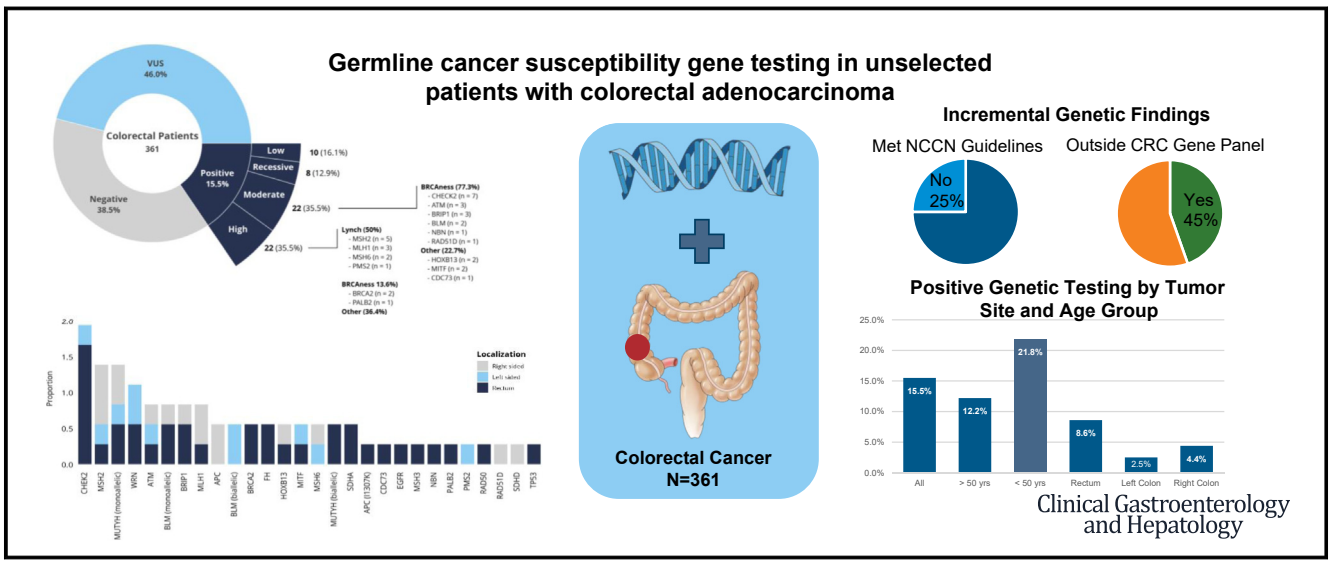
Germline Cancer Susceptibility Gene Testing in Unselected Patients With Colorectal Adenocarcinoma: A Multicenter Prospective Study

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BACKGROUND & AIMS: Hereditary factors play a role in the development of colorectal cancer (CRC). Identification of germline predisposition can have implications on treatment and cancer prevention. This study aimed to determine the prevalence of pathogenic germline variants (PGVs) in CRC patients using a universal testing approach, association with clinical outcomes, and the uptake of family variant testing.

METHODS: We performed a prospective multisite study of germline sequencing using a more than 80-gene next-generation sequencing platform among CRC patients (not selected for age or family history) receiving care at Mayo Clinic Cancer Centers between April 1, 2018, and March 31, 2020.

Abbreviations used in this paper: CRC, colorectal cancer; FVT, family variant testing; HRD, homologous recombination deficiency; INTERCEPT, Interrogating Cancer using Proactive genetic Testing; MMR, mismatch repair; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; OR, odds ratio; OS, overall survival; PARP, polyadenosine diphosphate ribose polymerase; PGV, pathogenic germline variant; VUS, variant of unknown significance.

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1542-3565/$36.00
https://doi.org/10.1016/j.cgh.2021.04.013

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RESULTS: **Of 361 patients, the median age was 57 years (SD, 12.4 y), 43.5% were female, 82% were white, and 38.2% had stage IV disease. PGVs were found in 15.5% (n = 56) of patients, including 44 in moderate- and high-penetrance cancer susceptibility genes. Thirty-four (9.4%) patients had incremental clinically actionable findings that would not have been detected by practice guideline criteria or a CRC-specific gene panel. Only younger age at diagnosis was associated with the presence of PGVs (odds ratio, 1.99; 95% CI, 1.12–3.56). After a median follow-up period of 20.7 months, no differences in overall survival were seen between those with or without a PGV ($P = .2$). Eleven percent of patients had modifications in their treatment based on genetic findings. Family cascade testing was low (16%).**

CONCLUSIONS: **Universal multigene panel testing in CRC was associated with a modest, but significant, detection of heritable mutations over guideline-based testing. One in 10 patients had changes in their management based on test results. Uptake of cascade family testing was low, which is a concerning observation that warrants further study.**

Keywords: Colorectal Cancer; Germline Testing; Lynch Syndrome; Homologous Recombination Deficiency.

Hereditary factors play a key role in the risk of developing several cancers including colorectal cancer (CRC).[1,2] Identification of genes related to the development or inheritance of cancer can have important implications in the selection of targeted treatments and in the screening of affected populations.[1,2] The prevalence of germline genomic alterations related to the development of colorectal carcinoma has been assessed in multiple studies with estimates of at least 10% of CRC patients. However, many of these studies have focused on registry populations, genetic testing company databases, high-risk cancer clinics, and the use of relatively small gene panels, which may have biased results or underestimated the true prevalence.[3–7] The emergence of targeted therapies, including the use of poly-adenosine diphosphate ribose polymerase (PARP) inhibitors for use in several tumors with germline homologous recombination deficiency (HRD) and the superior outcomes with anti-programmed cell death protein 1 pembrolizumab compared with chemotherapy in a randomized trial in metastatic microsatellite instability high CRC[2,8–11] has raised the question of whether more broad-based universal germline genetic testing has clinical implications in solid tumors.

We previously performed a prospective multisite study of germline genetic alterations among solid-tumor cancer patients at the Mayo Clinic Cancer Center (Rochester, MN; Jacksonville, FL; and Phoenix, AZ) and a community oncology practice (Eau Claire, WI) between April 1, 2018, and March 31, 2020. Patients were unselected for cancer type, stage of disease, family history of cancer, ethnicity, or age, and underwent germline testing using a more than 80-gene next-generation sequencing (NGS) platform. In this report, we evaluated only the patients with colorectal cancer recruited from the 3 Mayo Clinic Cancer Center sites (Rochester, MN; Jacksonville, FL; and Phoenix, AZ). We describe their clinical characteristics, prevalence, and the incremental yield of pathogenic genomic alterations, and the impact on clinical outcomes.

Methods

Patient Selection

From April 1, 2018, through March 31, 2020, there were 2984 adult (age, 18–85 y) patients with a new or active cancer diagnosis, confirmed pathologic diagnosis of carcinoma, seen in medical oncology, radiation oncology, dermatology, or surgical oncology clinics at any of the 3 Mayo Clinic destination Cancer Centers in Phoenix, AZ, Jacksonville, FL, or Rochester, MN, and English-speaking enrolled (Mayo Clinic Interrogating Cancer Etiology using Proactive Genetic Testing–Interrogating Cancer using Proactive genetic Testing [INTERCEPT] Program). Exclusion criteria were patients with hematologic malignancies and those undergoing surveillance after curative cancer. Patients were recruited using central lists of daily oncology clinic visits by research coordinators at each site. Patients were not selected based on stage of disease, family history of cancer, ethnicity, age at diagnosis, multifocal tumors, or personal history of multiple malignancies. Details of the INTERCEPT study and full cohort have been described previously.[12] The current study describes the cohort of 361 patients with a diagnosis of colorectal adenocarcinoma (2 cases were excluded from the original INTERCEPT cohort owing to a lack of complete medical records allowing for comprehensive in-house review of the pathologic diagnosis). Enrollment for each major cancer site was capped at approximately 300 to allow for sufficiently powered comparisons across cancer sites for the primary analysis.

All patients viewed a standardized pretest education video and were offered additional pretest genetic counseling if desired. Germline sequencing using an NGS panel of 83 genes (84 genes as of July 2019) on the Invitae Multi-Cancer panel was offered at no cost (Appendix 1). All test results, along with available pedigree information, were reviewed by a certified genetic counselor, disclosed to the patient, and those with pathogenic germline variants (PGVs) were invited for

What You Need to Know

Background

Hereditary factors play a role in the development of colorectal cancer (CRC). The identification of germline predisposition can have important implications on cancer prevention and possibly treatment

Findings

This was a multicenter prospective study of universal germline genetic testing in CRC patients not selected for age at diagnosis, cancer stage, or family history of cancer. Among 361 patients with CRC, 15.5% had pathogenic mutations identified, of which 25% would not have been detected using standard clinical criteria for genetics evaluation. Eleven percent of patients had modifications in their treatment based on genetic findings, however, there was no difference in overall survival between those with or without a pathogenic germline mutation

Implications for patient care

Our data support the use of broad universal multigene panel testing in CRC patients over a targeted approach based on clinical guidelines. One in 10 patients will have modifications to their clinical management plan based on the genetic findings. Cascade family variant testing is underused and can be improved to increase the potential for cancer prevention in the population.

genetic counseling. Patients with a variant of unknown significance (VUS) or negative test results were informed of their findings via electronic medical record portal message, telephone, or certified letter. Genetic counselors provided general cancer prevention screening recommendations based on family history if genetic test results were nondiagnostic.

Clinical, demographic, family history data, and pathologic information were collected on all patients from either medical records or self-administered electronic questionnaires. Race/ethnicity (ancestry) was determined by patient self-report. Family history information was collected using an electronic pedigree tool (CancerGene Connect, Invitae Labs, San Francisco, CA).

This study was approved by the Mayo Clinic Institutional Review Board (18-000326). All patients provided written informed consent. Data were de-identified except to investigators of the study.

Sequencing, Variant Calling, and Result Reporting

Full gene sequencing, deletion/duplication analysis, and variant interpretation were performed at Invitae (San Francisco, CA) as previously described (Appendix 2).[13–15] PGVs were classified as high (relative risk, >4), intermediate (relative risk, 2–4), or low (relative risk, <2) penetrance, or recessive medically actionable mutations.

Comparison of Guideline-Based Testing

The National Comprehensive Cancer Network (NCCN), National Society of Genetic Counselors, and American College of Medical Genetics guidelines (2018 and 2020) were used to determine whether genetic testing was indicated for a patient.[16] A PGV was considered incremental if it was detected based on the universal testing performed in this study and would not have been identified based on genetic testing/referral criteria of the 2018/2020 NCCN/National Society of Genetic Counselors/American College of Medical Genetics guidelines or was a gene outside those recommended on a guideline-based panel, such as the Invitae colorectal cancer guideline–based panel (*APC*, *AXIN2*, *BMPR1A*, *CHEK2*, *EPCAM*, *GREM1*, *MLH1*, *MSH2*, *MSH3*, *MSH6*, *MUTYH*, *NTHL1*, *PMS2*, *POLD1*, *POLE*, *PTEN*, *RPS20*, *SMAD4*, *STK11*, and *TP53*).

Family Variant Testing

Cascade family variant testing (FVT) at no cost was offered to all blood relatives of affected participants with PGV within a 90-day window of their finalized test result report. At the time of genetic counseling, patients were informed of the FVT program offered by Invitae and assisted in communicating this information to their relatives using a standardized template letter and an online video describing the risks and benefits of genetic testing.

Statistical Analysis

Demographic and clinical characteristics of the cohort are presented using descriptive statistics. The prevalence of PGV and VUS were reported in the cohort. Categoric variables were compared using the Pearson chi-square test. Rates of incremental findings were compared between subgroups by the Pearson chi-square test, and proportions of germline findings were compared by stage of disease using the chi-squared test. *P* values less than .05 were considered statistically significant. All statistical tests were 2-sided. Univariate logistic regression models were used to predict pathogenic germline mutation. Recurrence-free survival was considered the time from surgery to first disease recurrence and overall survival was considered the time from diagnosis to death or last date of follow-up evaluation.

Results

Cohort Characteristics

The distribution of sex, age, comorbidities, stage, and location of disease in these patients is shown in Table 1,

Table 1. Clinical and Demographic Characteristics of Included Patients

All	Arizona (N = 215)	Florida (N = 64)	Rochester (N = 82)	Total (N = 361)	*P* value
Sex					.665[a]
Male	123 (57.2%)	33 (51.6%)	48 (58.5%)	204 (56.5%)	
Female	92 (42.8%)	31 (48.4%)	34 (41.5%)	157 (43.5%)	
Age, *y*					.928[b]
Mean (SD)	56.9 (13.1)	56.3 (11.7)	56.4 (11.2)	56.7 (12.4)	
Median	58.0	56.0	52.0	57.0	
Range	24.0–80.0	33.0–79.0	27.0–78.0	24.0–80.0	
Race					.010[a]
White	167 (77.7%)	54 (84.4%)	75 (91.5%)	296 (82.0%)	
Hispanic/Latino	16 (7.4%)	0 (0.0%)	0 (0.0%)	16 (4.4%)	
Black/African American	6 (2.8%)	6 (9.4%)	3 (3.7%)	15 (4.2%)	
Asian	12 (5.6%)	2 (3.1%)	2 (2.4%)	16 (4.4%)	
American Indian/Alaskan Native	5 (2.3%)	0 (0.0%)	0 (0.0%)	5 (1.4%)	
Other	9 (4.2%)	2 (3.1%)	2 (2.4%)	13 (3.6%)	
Smoking					.635[a]
Yes	74 (34.4%)	26 (40.6%)	28 (34.1%)	128 (35.5%)	
No	141 (65.6%)	38 (59.4%)	54 (65.9%)	233 (64.5%)	
Body mass index ≥30					.013[a]
Yes	55 (25.6%)	17 (26.6%)	35 (42.7%)	107 (29.6%)	
No	160 (74.4%)	47 (73.4%)	47 (57.3%)	254 (70.4%)	
Diabetes mellitus					.201[a]
Yes	15 (7.0%)	8 (12.5%)	4 (4.9%)	27 (7.5%)	
No	200 (93.0%)	56 (87.5%)	78 (95.1%)	334 (92.5%)	
Hypertension					.350[a]
Yes	68 (31.6%)	26 (40.6%)	25 (30.5%)	119 (33.0%)	
No	147 (68.4%)	38 (59.4%)	57 (69.5%)	242 (67.0%)	
Family history of CRC in first-degree relative[c]					.242[a]
Yes	8 (3.7%)	5 (7.8%)	3 (3.7%)	16 (4.4%)	
No	102 (47.4%)	30 (46.9%)	30 (36.6%)	162 (44.9%)	
Unknown	105 (48.8%)	29 (45.3%)	49 (59.8%)	183 (50.7%)	
Clinical stage, AJCC 8th ed					.161[a]
I	8 (3.7%)	3 (4.7%)	8 (9.8%)	19 (5.3%)	
II	42 (19.5%)	15 (23.4%)	15 (18.3%)	72 (19.9%)	
III	85 (39.5%)	16 (25.0%)	31 (37.8%)	132 (36.6%)	
IV	80 (37.2%)	30 (46.9%)	28 (34.1%)	138 (38.2%)	
Tumor localization					.166[a]
Colon	133 (61.9%)	43 (67.2%)	43 (52.4%)	219 (60.7%)	
Rectal	82 (38.1%)	21 (32.8%)	39 (47.6%)	142 (39.3%)	
Colon					.545[a]
Right	57 (42.9%)	13 (30.2%)	19 (44.2%)	89 (40.6%)	
Transverse	6 (4.5%)	2 (4.7%)	3 (7.0%)	11 (5.0%)	
Left	70 (52.6%)	28 (65.1%)	21 (48.8%)	119 (54.3%)	
Rectal					.150[a]
Lower rectum	39 (47.6%)	4 (19.0%)	20 (51.3%)	63 (44.4%)	
Middle rectum	20 (24.4%)	8 (38.1%)	10 (25.6%)	38 (26.8%)	
Rectosigmoid junction	23 (28.0%)	9 (42.9%)	9 (23.1%)	41 (28.9%)	
Germline result					.807[a]
Positive	32 (14.9%)	13 (20.3%)	11 (13.4%)	56 (15.5%)	
Negative	84 (39.1%)	22 (34.4%)	33 (40.2%)	139 (38.5%)	
VUS	99 (46.0%)	29 (45.3%)	38 (46.3%)	166 (46.0%)	
Lynch MMR genes[d]					.876[a]
Yes	7 (3.3%)	2 (3.1%)	2 (2.4%)	11 (3.1%)	
HRD genes[e]					.230[a]
Yes	11 (5.1%)	5 (7.8%)	7 (8.3%)	23 (6.4%)	

Table 1. Continued

All	Arizona (N = 215)	Florida (N = 64)	Rochester (N = 82)	Total (N = 361)	*P* value
Deceased					.026[a]
Yes	36 (16.7%)	8 (12.5%)	4 (4.9%)	48 (13.3%)	
No	179 (83.3%)	56 (87.5%)	78 (95.1%)	313 (86.7%)	

AJCC, American Joint Committee on Cancer; CRC, colorectal cancer; HRD, homologous recombination deficiency; MMR, mismatch repair; SD, standard deviation; VUS, variant of unknown significance.
[a]Pearson chi-squared test.
[b]Linear model analysis of variance.
[c]Limited to participants with a pedigree available (N = 178).
[d]Lynch MMR genes are as follows: *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*.
[e]HRD genes are as follows: *ATM*, *BAP1*, *BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *NBN*, *PALB2*, *RAD50*, *RAD51C*, *RAD51D*, and *WRN*.

stratified by site of enrollment. The median age was 57 years at diagnosis, and 43.5% were female. Thirty-five percent of patients were ever smokers, 29.6% had a body mass index greater than 30, and 7.5% had type 2 diabetes. The proportion of patients with stages I, II, III, and IV disease at the time of enrollment were 5.3%, 19.9%, 36.6%, and 38.2%, respectively. The primary location of the tumor was rectal in 39.3%, and in the colon group 40.6% were right-sided. Race and ethnicity distributions included 4.4% Hispanic/Latino, 4.2% black/African American, and 4.4% Asian. Detailed pedigree information was available for 178 patients, for whom a family history of CRC in a first-degree relative was reported in 9.0% of participants. Clinical characteristics of the cohort stratified by age is shown in Supplementary Table 1.

Variant Detection and Clinical Outcomes

Of the 361 patients undergoing germline analysis, 56 patients (15.5%) harbored 62 pathogenic/likely pathogenic germline variants, of whom 5 patients had more than 1 PGV detected (Figure 1). PGVs could be stratified into those with high (n = 21), moderate (n = 21), or low (n = 7) penetrance, and 7 were carriers of variants associated with recessive syndromes (Supplementary Tables 2 and 8). The most common pathogenic variants were found in *CHEK2* (1.9%), *MSH2* (1.4%), and *MUTYH* (monoallelic) (1.4%). A molecular diagnosis of Lynch syndrome (PGV in *MSH2*, *MLH1*, *MSH6*, and *PMS2*) was confirmed in 11 (3.1%) patients, and 23 (6.4%) patients had a mutation in a gene associated with HRD (*ATM*, *BAP1*, *BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *NBN*,

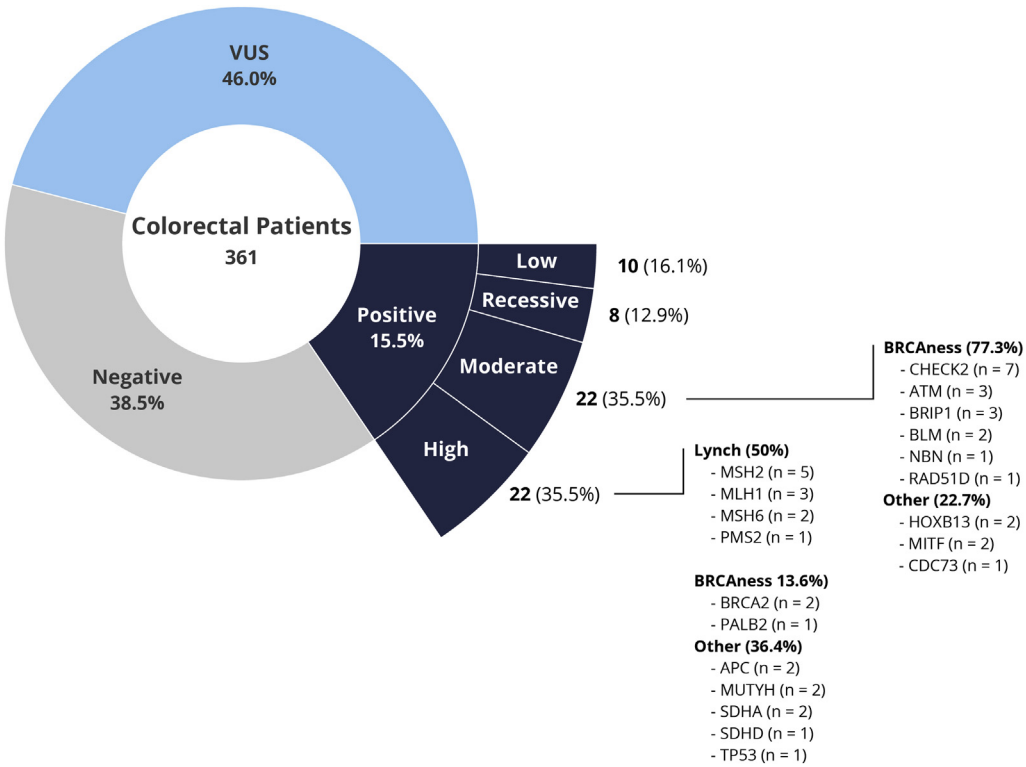


Figure 1. Distribution of germline testing results. VUS, variant of unknown significance.

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PALB2, *RAD50*, *RAD51C*, *RAD51D*, and *WRN*). More than half (54%) of Lynch syndrome cases were observed in patients with right-sided colon cancer. Figure 2 shows the distribution of PGVs by gene and tumor site. Table 2 shows the distribution of sex, age, ancestry, location, and stage stratified by mutation type. A higher proportion of patients with PGVs had no history of smoking, diabetes, or hypertension, and had a lower body mass index (<30). The rate of PGVs was higher in patients with rectal cancer than those with colon cancer ($P = .008$). The rate of PGVs in those less 50 years of age was 21.8% vs 12.2% in those ≥50 years of age ($P = .059$). Variants of uncertain significance were found in 166 patients (46%). The PGV rate by stage of cancer was 10.5% in stage I, 23.6% in stage II, 15.2% in stage III, and 12.3% in stage IV, and was not statistically different ($P = .168$). Those with a family history of CRC had a high rate of PGVs (31%). Nearly 15% of patients who had a PGV had no family history of CRC. Eighteen percent of the recruited population was of non-white race or ethnicity and the prevalence of PGVs was 12.3% and VUS was 55.4%. Figure 3 shows the specific type of PGV (deletion, insertion, duplication, or missense) listed by gene and tumor location. The majority of PGVs were missense mutations (67%), followed by deletion (24.2%), duplication (6.5%), and insertion (1.6%).

Overall survival (OS) was similar for those with and without a PGV ($P = .2$). Similarly, no differences in OS were noted in patients by tumor location (colon vs rectum) or presence of PGVs in Lynch or HRD genes. Early stages of disease (stages 1–3 vs 4) were associated with improved survival rates ($P < .001$). Kaplan–Meier plots for overall survival are shown in Supplementary Figure 1.

Logistic regression analysis showed that a younger age at diagnosis (age <50 y) was associated with an increased likelihood of having a PGV (odds ratio [OR], 1.99; 95% CI, 1.12–3.56), as was rectal (OR, 3.41; 95% CI, 1.61–7.92) and right colon (OR, 2.33; 95% CI, 0.99–5.75) location compared with left colon location. Sex, stage of cancer, family history of cancer in a first-degree relative, and CEA level were not associated with a higher likelihood of having a PGV (Table 3).

Application of Clinical Genetic Referral Criteria

Thirty-four cases had incremental clinically actionable findings that would not have been detected by phenotype or family history–based testing criteria using the 2018 NCCN guidelines (Supplementary Tables 3 and 4), or were outside of the genes recommended on a 20-gene panel of CRC-associated genes (Supplementary Table 5).[10] This represents 9.4% of the 361 cases overall and 60.7% of the 56 patients with PGV. Of the 34 patients with incremental findings, 8 (23.5%), 15 (44.1%), 5 (14.7%), and 6 (17.6%) patients carried high-, moderate-, or low-penetrance variants, and recessive alleles, respectively. Incremental mutations were found more often in patients with colorectal cancer diagnosed at an older age (≥50 vs <50 y; $P < .0017$) (Supplementary Table 6). Use of the 2020 NCCN guidelines resulted in the same 34 patients with incremental findings.

Clinical Implications of Pathogenic Germline Variants

Of the 56 patients found to have a PGV, 10.7% had clinically actionable management and treatment changes after detection of the PGV (Supplementary Table 7). These can be broadly categorized as need for surgery (n = 2) and targeted therapy (n = 4). One patient with a *CHEK2* mutation underwent surgical management involving hysterectomy and bilateral salpingo-oophorectomy, which was not consistent with guideline management, however, because of extenuating circumstances specific to the patient's tumor and treatment plan, it was performed after multidisciplinary discussion at the local tumor board.

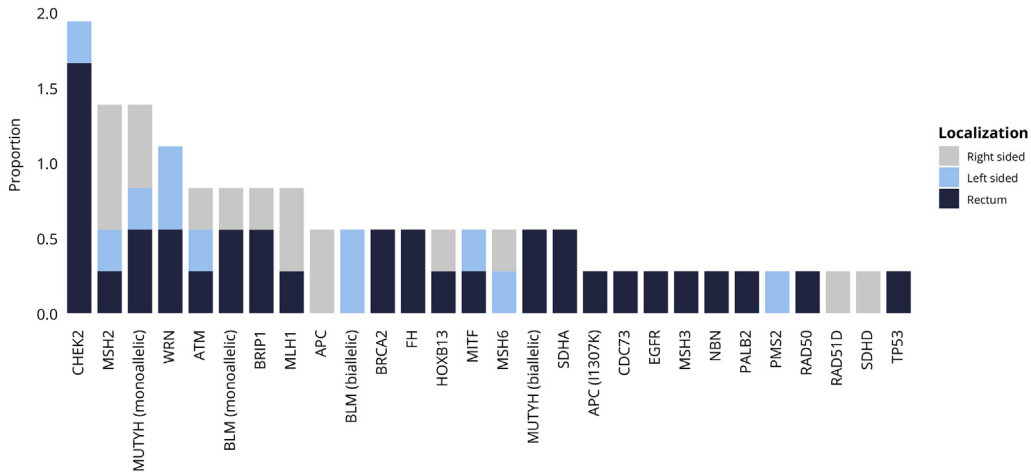


Figure 2. Distribution of pathogenic germline variants and localization of primary tumor.

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Table 2. Clinical and Demographic Characteristics Based on Germline Testing

	Positive (N = 56)	Negative (N = 139)	VUS (N = 166)	Total (N = 361)	*P* value
All patients	56 (15.5%)	139 (38.5%)	166 (46.0%)	361	
Sex					.824[a]
Male	33 (16.2%)	80 (39.2%)	91 (44.6%)	204	
Female	23 (14.6%)	59 (37.6%)	75 (47.8%)	157	
Age, *y*					.111[b]
Mean (SD)	53.5 (14.3)	57.3 (11.8)	57.2 (12.1)	56.7 (12.4)	
Median	50.5	58.0	57.5	57.0	
Range	24.0–80.0	24.0–79.0	31.0–80.0	24.0–80.0	
Race					.765[a]
White	48 (16.2%)	118 (39.9%)	130 (43.9%)	296	
Hispanic/Latino	3 (18.8%)	5 (31.2%)	8 (50.0%)	16	
Black/African American	1 (6.7%)	3 (20.0%)	11 (73.3%)	15	
Asian	2 (12.5%)	6 (37.5%)	8 (50.0%)	16	
American Indian/Alaskan Native	0 (0.0%)	2 (40.0%)	3 (60.0%)	5	
Other	2 (15.4%)	5 (38.5%)	6 (46.2%)	13	
Family history of CRC in first-degree relative[c]					.383[a]
Yes	5 (31.2%)	6 (37.5%)	5 (31.2%)	16	
No	24 (14.8%)	66 (40.7%)	72 (44.4%)	162	
Unknown	27 (14.8%)	67 (36.6%)	89 (48.6%)	183	
Clinical stage, AJCC 8th ed					.237[a]
I	2 (10.5%)	10 (52.6%)	7 (36.8%)	19	
II	17 (23.6%)	27 (37.5%)	28 (38.9%)	72	
III	20 (15.2%)	53 (40.2%)	59 (44.7%)	132	
IV	17 (12.3%)	49 (35.5%)	72 (52.2%)	138	
Smoking					.827[a]
Yes	19 (14.8%)	52 (40.6%)	57 (44.5%)	128	
No	37 (15.9%)	87 (37.3%)	109 (46.8%)	233	
Body mass index ≥30					.100[a]
Yes	11 (10.3%)	39 (36.4%)	57 (53.3%)	107	
No	45 (17.7%)	100 (39.4%)	109 (42.9%)	254	
Diabetes mellitus					.818[a]
Yes	5 (18.5%)	9 (33.3%)	13 (48.1%)	27	
No	51 (15.3%)	130 (38.9%)	153 (45.8%)	334	
Hypertension					.673[a]
Yes	19 (16.0%)	42 (35.3%)	58 (48.7%)	119	
No	37 (15.3%)	97 (40.1%)	108 (44.6%)	242	
Tumor localization					.016[a]
Colon	25 (11.4%)	84 (38.4%)	110 (50.2%)	219	
Rectal	31 (21.8%)	55 (38.7%)	56 (39.4%)	142	
Colon					.142[a]
Right	15 (16.9%)	32 (36.0%)	42 (47.2%)	89	
Transverse	1 (9.1%)	2 (18.2%)	8 (72.7%)	11	
Left	9 (7.6%)	50 (42.0%)	60 (50.4%)	119	
Rectal					.223[a]
Lower rectum	17 (27.0%)	27 (42.9%)	19 (30.2%)	63	
Middle rectum	8 (21.1%)	11 (28.9%)	19 (50.0%)	38	
Rectosigmoid junction	6 (14.6%)	17 (41.5%)	18 (43.9%)	41	
CEA, *ng/mL*					.454[a]
≤5	33 (16.6%)	73 (36.7%)	93 (46.7%)	199	
>5	17 (12.0%)	58 (40.8%)	67 (47.2%)	142	
Missing	6	8	6	20	
Overall survival, *d*					.289[b]
Mean (SD)	799.2 (555.6)	977.1 (1085.3)	835.2 (810.3)	884.2 (897.4)	
Median	655.5	627.0	614.5	630.0	
Range	55.0–2621.0	6.0–6328.0	50.0–5996.0	6.0–6328.0	

Table 2. Continued

	Positive (N = 56)	Negative (N = 139)	VUS (N = 166)	Total (N = 361)	*P* value
Recurrence-free survival, *d*					.140[b]
Mean (SD)	648.8 (494.2)	1054.8 (1355.0)	875.6 (918.7)	907.7 (1072.1)	
Median	573.5	481.0	473.0	489.0	
Range	55.0–1912.0	8.0–6328.0	3.0–4030.0	3.0–6328.0	

AJCC, American Joint Committee on Cancer; CRC, colorectal cancer; SD, standard deviation; VUS, variant of unknown significance.
[a]Pearson chi-squared test.
[b]Linear model analysis of variance.
[c]Limited to participants with a pedigree available (N = 178).

Family Variant Cascade Testing

No-cost FVT was offered to all blood relatives of affected participants. Only 9 (16%) patients with PGVs had family members undergo FVT within a 3-month window of their test result. The median number of family members tested was 2.0, with a range of 1 to 4. Almost half (46.4%) of family members tested were relatives of patients with stage IV disease.

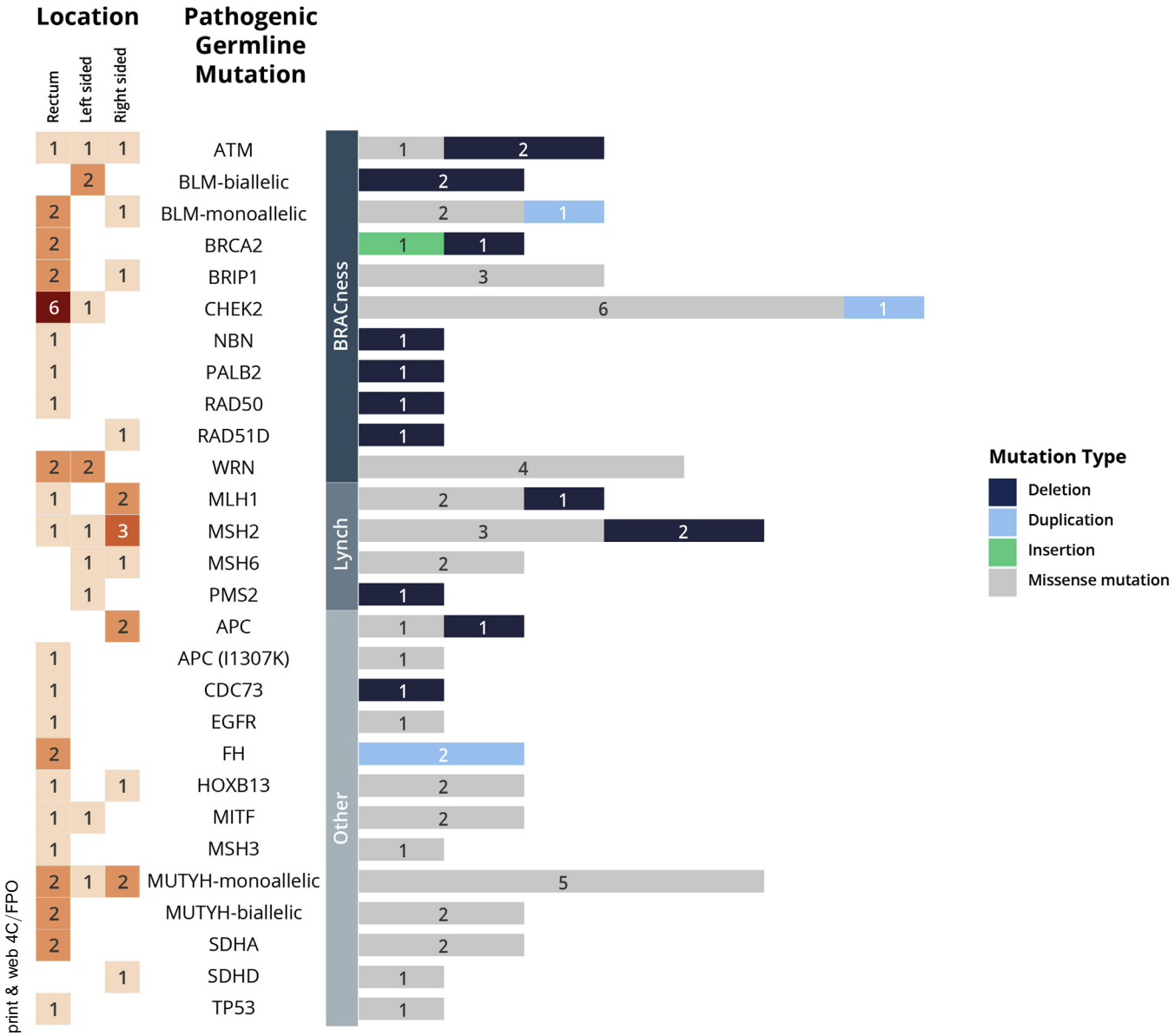


Figure 3. Type of mutations found in pathogenic germline variant cases.

Table 3. Logistic Regression ORs and 95% CIs of Patient and Tumor Predictors of Pathogenic/Likely Pathogenic Mutation

Characteristic	OR	95% CI	*P*
Age group, *y*			
≥50	**1.0 (ref)**		
<50	**1.996**	**1.12–3.56**	**.019**
Sex			
Male	1.0 (ref)		
Female	0.89	0.49–1.58	.691
Cancer stage, AJCC 8th ed			
Stage I	1.0 (ref)		
Stage II	2.63	0.66–17.62	.226
Stage III	1.52	0.39–10.04	.595
Stage IV	1.19	0.30–7.95	.822
Dichotomize cancer stage			
Early (stages 0–3)	1.0 (ref)		
Advanced (stage 4)	0.66	0.35–1.21	.189
Family history of cancer in first-degree relative			
No	1.0 (ref)		
Yes	1.60	0.90–2.89	.109
Tumor location			
Colon	**1.0 (ref)**		
Rectum	**2.17**	**1.22–3.88**	**.009**
Tumor location			
Left colon	**(ref)**		
Right/transverse colon	**2.33**	**0.99–5.75**	**.055**
Rectum	**3.41**	**1.61–7.92**	**.002**
CEA, *ng/mL*			
<5	1.0 (ref)		
≥5	0.68	0.36–1.27	.237

AJCC, American Joint Committee on Cancer; CI, confidence interval; OR, odds ratio.

Discussion

In this multisite prospective study of 361 unselected CRC patients, universal germline genetic testing found that approximately 1 in 6 CRC patients (15.5%) harbor a PGV of which more than 50% would not have been detected using standard practice guidelines or a guideline-specific gene panel. No differences in disease-specific outcomes were observed among patients with or without a PGV. Nearly 10% of patients had modifications in their treatment based on the findings, and cascade testing was underused even when cost was not a barrier.

The prevalence of pathogenic germline mutations in CRC has ranged between 5% and 18% in the literature.[1,3,5,17] The difference in the prevalence of germline PGV could be owing to many factors. First, the median age of patients can directly influence the percentage of positive results; a retrospective analysis of 430 patients younger than age 50 years resulted in a PGV rate of 18%.[17] Similarly, in our study, 22% of patients younger than age 50 years were detected with a PGV. Second, the number of genes evaluated in the panel can omit genes that are not associated commonly with colon malignancy/polyposis. Germline testing using a panel of 25 genes was evaluated in 1058 unselected patients with CRC and found nearly 10% had a PGV.[3] Finally, prior retrospective studies can suffer from selection bias, considering that germline testing would be more common in patients with a family history of cancer, younger age, and recruitment bias from a cancer genetics clinic or research registry. These are inherent strengths of our study, including prospective recruitment from multiple clinics and cancer centers, and additionally the use of a broader NGS panel.

Equally important to the discovery of a PGV in a CRC patient is the potential to share these findings with their relatives. Targeted testing may allow for earlier disease detection and prevention in those who are positive for the familial mutation. Family cascade testing remained low (16%) even though it was available at no cost. This result is consistent with several other studies that have found low adherence to cascade family testing. In a study conducted in Singapore in 183 patients, the uptake of free cascade testing was 21.6%.[18] A low rate of family testing also was observed in 2 studies examining patients with hereditary gynecologic cancer and Lynch syndrome.[19,20] Although the majority (87%) of patients were comfortable sharing genetic test results with close relatives, only 40% of first-degree relatives underwent genetic testing.[19] An online approach to low-cost cascade testing also was impacted by low rates of participation.[21] There likely are several factors associated with the low uptake outside of financial barriers, including poor understanding of the importance of testing or difficulties interpreting the reports, distance between relatives, family dynamics including communication barriers, shortage of genetics professions and long wait times, variable insurance coverage for genetic counseling, other social/economic restraints, fear of discrimination, and fear of eventual procedures related to the findings. The reliance on patient-driven cascade testing as a result of privacy laws with little support and follow-up evaluation from providers likely is a major barrier in the United States. To help support and standardize the disclosure of results to relatives, clinician-provided education material such as letters, brochures, websites, and videos can be considered.[19] In addition, providing patients with an annotated copy of their family tree indicating which members should receive genetic testing information may help ensure this information is shared with those who may benefit.[20] Finally, options that empower the clinician or testing laboratory to reach out to relatives directly may be fruitful,[21] however, this is contrary to current US privacy laws. The future also may include artificial intelligence–powered, Health Insurance Portability and Accountability Act–compliant chatbots to screen for heritable CRC syndromes and engage family members to increase cascade testing uptake.

A total of 11 (3.1%) patients had PGV in mismatch repair genes (MMR), confirming a molecular diagnosis of Lynch syndrome. Most of these patients presented with right-sided colon cancer (54%). The prevalence of Lynch syndrome in Western and Eastern populations ranges between 1% and 5% and is similar to our study.[22,23] Interestingly, 6.4% of patients with CRC had a mutation in genes related to HRD, with a higher prevalence seen in patients with rectal cancer (70%). In our cohort, we were able to evaluate disease outcomes including overall survival based on the presence of PGV and more specifically the type of mutation (HRD-associated gene or Lynch syndrome). No differences in OS were observed between patients with or without a PGV, including in those with MMR or HRD-associated gene mutations. Some studies have found that patients with advanced CRC carrying germline mutations on HRD genes were associated with better disease outcomes or survival.[24,25] The interpretation of these results should be taken with caution because a longer follow-up period likely is required because the vast majority of our cohort did not experience disease recurrence or death.

The discovery of PGV in a CRC patient will have therapeutic implications. For example, the presence of a MMR may help guide treatment decision making for the use of programmed cell death protein 1/PDL1 inhibitors in those with metastatic CRC.[2] Pembrolizumab improved the response rate and progression-free survival compared with chemotherapy in metastatic microsatellite instability high CRC in first-line therapy, in a phase III trial, with fewer treatment-associated adverse events.[2] Moreover, HRD-associated gene defects as a biomarker may have therapeutic implications including the use of topoisomerase inhibitors and PARP inhibitors.[5,26–28] There have been recent reports of metastatic CRC patients refractory to standard-line chemotherapy who harbor germline mutations in DNA damage response genes—including *CHEK2* and *ATM*—showing preliminary clinical response to PARP inhibitors and platinum therapy.[26,29] Currently, multiple tumor agnostic trials including solid tumors with mutations in genes related to DNA damage response are being developed (NCT04276376, NCT04657068, and NCT04564027). In our cohort of 58 CRC patients with P/LP variants, 35 (60%) potentially were eligible for approved precision therapy and/or clinical treatment trials. We identified nearly 11% of CRC patients with PGV in whom clinical management and treatment changes were made based on the detected PGV, including 7% who were started on targeted therapies or clinical trials. This is consistent with a prior study from New York that reported discussion of initiation of targeted therapy in 18% of their advanced cancer patients with PGV.[12] In addition to phenotypic characteristics (which may be muted in some patients) and tumor testing results, universal genetic testing will allow us to ensure all cancer patients who qualify for targeted therapy are identified. Multidisciplinary care between surgeons, oncologists, and geneticists is critical to ensure guideline-adherent modifications in care are recommended.

This study had limitations including the demographic characteristics of patients seen at the multiple Mayo Clinic sites participating, which may not completely reflect those in other regions of the country or other countries.[30] Family history of cancer was obtained through patient self-report or review of medical records and was not complete. Because of the relatively short follow-up period, most patients are still alive, liming the utility of survival analysis to address outcomes related to PGV in the MMR and HRD genes and response to targeted therapies. The study was not able to track if blood relatives underwent cascade testing outside of Invitae Laboratories or if they underwent full panel testing at another laboratory separately. The interpretation of the study and applicability of the results in wider heterogeneous populations should be taken with caution. Furthermore, long-term follow-up evaluation has not been conducted and will be necessary to address the real impact of germline PGV in disease-specific outcomes, on cancer decision making, and morbidity of screening and other invasive procedures.

Conclusions

In this multisite prospective cohort study of patients diagnosed with CRC, unselected for family cancer history, we found that universal multigene panel testing was associated with an increased detection of clinically actionable, heritable mutations over that predicted based on clinical guidelines. Ten percent of patients with PGV had modifications in their treatment. Family variant testing remained low, although the financial barrier of such testing was not present in this study, suggesting the importance of nonfinancial barriers to this process. In addition to Lynch syndrome, more than 6% of patients had mutations in genes related to homologous recombination repair, particularly in rectal tumors, suggesting the possibility of additional therapeutic trials for those with advanced cancer. The application of broader germline testing in CRC patients is feasible and can add valuable insights into the development of personalized treatment strategies.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2021.04.013.

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Acknowledgments
The authors thank the following persons for their assistance with this project: Sydney Welp, Jessie Fox, Plush Gutierrez, Sara Hernandez, Sharon Levy, Eric Nelson, Anne Bofferding, Arta Palaj, Lorelei Bandel, Megan Mulcahy, Rachel Colburn, and David Upjohn.

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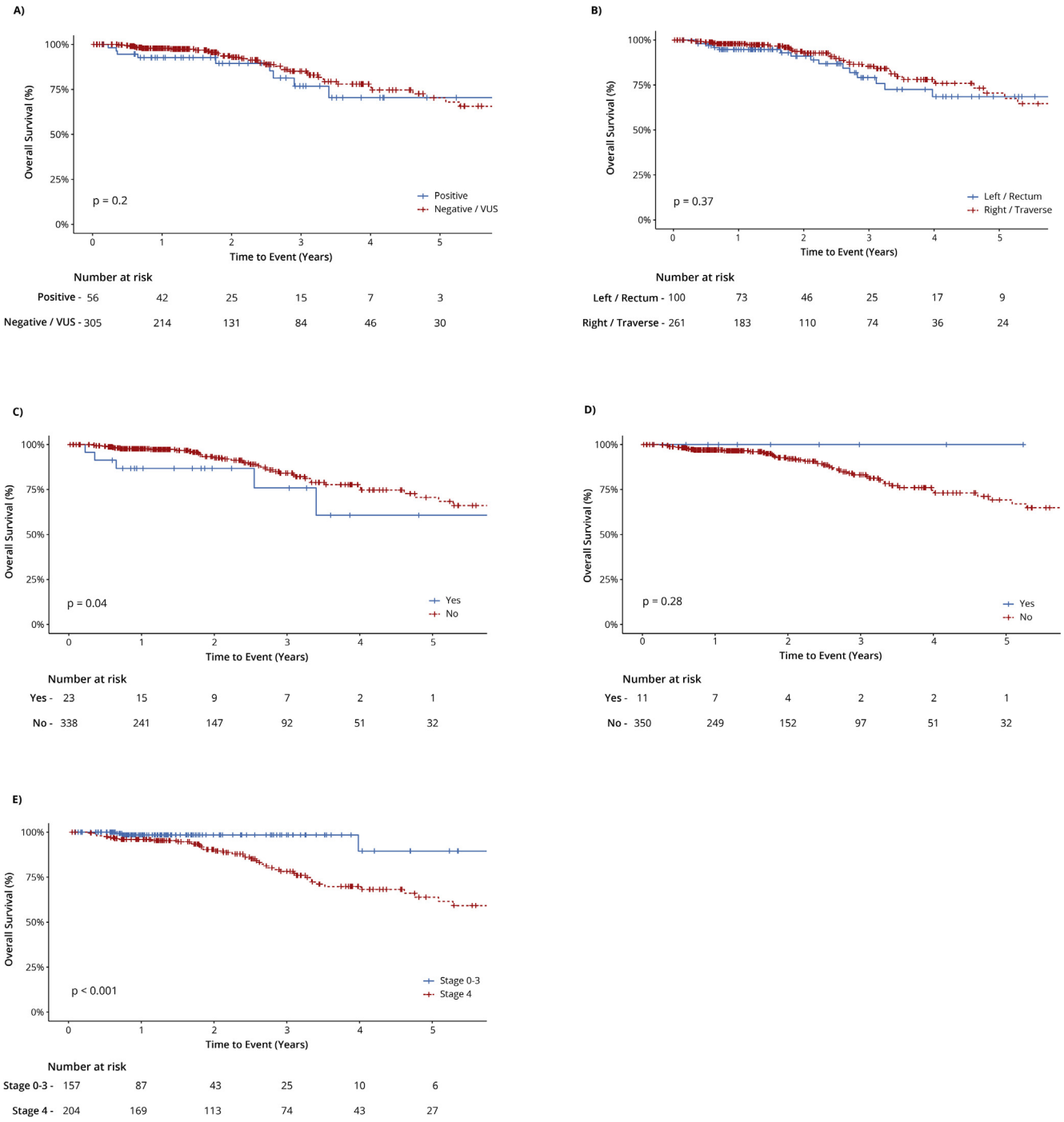
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Conflicts of interest

These authors disclose the following: N. Jewel Samadder is a consultant for Jansen Research, Recursion Pharmaceuticals, and Cancer Prevention Pharmaceuticals; and E. D. Esplin and R. L. Nussbaum are employees and stockholders of Invitae. The remaining authors disclose no conflicts.

Funding

Support for this project was provided by the Mayo Transform the Practice Grant, the Mayo Clinic Center for Individualized Medicine, Desert Mountain Members' Care Foundation, David and Twila Woods Foundation, and a Faculty Career Development Award from the Gerstner Foundation (N.J.S.). The funding sources did not play a role in the design, conduct, or reporting of the study or in the decision to submit the manuscript for publication.



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Supplementary Figure 1. Kaplan–Meier plots for overall survival. (*A*) Gene mutation type: pathogenic germline variants (PGV) vs negative/variant of unknown significance (VUS); (*B*) tumor location: left/rectum vs right; (*C*) homologous recombination deficiency gene status: PGV/VUS vs none; (*D*) Lynch mismatch repair (MMR) gene status: PGV/VUS vs none; and (*E*) cancer stages: 1 to 3 vs 4.

Supplementary Table 1. Clinical and Demographic Characteristics of Included Patients, Stratified by Age of CRC Diagnosis

	Age <50 y (N = 124)	Age ≥50 y (N = 237)	All CRC (N = 361)	*P* value
Sex				.257[a]
Male	65 (52.4%)	139 (58.6%)	204 (56.5%)	
Female	59 (47.6%)	98 (41.4%)	157 (43.5%)	
Age, *y*				<.001[b]
Mean (SD)	43.2 (5.9)	63.7 (8.4)	56.7 (12.4)	
Median	45.0	64.0	57.0	
Range	24.0–49.0	50.0–80.0	24.0–80.0	
Race (grouped)				.575[a]
White	98 (79.0%)	198 (83.5%)	296 (82.0%)	
Hispanic/Latino	6 (4.8%)	10 (4.2%)	16 (4.4%)	
Black/African American	8 (6.5%)	7 (3.0%)	15 (4.2%)	
Asian	7 (5.6%)	9 (3.8%)	16 (4.4%)	
American Indian/Alaskan Native	1 (0.8%)	4 (1.7%)	5 (1.4%)	
Other	4 (3.2%)	9 (3.8%)	13 (3.6%)	
Smoking				<.001[a]
Yes	27 (21.8%)	101 (42.6%)	128 (35.5%)	
No	97 (78.2%)	136 (57.4%)	233 (64.5%)	
Body mass index >30				.586[a]
Yes	39 (31.5%)	68 (28.7%)	107 (29.6%)	
No	85 (68.5%)	169 (71.3%)	254 (70.4%)	
Diabetes mellitus				<.001[a]
Yes	1 (0.8%)	26 (11.0%)	27 (7.5%)	
No	123 (99.2%)	211 (89.0%)	334 (92.5%)	
Hypertension				<.001[a]
Yes	21 (16.9%)	98 (41.4%)	119 (33.0%)	
No	103 (83.1%)	139 (58.6%)	242 (67.0%)	
Family history of CRC in first-degree relative[c]				.960[a]
Yes	6 (4.8%)	10 (4.2%)	16 (4.4%)	
No	55 (44.4%)	107 (45.1%)	162 (44.9%)	
Unknown	63 (50.8%)	120 (50.6%)	183 (50.7%)	
Clinical stage: AJCC 8th ed at diagnosis				.040[a]
I	1 (0.8%)	18 (7.6%)	19 (5.3%)	
II	26 (21.0%)	46 (19.4%)	72 (19.9%)	
III	44 (35.5%)	88 (37.1%)	132 (36.6%)	
IV	53 (42.7%)	85 (35.9%)	138 (38.2%)	
Tumor localization				.158[a]
Colon	69 (55.6%)	150 (63.3%)	219 (60.7%)	
Rectal	55 (44.4%)	87 (36.7%)	142 (39.3%)	
Colon				.162[a]
Right	24 (34.8%)	65 (43.3%)	89 (40.6%)	
Traverse	6 (8.7%)	5 (3.3%)	11 (5.0%)	
Left	39 (56.5%)	80 (53.3%)	119 (54.3%)	
Rectal				.189[a]
Lower rectum	20 (36.4%)	43 (49.4%)	63 (44.4%)	
Middle rectum	19 (34.5%)	19 (21.8%)	38 (26.8%)	
Rectosigmoid junction	16 (29.1%)	25 (28.7%)	41 (28.9%)	
Germline result				.059[a]
Positive	27 (21.8%)	29 (12.2%)	56 (15.5%)	
Negative	44 (35.5%)	95 (40.1%)	139 (38.5%)	
VUS	53 (42.7%)	113 (47.7%)	166 (46.0%)	
Lynch MMR genes[d]				.002[a]
Yes	10 (8.1%)	1 (0.4%)	11 (3.1%)	
HRD genes[e]				.093[a]
Yes	8 (6.5%)	15 (6.3%)	23 (6.4%)	

Supplementary Table 1. Continued

	Age <50 y (N = 124)	Age ≥50 y (N = 237)	All CRC (N = 361)	*P* value
Deceased				.015[a]
Yes	9 (7.3%)	39 (16.5%)	48 (13.3%)	
No	115 (92.7%)	198 (83.5%)	313 (86.7%)	

AJCC, American Joint Committee on Cancer; CRC, colorectal cancer; HRD, homologous recombination deficiency; MMR, mismatch repair; VUS, variant of unknown significance.
[a]Pearson chi-squared test.
[b]Linear model analysis of variance.
[c]Limited to participants with a pedigree available (N = 178).
[d]Lynch MMR genes are as follows: *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*.
[e]HRD genes are as follows: *ATM*, *BAP1*, *BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *NBN*, *PALB2*, *RAD50*, *RAD51C*, *RAD51D*, and *WRN*.

Supplementary Table 2. Distribution of the 62 PGV by Penetrance Status

	Positive gene	Total (n = 62)
High penetrance	*APC*	2 (3.2%)
	BRCA2	2 (3.2%)
	MLH1	3 (4.8%)
	MSH2	5 (8.1%)
	MSH6	2 (3.2%)
	MUTYH-biallelic	2 (3.2%)
	PALB2	1 (1.6%)
	PMS2	1 (1.6%)
	SDHA	2 (3.2%)
	SDHD	1 (1.6%)
	TP53	1 (1.6%)
Moderate penetrance	*ATM*	3 (4.8%)
	BLM-biallelic	2 (3.2%)
	BRIP1	3 (4.8%)
	CDC73	1 (1.6%)
	CHEK2	7 (11.3%)
	HOXB13	2 (3.2%)
	MITF	2 (3.2%)
	NBN	1 (1.6%)
	RAD51D	1 (1.6%)
Low penetrance	*APC* (I1307K)	1 (1.6%)
	EGFR	1 (1.6%)
	MUTYH-monoallelic	5 (8.1%)
	RAD50	1 (1.6%)
Recessive	*BLM*-monoallelic	3 (4.8%)
	FH	2 (3.2%)
	MSH3	1 (1.6%)
	WRN	4 (6.5%)

PGV, pathogenic germline variant.

Supplementary Table 3. Incidence of Findings Not Predicted by 2020 Clinical Guidelines: Incremental Findings

	Patients with PGV (N = 56)
Did they meet 2020 NCCN/NSGC/ACMG testing guidelines?	
Yes	42 (75.0%)
NCCN	42
NSGC/ACMG	35
No	**14 (25.0%)**
Was the PGV outside of the genes on the colorectal cancer guideline–based gene panel?[a]	
Yes	**25 (44.6%)**
No	31 (55.4%)

ACMG, American College of Medical Genetics; NCCN, National Comprehensive Cancer Network; NSGC, National Society of Genetic Counselors; PGV, pathogenic germline variant.
[a]Colorectal cancer guideline panel genes included *APC*, *AXIN2*, *BMPR1A*, *CHEK2*, *EPCAM*, *GREM1*, *MLH1*, *MSH2*, *MSH3*, *MSH6*, *MUTYH*, *NTHL1*, *PMS2*, *POLD1*, *POLE*, *PTEN*, *RPS20*, *SMAD4*, *STK11*, and *TP53*.

Supplementary Table 4. Of Those Who Met a Screening Guideline, Which Guidelines Were Met?

	2018 (N = 42)	2020 (N = 42)	*P* value
NCCN			
No	0 (0.0%)	0 (0.0%)	
Yes	42 (100.0%)	42 (100.0%)	
NSGC/ACMG			.763
No	7 (16.7%)	6 (14.3%)	
Yes	35 (83.3%)	36 (85.7%)	

ACMG, American College of Medical Genetics; NCCN, National Comprehensive Cancer Network; NSGC, National Society of Genetic Counselors.

Supplementary Table 5. Description of the CRC Cases That Were Incremental PGV Using Guidelines and Guideline-Based Gene Panel

Case	Age, *y*	Sex	Tumor location	Stage	Gene	Incremental PGV	Did they meet 2018 NCCN/ NSGC/ACMG testing guidelines	Was the PGV outside of the genes included on a guideline based panel?[a]
1	40	Male	Rectum	4	*TSC2* (VUS), *CDC73* (positive)	**Yes**	Yes	Yes
2	44	Male	Rectum	3	*BRCA2* (positive), *BRCA1* (VUS), *WRN* (VUS)	**Yes**	Yes	Yes
3	73	Female	Right-sided	4	*RAD51D* (positive)	**Yes**	No	Yes
4	45	Female	Rectum	4	*BRIP1* (positive)	**Yes**	Yes	Yes
5	48	Male	Rectum	4	*BRCA2* (positive), *ATM* (VUS)	**Yes**	Yes	Yes
6	61	Male	Rectum	3	*MUTYH* (positive)	**Yes**	No	No
7	72	Male	Rectum	2	*CHEK2* (positive), *NF1* (VUS)	**Yes**	No	No
8	71	Male	Left-sided	4	*MITF* (positive), *BRCA2* (VUS), *NBN* (VUS), *POLD1* (VUS)	**Yes**	Yes	Yes
9	51	Male	Right-sided	4	*ATM* (positive)	**Yes**	Yes	Yes
10	56	Female	Rectum	3	*CHEK2* (positive), *BRIP1* (VUS)	**Yes**	No	No
11	79	Female	Rectum	3	*CHEK2* (positive), *POLE* (VUS)	**Yes**	No	No
12	72	Female	Rectum	3	*FH* (positive)	**Yes**	No	Yes
13	68	Female	Right-sided	2	*BRIP1* (positive), *AXIN2* (VUS)	**Yes**	No	Yes
14	47	Female	Rectum	4	*HOXB13* (increased risk allele), *AXIN2* (VUS), *PALB2* (VUS), *RECQL4* (VUS)	**Yes**	Yes	Yes
15	62	Male	Rectum	2	*WRN* (positive), *BRIP1* (VUS)	**Yes**	Yes	Yes
16	27	Male	Rectum	3	*RAD50* (positive), *MSH2* (positive)	**Yes**	Yes	Yes
17	76	Male	Right-sided	3	*HOXB13* (increased risk allele)	**Yes**	Yes	Yes
18	49	Female	Rectum	2	*PALB2* (positive)	**Yes**	Yes	Yes
19	63	Male	Left-sided	1	*WRN* (positive)	**Yes**	Yes	Yes
20	58	Male	Right-sided	3	*MUTYH* (positive), *WRN* (VUS)	**Yes**	No	No
21	77	Male	Left-sided	2	*CHEK2* (positive), *APC* (VUS)	**Yes**	No	No
22	49	Male	Rectum	4	*MUTYH* (positive), *FH* (positive), *CEBPA* (VUS)	**Yes**	Yes	Yes
23	48	Male	Left-sided	2	*PMS2* (positive), *WRN* (positive)	**Yes**	Yes	Yes
24	75	Female	Right-sided	2	*SDHD* (positive), *FLCN* (VUS)	**Yes**	Yes	Yes
25	59	Male	Rectum	2	*NBN* (positive), *BMPR1A* (VUS)	**Yes**	No	Yes
26	61	Female	Right-sided	3	*BLM* (positive)	**Yes**	No	Preliminary evidence
27	62	Female	Rectum	2	*MITF* (positive)	**Yes**	Yes	Yes
28	46	Male	Rectum	4	*SDHA* (positive)	**Yes**	Yes	Yes
29	57	Male	Rectum	2	*BLM* (positive)	**Yes**	No	Preliminary evidence
30	48	Male	Rectum	4	*SDHA* (positive)	**Yes**	Yes	Yes
31	56	Male	Rectum	3	*APC* (increased risk allele), *MSH6* (VUS), *RAD50* (VUS)	**Yes**	No	No
32	48	Female	Rectum	4	*EGFR* (positive)	**Yes**	Yes	Yes

Supplementary Table 5. Continued

Case	Age, *y*	Sex	Tumor location	Stage	Gene	Incremental PGV	Did they meet 2018 NCCN/ NSGC/ACMG testing guidelines	Was the PGV outside of the genes included on a guideline based panel?[a]
33	65	Female	Rectum	3	*BRIP1* (positive), *RECQL4* (VUS), *WT1* (VUS)	**Yes**	No	Yes
34	50	Male	Rectum	2	*FH* (VUS), *WRN* (positive)	**Yes**	Yes	Yes
35	49	Male	Right-sided	2	*MSH2* (positive), *WRN* (VUS)	No	Yes	No
36	43	Male	Rectum	3	*CHEK2* (positive), *FLCN* (VUS)	No	Yes	No
37	33	Female	Rectum	3	*CHEK2* (positive)	No	Yes	No
38	33	Female	Right-sided	3	*APC* (positive), *POLE* (VUS)	No	Yes	No
39	72	Female	Left-sided	2	*MUTYH* (positive), *POLD1* (VUS)	No	Yes	No
40	40	Female	Left-sided	4	*MSH6* (positive)	No	Yes	No
41	56	Male	Right-sided	4	*APC* (positive)	No	Yes	No
42	44	Male	Left-sided	2	*MSH2* (positive)	No	Yes	No
43	46	Male	Right-sided	1	*MSH2* (positive), *APC* (VUS)	No	Yes	No
44	64	Female	Rectum	4	*ATM* (positive)	No	Yes	Preliminary evidence
45	49	Male	Right-sided	2	*MLH1* (positive)	No	Yes	No
46	80	Female	Rectum	4	*MSH3* (positive)	No	Yes	Preliminary evidence
47	27	Female	Right-sided	2	*MSH6* (positive)	No	Yes	No
48	68	Male	Left-sided	3	*ATM* (positive), *NF1* (VUS), *PTCH1* (VUS)	No	Yes	Preliminary evidence
49	41	Female	Right-sided	2	*MUTYH* (positive), *ATM* (VUS), *POLE* (VUS)	No	Yes	No
50	36	Male	Rectum	3	*MLH1* (positive)	No	Yes	No
51	57	Male	Rectum	3	*CHEK2* (positive)	No	Yes	No
52	52	Male	Right-sided	3	*MSH2* (positive)	No	Yes	No
53	27	Male	Right-sided	3	*MLH1* (positive), *ALK* (VUS), *ATM* (VUS), *RAD51D* (VUS)	No	Yes	No
54	45	Female	Rectum	4	*BLM* (positive), *MUTYH* (positive), *MUTYH* (positive), *TERT* (VUS)	No	Yes	Preliminary evidence
55	24	Female	Left-sided	4	*BLM* (positive), *BLM* (positive)	No	Yes	Preliminary evidence
56	47	Male	Rectum	3	*TP53* (positive), *APC* (VUS)	No	Yes	No

ACMG, American College of Medical Genetics; CRC, colorectal cancer; NCCN, National Comprehensive Cancer Network; NSGC, National Society of Genetic Counselors; PGV, pathogenic germline variant; VUS, variant of unknown significance.

[a]CRC guideline panel genes include the following: *APC*, *AXIN2*, *BMPR1A*, *CHEK2*, *EPCAM*, *GREM1*, *MLH1*, *MSH2*, *MSH3*, *MSH6*, *MUTYH*, *NTHL1*, *PMS2*, *POLD1*, *POLE*, *PTEN*, *RPS20*, *SMAD4*, *STK11*, and *TP53*.

Supplementary Table 6. Participant and Tumor Characteristics by Incremental Vs Nonincremental PGM

	Nonincremental (N = 23)	Incremental PGV (N = 34)	Total (N = 56)	*P* value
Sex				.592[a]
Male	12 (54.5%)	21 (61.8%)	33 (58.9%)	
Female	10 (45.5%)	13 (38.2%)	23 (41.1%)	
Age				**.004**[b]
Median (SD)	45.5 (14.8)	57.5 (12.3)	50.5 (14.3)	
Age <50 y	15 (68.2%)	12 (35.3%)	27 (48.2%)	**.016**[a]
Age ≥50 y	7 (31.8%)	22 (64.7%)	29 (51.8%)	
Ancestry				.146[a]
White	17 (77.3%)	31 (91.2%)	48 (85.7%)	
Non-white	5 (22.7%)	3 (8.8%)	8 (14.3%)	
Family history of CRC in first-degree relatives[c]				.303[a]
Yes	3 (13.6%)	2 (5.9%)	5 (8.9%)	
No	11 (50.0%)	13 (38.2%)	24 (42.9%)	
Unknown	8 (36.4%)	19 (55.9%)	27 (48.2%)	
Lynch MMR genes				.001[a]
Yes	9 (40.9%)	2 (5.9%)	11 (19.6%)	
No	13 (59.1%)	32 (94.1%)	45 (80.4%)	
BRCA 1 and 2 genes				.247[a]
Yes	0 (0.0%)	2 (5.9%)	2 (3.6%)	
No	22 (100.0%)	32 (94.1%)	54 (96.4%)	
Primary cancer				.071[a]
Right colon	9 (40.9%)	7 (20.6%)	16 (28.6%)	
Left colon	5 (22.7%)	4 (11.8%)	9 (16.1%)	
Rectum	8 (36.4%)	23 (67.6%)	31 (55.4%)	
Cancer stage				.788[a]
Early stages (1–2)	7 (31.8%)	12 (35.3%)	19 (33.9%)	
Late stages (3–4)	15 (68.2%)	22 (64.7%)	37 (66.1%)	

CRC, colorectal cancer; MMR, mismatch repair.
[a]Pearson chi-squared test.
[b]Kruskal–Wallis rank-sum test.
[c]Limited to patients with a pedigree available.

Supplementary Table 7. Clinical Implications of Patients With High and Moderate Penetrance PGV

Patient	Age, *y*	Sex	Cancer type	PGV	Clinical action	Description	Other characteristics
1	44	Male	Colorectal	*MSH2*	Targeted therapy	Pembrolizumab	IHC + (loss of MSH2 and weak expression of MSH6)
2	33	Female	Colorectal	*APC*	Surgery	Total colectomy	Colonoscopy found >200 polyps
3	40	Female	Colorectal	*MSH6*	Targeted therapy	Nivolumab + ipilimumab	Negative IHC
4	48	Male	Colorectal	*PMS2*	Targeted therapy	Nivolumab + ipilimumab	IHC + (loss of PMS2 and MSH6)
5	52	Male	Colorectal	*MSH2*	Targeted therapy	Pembrolizumab, nivolumab, ipilimumab	IHC + (loss of MSH2 and MSH6)
6	34	Female	Colorectal	*CHEK2*	Surgery	Hysterectomy + BSO	Not applicable

BSO, bilateral salpingo-oophorectomy; PGV, pathogenic germline variant.

Supplementary Table 8. Distribution of Recessive PGV

	Recessive gene variants identified	
Patient	Gene 1	Gene 2
1	*FH*	
2	*WRN*	
3	*WRN*	
4	*FH*	*MUTYH*
5	*WRN*	
6	*MSH3*	
7	*BLM*	
8	*BLM*	
9	*MUTYH* (biallelic)	*BLM*
10	*WRN*	

Appendix 1. Invitae Multigene Panel List by Penetrance Category

High penetrance	Moderate penetrance
ALK, *APC*, *AXIN2*, *BAP1*, *BMPR1A*, *BRCA1*, *BRCA2*, *CDH1*, *CDK4*, *CDKN1C*, *CDKN2A*, *DICER1*, *EPCAM*, *FH* (biallelic), *FLCN*, *GATA2*, *HRAS*, *MEN1*, *MET*, *MLH1*, *MSH2*, *MSH6*, *MUTYH* (biallelic), *NF2*, *PALB2*, *PHOX2B*, *PMS2*, *PTCH1*, *PTEN*, *RB1*, *RET*, *RUNX1*, *SDHA*, *SDHB*, *SDHD*, *SDHC*, *SMAD4*, *SMARCA4*, *SMARCB1*, *SMARCE1*, *STK11*, *SUFU*, *TERC*, *TERT*, *TP53*, *TSC1*, *TSC2*, *VHL*	*AIP*, *ATM*, *BLM* (biallelic), *BRIP1*, *CASR*, *CDC73*, *CDKN1B*, *CEBPA*, *CHEK2*, *GREM1*, *HOXB13*, *MAX*, *MITF*, *NBN*, *NF1*, *POLD1*, *POLE*, *POT1*, *PRKAR1A*, *RAD51C*, *RAD51D*, *RECQL4* (biallelic), *SDHAF2*, *TMEM127*, *WT1*
Low penetrance	**Recessive conditions**
APC (I1307K), *BARD1*, *CTNNA1*, *EGFR*, *KIT*, *MUTYH* (monoallelic), *PDGFRA*, *RAD50*	*BLM* (monoallelic), *DIS3L2*, *FH*, *MSH3*, *NTHL1*, *RECQL4*, *WRN* X-linked conditions *GPC3*

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Appendix 2. Methods for Sequencing and Determination of Pathogenicity of Variants

Patients in the study underwent clinical genetic testing at Invitae, a CLIA-certified, NYS-certified, and CAP-accredited genetic diagnostics laboratory. Cancer genetic testing at Invitae comprehensively analyzes patients via full-gene sequencing (including coding exons, 10–20 base pairs of adjacent intronic sequencing on either side of the coding exons, and select noncoding variants) and deletion-duplication analysis for single-nucleotide variants, indels, copy number variants, and splice site variants. Based on validation study results, this assay achieves more than 99% analytical sensitivity and specificity for single-nucleotide variants, insertions and deletions shorter than 15 bp in length, and exon-level deletions and duplications. Invitae's methods also detect insertions and deletions larger than 15 bp but smaller than a full exon, but sensitivity for these may be marginally reduced. Invitae's deletion/duplication analysis determines copy number at a single exon resolution at virtually all targeted exons.

Genomic DNA was extracted from whole blood using a QiaSymphony (Qiagen, Hilden, Germany). NGS and quality control were performed on an Illumina platform as previously described.[1,2] Reads were aligned to the reference human genome sequence GRCh37 using Novoalign (Novocraft Technologies, Selangor, Malaysia). Small indels and single-nucleotide variants were analyzed using the Genome Analysis Toolkit. Copy number variant calls were performed using CNVitae.[3] Large structural variants were detected using split-read analysis as described previously.[4]

The pathogenicity of candidate variants, including structural variants, was established and they were classified as pathogenic or likely pathogenic if they involved large genomic events or conferred a truncating initiation codon or splice donor/acceptor effect, if functional data showed an impact on protein function, or if pathogenicity was otherwise reported in published literature.[5] Validation of PGMs observed was performed in accordance with Invitae standard operating practices, wherein orthogonal technology was used to validate pathogenic and likely pathogenic variants via Sanger sequencing or Multiplex Ligation-Dependent Probe Amplification.[6] Confirmed variants then were investigated using a refinement of American College of Medical Genetics and Genomics criteria (Sherloc).[7]

Genes in which PGMs were identified were organized into high, intermediate, and low penetrance categories based on the relative risk of cancer associated with PGMs in each gene, based on evidence in the literature. Examples include high-penetrance genes such as *BRCA1* and *BRCA2*, with a relative risk of 5 to 9[8]; moderate-penetrance genes such as *CHEK2*, with a relative risk of 3.3[9]; low-penetrance genes such as *BARD1*, with a relative risk of less than 2[10]; and recessive genes, such as *WRN*.[11] In some cases specific PGMs (eg, those occurring more frequently in certain populations) were classified individually based on the relative risk of cancer that has been established for these particular variants, an example of which is *APC* I1307K, with a relative risk of approximately 2 in the Ashkenazi Jewish population.[12]

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