afs*9	MMR-P	Wild type	None	CV (mother, 58)	<i>ATM</i> c.1703G>T p.R568I
33/F	Sigmoid colon	IV	Non-Hispanic white	<i>ATM</i> c.7638_7646del p.R2547_S2549del	MMR-P	Mutation (G12D)	None	CV (sister), MEL (mat grandmother; mat aunt), LG (mat uncle, 50), UNK (pat grandmother)	<i>RAD51C</i> c.784T>G p.L262V
39/F	Ascending colon	IV	Non-Hispanic white	<i>ATM</i> c.4632_4635del p.Y1544X	MMR-P	Mutation (NOS)	None	None	<i>BRD1</i> c.1757G>T p.S586I
42/M	Cecum	IIA	Non-Hispanic white	<i>ATM</i> c.3760del p.V1254Ffs*2	MMR-D and MSI-H	Wild type	None	CRC (pat uncle, 79), OV (pat grandmother, 75), LIV (pat grandfather, 42), LG (mat grandfather, 72)	None
47/F	Rectum	IV	Asian	<i>ATM</i> c.802C>T p.Q268X	MMR-P and MSS	Wild type	None	None	None
47/F	Rectum	IV	Non-Hispanic white	<i>ATM</i> c.790del p.Y264Ifs*12	MMR-P	Mutation (G12A)	None	BR (mother, 70), CRC (pat grandfather, 68), UNK (mat aunt, 60)	None
52/M	Rectum	IV	Non-Hispanic white	<i>ATM</i> c.5570C>A p.S1857X	np	Wild type	None	None	None
61/F	Rectum	IIA	Non-Hispanic white	<i>ATM</i> c.2413C>T p.R805X	MMR-P	Wild type	None	PR (father, 67; uncle), CRC (mat uncle)	None
67/M	Cecum	IV	Non-Hispanic white	<i>ATM</i> c.2250G>A p.K750K	MMR-P	Mutation (G13D)	None	None	None

(continued on following page)

Table A2. Clinical Characteristics of Patients With Moderate-Penetrance Germline Mutations (continued)

Age, years/sex	Tumor Site	Stage	Ethnicity	Pathogenic Germline Mutation	MSI/MMR Status	KRAS Status	Other Personal History of Cancer (age at diagnosis, years)	Family History of Cancer (relation, age if known, years)	Germline VUS
73/F	Cecum	I	Non-Hispanic white	<i>ATM</i> c.3480_3492dup p.S1165Gfs*5	np	Mutation (G12D)	None	CRC (mother, 35), BR (mother)	<i>ATM</i> c.7999A>G p.M2667V
62/M	Rectum	IIIA	Non-Hispanic white	<i>BARD1</i> del exons 5-6	np	Wild type	PR (59)	MEL (brother, 30), PR (mat uncle, 60)	<i>MSH2</i> c.1223A>T p.Y408F
48/M	Cecum	IV	Non-Hispanic white	<i>BRIP1</i> c.2990_2993del p.1997Rfs*61	MMR-P	Mutation (G12C)	None	KID (mother, 60), UNK (mat aunt, 78)	<i>ATM</i> c.5971G>A p.E1991K
56/M	Hepatic flexure	IV	Non-Hispanic white	<i>BRIP1</i> c.2379+1G>T	np	np	None	MCC (father, 70), LG (pat uncle, 85), UNK (pat aunt, 70)	None
57/F	Rectum	I	Non-Hispanic white	<i>BRIP1</i> c.1970del p.G657Vfs*31	np	np	None	LG (father, 52), PR (brother, 64)	None
50/F	Rectum	IIIB	Non-Hispanic white	<i>NBN</i> c.657_661del p.K219Nfs*16	MMR-P and MSS	Wild type	None	LG (mother, 47; mat aunt), LEU (brother), LYM (mat grandmother, 92)	None
76/F	Ascending colon	IIIC	Non-Hispanic white	<i>NBN</i> c.1142del p.P381Qfs*23	np	np	None	CRC (father, 56)	None

NOTE: Relatives (e.g., aunts, uncles, grandparents) were only listed as being from the proband's maternal or paternal lineage if this was specified in the medical record.

Abbreviations: BL, bladder cancer; BR, breast cancer; CLL, chronic lymphocytic leukemia; CNS, brain cancer; CRC, colorectal cancer; ESO, esophageal cancer; HL, Hodgkin lymphoma; HN, head and neck cancer; KID, kidney cancer; LEU, leukemia; LG, lung cancer; LIV, liver cancer; LYM, lymphoma, not otherwise specified; mat, maternal; MCC, Merkel cell carcinoma; MEL, melanoma; MM, multiple myeloma; MMR, mismatch repair; MMR-D, mismatch repair deficient; MMR-P, mismatch repair proficient; MSI, microsatellite instability; MSI-H, high-level microsatellite instability; MSS, low-level microsatellite stability; NHL, non-Hodgkin lymphoma; NOS, not otherwise specified; np, not performed; OV, ovarian cancer; pat, paternal; PR, prostate cancer; STO, stomach cancer; TES, testicular cancer; THY, thyroid cancer; UNK, unknown cancer diagnosis; UT, uterine cancer; VUS, variant of uncertain significance.

*An additional four participants with concurrent high-penetrance mutations are presented in Table 3 only.

†Participant also is listed in Appendix Table A5 because of his tumor's MMR-D and a germline Lynch syndrome VUS.

Table A3. Factors That Predict the Presence of a Non-Lynch Syndrome Cancer Susceptibility Gene Mutation in Patients With CRC (versus patients with mutation-negative CRC)

Characteristic	OR (95% CI)
Age at first CRC diagnosis (per 10 years)	0.93 (0.76 to 1.15)
Number of lifetime CRC diagnoses	
1	1.0 (Reference)
>1	3.70 (1.31 to 10.49)
Personal history of non-CRC diagnosis*	
No	1.0 (Reference)
Yes	1.76 (0.95 to 3.27)
<i>KRAS</i> mutation status	
<i>KRAS</i> wild type	1.0 (Reference)
<i>KRAS</i> G12C mutation	4.58 (1.76 to 11.92)
Other <i>KRAS</i> mutation	0.79 (0.42 to 1.48)
Missing <i>KRAS</i> status	0.75 (0.41 to 1.39)
Any first-degree relative with CRC	
No	1.0 (Reference)
Yes	1.22 (0.59 to 2.51)
Any first-degree relative with breast cancer	
No	1.0 (Reference)
Yes	1.84 (0.99 to 3.40)
Any first-degree relative with ovarian cancer	
No	1.0 (Reference)
Yes	3.06 (0.97 to 9.64)

Abbreviations: CRC, colorectal cancer; OR, odds ratio.

*Excludes cutaneous basal and squamous cell carcinomas.

Germline Mutations in Individuals With Colorectal Cancer

Table A4. Germline Variants of Uncertain Significance Detected by a 25-Gene Panel Among 1,058 Participants With Colorectal Cancer

Subject ID	Gene	HGVS Alteration Name
COLB3735-3	<i>APC</i>	c.1223A>G (p.H408R)
COLB5623-3	<i>APC</i>	c.1589T>C (p.V530A)
COLB5803-3	<i>APC</i>	c.1631T>C (p.I544T)
COLB4015-3	<i>APC</i>	c.166G>C (p.E56Q)
COLB3843-3	<i>APC</i>	c.1825G>A (p.V609I)
COLB3958-3	<i>APC</i>	c.1825G>A (p.V609I)
COLB5005-3	<i>APC</i>	c.1895T>C (p.I632T)
COLB3393-3	<i>APC</i>	c.2204C>T (p.A735V)
COLB3198-3	<i>APC</i>	c.2438A>G (p.N813S)
COLB4360-3	<i>APC</i>	c.3341G>A (p.R1114Q)
COLB3180-3	<i>APC</i>	c.3352A>G (p.N1118D)
COLB4036-3	<i>APC</i>	c.3625G>A (p.E1209K)
COLB5668-3	<i>APC</i>	c.3739G>A (p.A1247T)
COLB4174-3	<i>APC</i>	c.3875C>T (p.T1292M)
COLB3534-3	<i>APC</i>	c.4072G>A (p.A1358T)
COLB5905-3	<i>APC</i>	c.4072G>A (p.A1358T)
COLB5818-3	<i>APC</i>	c.442C>A (p.L148I)
COLB4045-3	<i>APC</i>	c.5752A>G (p.I1918V)
COLB3876-3	<i>APC</i>	c.5879C>T (p.P1960L)
COLB3357-3	<i>APC</i>	c.5891C>T (p.S1964F)
COLB5405-3	<i>APC</i>	c.5912C>G (p.S1971C)
COLB3606-3	<i>APC</i>	c.5975C>T (p.P1992L)
COLB3396-3	<i>APC</i>	c.6365ins3 (p.A2122ins)
COLB4937-3	<i>APC</i>	c.6365ins3 (p.A2122ins)
COLB2792-3	<i>APC</i>	c.640G>A (p.A214T)
COLB5686-3	<i>APC</i>	c.647G>A (p.R216Q)
COLB3023-3	<i>APC</i>	c.6724A>G (p.S2242G)
COLB5803-3	<i>APC</i>	c.6873A>T (p.Q2291H)
COLB3979-3	<i>APC</i>	c.7415C>T (p.A2472V)
COLB2594-3	<i>APC</i>	c.7468G>A (p.D2490N)
COLB4872-3	<i>APC</i>	c.7471A>G (p.M2491V)
COLB5731-3	<i>APC</i>	c.7625A>G (p.N2542S)
COLB5737-3	<i>APC</i>	c.7667C>T (p.S2556L)
COLB3561-3	<i>APC</i>	c.8389A>G (p.S2797G)
COLB3885-3	<i>APC</i>	IVS5-8T>A
COLB2873-3	<i>APC</i>	IVS7-3T>C
COLB5911-3	<i>ATM</i>	c.1444A>C (p.Lys482Gln)
COLB5301-3	<i>ATM</i>	c.1703G>T (p.Arg568Ile)
COLB5911-3	<i>ATM</i>	c.1703G>T (p.Arg568Ile)
COLB5818-3	<i>ATM</i>	c.1960C>A (p.Gln654Lys)
COLB3843-3	<i>ATM</i>	c.2096A>G (p.Glu699Gly)
COLB4183-3	<i>ATM</i>	c.2207C>T (p.Ala736Val)
COLB4333-3	<i>ATM</i>	c.2428A>G (p.Lys810Glu)
COLB4279-3	<i>ATM</i>	c.2467-8C>G
COLB3267-3	<i>ATM</i>	c.2492A>G (p.Asp831Gly)
COLB5340-3	<i>ATM</i>	c.2581T>C (p.Tyr861His)
COLB5686-3	<i>ATM</i>	c.2608A>G (p.Asn870Asp)
COLB3453-3	<i>ATM</i>	c.2804C>T (p.Thr935Met)
COLB3988-3	<i>ATM</i>	c.2804C>T (p.Thr935Met)
COLB3681-3	<i>ATM</i>	c.2819A>C (p.Lys940Thr)
COLB5458-3	<i>ATM</i>	c.2945G>A (p.Arg982His)
COLB4447-3	<i>ATM</i>	c.3295G>A (p.Asp1099Asn)
COLB4672-3	<i>ATM</i>	c.4768C>T (p.Leu1590Phe)
COLB2972-3	<i>ATM</i>	c.4909+3G>A
COLB5328-3	<i>ATM</i>	c.5080G>A (p.Ala1694Thr)
COLB3576-3	<i>ATM</i>	c.5089A>G (p.Thr1697Ala)
COLB3630-3	<i>ATM</i>	c.5435C>T (p.Ala1812Val)
COLB2702-3	<i>ATM</i>	c.5728C>A (p.Leu1910Ile)
COLB5221-3	<i>ATM</i>	c.5971G>A (p.Glu1991Lys)
COLB3582-3	<i>ATM</i>	c.6176C>T (p.Thr2059Ile)
COLB4765-3	<i>ATM</i>	c.6226A>G (p.Ile2076Val)
COLB3534-3	<i>ATM</i>	c.6652A>C (p.Ser2218Arg)
COLB3050-3	<i>ATM</i>	c.6860G>A (p.Gly2287Glu)

(continued on following page)

Table A4. Germline Variants of Uncertain Significance Detected by a 25-Gene Panel Among 1,058 Participants With Colorectal Cancer (continued)

Subject ID	Gene	HGVS Alteration Name
COLB2726-3	<i>ATM</i>	c.6860G>C (p.Gly2287Ala)
COLB2825-3	<i>ATM</i>	c.6919C>T (p.Leu2307Phe)
COLB2963-3	<i>ATM</i>	c.6919C>T (p.Leu2307Phe)
COLB3867-3	<i>ATM</i>	c.6919C>T (p.Leu2307Phe)
COLB5431-3	<i>ATM</i>	c.6919C>T (p.Leu2307Phe)
COLB3585-3	<i>ATM</i>	c.7187C>G (p.Thr2396Ser)
COLB2669-3	<i>ATM</i>	c.7190A>G (p.Gln2397Arg)
COLB4555-3	<i>ATM</i>	c.7235A>G (p.Asn2412Ser)
COLB4012-2	<i>ATM</i>	c.7375C>G (p.Arg2459Gly)
COLB5594-3	<i>ATM</i>	c.7390T>C (p.Cys2464Arg)
COLB5680-3	<i>ATM</i>	c.7429G>A (p.Gly2477Arg)
COLB5176-3	<i>ATM</i>	c.7475T>G (p.Leu2492Arg)
COLB3459-3	<i>ATM</i>	c.7552C>T (p.Pro2518Ser)
COLB4142-3	<i>ATM</i>	c.7818A>G (p.Ile2606Met)
COLB4139-3	<i>ATM</i>	c.7999A>G (p.Met2667Val)
COLB3549-3	<i>ATM</i>	c.8071C>T (p.Arg2691Cys)
COLB5364-3	<i>ATM</i>	c.8113G>A (p.Val2705Ile)
COLB4570-3	<i>ATM</i>	c.8192T>A (p.Val2731Asp)
COLB4682-3	<i>ATM</i>	c.8560C>T (p.Arg2854Cys)
COLB5325-3	<i>ATM</i>	c.8560C>T (p.Arg2854Cys)
COLB5579-3	<i>ATM</i>	c.8560C>T (p.Arg2854Cys)
COLB4866-3	<i>ATM</i>	c.8734A>G (p.Arg2912Gly)
COLB4949-3	<i>ATM</i>	c.9079dup (p.Ser3027Lysfs*36)
COLB5541-3	<i>ATM</i>	c.9079dup (p.Ser3027Lysfs*36)
COLB3744-3	<i>ATM</i>	c.9086G>A (p.Gly3029Asp)
COLB4327-3	<i>BARD1</i>	c.1223T>C (p.Met408Thr)
COLB4066-3	<i>BARD1</i>	c.1283_1285del (p.Gly428del)
COLB3660-3	<i>BARD1</i>	c.1568T>C (p.Val523Ala)
COLB4103-3	<i>BARD1</i>	c.1586G>A (p.Arg529Gln)
COLB4109-3	<i>BARD1</i>	c.1714C>T (p.Leu572Phe)
COLB3444-3	<i>BARD1</i>	c.1757G>T (p.Ser586Ile)
COLB3207-3	<i>BARD1</i>	c.1793C>A (p.Thr598Asn)
COLB5244-3	<i>BARD1</i>	c.1801G>A (p.Asp601Asn)
COLB4228-3	<i>BARD1</i>	c.1918C>A (p.Leu640Ile)
COLB5680-3	<i>BARD1</i>	c.2155A>G (p.Thr719Ala)
COLB4015-3	<i>BARD1</i>	c.2230A>G (p.Asn744Asp)
COLB3904-3	<i>BARD1</i>	c.33G>T (p.Gln11His)
COLB4922-3	<i>BARD1</i>	c.33G>T (p.Gln11His)
COLB5038-3	<i>BARD1</i>	c.33G>T (p.Gln11His)
COLB5716-3	<i>BARD1</i>	c.33G>T (p.Gln11His)
COLB3504-3	<i>BARD1</i>	c.346C>T (p.His116Tyr)
COLB4174-3	<i>BARD1</i>	c.346C>T (p.His116Tyr)
COLB4168-3	<i>BARD1</i>	c.568G>A (p.Asp190Asn)
COLB2762-3	<i>BMPR1A</i>	c.1327C>T (p.Arg443Cys)
COLB2810-1	<i>BMPR1A</i>	c.1327C>T (p.Arg443Cys)
COLB4826-3	<i>BMPR1A</i>	c.1333A>G (p.Ile445Val)
COLB5262-3	<i>BMPR1A</i>	c.1348G>A (p.Val450Met)
COLB4450-3	<i>BMPR1A</i>	c.1433G>A (p.Arg478His)
COLB4210-3	<i>BMPR1A</i>	c.430+10C>T
COLB4957-3	<i>BMPR1A</i>	c.478A>G (p.Met160Val)
COLB4723-3	<i>BMPR1A</i>	c.499A>G (p.Met167Val)
COLB3967-3	<i>BMPR1A</i>	c.733T>A (p.Tyr245Asn)
COLB4252-3	<i>BMPR1A</i>	c.760C>T (p.Arg254Cys)
COLB5887-3	<i>BMPR1A</i>	c.-8A>G
COLB4408-3	<i>BMPR1A</i>	c.901T>A (p.Ser301Thr)
COLB4085-3	<i>BMPR1A</i>	c.943G>A (p.Gly315Arg)
COLB3907-3	<i>BRCA1</i>	c.1469A>T (p.K450N)
COLB3789-3	<i>BRCA1</i>	c.1665G>C (p.D516H)
COLB3985-3	<i>BRCA1</i>	c.2158del3 (p.K680del)
COLB5497-3	<i>BRCA1</i>	c.3028A>G (p.K970R)
COLB5920-3	<i>BRCA1</i>	c.3652G>A (p.S1178N)
COLB4177-3	<i>BRCA1</i>	c.3664A>T (p.Q1182L)
COLB2966-3	<i>BRCA1</i>	c.4281C>G (p.Q1388E)

(continued on following page)

Germline Mutations in Individuals With Colorectal Cancer

Table A4. Germline Variants of Uncertain Significance Detected by a 25-Gene Panel Among 1,058 Participants With Colorectal Cancer (continued)

Subject ID	Gene	HGVS Alteration Name
COLB4279-3	<i>BRCA2</i>	c.1129G>T (p.D301Y)
COLB5943-3	<i>BRCA2</i>	c.3388G>C (p.D1054H)
COLB3342-3	<i>BRCA2</i>	c.3823G>A (p.D1199N)
COLB5629-3	<i>BRCA2</i>	c.4282G>T (p.D1352Y)
COLB4165-3	<i>BRCA2</i>	c.463A>G (p.I79V)
COLB3282-3	<i>BRCA2</i>	c.5170G>A (p.A1648T)
COLB5405-3	<i>BRCA2</i>	c.5393G>C (p.S1722T)
COLB5701-3	<i>BRCA2</i>	c.7844A>G (p.Q2539R)
COLB4339-3	<i>BRCA2</i>	c.8927A>T (p.D2900V)
COLB5431-3	<i>BRCA2</i>	c.9944C>A (p.S3239Y)
COLB4878-3	<i>BRCA2</i>	IVS2+4T>C
COLB4480-3	<i>BRCA2</i>	IVS7-3C>T
COLB3858-3	<i>BRIP1</i>	c.1105T>C (p.Tyr369His)
COLB4279-3	<i>BRIP1</i>	c.1255C>T (p.Arg419Trp)
COLB4123-3	<i>BRIP1</i>	c.1655T>C (p.Ile552Thr)
COLB3708-3	<i>BRIP1</i>	c.1684A>G (p.Ile562Val)
COLB3729-3	<i>BRIP1</i>	c.1735C>T (p.Arg579Cys)
COLB2939-3	<i>BRIP1</i>	c.1831G>A (p.Val611Ile)
COLB3231-3	<i>BRIP1</i>	c.1871C>T (p.Ser624Leu)
COLB4975-3	<i>BRIP1</i>	c.2492+5G>A
COLB4405-3	<i>BRIP1</i>	c.2494T>G
COLB4405-3	<i>BRIP1</i>	c.2786T>C (p.Leu929Pro)
COLB5896-3	<i>BRIP1</i>	c.3149C>A (p.Thr1050Asn)
COLB5842-3	<i>BRIP1</i>	c.415T>G (p.Ser139Ala)
COLB4615-3	<i>BRIP1</i>	c.728T>C (p.Ile243Thr)
COLB5620-3	<i>BRIP1</i>	c.778A>G (p.Thr260Ala)
COLB3216-3	<i>BRIP1</i>	c.790C>T (p.Arg264Trp)
COLB3759-3	<i>BRIP1</i>	c.790C>T (p.Arg264Trp)
COLB5391-3	<i>BRIP1</i>	c.790C>T (p.Arg264Trp)
COLB5737-3	<i>BRIP1</i>	c.83T>A (p.Met28Lys)
COLB5692-3	<i>CDH1</i>	c.1115C>T (p.Pro372Leu)
COLB4889-3	<i>CDH1</i>	c.1138-3C>T
COLB3348-3	<i>CDH1</i>	c.1214A>G (p.Asn405Ser)
COLB3958-3	<i>CDH1</i>	c.1244T>C (p.Ile415Thr)
COLB4033-3	<i>CDH1</i>	c.164T>G (p.Val55Gly)
COLB2642-3	<i>CDH1</i>	c.2104G>A (p.Glu702Lys)
COLB5292-3	<i>CDH1</i>	c.2104G>A (p.Glu702Lys)
COLB5403-3	<i>CDH1</i>	c.2249A>G (p.Asp750Gly)
COLB3967-3	<i>CDH1</i>	c.2369C>T (p.Thr790Ile)
COLB5573-3	<i>CDH1</i>	c.2413G>A (p.Asp805Asn)
COLB5674-3	<i>CDH1</i>	c.2413G>A (p.Asp805Asn)
COLB5185-3	<i>CDH1</i>	c.2638G>A (p.Glu880Lys)
COLB5467-3	<i>CDH1</i>	c.394G>A (p.Val132Ile)
COLB3330-3	<i>CDH1</i>	c.655C>T (p.Pro219Ser)
COLB3195-3	<i>CDH1</i>	c.88C>A (p.Pro30Thr)
COLB5782-3	<i>CDH1</i>	c.88C>A (p.Pro30Thr)
COLB2720-3	<i>CDH1</i>	c.8C>G (p.Pro3Arg)
COLB2648-3	<i>CDK4</i>	c.122A>G (p.Asn41Ser)
COLB3318-3	<i>CDK4</i>	c.349A>G (p.Ile117Val)
COLB3958-3	<i>CDKN2A (p14ARF)</i>	c.217A>C (p.Ser73Arg)
COLB4426-3	<i>CDKN2A (p14ARF)</i>	c.268C>G (p.Arg90Gly)
COLB4943-3	<i>CDKN2A (p14ARF)</i>	c.316G>A (p.Gly106Arg)
COLB5797-3	<i>CDKN2A (p16)</i>	5'UTR-2G>A
COLB3123-3	<i>CDKN2A (p16)</i>	5'UTR-33G>C
COLB3618-3	<i>CDKN2A (p16)</i>	5'UTR-33G>C
COLB4039-3	<i>CDKN2A (p16)</i>	5'UTR-33G>C
COLB4327-3	<i>CDKN2A (p16)</i>	5'UTR-33G>C
COLB4297-3	<i>CDKN2A (p16)</i>	c.170C>T (p.A57V)
COLB5591-3	<i>CDKN2A (P16)</i>	c.206A>G (p.E69G)
COLB4243-3	<i>CDKN2A (p16)</i>	c.373G>C (p.D125H)
COLB3372-3	<i>CDKN2A (p16)</i>	c.89C>T (p.A30V)
COLB4531-2	<i>CHEK2</i>	c.1160C>G (p.Thr387Ser)
COLB2588-3	<i>CHEK2</i>	c.1183G>C (p.Val395Leu)

(continued on following page)

Table A4. Germline Variants of Uncertain Significance Detected by a 25-Gene Panel Among 1,058 Participants With Colorectal Cancer (continued)

Subject ID	Gene	HGVS Alteration Name
COLB5881-3	<i>CHEK2</i>	c.1283C>T (p.Ser428Phe)
COLB3213-3	<i>CHEK2</i>	c.1343T>G (p.Ile448Ser)
COLB4207-3	<i>CHEK2</i>	c.1343T>G (p.Ile448Ser)
COLB4522-3	<i>CHEK2</i>	c.1343T>G (p.Ile448Ser)
COLB4943-3	<i>CHEK2</i>	c.1343T>G (p.Ile448Ser)
COLB3192-3	<i>CHEK2</i>	c.1427C>T (p.Thr476Met)
COLB5647-3	<i>CHEK2</i>	c.1471A>T (p.Met491Leu)
COLB5585-3	<i>CHEK2</i>	c.14C>T (p.Ser5Leu)
COLB4003-3	<i>CHEK2</i>	c.1556G>A (p.Arg519Gln)
COLB2840-3	<i>CHEK2</i>	c.1582G>A (p.Glu528Lys)
COLB3312-3	<i>CHEK2</i>	c.190G>A (p.Glu64Lys)
COLB3059-3	<i>CHEK2</i>	c.305G>T (p.Gly102Val)
COLB3642-3	<i>CHEK2</i>	c.349A>G (p.Arg117Gly)
COLB3624-3	<i>CHEK2</i>	c.470T>C (p.Ile157Thr)
COLB3934-3	<i>CHEK2</i>	c.470T>C (p.Ile157Thr)
COLB4796-3	<i>CHEK2</i>	c.470T>C (p.Ile157Thr)
COLB5425-3	<i>CHEK2</i>	c.470T>C (p.Ile157Thr)
COLB5902-3	<i>CHEK2</i>	c.470T>C (p.Ile157Thr)
COLB5800-3	<i>CHEK2</i>	c.505T>A (p.Phe169Ile)
COLB2717-3	<i>CHEK2</i>	c.715G>A (p.Glu239Lys)
COLB5179-3	<i>CHEK2</i>	c.751A>T (p.Ile251Phe)
COLB5425-3	<i>CHEK2</i>	c.949A>G (p.Lys317Glu)
COLB5361-3	<i>CHEK2</i>	c.952C>T (p.Arg318Cys)
COLB4931-2	<i>CHEK2</i>	dup exons 2-14
COLB2921-3	<i>MLH1</i>	c.1202G>A (p.S401N)
COLB4866-3	<i>MLH1</i>	c.1253A>G (p.D418G)
COLB5425-3	<i>MLH1</i>	c.1273A>G (p.R425G)
COLB4651-3	<i>MLH1</i>	c.1731G>T (p.S577S)
COLB4597-3	<i>MLH1</i>	c.1850A>C (p.K617T)
COLB4689-3	<i>MLH1</i>	c.1976G>A (p.R659Q)
COLB5704-3	<i>MLH1</i>	c.1976G>A (p.R659Q)
COLB5749-3	<i>MLH1</i>	c.691A>G (p.I231V)
COLB3071-3	<i>MLH1</i>	c.790C>T (p.H264Y)
COLB3083-3	<i>MLH1</i>	c.861C>G (p.N287K)
COLB5533-3	<i>MLH1</i>	IVS10-18del4
COLB2669-3	<i>MLH1</i>	IVS16-2A>G
COLB4872-3	<i>MSH2</i>	5'UTR-3G>C
COLB4996-3	<i>MSH2</i>	5'UTR-3G>C
COLB5151-3	<i>MSH2</i>	c.116G>C (p.R39P)
COLB2675-3	<i>MSH2</i>	c.1172C>A (p.A391D)
COLB2951-3	<i>MSH2</i>	c.1223A>T (p.Y408F)
COLB2834-3	<i>MSH2</i>	c.1273G>A (p.E425K)
COLB5289-3	<i>MSH2</i>	c.1760G>A (p.G587D)
COLB3603-3	<i>MSH2</i>	c.1847C>G (p.P616R)
COLB4838-3	<i>MSH2</i>	c.247A>G (p.M83V)
COLB2807-3	<i>MSH2</i>	c.2726A>G (p.K909R)
COLB3810-3	<i>MSH2</i>	c.274C>G (p.L92V)
COLB3510-3	<i>MSH2</i>	c.79C>A (p.P27T)
COLB2924-2	<i>MSH6</i>	c.1054G>A (p.V352I)
COLB4297-3	<i>MSH6</i>	c.1239G>C (p.W413C)
COLB4079-3	<i>MSH6</i>	c.1844G>T (p.C615F)
COLB4456-3	<i>MSH6</i>	c.1844G>T (p.C615F)
COLB4351-3	<i>MSH6</i>	c.1988C>G (p.T663S)
COLB3417-3	<i>MSH6</i>	c.2425G>A (p.V809I)
COLB4872-3	<i>MSH6</i>	c.3107T>G (p.L1036R)
COLB4996-3	<i>MSH6</i>	c.3107T>G (p.L1036R)
COLB4495-3	<i>MSH6</i>	c.3386G>A (p.C1129Y)
COLB3507-3	<i>MSH6</i>	c.3787C>T (p.R1263C)
COLB3201-3	<i>MSH6</i>	c.3800T>C (p.M1267T)
COLB3651-3	<i>MSH6</i>	c.3800T>C (p.M1267T)
COLB4525-3	<i>MSH6</i>	c.383G>T (p.R128L)
COLB5277-3	<i>MSH6</i>	c.3850A>G (p.T1284A)
COLB5403-3	<i>MSH6</i>	c.4065ins4

(continued on following page)

Germline Mutations in Individuals With Colorectal Cancer

Table A4. Germline Variants of Uncertain Significance Detected by a 25-Gene Panel Among 1,058 Participants With Colorectal Cancer (continued)

Subject ID	Gene	HGVS Alteration Name
COLB2639-3	<i>MSH6</i>	c.4080ins4
COLB3216-3	<i>MSH6</i>	c.628G>T (p.V210L)
COLB4267-3	<i>MSH6</i>	c.643G>C (p.V215L)
COLB3056-3	<i>MSH6</i>	c.942C>G (p.S314R)
COLB2939-3	<i>MSH6</i>	IVS3-14T>G
COLB3867-3	<i>MSH6</i>	IVS8+5G>A
COLB2603-3	<i>MUTYH</i>	c.1234C>T (p.R412C)
COLB3171-3	<i>MUTYH</i>	c.1234C>T (p.R412C)
COLB3453-3	<i>MUTYH</i>	c.1234C>T (p.R412C)
COLB3834-3	<i>MUTYH</i>	c.1234C>T (p.R412C)
COLB4057-3	<i>MUTYH</i>	c.1234C>T (p.R412C)
COLB4210-3	<i>MUTYH</i>	c.1234C>T (p.R412C)
COLB4492-3	<i>MUTYH</i>	c.1234C>T (p.R412C)
COLB5074-3	<i>MUTYH</i>	c.1234C>T (p.R412C)
COLB5797-3	<i>MUTYH</i>	c.1234C>T (p.R412C)
COLB2972-3	<i>MUTYH</i>	c.1259C>T (p.T420M)
COLB4698-3	<i>MUTYH</i>	c.1351C>G (p.P451A)
COLB4805-3	<i>MUTYH</i>	c.1378C>T (p.R460C)
COLB4615-3	<i>MUTYH</i>	c.1525C>T (p.R509C)
COLB4543-3	<i>MUTYH</i>	c.203C>A (p.S68Y)
COLB4444-3	<i>MUTYH</i>	c.565C>T (p.R189C)
COLB3904-3	<i>MUTYH</i>	c.607C>T (p.R203C)
COLB3393-3	<i>MUTYH</i>	c.625A>G (p.I209V)
COLB5563-3	<i>MUTYH</i>	c.625A>G (p.I209V)
COLB2672-3	<i>MUTYH</i>	c.779G>A (p.R260Q)
COLB3312-3	<i>MUTYH</i>	c.779G>A (p.R260Q)
COLB2627-3	<i>MUTYH</i>	c.838C>T (p.R280C)
COLB4375-3	<i>MUTYH</i>	c.883C>T (p.R295C)
COLB4273-3	<i>MUTYH</i>	c.958C>G (p.P320A)
COLB3964-3	<i>MUTYH</i>	c.971del2insGC
COLB2993-3	<i>MUTYH</i>	IVS14-17C>G
COLB3747-3	<i>MUTYH</i>	IVS15-14C>G
COLB4177-3	<i>NBN</i>	c.1124+6G>T
COLB3357-3	<i>NBN</i>	c.1222A>G (p.Lys408Glu)
COLB5497-3	<i>NBN</i>	c.1222A>G (p.Lys408Glu)
COLB4273-3	<i>NBN</i>	c.1262T>C (p.Leu421Ser)
COLB5226-3	<i>NBN</i>	c.1465C>G (p.Leu489Val)
COLB4447-3	<i>NBN</i>	c.1690G>A (p.Glu564Lys)
COLB5340-3	<i>NBN</i>	c.1690G>A (p.Glu564Lys)
COLB2912-3	<i>NBN</i>	c.1720T>A (p.Leu574Ile)
COLB5194-3	<i>NBN</i>	c.1720T>A (p.Leu574Ile)
COLB5494-3	<i>NBN</i>	c.1720T>A (p.Leu574Ile)
COLB3717-3	<i>NBN</i>	c.1912T>C (p.Ser638Pro)
COLB3231-3	<i>NBN</i>	c.260T>C (p.Phe87Ser)
COLB3330-3	<i>NBN</i>	c.278C>T (p.Ser93Leu)
COLB3339-3	<i>NBN</i>	c.278C>T (p.Ser93Leu)
COLB2720-3	<i>NBN</i>	c.321-3C>T
COLB3486-3	<i>NBN</i>	c.415A>G (p.Thr139Ala)
COLB2957-3	<i>NBN</i>	c.456G>A (p.Met152Ile)
COLB3390-3	<i>NBN</i>	c.511A>G (p.Ile171Val)
COLB4042-3	<i>NBN</i>	c.511A>G (p.Ile171Val)
COLB4993-3	<i>NBN</i>	c.511A>G (p.Ile171Val)
COLB5229-3	<i>NBN</i>	c.511A>G (p.Ile171Val)
COLB2831-3	<i>NBN</i>	c.643C>T (p.Arg215Trp)
COLB3372-3	<i>NBN</i>	c.643C>T (p.Arg215Trp)
COLB3393-3	<i>NBN</i>	c.643C>T (p.Arg215Trp)
COLB3522-3	<i>NBN</i>	c.643C>T (p.Arg215Trp)
COLB3891-3	<i>NBN</i>	c.643C>T (p.Arg215Trp)
COLB4213-3	<i>NBN</i>	c.643C>T (p.Arg215Trp)
COLB4402-3	<i>NBN</i>	c.643C>T (p.Arg215Trp)
COLB5683-3	<i>NBN</i>	c.643C>T (p.Arg215Trp)
COLB5262-3	<i>NBN</i>	c.671G>A (p.Gly224Glu)
COLB5470-3	<i>NBN</i>	c.937G>T (p.Ala313Ser)

(continued on following page)

Table A4. Germline Variants of Uncertain Significance Detected by a 25-Gene Panel Among 1,058 Participants With Colorectal Cancer (continued)

Subject ID	Gene	HGVS Alteration Name
COLB4109-3	<i>NBN</i>	del exons 15-16
COLB4765-3	<i>PALB2</i>	c.1085T>G (p.L362R)
COLB4528-3	<i>PALB2</i>	c.1273G>A (p.V425M)
COLB2903-3	<i>PALB2</i>	c. ¹³ C>T (p.P5S)
COLB4925-3	<i>PALB2</i>	c.1454C>T (p.T485I)
COLB4799-3	<i>PALB2</i>	c.1637T>C (p.V546A)
COLB3708-3	<i>PALB2</i>	c.1742T>C (p.L581S)
COLB5391-3	<i>PALB2</i>	c.1987C>T (p.R663C)
COLB3474-3	<i>PALB2</i>	c.2422G>A (p.G808R)
COLB4339-3	<i>PALB2</i>	c.2743G>A (p.A915T)
COLB3023-3	<i>PALB2</i>	c.37G>A (p.E13K)
COLB2888-2	<i>PMS2</i>	5'UTR-7T>C
COLB4747-3	<i>PMS2</i>	5'UTR-7T>C
COLB5443-3	<i>PMS2</i>	5'UTR-7T>C
COLB4276-3	<i>PMS2</i>	c.1004A>G (p.N335S)
COLB3071-3	<i>PMS2</i>	c.1012C>G (p.P338A)
COLB5247-3	<i>PMS2</i>	c.1211C>G (p.P404R)
COLB4465-3	<i>PMS2</i>	c.1490G>A (p.G497D)
COLB5077-3	<i>PMS2</i>	c.1490G>A (p.G497D)
COLB5461-3	<i>PMS2</i>	c.1510G>C (p.E504Q)
COLB3243-3	<i>PMS2</i>	c.1567T>A (p.S523T)
COLB3861-3	<i>PMS2</i>	c.1610ins18
COLB5100-3	<i>PMS2</i>	c.1632C>A (p.D544E)
COLB4045-3	<i>PMS2</i>	c.1753C>A (p.L585I)
COLB3645-3	<i>PMS2</i>	c.2012C>T (p.T671M)
COLB5035-3	<i>PMS2</i>	c.2012C>T (p.T671M)
COLB4273-3	<i>PMS2</i>	c.2395C>T (p.R799W)
COLB4354-3	<i>PMS2</i>	c.2534A>T (p.H845L)
COLB3186-3	<i>PMS2</i>	c.320G>A (p.R107Q)
COLB5373-3	<i>PMS2</i>	c.487T>C (p.F163L)
COLB3489-3	<i>PMS2</i>	c.497T>C (p.L166P)
COLB5446-3	<i>PMS2</i>	c.497T>C (p.L166P)
COLB3369-3	<i>PMS2</i>	c.53T>C (p.I18T)
COLB3705-2	<i>PMS2</i>	c.53T>C (p.I18T)
COLB5226-3	<i>PMS2</i>	c.53T>C (p.I18T)
COLB3729-3	<i>PMS2</i>	c.86G>C (p.G29A)
COLB4863-3	<i>PMS2</i>	c.86G>C (p.G29A)
COLB3032-3	<i>PMS2</i>	c.994G>A (p.V332I)
COLB5869-3	<i>PMS2</i>	IVS4+6A>G
COLB4408-3	<i>PMS2</i>	IVS4-5C>G
COLB3459-3	<i>PTEN</i>	c.882T>G (p.Ser294Arg)
COLB4177-3	<i>RAD51C</i>	c.-19G>T
COLB4561-3	<i>RAD51C</i>	c.257C>T (p.Thr86Ile)
COLB5367-3	<i>RAD51C</i>	c.329T>G (p.Ile110Ser)
COLB3654-3	<i>RAD51C</i>	c.512A>G (p.Asp171Gly)
COLB4282-3	<i>RAD51C</i>	c.718A>T (p.Ile240Leu)
COLB3432-3	<i>RAD51C</i>	c.778C>T (p.Arg260Trp)
COLB4857-3	<i>RAD51C</i>	c.784T>G (p.Leu262Val)
COLB3375-3	<i>RAD51C</i>	c.790G>A (p.Gly264Ser)
COLB3534-3	<i>RAD51C</i>	c.790G>A (p.Gly264Ser)
COLB4405-3	<i>RAD51C</i>	c.790G>A (p.Gly264Ser)
COLB4683-3	<i>RAD51C</i>	c.790G>A (p.Gly264Ser)
COLB5538-3	<i>RAD51C</i>	c.790G>A (p.Gly264Ser)
COLB5185-3	<i>RAD51D</i>	c.196G>A (p.Val66Met)
COLB5340-3	<i>RAD51D</i>	c.196G>A (p.Val66Met)
COLB3931-3	<i>RAD51D</i>	c.208G>A (p.Asp70Asn)
COLB3720-3	<i>RAD51D</i>	c.26G>C (p.Cys9Ser)
COLB2609-3	<i>RAD51D</i>	c.433C>T (p.Arg145Cys)
COLB4336-3	<i>RAD51D</i>	c.739-3C>T
COLB4555-3	<i>SMAD4</i>	c.1123G>A (p.Ala375Thr)
COLB3504-3	<i>SMAD4</i>	c.1573A>G (p.Ile525Val)
COLB3846-3	<i>SMAD4</i>	c.1573A>G (p.Ile525Val)

(continued on following page)

Germline Mutations in Individuals With Colorectal Cancer

Table A4. Germline Variants of Uncertain Significance Detected by a 25-Gene Panel Among 1,058 Participants With Colorectal Cancer (continued)

Subject ID	Gene	HGVS Alteration Name
COLB4018-3	<i>SMAD4</i>	c.1573A>G (p.Ile525Val)
COLB5866-3	<i>SMAD4</i>	c.1573A>G (p.Ile525Val)
COLB5086-3	<i>SMAD4</i>	c.535A>G (p.Ile179Val)
COLB5050-3	<i>SMAD4</i>	c.566G>A (p.Arg189His)
COLB5345-3	<i>SMAD4</i>	c.677C>T (p.Ala226Val)
COLB5238-3	<i>SMAD4</i>	c.845A>C (p.His282Pro)
COLB2870-2	<i>SMAD4</i>	c.905-19_905-17del
COLB2711-3	<i>STK11</i>	c.1012G>A (p.Val338Met)
COLB2975-3	<i>STK11</i>	c.1180G>C (p.Gly394Arg)
COLB4492-3	<i>STK11</i>	c.1189G>T (p.Ala397Ser)
COLB3834-3	<i>STK11</i>	c.1211C>T (p.Ser404Phe)
COLB4423-3	<i>STK11</i>	c.1211C>T (p.Ser404Phe)
COLB5097-3	<i>STK11</i>	c.1211C>T (p.Ser404Phe)
COLB3005-3	<i>STK11</i>	c.291G>A (p.Lys97Lys)
COLB5185-3	<i>STK11</i>	c.465-19G>A
COLB4396-3	<i>STK11</i>	c.841C>A (p.Pro281Thr)
COLB3366-3	<i>STK11</i>	c.937C>T (p.His313Tyr)
COLB3684-3	<i>STK11</i>	c.991C>T (p.Arg331Trp)
COLB4880-3	<i>STK11</i>	c.991C>T (p.Arg331Trp)
COLB4423-3	<i>STK11</i>	c.992G>A (p.Arg331Gln)
COLB3141-3	<i>TP53</i>	c.1079G>C (p.Gly360Ala)
COLB4883-3	<i>TP53</i>	c.394A>G (p.Lys132Glu)
COLB3384-3	<i>TP53</i>	c.640C>A (p.His214Asn)
COLB4142-3	<i>TP53</i>	c.704A>G (p.Asn235Ser)
COLB5582-3	<i>TP53</i>	c.704A>G (p.Asn235Ser)
COLB4156-3	<i>TP53</i>	c.715A>G (p.Asn239Asp)
COLB5683-3	<i>TP53</i>	c.783-1G>A
COLB3780-3	<i>TP53</i>	c.839G>C (p.Arg280Thr)
COLB3177-3	<i>TP53</i>	c.847C>T (p.Arg283Cys)
COLB4654-3	<i>TP53</i>	c.847C>T (p.Arg283Cys)
COLB4756-3	<i>TP53</i>	c.869G>A (p.Arg290His)

Abbreviation: HGVS, Human Genome Variation Society

Table A5. Clinical Characteristics of Patients With MMR-D CRC and Germline VUS in DNA MMR Genes

Age, years/ sex	Tumor Site	Stage	Ethnicity	MSI Status	MMR IHC Results	KRAS Status	BRAF Status	MLH1 Promoter Hypermethylation	Other Personal History of Cancer (age at diagnosis, years)	Family History of Cancer (relation, age if known, years)	Germline Results
36/M	Cecum	IIA	Non-Hispanic white	MSI-H	MSH2/MSH6 intact; MLH1/PMS2 absent	Mutation (G13D)	Wild type	np	None	CRC (father, 45), NHL (grandmother, 77)	MLH1 c.1976G>A p.R659Q (VUS)
37/F	Cecum	IIIB	Non-Hispanic black	np	MSH2/MSH6 intact; MLH1/PMS2 absent	Wild type	np	np	None	LG (mat uncle, 69), UNK (grandmother)	PMS2 c.497T>C p.L166P (VUS)
41/F	Transverse colon	IIIB	Asian	np	MSH2/MSH6 intact; MLH1/PMS2 absent	Wild type	Wild type	np	None	PAN (father, 39), CRC (pat aunt, 40; pat uncle, 35; pat uncle, 40; pat uncle, 38), OV (pat cousin, 35)	MLH1 c.1731G>T p.S577S (VUS)
53/M	Ascending colon	IIA	Non-Hispanic white	np	MLH1/PMS2 intact; MSH2/MSH6 absent	Mutation (G13D)	Wild type	np	None	BR (mother), LEU (pat grandfather)	MSH6 c.1239G>C p.W413C (VUS) and CDKN2A c.170C>T p.A57V (VUS)
59/M*	Transverse colon (x2)	IIA	Non-Hispanic white	MSI-H	MLH1, MSH2, MSH6 intact; PMS2 absent	np	np	np	Synchronous transverse colon cancer; same MMR IHC pattern	CRC (father, 62; pat uncle, 60), BR (mother, 85), UNK (mat grandfather)	PMS2 c.2534A>T p.H845L (VUS) and APC I1307K
80/F	Ascending colon	IIA	Non-Hispanic white	np	MLH1 absent; MSH2, MSH6, PMS2 not tested	np	np	np	None	MM (father, 60), LG (son, 55)	PMS2 c.2395C>T p.R799W (VUS), MLTTH c.968C> G p.P320A (VUS), and NBN c.1262T>C p.L421S (VUS)

NOTE: Relatives (e.g., aunts, uncles, grandparents) were only listed as being from the proband's maternal or paternal lineage if this was specified in the medical record.

Abbreviations: BR, breast cancer; CRC, colorectal cancer; IHC, immunohistochemistry; LEU, leukemia; LG, lung cancer; mat, maternal; MM, multiple myeloma; MSI, microsatellite instability; MSI-H, high-level microsatellite instability; MMR, mismatch repair; MMR-D, mismatch repair deficient; NHL, non-Hodgkin lymphoma; np, not performed; OV, ovarian cancer; PAN, pancreatic cancer; pat, paternal; UNK, unknown cancer diagnosis; VUS, variant of uncertain significance.

*Participant also is listed in Table A2 because of the presence of a concurrent pathogenic moderate-penetrance germline mutation.