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ORIGINAL RESEARCH

Genetic Testing Reveals Germline Mutations Among Patients Undergoing Surgery for Colorectal Carcinoma in a Community Hospital Setting

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Introduction: Defined germline mutations contribute to 5% to 10% of cases of colorectal carcinoma (CRC). While protocols for universal tumor screening have been adopted to detect mismatch repair (MMR) protein deficiency, widespread multigene panel testing has not been achieved. Barriers to implementing testing protocols may occur in community settings.

Methods: A total of 160 patients presenting for surgical management of CRC between 2011 and 2020 were considered for retrospective analysis in a single-surgeon, single-institution, community-based cohort. The rate of multigene panel testing and prevalence of germline mutations were calculated, and patient characteristics were assessed.

Results: A total of 32/160 (20%) patients underwent multigene panel testing, with 14/160 (9%) patients having germline mutations. While 88% of patients underwent panel testing after CRC diagnosis, 43% of these patients would have met testing criteria before diagnosis. Among the patients meeting criteria before diagnosis, 50% were found to carry a germline mutation.

Discussion: The prevalence of germline mutations was similar to previously reported values, while the rate of multigene panel testing was higher than previously reported. These results may be unique to the study setting or result from multidisciplinary conference discussion. A significant number of patients with abnormal panel testing were not tested before CRC diagnosis, despite meeting the criteria. This finding represents a missed opportunity for risk stratification and underscores the importance of addressing testing barriers in the primary care setting.

Conclusions: Primary care providers and oncologists in community hospitals must remain cognizant of changing guidelines as multigene panel testing becomes increasingly available.

Keywords: colorectal carcinoma, genetic screening, community hospital, risk stratification

Colorectal carcinoma (CRC) is the third leading cause of cancer-related death worldwide.[1] While most cases of CRC occur sporadically, heritable factors may be identified in 20% to 30% of cases, with 5% to 10% of cases specifically associated with a defined genetic syndrome.[2] Colon and rectal cancers represent a heterogenous group of malignancies with diverse molecular and genetic underpinnings. Interestingly, specific genetic changes are associated with tumor site[3] and distinct tumor behaviors, which inform prognosis and options for targeted therapies. In some cases, germline mutations give rise to familial cancer syndromes with greater lifetime risk of developing CRC. Most common among these are Lynch Syndrome, characterized by mutations in mismatch repair (MMR), and Familial Adenomatous Polyposis, characterized by *Adenomatous Polyposis Coli* genes. Recognizing patients with such syndromes is particularly important, as future risk for patients and their family members can be subsequently stratified.

While many health systems have implemented a protocol for universal screening of CRC tumor tissue

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for MMR protein deficiency, the decision to screen patients for inherited genetic changes using next-generation sequencing is more complex. Regulatory bodies, such as the National Comprehensive Cancer Network (NCCN), have provided guidance on which patients with CRC should undergo such testing. Although rates of multigene panel testing are increasing, many barriers limit the number of qualifying patients who actually undergo testing.[4, 5] These barriers may be even more pronounced in the community hospital setting.[6] We hypothesize that: (1) the rate of genetic testing for patients with CRC at our community-based institution may be lower than in the academic setting; (2) the prevalence of germline mutations may be lower in our cohort due to testing barriers coupled with a tendency for younger, more complex patients (who may be more likely to exhibit germline mutations) to be referred to large specialty centers; (3) a significant proportion of patients undergoing genetic testing after their cancer diagnosis would not have met testing criteria before diagnosis.

METHODS

A total of 160 patients who underwent surgical resection of the colon or rectum for a diagnosis of CRC between 2011 and 2020 were considered for inclusion in the retrospective analysis. Surgeries were performed by a single surgeon (KC), who is responsible for all elective CRC surgeries at Mercy Hospital in Portland, Maine. Characteristics of these patients, including basic demographics, cancer location and stage, results of MMR deficiency testing, and results of multigene panel testing, were abstracted from a colorectal surgery database that is prospectively maintained. This database was approved by the institutional review board at Mercy Hospital (protocol 137) for research purposes, and patients enrolled after the approval date provided informed consent. Additional chart review was performed to obtain detailed data on family history.

As part of routine pathologic analysis, staining of tumor tissue for MMR proteins was performed, and tumors with MMR protein deficiency reflexed to *MLH1* promoter hypermethylation and *BRAF* mutation studies. MMR deficiency can occur in sporadic cases of CRC, specifically with lost *MLH1* expression due to somatic hypermethylation of the *MLH1* promoter or with *BRAF* V600E mutations.[7] Hence, patients with either *MLH1* promoter hypermethylation or *BRAF* V600E mutations were considered to have MMR deficiency that was somatic in nature. In these cases, further testing for germline mutations was not recommended.

Cases were presented at an institutional multidisciplinary cancer conference either at the time of diagnosis or after surgery, at which time genetic testing considerations were discussed. Patients who met NCCN criteria for multigene panel testing based on MMR status, personal/family history, or polyp burden[8] were considered appropriately. The cost of testing varied depending on the patient's health insurance plan. Patients facing prohibitively high out-of-pocket costs, including those without insurance, were helped with applying for financial assistance from companies offering testing; this cost was often forgiven. Following this counseling, patients decided whether to proceed with multigene panel testing. This process of selecting patients for multigene panel testing is summarized in Figure 1.

For each of the 32 patients undergoing multigene panel testing, chart review was conducted to assess (1) when testing was conducted relative to CRC diagnosis and (2) whether patients would have met any NCCN criteria for multigene panel testing unrelated to the index cancer diagnosis.[8] These patients are described as having met diagnosis-independent criteria (DIC). Descriptive statistics only were used in data analysis.

RESULTS

In this community-based cohort of 160 patients presenting for surgical management of CRC, 32 (20%) underwent multigene panel testing for germline mutations. The prevalence of germline mutations in this cohort was 14/160 (9%) in the overall population with CRC, and 14/32 (44%) in those undergoing multigene panel testing. Compared with 128 patients who did not undergo testing, or had negative results, patients with germline mutations were more likely to have right-sided tumors (positive: 50%; negative/untested 40%) and were younger (positive: 59 years; negative/untested: 66 years) at the time of surgery (Table1).

In total, 28/32 (88%) patients undergoing multigene panel testing were tested only after the diagnosis of CRC was given. Testing was positive for 3 patients who were tested before diagnosis and for 1 patient for whom timing could not be determined. The decision to pursue testing was based on results from universal screening for MMR protein deficiency in

11/32 (34%) patients ultimately undergoing testing. Among these patients, 6/11 (55%) harbored a germline mutation. Among patients undergoing testing based on other factors, 8/21 (38%) patients harbored a germline mutation. Among 28 patients tested after CRC diagnosis, 12 (43%) would have qualified before CRC diagnosis (ie, met DIC). Among these 12 patients, 6 tested positive for germline mutations (50%) compared to 4/16 (25%) patients who did not meet DIC before diagnosis (Figure 2). Factors causing these specific patients to meet DIC are listed in Table 1. Overall, prior history of cancer was the most common reason for meeting DIC.

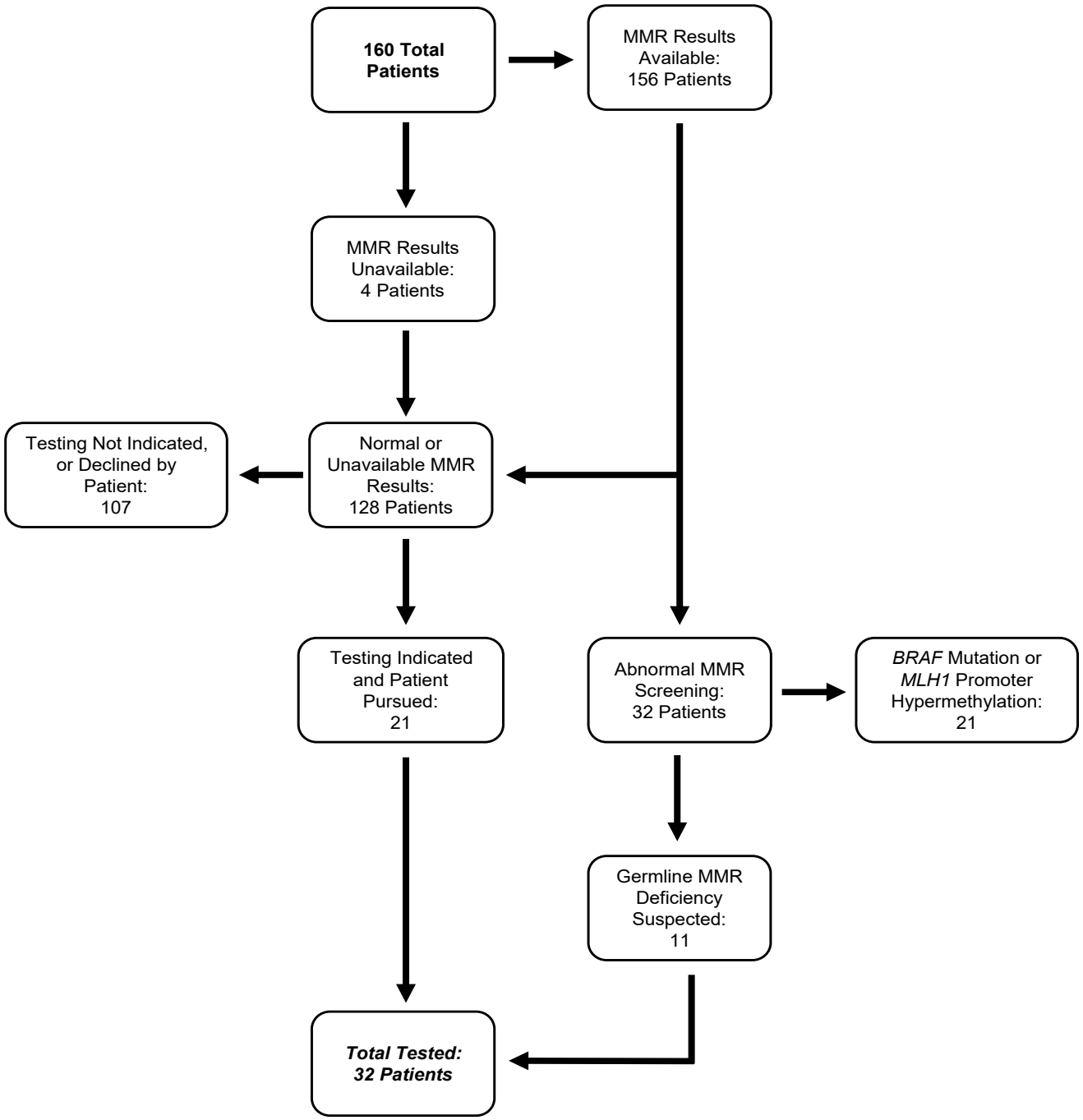

Figure 1: Summary of patient selection for multigene panel testing among the total population presenting for surgical management of colorectal carcinoma and breakdown of the corresponding number of patients in each sub-cohort. Patients were selected for germline mutation based upon either abnormal universal screening for MMR protein deficiency without abnormal BRAF/MLH1 promoter hypermethylation reflex testing, or other unrelated clinical factors.
MMR: mismatch repair

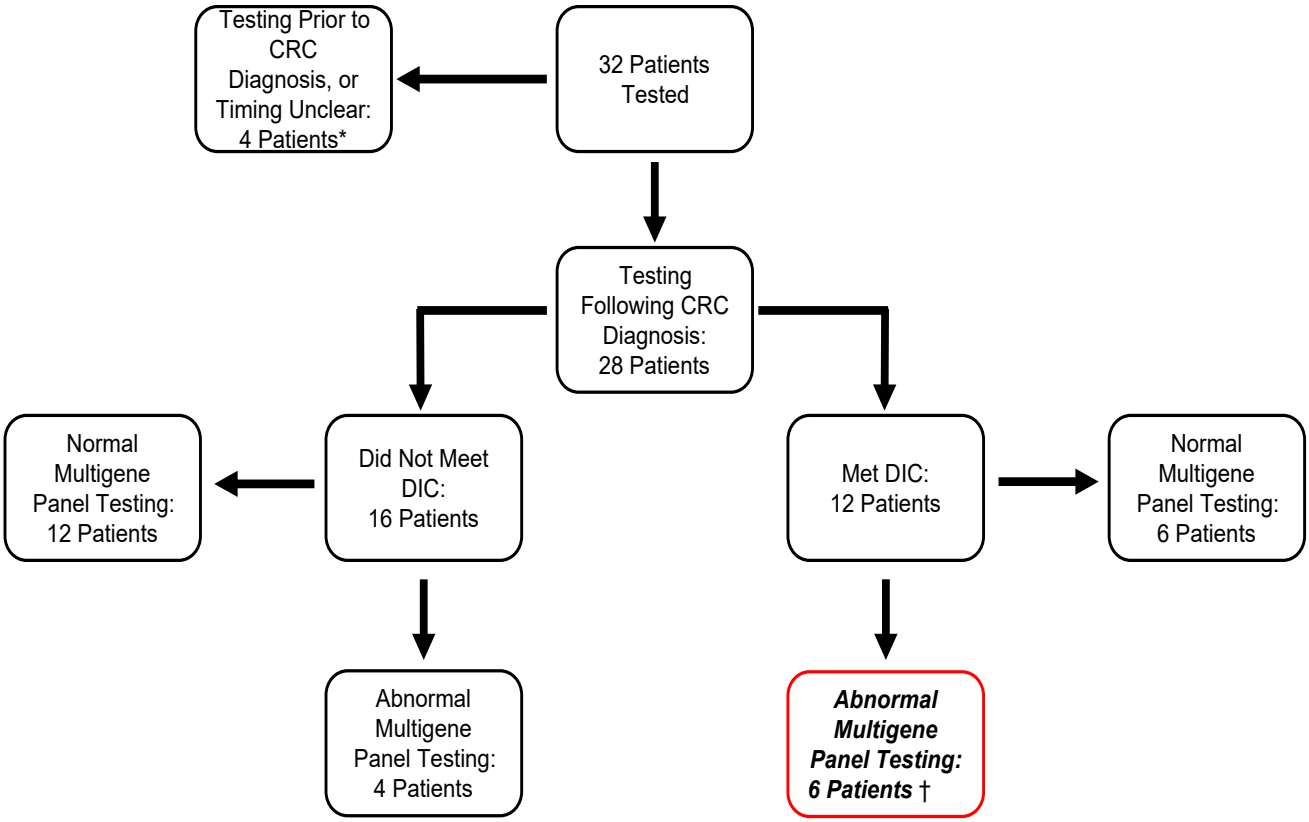

Figure 2: Summary of the timing of testing relative to colorectal carcinoma diagnosis for 32 patients undergoing multigene panel testing and results of this testing stratified by whether or not patients met diagnosis-independent criteria for testing.

* Each of these patients ultimately exhibited abnormalities on multigene panel testing.

† These patients represent missed opportunities to identify germline mutation and subsequently risk stratify prior to diagnosis.

CRC: colorectal carcinoma; DIC: diagnosis-independent criteria

DISCUSSION

In our community-based cohort of patients undergoing surgery for CRC, the prevalence of identified germline mutations was 9%, within the range of previously reported values.[2] Among all patients with CRC, 20% underwent multigene panel testing. This value is higher than that reported in another study among 4 academic centers, wherein 8.5% of patients with CRC underwent panel testing.[9] These findings suggest that our patients did not face disproportionate barriers to testing relative to the cohort based in a tertiary care center, or that those barriers were addressed. The relatively high percentage of patients tested may be because of the routine discussion of cases with a genetic testing coordinator through multidisciplinary conference, or that this was a single-surgeon series, limiting variability in practice."

Patients with germline mutations were younger at the time of surgery than those not harboring such mutations. However, these patients were, on average, older than those in another study reporting a mean age at diagnosis of less than 40 years for those with germline mutations.[10] Importantly, however, this study showed that only 1 in 5 patients diagnosed under the age of 50 years carried such a mutation, suggesting a significant role for other factors in the development of early onset CRC.[10] Our discrepant finding may be due to a small sample size, or it may better reflect the demographics of patients who present for care in a community setting. In either case, we recommend considering heritable factors in all patients presenting with CRC, regardless of age at diagnosis.

The minority of patients undergoing multigene panel testing in our cohort were tested for germline mutations based on universal screening for MMR protein deficiency (34%). However, these patients

Table 1. General and Genetic Tumor Characteristics for All patients with Abnormalities on Multigene Panel Testing

ID	Patient age, y	Tumor location	Tumor stage	Reason for testing†	Multigene panel result	Met DIC	Reason for meeting DIC
1	62	Cecum	I	Other factors	*MUTYH* x2	Yes	Polyps
2	63	Cecum	IIC	MMR screening	VUS in *NBN*	No	NA
3*	69	Cecum	I	Other factors	*MSH6*	Yes	Prior cancer
4	64	Right colon	I	Other factors	VUS in *ATM*	No	NA
5	68	Rectum	IIIB	MMR screening	*MUTYH*	No	NA
6	43	Transverse colon	I	MMR screening	*MLH1*	Yes	Family history
7	64	Cecum	IIA	MMR screening	*MUTYH* Heterozygous	Yes	Family history
8*	42	Cecum	IIA	MMR screening	*PMS2*	Yes	Prior cancer
9	60	Transverse colon	IIC	MMR screening	*MLH1*/LYNCH	Yes	Family history
10	56	Right colon	IIIB	Other factors	*CHEK2*	Yes	Family history
11	66	Rectum	I	Other factors	*MSH6*	No	NA
12	72	Left colon	IIIB	Other factors	VUS in *MYH*	Yes	Prior cancer
13*	46	Sigmoid colon	IVB	Other factors	*p53*	Yes	Prior cancer
14*	48	Rectum	I	Other factors	VUS in *p53*	Yes	Prior cancer

CRC, colorectal carcinoma; DIC, diagnosis-independent criteria; MMR, mismatch repair; NA, not applicable; VUS, variant of uncertain significance.
*Multigene panel testing conducted before CRC diagnosis or timing could not be determined.
†Presented as either abnormal MMR protein deficiency screening with negative reflex testing for a BRAF mutation or MLH1 promoter hypermethylation (suggesting germline deficiency), or other clinical factors independent of MMR screening results.

were more likely (55%) to test positive for a germline mutation than patients tested based on other factors (38%). This finding is consistent with the observation that Lynch Syndrome, caused by MMR protein deficiency, is the most common heritable cause of CRC.

We found that 50% of cancers were right-sided in the cohort of patients with germline mutations compared to 40% of those without such mutations. This finding is consistent with previous models that suggest a continuum of carcinogenesis along the gastrointestinal tract. In these models, right-sided tumors exhibit a higher degree of *BRAF* mutations and microsatellite instability.[3] Based on these findings, CRC cases characterized by heritable factors, and in particular those demonstrating microsatellite instability, are more likely to be right-sided. Our findings were consistent with this model. In our cohort, 2 patients with mutations associated with Lynch Syndrome and 2 patients with *MUTYH* mutations, corresponding to a related syndrome, developed tumors in the right colon or cecum.

A minority (43%) of patients who underwent genetic testing after CRC diagnosis would have qualified for testing based on other criteria. An opportunity to risk stratify these patients may have been missed either because historical information was not collected or updated before diagnosis, or because this information was not subsequently applied to existing guidelines. This

missed opportunity underscores the importance of an approach to genetic counseling that is holistic, but also dynamic and adaptable to changing personal history, family history, and guidelines. In some cases, the failure to properly screen and test patients with indications may have clinically significant implications. Considering only patients who underwent testing after CRC diagnosis among our cohort, those who met criteria before diagnosis were more likely to subsequently test positive for a germline mutation (50%) than those who did not meet criteria before diagnosis (25%) (Figure 2). For the 6 patients with germline mutations who met criteria before diagnosis, earlier genetic testing and risk stratification may have resulted in diagnosis at a less advanced stage and consequently changed management and prognosis.

In addition to risk stratification and enhanced cancer screening, identifying inherited cancer syndromes before cancer diagnosis supports patient counseling regarding future health risks, fertility, and other important considerations. These benefits highlight the importance of attempting to identify these patients in the primary care setting. While our study was not designed to evaluate the presence of pre-diagnosis testing barriers faced by our patients, we suspect several factors may have contributed to the missed opportunities to identify mutation carriers before diagnosis. Consistently identifying patients for multigene panel testing in the primary care setting requires that patients have a primary care doctor, personal and family history of malignancy are updated and reviewed often, and primary care providers remain up-to-date on the indications for panel testing. These challenges are exacerbated by experts who do not agree on which patients should qualify for multigene panel testing. A position statement on multigene panel testing from the Collaborative Group of the Americas on Inherited Gastrointestinal Cancer outlines patients who are most likely to benefit from testing based on available evidence.[4] While the NCCN guidelines currently provide similar suggestions,[8] widespread consensus and implementation of corresponding protocols has yet to be achieved. Even among patients who are appropriately counseled on genetic risk and eligibility for testing, patient-specific factors (eg, insurance status, out-of-pocket expenses, education level) may influence the decision to proceed. Our institution has seen some success in identifying patients for genetic testing by eliciting a focused personal and family history from patients when they present for breast or CRC screening, which reduces the burden on primary care providers.

Primary care providers face challenges in identifying and managing patients at increased heritable risk for cancer. These challenges, including provider-, institution-, and society-specific barriers, were documented even before widespread availability of next-generation sequencing.[11] In the case of multigene panel testing, implementing protocols for risk-assessment, testing, results interpretation, and counseling can be difficult, even in subspecialty centers. One study used a standardized tool to assess clinical risk for CRC at an open-access colonoscopy practice. The study demonstrated a low rate of referral to cancer genetics centers for patients identified as high-risk and a low rate of patient follow-through, even in cases when a referral was completed.[12] A number of factors, similar to those encountered in the primary care setting, likely contribute to this phenomenon. One particular concern for any provider ordering multigene panel testing is whether identified mutations will even alter clinical decision-making. Our findings indicate that screening unaffected individuals in our community-based cohort could have identified patients at high risk before cancer developed or earlier in the course of disease. While our study was not designed to determine whether earlier diagnosis would have improved outcomes, an analogous study among patients with breast cancer demonstrated changes in medical management for 58% of patients with identified mutations on multigene panel testing.[13] If multigene panel testing can provide clinically useful information, ordering providers must also be capable of applying results to the care of patients at high risk, another obstacle to widespread implementation of such practices.

Whether in the primary care setting, or a subspecialty clinic, addressing challenges with systems-based practices may help improve availability of next-generation sequencing technology to patients presenting in the community setting. To this end, several suggestions have been proposed, including collaboration between non-genetics and genetics providers,[14] multidisciplinary care coordination in a single visit,[15] workflow changes,[16] risk-assessment training and education, practice-based support, and opportunities to enroll patients in research trials after identifying uncharacterized mutations.[5] While our cohort of patients appeared to face

significant barriers to testing before CRC diagnosis, they did not face such limitations after diagnosis. This result is likely due to the comprehensive and multidisciplinary approach to genetic risk-assessment adopted by our institution.

Our study is limited by the retrospective nature, small sample size, and single-surgeon/single-institution population, each of which limits generalizability of our results. In addition, due to the limitations inherent to using retrospective data, we were unable to determine what proportion of patients may have qualified for genetic testing but ultimately remained untested due to patient preference or financial constraints. A chart review of the untested cohort revealed only one patient who declined and a few references to the financial burden of testing. These issues, therefore, do not seem to be common, which is supported by the relatively high rate of testing in this cohort compared with previous reports. Despite these limitations, we were able to document a relatively high rate of mutation carriers in our population, some of whom would have met criteria for genetic testing before their cancer diagnosis. This notion highlights the need for improved practices in genetic testing both in primary care and subspecialty settings.

CONCLUSIONS

The prevalence of germline mutations among our community-based cohort of patients undergoing surgery for CRC is similar to previously published population-wide values. Patients with germline mutations were younger, more likely to have right-sided tumors, and could not always be identified by genetic testing criteria other than the index cancer diagnosis. In some cases, however, patients had indications for genetic testing before diagnosis, representing a missed opportunity to identify germline mutations before CRC developed or earlier in the disease course. These results underscore that subspecialty and primary care providers in community settings need to remain cognizant of changing guidelines. They also need to advocate for systems-based practices that address limitations to implementing genetic counseling and screening among patients presenting for care, both with and without a cancer diagnosis. These efforts will become increasingly important as targeted therapies and personalized medicine become more prevalent.

Conflict of Interest: none

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