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Genetics in Gastroenterology Practice (B Katona, Section Editor)

# Genetic Testing Use and Expectations in Early Onset Colorectal Cancer

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**Abbreviations** *CRC* Colorectal cancer · *EOCRC* Early-onset colorectal cancer · *FAP* Familial adenomatous polyposis · *PCR* Polymerase chain reaction · *DNA* Deoxyribonucleic acid · *NCI* National Cancer Institute · *MSS* Microsatellite stable · *HNPCC* Hereditary nonpolyposis colorectal cancer · *MSTF* Multi-Society Task Force · *NCCN* National Comprehensive Cancer Network

## Abstract

*Purpose of review* A substantial proportion of colorectal cancer (CRC) diagnosed under age 50, or early-onset colorectal cancer (EOCRC), is associated with a hereditary cancer syndrome. It is of utmost importance to identify these patients to customize cancer treatment options, decrease the risk of metachronous cancers, and facilitate testing within the family to identify carriers. The purpose of this paper is to review the evolution of genetic evaluation in patients with EOCRC, review current best practices, and describe areas ripe for future work and research.

*Recent findings* Fourteen to 25% of all EOCRCs are associated with a pathogenic germline variant. These variants are found in genes typically associated with EOCRC, such as Lynch syndrome or biallelic *MUTYH*, as well as genes that are not necessarily congruent with EOCRC phenotypes such as polyposis syndromes or hereditary breast and ovarian cancer syndromes.

*Summary* Professional societies now recommend comprehensive multigene panel testing in all patients with EOCRC, regardless of personal history, family history, or tumor characteristics.

## Introduction

The incidence and mortality associated with early age onset colorectal cancer (EOCRC), or CRC diagnosed in patients under the age of 50, are increasing [1•, 2] and are currently the second leading cause of cancer-related mortality in patients under age 50. If current trends continue, by the year 2030, 10% of all colon cancers and 22% of all rectal cancers in the USA are expected to be diagnosed in patients younger than age 50 years [3].

Although the etiology of the rise of the EOCRC burden is unknown [4, 5], a substantial proportion of cancers diagnosed in young patients is driven by hereditary cancer syndromes caused by pathological germline variants in cancer predisposition genes. It is important to identify patients whose CRC may have been driven by a hereditary syndrome in order to offer customized cancer treatments, such as surgical approaches and chemotherapy options. Furthermore, prevention strategies, including chemoprevention, screening examinations, and prophylactic surgery, can significantly reduce the risk of metachronous cancers [6, 7]. Finally, given established inheritance patterns of these syndromes, at-risk individuals can be identified within the family and prevention strategies can be implemented in all carriers.

Although young patients with CRC have long been recognized as a high risk for hereditary syndromes, our approach to identifying syndromes among CRC patients has changed substantially in recent years. The purpose of this paper is to review the evolution of genetic evaluation in patients with EOCRC, review current best practices, and describe areas ripe for future work and research.

# Tumor-based screening in EOCRC

Historically, the main syndrome under consideration for those with EOCRC and a "non-polyposis" phenotype was Lynch syndrome (LS). A detailed review of the approach to genetic evaluation of polyposis syndromes can be found elsewhere [8–10] and in the accompanying papers in this series about oligopolyposis and familial adenomatous polyposis (FAP). LS is the most common hereditary CRC syndrome, accounting for 2–4% of all CRC diagnoses [11]. LS is characterized by a multiplicity of multi-organ cancers that occur at young ages [6, 12–16].

LS is caused by an autosomal dominant inheritance of a pathogenic variant in one of the mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) or *EPCAM*—which is immediately upstream of *MSH2* and deletion of the termination codon of *EPCAM* causes epigenetic silencing of *MSH2*. Defective MMR results in microsatellite instability (MSI), caused by insertions and deletions in simple repetitive sequences within the tumor DNA (microsatellites) [17]. Defective MMR function causes MSI in LS, but is also seen with double somatic inactivation of DNA MMR genes (Lynch-like syndrome [18]), or because of *MLH1* promoter hypermethylation in sporadic tumors (accounting for approximately 12% of all CRCs) [19]. These tumor characteristics have been leveraged to perform tumor-based screening for LS by assessing for the absence of MMR protein in tumor tissue by immunohistochemistry (IHC) and probing

for mutations in microsatellite DNA fragments via polymerase chain reaction (PCR).

# Tumor screening options and test characteristics

Tumor-based screening for defective DNA MMR activity, either using IHC or by PCR, can be performed in tissue acquired via endoscopic biopsy or on surgical resection specimens. It can be completed on archived, formalin-fixed specimens. It is important to note that tumor-based testing is a screening method and is not diagnostic of germline pathogenic variants, since a majority of those CRCs with MSI are not associated with LS. Thus, abnormalities in tumor-based screening require follow-up genetic counseling and germline genetic testing.

## Microsatellite instability testing

This method involves PCR amplification of DNA markers in tumor and surrounding normal tissue. In 1996, the Early Detection Branch of the National Cancer Institute (NCI) convened an international group who synthesized the emerging data on the clinical and pathologic characteristics of LS (then termed hereditary nonpolyposis colorectal cancer (HNPCC)). The group came to a consensus on clinical criteria to identify CRC patients at risk for LS, the Bethesda Guidelines, and agreed that tumor-based screening using MSI markers should be applied in these patients [20]. Members of the group re-convened in 1997 and recommended a panel of mono- (*BAT-25, BAT-26)* and di-nucleotide (*D2S123*, *D5S346*, *D17S250*) PCR markers [21]. The group proposed that tumors with two or more of the five mutated markers be termed MSI-high (MSI-H), those with one mutated marker be termed MSI-low (MSI-L) and those with no mutated markers be termed microsatellite stable (MSS) [21]. Since then, multiple additional markers have been used and most laboratories have their own customized panels [22•] also including mononucleotide markers, as more recent studies have demonstrated that mononucleotides are more sensitive and specific than dinucleotides [23, 24]. There is some variability between labs since each lab defines how many markers are required to distinguish between MSI-H and MSI-L.

Historical studies reporting the performance of MSI testing to detect LS patients are difficult to interpret and apply to current practice. A recent systematic review conducted by Coelho et al. [[22•] included ten studies and reported a sensitivity of MSI ranging from 67% (95% CI 47, 83) to 100% (95% CI 94, 100), when MSI-L was considered MSS. Three of the studies reported specificity ranging from 61.1% (95% CI 57.0–65.1) to 87.8% (95% CI 73.8–95.9). This wide range in performance was likely due to variable microsatellite targets used in each study and differences in how many mutant markers were required to define a tumor MSI-H. As expected, the studies reporting lower sensitivity required more markers for MSI-H and had higher specificity. It is also important to note that the approach to germline testing in the cohorts was variable and none of the studies included germline testing for *PMS2*. Some studies performed germline testing in all CRC patients, whereas others only performed germline testing in those with MSI-H and MSI-L tumors and a random sample of the MSS tumors. Based on these factors, Coelho et al. were unable to pool the data to provide overall test

characteristics. Despite these variabilities in testing approach that preclude pooling data, contemporary MSI panels are reported to have a 90–95% sensitivity for detection of LS carriers [25, 26].

## Immunohistochemistry for MMR protein loss

This method involves histologic antibody staining for MMR proteins within tumor tissue and adjacent normal tissue (as an internal control). IHC panels most commonly include four antibodies (*MLH1*, *MSH2*, *MSH6*, and *PMS2*). Given the heterodimeric association between MLH1/PMS2 and MSH2/MSH6 (in which MLH1 stabilizes the PMS2 protein and MSH2 stabilizes the MSH6 protein), the loss of MLH1 is generally accompanied by loss of *PMS2* and the loss of *MSH2* is accompanied by loss of *MSH6*. However, isolated losses of MSH6 and PMS2 can be seen, since MLH1 and MSH2 proteins can associate with other proteins as stable heterodimers). If nuclear staining is present for all MMR proteins, this suggests that the MMR system is intact and there is a low likelihood of LS. However, if there is an absence of staining, this may be because of germline loss of MMR function with a second hit, double somatic mutations, or acquired hypermethylation of the promoters of both alleles of *MLH1*. Interpretation and appropriate follow-up of MMR protein loss is discussed in detail elsewhere [6].

It is important to note that a small proportion of MSI-H LS tumors show normal IHC. This may be due to germline variants that express a stable, but functionally inactive protein that can be detected by antibody testing [27]. Finally, incomplete tissue fixation can result in technical failure to complete IHC. Palomaki et al. reported a failure rate of 4.4% [28]. Also, some experience on the part of the pathologist is required since the DNA MMR proteins are expressed mainly during DNA replication; therefore, the proteins are most abundant at the base of the colonic crypts and may not be detectable in a superficial biopsy from the top of the crypt.

Snowsill et al. [29] summarized the performance of IHC for the detection of LS patients based on seven studies. The reported sensitivity ranged from 80.8% (95% CI 60.6–93.4) to 100% (95% CI 81.5–100.0). Similar to MSI testing, there was variability in the population of patients studied, the IHC testing performed (for instance, only two studies included staining for PMS2), and the germline testing performed.

Due to staining variability or patchiness and operator dependence in interpretation [26], professional societies recommend that IHC should be performed by a reference laboratory with appropriate quality control measures [6, 30]. Under these conditions, IHC has a > 90% sensitivity for detection of LS carriers [25, 26].

# Patient selection for tumor testing

Tumor-based assessment for EOCRC was established well before reliable germline testing became available. Liu et al. first described the rates of MSI in CRC based on age in 1995. They reported that 58% (18/31) of patients ≤ 35 years old had MSI-H tumors compared to 12% (19/158) of patients > 35 [31]. This study was performed prior to the standardization of MSI testing. Since then, studies including diverse cohorts of EOCRC have reported MSI by PCR in 10–37% [32–39] of cases and absence of MMR in 10–43% [35–40] of EOCRC.

As previously discussed, the NCI workshop convened in 1996 developed the Bethesda Guidelines to identify CRC patients who should undergo tumor-based screening for LS [20]. These guidelines included the family history elements from the Amsterdam criteria developed by the International Collaborative Group on HNPCC [41] as well as specific pathologic characteristics in the tumor. These guidelines were further revised in 2004 [42] (Table 1).

In 2005, Pinol et al. [43] reported the test characteristics of applying the Revised Bethesda Guidelines for detecting LS patients. They performed PCR for MSI and IHC for MLH1/MSH2 on 1222 patients diagnosed with CRC and subsequent germline testing for *MLH1*/*MSH2* in patients with abnormal tumor testing. Of the eleven patients with a pathogenic germline variant in *MLH1* or *MSH2*, 10 met the Revised Bethesda Guidelines, 10 were microsatellite unstable (MSI-H), and all had an absence of MMR on IHC. Similarly, Hampel et al. [44] performed PCR for MSI testing and IHC for all four MMR proteins on 1066 CRCs. For those with abnormal tumor testing, germline testing was performed. Of the 23 patients with pathogenic germline variants, 18 met the Revised Bethesda Criteria. Based on these studies reporting 78–91% sensitivity of the revised Bethesda Criteria in detecting germline LS variants, multiple professional societies supported the use of the Bethesda Criteria to identify CRC patients who should undergo tumor-based screening with MSI or IHC and subsequent germline testing if tumor-based screening was abnormal [6, 45].

Although the Revised Bethesda Criteria perform well in the detection of LS, there has been accumulating data that these criteria are not consistently applied in practice, and thus, hereditary patients go unrecognized. Even in an integrated health care system such as the Kaiser system, Cross et al. [46] reported that only 11% of CRC patients from 2004 to 2009 who clearly met Revised Bethesda Criteria were screened for LS. Similarly, Mittal et al. reported that only 15% of CRC patients who met Bethesda Criteria were referred for genetic evaluation at two large Veterans Affairs Medical Centers from 2010 to 2016 [47]. Karlitz et al. [48] reported that only 23% of CRCs diagnosed in patients under the age of 50 in Louisiana in 2011 had MSI and/or IHC testing. Thus, the poor application of

**Table 1. Clinical criteria for evaluation of Lynch syndrome**

| Amsterdam II criteria [41] | Revised Bethesda Guidelines [42] |
|---|---|
| At least three relatives with a Lynch-associated cancer (CRC, endometrial, small bowel, ureter, renal pelvis) | CRC diagnosed at age 50 or younger |
| Two or more successive generations affected | Presence of synchronous or metachronous Lynch-associated cancer, regardless of age |
| One or more relatives diagnosed before the age of 50 | CRC with Lynch-like histology (tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation or medullary growth pattern) in patient younger than 60 |
| One should be the first-degree relative of the other two | CRC in a patient with at least 1 first-degree relative with Lynch-associated cancer diagnosed at age 50 or younger |
| Familial adenomatous polyposis should be excluded<br>Tumors should be verified by pathologic examination | CRC in a patient with two or more first- or second-degree relatives with a Lynch-associated tumor, regardless of age |

*CRC* colorectal cancer

the Bethesda Criteria limits their utility in the identification of hereditary syndromes among patients with EOCRC.

Acknowledging the limitations of relying on recognition and application of clinical criteria to identify CRC patients who would benefit from tumor based screening for LS, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group recommended screening all newly diagnosed CRC cases for LS via tumor testing (MSI and/or IHC for MMR deficiency) in 2009 [49]. This recommendation was further endorsed by the National Comprehensive Cancer Network in 2013 [50], the US Multi-Society Task Force (MSTF) in 2014 [6], and most recently the US pathology societies in 2017 [30].

## Tumor testing limitations

Although tumor-based screening via MSI and/or IHC is an effective way to identify EOCRC patients who have the most common hereditary syndrome, LS, there are several important limitations. As discussed below, there are multiple other germline variants found in EOCRC patients that tumor testing is not designed to screen for. In the longer term, each syndrome carries different colonic and extra-colonic cancer risks that require customized surveillance and risk reduction programs [7].

Tumor-based screening is a multi-step process, spanning from tissue acquisition, tissue analysis via MSI and/or IHC, result interpretation, and ultimately referral and completion of genetic counseling/testing. The details of this complex process are reviewed elsewhere [6, 28, 44], however require dedicated personnel and infrastructure to adequately complete, interpret, and act upon. Noll et al. [51] conducted a survey study of 442 US gastroenterologists aimed to understand the barriers to LS screening. Only 33% of respondents reported that gastroenterologists should be responsible for requesting MSI/IHC and only 46% reported that MSI/IHC should be performed on all CRCs. Cost, lack of familiarity with how to interpret tumor testing, and access to genetic counseling were the most commonly cited barriers to completing tumor-based screening. Likely because of these challenges, Beamer et al. [52] reported that in 2012, 71% of National Cancer Institute Comprehensive Cancer Centers were conducting universal tumor screening for LS, whereas only 36% of community hospital comprehensive cancer centers and 15% of community hospital cancer programs were doing so.

## Spectrum of germline variants in EOCRC

As continued efforts are underway to improve the adoption of universal tumor screening, advancing technology has allowed direct assessment of the germline in patients with EOCRC. The direct germline approach overcomes some of the challenges faced with interpretation and follow-up of tumor-based screening discussed above. Furthermore, a recent shift towards multigene panel germline testing, as reviewed by Powers et al. [53], has uncovered a wide variability in syndrome phenotypes, such that patients with pathogenic variants may not fit previously established clinical and/or family history criteria.

A series of studies have shed light on the spectrum of germline variants in EOCRC patients (Table 2). In 2015, Mork et al. [54] performed germline genetic

**Table 2. Pathological germline variants in early age onset colorectal cancer**

| Study | Cohort characteristics | Genes tested | Pathogenic or likely pathogenic variants N (%) | Germline variants found (N) | Variants of uncertain significance N (%) |
|---|---|---|---|---|---|
| Mork et al. [54] 2009–2013 Single-center US | < 35 years old: $n = 193$ Mean age 29 | Phenotype-driven; all patients had been referred for genetic counseling | 44/193 (22.8) | *APC* (13), biallelic *MUTYH* (2), CMMRD (2), *TP53* (1), monoallelic *MUTYH* (1), *MLH1* (6), *MSH2* (5), *MSH6* (2), *PMS2* (1), *EPCAM* (1) | 6/193 (3.1) |
| Yurgelun et al. [55] 2008–2014 Single-center US | < 50 years old: $n = 336$ Median age 56 (from 1058 consecutive CRC patients of all ages) | 25-gene panel | 52 variants in 47 patients/336 (14.0) | *MLH1* (13), *MSH2* (4), *MSH6* (3), *PMS2* (2), *APC* (3), *BRCA1* (2), *BRCA2* (4), *APC* I1307K (4), biallelic *MUTYH* (3), monoallelic *MUTYH* (6), *BRIP1* (1), *ATM* (6), *CHEK2* (1) | 479/1260 (38.0)‖ |
| Pearlman et al. [56] 2013–2016 Multi-center US | < 50 years old: $n = 450$ Mean age 43 | 25-gene panel | 75 variants in 72 patients/450 (16.0) | *MLH1* (13), *MSH2* (17), *MSH6* (2), *PMS2* (6), *APC* (6), *APC* I1307K (4), biallelic *MUTYH* (4), monoallelic *MUTYH* (8), *SMAD4* (1), *BRCA1* (2), *BRCA2* (4), *ATM* (4), *CHEK2* (1), *PALB2* (2), *CDKN2A* (1) | 145/450 (32.2)¥ |
| Stoffel et al. [57] 1998–2015 Single-center US | < 50 years old: $n = 430$ Mean age 40; (retrospectively selected from genetic counseling service) | 124 or 67 gene panel | 79/315 (25.1) | *MSH2* (25), *MLH1* (24), *MSH6* (5), *PMS2* (2), *APC* (10), *MUTYH* (8), *SMAD4* (2), *BRCA1* (1), *TP53* (1), *CHEK2* (1) | 21/315 (6.7) |
| Toh et al. [58] 2014–2016 Single-center Singapore | < 50 years old: $n = 88$ Mean age 41 | 64-gene panel, excluding MMR genes | 12/88 (13.6) | *APC* (4), monoallelic *MUTYH* (2), *ATM* (1), *BRCA2* (1), *NTHL1* (1), *PALB2* (1), *WRN* (2) | 236 VUS in 88 patients ¥ |

*MSI-H* microsatellite unstable, *MMR* mismatch repair, *US* United States, *CMMRD* Constitutional Mismatch Repair Deficiency

*Only reported MMR

†Reported as MSI-H and/or MMR

‖Includes total cohort of patients (data on only < 50 cohorts not available)

¥Patients had more than one variant. The study did not report the number of patients with at least 1 variant

‡Excluded patients with MMR-deficient tumors

testing in 193 patients ≤ 35 years old referred for genetic counseling between 2009 and 2013. 34% (66/193) had a germline variant consistent with a hereditary cancer syndrome; 22 had LS, 16 had FAP, two had biallelic *MUTYH*, two had biallelic MMR variants (constitutional mismatch repair deficiency), and one had a pathogenic variant in *TP53*. Although this was a highly selected, risk-enriched population, this study highlighted that relying on syndrome phenotypes for genetic risk assessment can miss hereditary syndromes in EOCRC patients.

A series of subsequent studies have demonstrated that EOCRC patients have a wide spectrum of germline variants, including in genes not typically associated with CRC. In 2017, Yurgelun et al. [55••] reported results of 25-gene panel germline testing in 1058 consecutive and unselected CRC patients including 336 under age 50. Fourteen percent (47/336) of the EOCRC patients had a pathogenic variant, including six *BRCA1/2* variants, six *ATM* variants, and one *BRIP1* variant. Pearlman et al. [56••] conducted similar multi-gene panel testing in 450 EOCRC patients from a consortium of 51 Ohio hospitals. Sixteen percent (72/450) of patients had a pathogenic germline variant. As expected, 38 (8.4%) had a LS gene variant. Phenotypically unexpected results included eleven patients with polyposis gene variants (six with FAP, four with biallelic *MUTYH*, and one with *SMAD4*), six with *BRCA1/2*, four with *ATM*, two with *PALB2*, and one each of *CHEK2* and *CDKN2A*. Figure 1 summarizes the

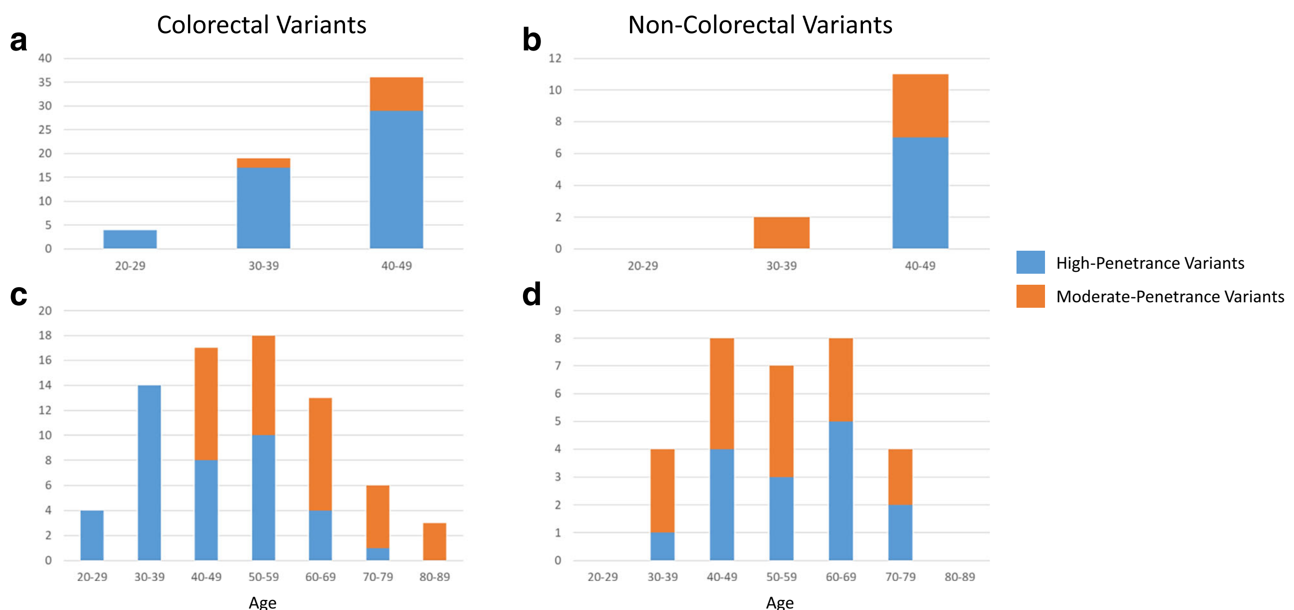


**Fig. 1.** Germline variants (*n*) by age and penetrance. **a** Colorectal variants from Pearlman et al. [56], **b** non-colorectal variants from Pearlman et al. [56], **c** colorectal variants from Yurgelun et al. [55], and **d** non-colorectal variants from Yurgelun et al. [55] High-penetrance colorectal genes included *MLH1, MSH2, MSH6, PMS2, APC, biallelic MUTYH,* and *SMAD4*. Moderate-penetrance colorectal genes included *APC I1307K*, monoallelic *MUTYH*. High-penetrance non-colorectal genes included *BRCA1, BRCA2, CDKN2A, PALB2,* and *TP53*. Moderate penetrance non-colorectal genes included *ATM, CHEK2, BARD1, BRIP1,* and *NBN*. Note: The *Y*-axis represents the number of patients with a variant. Some patients had more than one variant; the higher-penetrance variant is represented. Adapted from Boland, Goel & Patel [59]

pathogenic variants by CRC risk found in EOCRC patients from the Yurgelun et al. and Pearlman et al. cohorts. Stoffel et al. reported that 25% (79/315) EOCRC patients who had been selected for germline testing had a pathogenic variant. Again, twenty patients had phenotypically unexpected polyposis variants and one each had *BRCA1*, *TP53*, and *CHEK2* variants. In 2018, Toh et al. reported whole-exome sequencing analyzed for targeted cancer-associated genes from 88 EOCRC patients; however, MMR variant carriers were excluded [58]. In addition to six polyposis patients, they found six additional patients with variants in *ATM*, *BRCA2*, *PALB2*, *NTHL1*, and *WRN* (2). These studies demonstrate that EOCRC patients have a wide spectrum of pathogenic germline variants, often without manifesting the expected phenotype or expected family history.

In addition to traditionally non-CRC risk genes, it is important to note that some of the pathogenic variants identified in these EOCRC patients were "moderate" risk genes. For instance, Yurgelun et al. reported that 15% (16/106) of the variants for their entire (all age) cohort were in moderate penetrance genes. Twenty-one percent (15/72) of the variants found in the Pearlman et al. study and were in moderate penetrance genes. It is unclear if these germline variants drove the EOCRC or were coincidental findings. There is limited data on the long-term colonic and extra-colonic cancer risks associated with these moderate penetrance genes, and accordingly, professional guidelines are evolving on how best to minimize metachronous cancer risk for these patients [25].

These studies also demonstrated high rates of variants of uncertain significance (VUS), or variants where there is limited or conflicting information regarding pathogenicity. Yurgelun et al. reported that 38% of their entire cohort had at least one VUS and Pearlman et al. found a VUS in 32% of their patients.

Another important finding from these studies was that a substantial proportion of patients with variants did not meet the expected family history criteria. Mork et al. reported that 19% of the patients diagnosed with a hereditary syndrome did not have a family history consistent with the identified syndrome. Similarly, 19% and 26% of patients with a pathogenic variant in the Yurgelun and Pearlman cohorts, respectively, had no family history of cancer.

The spectrum of potential germline findings in EOCRC patients highlights the importance of pre- and post-testing genetic counseling. Although it is not feasible to review every possible expected or unexpected result from a multigene panel, genetic specialists can prepare patients for potentially unexpected results and the associated implications for themselves and their family members.

# Approach to a genetic evaluation in EOCRC

## Who should be evaluated?

All patients diagnosed with CRC, regardless of age, personal history, family history, or tumor characteristics, should have their tumors screened for LS with MSI, IHC, or both. For patients over age 50, those who meet clinical criteria for genetic testing based on family history and those who have MSI-H tumors and/or IHC patterns consistent with a possible germline variant should be referred for genetic counseling and genetic testing. This universal approach to tumor screening was first proposed by EGAPP in 2009 [49] and was adopted by the

NCCN in 2013, the MSTF in 2014 [6], and the pathology societies in 2017 [30].

In addition to screening all CRC patients for LS with tumor-based testing, all patients with CRC diagnosed under age 50, regardless of personal history, family history, or tumor characteristics should receive genetic counseling and be offered direct multi-gene panel germline genetic testing. The NCCN has supported this approach since 2017 [25, 60, 61].

## When should testing take place?

It is ideal to perform a genetic evaluation in EOCRC patients as early in the diagnostic workup as possible for several reasons. Most pressing, the presence of a hereditary condition may influence treatment options. For instance, in CRC patients with LS, a more extended colectomy is recommended given the increased lifetime risk of metachronous CRC [62, 63]. Furthermore, the presence of MSI provides prognostic information and customized treatment options for those with more advanced disease [64]. Patients with CRC and FAP due to pathogenic variants in the *APC* gene may be at increased risk for perioperative complications related to desmoid disease; thus, knowledge of a germline variant may influence the surgical approach [65]. The presence of a hereditary condition may also affect family planning decisions, especially when systemic therapies that impact fertility, such as chemotherapy and radiation, are needed [66]. Finally, it is also important to note that neoadjuvant treatment, which is currently the standard of care for non-stage I rectal cancer, can change the results of IHC. Goldstein et al. reported that neoadjuvant treatment can cause loss of previously intact MMR protein, particularly MSH6 [67].

It is therefore ideal to perform tumor-based screening on tissue obtained at the time of endoscopic diagnosis, rather than wait for surgical resection. Multiple studies have shown equivalent test characteristics when tumor-based testing is performed on endoscopic biopsies compared to surgical resection specimens [68, 69]. It is important to include samples of normal tissue so that there is a sufficient sample to run internal controls for IHC staining and compare MSI markers in normal vs tumor tissue.

Those with abnormal tumor-based screening, and all patients with EOCRC should be promptly referred for genetic counseling and genetic testing. There is unfortunately a shortage of genetic specialists in the USA which may contribute to delays in genetic evaluation [70]. Furthermore, in the midst of cancer staging and treatment, genetic referrals are often overlooked and even when placed, are deferred by patients because of the competing health demands of cancer treatment [71].

There are multiple practical approaches to overcoming barriers to timely genetic evaluation of EOCRC patients. Tumor-based screening on endoscopy biopsy specimens can be reflexively completed instead of relying on an explicit request from the endoscopist. Similarly, electronic health record systems can be leveraged to prompt referrals for genetic counseling/testing in all patients diagnosed with CRC under age 50. For medical systems with a multidisciplinary infrastructure that reviews all new cancer diagnoses, such as tumor boards, genetic specialists can be incorporated into these groups to provide genetic counseling and complete genetic testing at the time of CRC diagnosis as the patient is completing all other staging and referrals. This integrated approach has significantly improved the collection of accurate family history and timely

completion of genetic counseling and genetic testing [72]. Telehealth genetic counseling and mailed saliva kit genetic testing can overcome travel and access barriers without compromising quality or patient satisfaction [73, 74].

## Testing selection

As reviewed by Powers et al. [53], multi-gene panel testing has emerged as the standard of care for most patients undergoing hereditary cancer risk assessment. There are multiple commercially available genetic testing panels and many options for the number of genes included on various panels. Given the established overlap in CRC and non-CRC syndrome phenotypes, we recommend that patients at minimum be offered panels that include genes for which there are evidence-based guidelines for cancer risk reduction, whether they are traditionally thought to be related to CRC or not. More comprehensive panels including genes where the exact cancer risks are not fully established can be considered, depending on a shared decision with the patient. We recommend testing through a Clinical Laboratory Improvement Amendment (CLIA)-certified laboratory. It is of utmost importance that the patient receives genetic counseling regarding the medical and non-medical implications of expected and unexpected results, including the high probability of a VUS.

## Future directions

There are multiple areas of active research pertaining to a genetic evaluation in EOCRC patients. There have been recent advances in tumor-based, next-generation DNA sequencing to simplify the current multi-step algorithms for LS screening. This method generates a comprehensive genetic profile for germline and somatic variants in tumors. Hampel et al. demonstrated that tumor sequencing had better sensitivity and equivalent specificity for the detection of LS carriers [75•]. Although medical oncologists are more routinely performing tumor sequencing for personalized treatment options, this approach has not yet been adopted in clinical practice for LS screening.

As the genes included in multi-gene panels expand to include genes where there is a paucity of evidence on lifetime cancer risks, there is an increasing need to understand the lifetime colonic and extra-colonic cancer risks in order to formulate a consensus on risk-reduction guidelines.

With the increasing use of colonoscopy for a variety of indications in all age groups [76], another area of needed research is to understand the genetic spectrum among patients whom have had CRC precursors or advanced colorectal polyps. Though the polyps are removed via endoscopic polypectomy, thereby interrupting the natural history of CRC development, the underlying genetic predisposition of the patient does not change.

# Conclusions

A substantial proportion of EOCRCs is associated with a germline pathogenic variant in a cancer predisposition gene. Traditional methods of relying on clinical and family history criteria and tumor-based screening are challenging to apply in clinical practice and do not capture all patients with a germline variant. Thus, current recommendations are to perform comprehensive germline genetic testing in all patients diagnosed with CRC under the age of 50. Patients

with EOCRC have pathogenic variants in genes traditionally associated with CRC, such as LS or biallelic *MUTYH* carriers, but a substantial proportion also carries unexpected variants in genes such as *BRCA1/2*. It is critically important to offer genetic counseling to prepare patients for potentially unexpected results. As tumor sequencing becomes more cost-effective, it may replace traditional MSI and IHC methods of tumor-based screening to serve as screening for germline variants.

## Compliance with Ethical Standards

**Conflict of Interest**
SGP: no conflicts of interest
CRB: honoraria from Ambry Genetics

**Human and Animal Rights and Informed Consent**
This article does not contain any studies with human or animal subjects performed by any of the authors.

## References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

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