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Low Frequency of Lynch Syndrome Among Young Patients With Non-Familial Colorectal Cancer

Ajay Goel^{*}, Takeshi Nagasaka^{*,‡}, Jennifer Spiegel[§], Richard Meyer[§], Warren E. Lichliter^{||}, and C. Richard Boland^{*}

^{*}Department of Internal Medicine, Division of Gastroenterology, Charles A Sammons Cancer Center, and Baylor Research Institute, Baylor University Medical Center, Dallas, Texas

[‡]Department of Gastroenterological Surgery and Surgical Oncology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

[§]Department of Pathology, Baylor University Medical Center, Dallas, Texas

^{||}Division of Colorectal Surgery, Baylor University Medical Center, Dallas, Texas

Abstract

BACKGROUND & AIMS—Colorectal cancer (CRC) is uncommon in individuals <50 years old. Lynch Syndrome is caused by germline mutations in DNA mismatch repair (MMR) genes and associated with early-onset CRC, but little is known about the proportion of young patients with apparently sporadic CRC who actually have Lynch Syndrome. We examined patterns of microsatellite instability (MSI) and expression of MMR genes among patients <50 years old with non-familial CRC (patients with more than family member with CRC).

METHODS—Neoplastic and matched normal tissues were collected from 75 CRC patients <50 years old (mean age=34.5 years) and analyzed using immunohistochemical analyses of MLH1, MSH2, MSH6, and PMS2. MSI and mutations in *BRAF* and *KRAS* were also analyzed.

RESULTS—Most cancers (72%) arose in the distal colon. MSI was detected in 21% of the samples and loss of one or more MMR proteins was observed in 21%. Interestingly, only 38% of the MMR-deficient CRCs lost either MLH1 or MSH2, whereas 63% of the MMR-deficient CRC samples lost either PMS2 or MSH6. All 11 CRC samples that had lost MSH2, MLH1, or PMS2 had MSI, but only 2 of the 5 tumors that lost only MSH6 had MSI. There were no *BRAF* mutations in any tumor.

CONCLUSIONS—In young patients with apparently sporadic CRC, most tumors arise in the distal colon; only 21% have features of Lynch Syndrome. Loss of MSH6 or PMS2 occurred in 13.3% of these tumors. Most tumors that lose MSH6 will not be detected in screens for MSI; CRC screening might be modified to identify more patients with Lynch Syndrome.

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Reprint requests. Address requests for reprints to: C. Richard Boland, Gastrointestinal Cancer Research Laboratory, Baylor University Medical Center, 3500 Gaston Avenue, Suite 250 Hoblitzelle, Dallas, Texas 75246. rick.boland@baylorhealth.edu; fax: (214) 818-9292; or Ajay Goel, Gastrointestinal Cancer Research Laboratory, Baylor University Medical Center, 3500 Gaston Avenue, Suite 250 Hoblitzelle, Dallas, Texas 75246. ajay.goel@baylorhealth.edu; fax: (214) 818-9292.

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Conflicts of interest

The authors disclose no conflicts.

Keywords

Colon Cancer; Young Patients; Familial Colorectal Cancer

Colorectal cancer (CRC) is a common disease with a 5.29% lifetime risk in the United States. The disease typically associated with aging. The median age for CRC from 2001–2005 according to the United States SEER database was 71 years; 4.8% of CRCs occurred by age 45, and 16.9% by age 55¹. The disease is uncommon in young people.

A number of specific diseases are associated with CRCs that occur before age 50. These include inflammatory bowel disease, familial adenomatous polyposis (FAP), and Lynch Syndrome. However, the relative contribution of each of these has not been systemically explored, although there are important implications associated with each of these. Ulcerative colitis and most cases of FAP are readily identified by their phenotypic features, and both require total proctocolectomies. However, Lynch Syndrome does not have a characteristic premorbid phenotype, and inadequate management typically occurs when the diagnosis is not known prior to treating the tumor.

Lynch Syndrome is a familial predisposition to cancers of the colon, rectum, uterus, ovary, stomach, and other organs². The CRCs have some phenotypically distinctive features. They occur at an average age between 40–50, and approximately two thirds of these tumors occur proximal to the splenic flexure³. The diagnosis of Lynch Syndrome is suspected when there is a family history that involves 3 or more individuals with CRC and at least one patient with CRC is <50 years old⁴, but the diagnosis is definitively established by finding a deleterious germline mutation in a DNA mismatch repair (MMR) gene⁵. Germline mutations in any of four DNA MMR genes (*MSH2*, *MLH1*, *MSH6* and *PMS2*) cause Lynch Syndrome, and this diagnosis has been definitely made in 2.2% of a population-based survey of CRC patients, using existing sequencing analysis⁶. However, as the analytical approaches to these genes become more sensitive, the proportion of all CRC patients who have Lynch Syndrome may increase to perhaps 3–4%.

If a patient is known to have Lynch Syndrome and develops CRC, the recommended strategy is to perform a subtotal colectomy, since the risk of metachronous tumors is so high⁷. One study reported a 40% risk of a second primary CRC within 7 years of the first tumor⁸. Also, women with Lynch Syndrome need to understand that their risk of endometrial cancer may be even higher than their risk of CRC⁹, underscoring the implications of making a correct and timely diagnosis. Moreover, there are important implications for the first-degree relatives of patients with Lynch Syndrome. Perhaps it is not surprising that the majority of the germline mutations were in the *MSH6* and *PMS2* genes, since individuals with identifiable family histories of CRC were systematically excluded from the analysis. However, this underscores the importance of considering this diagnosis, and the implications for family counseling, in young patients with these cancers.

However, this diagnosis is a particular challenge when there is no family history to rely upon (small families, inadequate information), or if there has never been a germline mutation documented in the family. One should have an increased suspicion for this disease when CRC occurs in a young individual. However, the likelihood of this diagnosis, and the optimal approach to screening for the disease, have never been established. This study, which is first of its kind, examined a cohort of CRC patients ≤50 years old, from which we excluded those with a family history suggestive of Lynch Syndrome and those with FAP or inflammatory bowel disease, and screened the tumor tissue for the characteristic features of defective DNA MMR. Interestingly, we found that only a relatively small percentage of them had Lynch

Syndrome, and that the distribution of genes involved was unexpected. Furthermore, those young patients with CRC who did not have Lynch Syndrome had a marked clustering of tumors in the distal colon. We found other unique features by looking for mutations in *BRAF* and *KRAS* genes. These findings have important implications for the care of young patients with CRC.

MATERIALS AND METHODS

Tissue specimens

This study began with a cohort of 85 patients ≤ 50 years old, with a primary CRC in which the surgical treatment occurred at the Baylor University Medical Center, Dallas between 1986 and 2004. Patients with diagnoses of inflammatory bowel disease or FAP, and patients who had more than one family member known to have CRC were excluded from analysis. None of the patients with other Lynch syndrome associated cancers were excluded from data analysis. Patients signed a protocol-specific informed consent for use of their tissues and Institutional Review Board approval was granted for the studies, which were performed on anonymized samples.

Microdissection and DNA amplification

Serial sections from formalin-fixed paraffin-embedded neoplastic and matched normal tissues (5 μ m) were stained for pathological analysis, and representative normal and tumor regions identified. Normal (non-tumor) control tissue was obtained from histologically normal mucosa or lymph nodes. Genomic DNA was isolated from the tissue microdomains after serial rehydration of the slides. The hydrated tissues were digested with Proteinase K, and followed by DNA extraction using the QIAamp DNA mini-kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions.

Microsatellite instability Analysis

Microsatellite analysis of the extracted DNA specimens was performed by polymerase chain reaction (PCR) amplification using a panel of five mononucleotide repeat markers in a pentaplex PCR10. The five markers in the pentaplex PCR system were mononucleotide repeats: BAT25, BAT26, NR21, NR24 and NR27, as previously described^{10, 11}, and allelic sizes were estimated using Genescan 2.1 software (Applied Biosystems). Microsatellite instability (MSI) was defined when 2 or more of the 5 markers showed allelic shifts; those with 1 were called microsatellite low (MSI-L), and with 0 mutated alleles as microsatellite stable (MSS).

MMR Protein Immunohistochemistry (IHC)

We examined the protein expression of MLH1, MSH2, PMS2, and MSH6 in all 75 tumor tissues by IHC staining using the DAKO EnVision System-HRP polymer system kit (Dako Cytomation Inc., Carpinteria, CA). One block of formalin-fixed, paraffin-embedded tumor tissue was selected per case. Before IHC staining, antigen retrieval was performed by immersing sections in a 10 mM concentration of citrate buffer, pH 6.0, and boiling in a pressure cooker for 5 min. Sections were thereafter incubated for an hour with appropriate dilutions of mouse monoclonal antibodies against MLH1 (clone 13271A, BD Pharmingen, San Diego, CA), MSH2 (clone FE11, Oncogene Research Products, Boston, MA), PMS2 (clone A37, BD Pharmingen San Diego, CA), and MSH6 protein (clone 44, BD Transduction Laboratories, Lexington, KY). The peroxidase reaction was developed using diaminobenzidine tetrachloride as the chromogen. Tumor cells were judged to be negative for protein expression only if they lacked staining in a sample in which non-neoplastic colonocytes and stroma cells were positively stained. Tumors lacking MLH1 or MSH2 expression (i.e., the "major" DNA MMR

protein products) characteristically lack PMS2 or MSH6 expression respectively (i.e., the “minor DNA MMR protein products), because of heterodimeric protein stabilization^{12, 13}. Hence, we classified tumors lacking both MLH1 and PMS2 expression as MLH1-deficient, and tumors lacking both MSH2 and MSH6 expression as MSH2-deficient. This was distinguished from those tumors with isolated absence of expression of PMS2 or MSH6, which suggest specific abnormalities in the *PMS2* and *MSH6* genes.

Analysis for mutations in *BRAF* and *KRAS*

Mutational analysis of *BRAF* and *KRAS* were performed by PCR and sequencing as previously reported¹⁴. Analysis was specifically focused on the V600E mutation of *BRAF*, and codons 12 and 13 of *KRAS*.

Statistical Analysis

We used logistic regression analysis to examine the performance of the different strategies to define MSI status in the MMR-deficient tumors. To examine the relationship between the markers, multivariate correlation and hierarchical clustering analysis were performed by using standardized absolute difference length between tumor allele and germline allele as described previously¹¹. Analyses were performed with the use of JMP software, version 6.0 (SAS Institute). All reported P values are two-sided and a P value of less than 0.05 was considered statistically significant.

RESULTS

Patient characteristics

A total of 75 patients had CRC, were ≤ 50 years of age, did not have inflammatory bowel disease, FAP, or a family history that suggested Lynch Syndrome, and the colonic resection specimens were available for analysis. The mean age of the patients was 34.5 years (95% CI: 33.2–35.8). Forty one of the patients were women (55%), and 34 were men (45%). Twenty one of the CRCs (28%) were located proximal to the splenic flexure, and 54 (72%) were distal. Forty four (58.6%) were located in the rectum or rectosigmoid colon. Only 5 (7%) reported CRC in a first degree relative.

MMR-deficiency in non-familial CRCs is limited to patients under age 40

For purposes of this study, DNA MMR deficiency was defined as the failure to normally express any one of the 4 DNA MMR proteins at IHC in the tumor cell nuclei when there was normal protein expression in the non-neoplastic tissues in the section. Sixteen CRCs (21%) showed DNA MMR deficiency, and the distributions by gender, location in the colon and family history are illustrated in Table 1. The largest proportion of the patients was between ages 31–40 (75% of the cohort), and we found that all 21 CRCs with MMR deficiency were ≤ 40 years in age; with 20% (11 of 56) of MMR-deficient CRCs occurring in patients between ages 31–40 and 31% (5 of 16) in patients younger than age 30 ($P=0.40$). Significant associations were found between MMR deficiency and a first-degree relative with CRC ($P=0.0009$).

Unique MMR gene expression patterns are present in younger non-familial CRCs

Lynch syndrome CRCs predominantly harbor mutations in major MMR genes, *MLH1* and *MSH2*, and consequently IHC analysis often reveals loss of expression for these proteins in these patients. Since the molecular pathogenesis of non-familial CRCs is unclear, we performed IHC analysis for all 4 MMR proteins in our cohort of patients. Of the 16 DNA MMR deficient tumors, 3 failed to express MLH1 and PMS2 (“MLH1 deficient”), and 3 failed to express MSH2 and MSH6 (“MSH2 deficient”), which are the most common DNA MMR abnormalities

(Supplementary Table 1). However, surprisingly, 10 showed an isolated failure to express MSH6 (n=5) or PMS2 (n=5).

MSI was found in 16 CRCs (21%), including 14/16 (88%) of those with DNA MMR deficiency, and 1/59 (2%) that were DNA MMR proficient. There was a high degree of correlation between DNA MMR deficiency at IHC and MSI ($P<0.0001$). The microsatellite markers mutated in each case are shown in Supplementary Table 1. Two of 5 MSH6-deficient tumors were MSS, but all of the other DNA MMR-deficient tumors were detected by MSI testing. Seven of the 59 (12%) DNA MMR-proficient tumors had alterations at 1 of the mononucleotide repeat markers, which is termed MSI-low, and these tumors are phenotypically distinct from those CRCs that are MSI-H¹⁵. The receiver operating curve (ROC) in Supplementary Figure 1 illustrates the sensitivity and specificity of the pentaplex MSI assay used for the detection of DNA MMR-deficiency in our cohort of CRCs.

Table 2 demonstrates the individual results of IHC, analysis for MSI, and the clinical variables for the 16 individuals with DNA MMR deficiency. Although none of the patients' family histories met the Amsterdam Criteria for Lynch Syndrome, 4 had CRC in a first degree relative, but had no other distinctive features. The patients with PMS2-deficiency had high levels of MSI, with 4–5 microsatellite markers mutated, but in the MSH6 patients, one had 4 mutated markers, two had 3 mutant markers, and 2 had none.

CRCs in non-familial younger patients are predominantly present in the distal colon

Another interesting pathological feature of the colorectal cancers in younger non-familial patients was that majority of the tumors occurred in the distal colon (72% distal vs 28% proximal; $P<0.0001$; Table 1 and Figure 1). Distal location of CRCs in these patients was independent of age, gender, and MMR protein expression status. However, patients with another CRC in the first-degree relative, and tumors with mutant *KRAS* tended to occur more frequently in the proximal colon.

***BRAF* V600E mutations do not occur in younger non-familial CRCs**

We next determined whether there were any significant differences in the mutational profiles of MAPK-signaling pathways genes, *KRAS* and *BRAF* in our group of non-familial CRCs. For these experiments, we determined the mutational analysis of V600E *BRAF* mutation, and codons 12 and 13 *KRAS* mutations in all CRCs. Table 1 and Figure 1 shows the results from mutational analysis of *BRAF* and *KRAS* mutations. None of the 75 young CRC patients' tumors had the V600E *BRAF* mutation. However, 21/75 (28%) had a *KRAS* mutation in codon 12 or 13. There was no relationship between *KRAS* mutations and MSI, DNA MMR-deficiency, patient age, or tumor location (Supplementary Table 2).

DISCUSSION

In this study, we have systematically assessed the clinicopathological and molecular features of a large cohort of early-onset non-familial CRCs. We found that 21% of individuals <50 years old with CRC had an abnormality in the DNA MMR system that suggested Lynch Syndrome. However, importantly, this study uncovered a number of unexpected aspects of CRC in young people. First, most people ≤50 years old with CRC who do not have more than one first degree relative with CRC do not have Lynch Syndrome. Second, we discovered that contrary to typical Lynch syndrome patients, majority of younger non-familial patients demonstrate unique and unexpected distribution of MMR gene involvement in which there is a frequent loss of expression of PMS2 and MSH6 proteins. Third, majority of these cancers arise in the distal colon. Fourth, these tumors do not harbor *BRAF* V600E mutations.

Our study revealed that most patients with early-onset CRCs and not more than one first degree relative with CRC do not have Lynch Syndrome, and just 21% had otherwise cryptic familial CRC. An earlier study that only used MSI testing (and used only one dinucleotide repeat and one mononucleotide repeat marker) suggested that 58% of patients <35 years of age had MSI, whereas only 12% of those 35–55 years old did. However, that case series was selected on the basis of possible hereditary cancer, there was no exclusion on the basis of family history, and the investigators were unable to identify the DNA MMR gene involved in most of the cases¹⁶. It would appear that if one is dealing with a CRC patient ≤50 years old without a suspicious family history, it is relatively unlikely that Lynch Syndrome is involved.

We found features of Lynch Syndrome in 21% of our cases, none of which had a family history that met the Amsterdam Criteria⁴. Most interesting, 10/16 had deficiency of either *PMS2* or *MSH6* in their CRCs, which is quite distinct from the usual distribution of Lynch Syndrome genes, in which *MSH2* and *MLH1* account for ~90% of identifiable families¹⁷. None of the *MSH2* or *MLH1* cases had positive family histories (as these had been excluded by the protocol). There are several possible explanations for this, including *de novo* mutations, misattribution of paternity, inadequate information about the actual family history, and simple chance. These cases would be found if one screened all CRCs from young patients by MSI or IHC.

However, 2/5 of the *MSH6*-deficient tumors showed no mutated microsatellites, and would be missed if one used a screening approach based upon MSI. The penetrance of CRC in Lynch Syndrome-*MSH6* type is known to be less than in Lynch Syndrome linked to *MSH2* or *MLH1*^{18, 19}. Finding absent *PMS2* expression in 5 of the 16 cases is somewhat less difficult to explain. The diagnosis of Lynch Syndrome-*PMS2* type is infrequently made and probably underestimated, as *PMS2* is the only Lynch Syndrome gene for which technical issues have prevented the development of a commercially-available diagnostic test²⁰. However, it has been reported that the isolated absence of *PMS2* expression in a CRC, which is highly suggestive of Lynch Syndrome, may be as common as Lynch Syndrome-*MSH2* type²¹. The families may be underestimated because of the low penetrance for CRC in this variety of the syndrome²².

Another unexpected finding is that CRCs in young people have a predilection for the distal colon, whether or not there is DNA MMR deficiency. In fact, 72% were distal to the splenic flexure, and nearly 60% were in the rectum or rectosigmoid colon. The clustering was slightly more skewed when there was MMR-deficiency, where 75% were located distally.

There are limitations to this study. First, we did not have germline mutational analysis in this cohort to demonstrate which patients actually had Lynch Syndrome. However, the loss of DNA MMR gene expression and concomitant MSI strongly implicate the role of defective MMR in the genesis of these tumors. Somatic mutation of *MSH2*, *MSH6* and *PMS2* is quite rare. The only DNA MMR gene for which somatic inactivation plays a role is *MLH1*; this could be an issue in no more than 3 of our cases. Second, the patients were drawn from the experience at a single institution, and the study is not population-based. However, the cases were not selected on the basis of a family history (these were actually excluded), and the numbers are sufficiently large to be convincing, and we had excellent concordance between the IHC and MSI data.

In summary, we studied a cohort of CRC patients 50 years old or younger, and found that only 21% had unsuspected Lynch Syndrome; the rest had no other explanation for their disease. Those that had Lynch Syndrome were more likely to have involvement of the “minor” DNA MMR proteins *MSH6* and *PMS2*, rather than the “major” proteins *MSH2* or *MLH1*. Lynch Syndrome-*MSH6* type may be missed if the screening algorithm relies entirely upon MSI testing. These results have important clinical implications for tailoring CRC screening and surveillance strategies in younger patients with unsuspected Lynch Syndrome in future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. US SEER Data Base. 2009. <http://seer.cancer.gov/statfacts/html/colorect.html>
2. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med* 2003;348:919–932. [PubMed: 12621137]
3. Vasen HF. Clinical description of the Lynch syndrome [hereditary nonpolyposis colorectal cancer (HNPCC)]. *Fam Cancer* 2005;4:219–225. [PubMed: 16136381]
4. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. *Gastroenterology* 1999;116:1453–1456. [PubMed: 10348829]
5. Boland CR. Evolution of the nomenclature for the hereditary colorectal cancer syndromes. *Fam Cancer* 2005;4:211–218. [PubMed: 16136380]
6. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Nakagawa H, Sotamaa K, Prior TW, Westman J, Panescu J, Fix D, Lockman J, Comeras I, de la CA. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med* 2005;352:1851–1860. [PubMed: 15872200]
7. Lindor NM, Petersen GM, Hadley DW, Kinney AY, Miesfeldt S, Lu KH, Lynch P, Burke W, Press N. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. *JAMA* 2006;296:1507–1517. [PubMed: 17003399]
8. Lin KM, Shashidharan M, Ternent CA, Thorson AG, Blatchford GJ, Christensen MA, Lanspa SJ, Lemon SJ, Watson P, Lynch HT. Colorectal and extracolonic cancer variations in MLH1/MSH2 hereditary nonpolyposis colorectal cancer kindreds and the general population. *Dis Colon Rectum* 1998;41:428–433. [PubMed: 9559626]
9. Watson P, Riley B. The tumor spectrum in the Lynch syndrome. *Fam Cancer* 2005;4:245–248. [PubMed: 16136385]
10. Suraweera N, Duval A, Reperant M, Vaury C, Furlan D, Leroy K, Seruca R, Iacopetta B, Hamelin R. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. *Gastroenterology* 2002;123:1804–1811. [PubMed: 12454837]
11. Goel A, Nagasaka T, Hamelin R, Boland CR. An optimized pentaplex PCR for detecting DNA mismatch repair-deficient colorectal cancers. *PLoS One* 2010;5:e9393. [PubMed: 20195377]
12. Boland CR, Koi M, Chang DK, Carethers JM. The biochemical basis of microsatellite instability and abnormal immunohistochemistry and clinical behavior in Lynch Syndrome: from bench to bedside. *Fam Cancer* 2008;7:41–52. [PubMed: 17636426]
13. Chang DK, Ricciardiello L, Goel A, Chang CL, Boland CR. Steady-state regulation of the human DNA mismatch repair system. *J Biol Chem* 2000;275:29178. [PubMed: 10979986]
14. Nishida N, Nagasaka T, Nishimura T, Ikai I, Boland CR, Goel A. Aberrant methylation of multiple tumor suppressor genes in aging liver, chronic hepatitis, and hepatocellular carcinoma. *Hepatology* 2008;47:908–918. [PubMed: 18161048]
15. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998;58:5248–5257. [PubMed: 9823339]

16. Liu B, Farrington SM, Petersen GM, Hamilton SR, Parsons R, Papadopoulos N, Fujiwara T, Jen J, Kinzler KW, Wyllie AH. Genetic instability occurs in the majority of young patients with colorectal cancer. *Nat Med* 1995;1:348–352. [PubMed: 7585065]
17. Peltomaki P. Lynch syndrome genes. *Fam Cancer* 2005;4:227–232. [PubMed: 16136382]
18. Kolodner RD, Tytell JD, Schmeits JL, Kane MF, Gupta RD, Weger J, Wahlberg S, Fox EA, Peel D, Ziogas A, Garber JE, Syngal S, Anton-Culver H, Li FP. Germ-line msh6 mutations in colorectal cancer families. *Cancer Res* 1999;59:5068–5074. [PubMed: 10537275]
19. Pinto C, Veiga I, Pinheiro M, Mesquita B, Jeronimo C, Sousa O, Fragoso M, Santos L, Moreira-Dias L, Baptista M, Lopes C, Castedo S, Teixeira MR. MSH6 germline mutations in early-onset colorectal cancer patients without family history of the disease. *Br J Cancer* 2006;95:752–756. [PubMed: 16940983]
20. Nakagawa H, Lockman JC, Frankel WL, Hampel H, Steenblock K, Burgart LJ, Thibodeau SN, de la CA. Mismatch repair gene PMS2: disease-causing germline mutations are frequent in patients whose tumors stain negative for PMS2 protein, but paralogous genes obscure mutation detection and interpretation. *Cancer Res* 2004;64:4721–4727. [PubMed: 15256438]
21. Truninger K, Menigatti M, Luz J, Russell A, Haider R, Gebbers JO, Bannwart F, Yurtsever H, Neuweiler J, Riehle HM, Cattaruzza MS, Heinemann K, Schar P, Jiricny J, Marra G. Immunohistochemical analysis reveals high frequency of PMS2 defects in colorectal cancer. *Gastroenterology* 2005;128:1160–1171. [PubMed: 15887099]
22. Senter L, Clendenning M, Sotamaa K, Hampel H, Green J, Potter JD, Lindblom A, Lagerstedt K, Thibodeau SN, Lindor NM, Young J, Winship I, Dowty JG, White DM, Hopper JL, Baglietto L, Jenkins MA, de la Chapelle A. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. *Gastroenterology* 2008;135:419–428. [PubMed: 18602922]

Figure 1.

The figure demonstrates the relationship between location of the tumor in the colon and various diagnostic features including DNA MMR-IHC status, MSI, *KRAS* and *BRAF* mutations. Purple bars represent frequency of the tumors occurring in the distal colon, while blue bars are indicative of proximal location. As shown in the figure, majority of the younger non-familial CRCs occur in the distal colon, and their location is independent of the MMR status.

Table 1

Clinico-pathological features and their relationship with the MMR status in young patients with non-familial colorectal cancers

Characteristic	All Patients (n=75)	Patients with MMR-Deficiency (n=16)	Patients with MMR-Proficiency (n=59)	P-value*
Age at Diagnosis; No. (%)				
> 40	3 (4)	0 (0)	3 (100)	0.4
31 – 40	56 (75)	11 (20)	45 (80)	
≤ 30	16 (21)	5 (31)	11 (69)	
Gender; No. (%)				
Female	14 (55)	8 (20)	33 (80)	0.67
Male	34 (45)	8 (24)	26 (76)	
Location of tumor relative to Splenic Flexure; No. (%)				
Proximal	21 (28)	4 (25)	17 (29)	0.001
Distal	54 (72)	12 (75)	42 (71)	
Existence of a CRC in the First Degree Relative; No. (%)				
yes	5 (7)	4 (80)	1 (20)	0.0009
no	70 (93)	12 (75)	58 (98)	
MSI Status; No. (%)				
MSI-High	13 (17)	13 (100)	0 (0)	<0.0001
MSI-Low	3 (4)	1 (33)	2 (67)	
MSS	59 (79)	2 (3)	57 (97)	
KRAS/BRAF Mutation Status; No. (%) **				
BRAF mutant	0 (0)	0 (0)	0 (0)	0.90***
KRAS mutant	18 (27)	4 (22)	14 (78)	
Wild-type, both genes	48 (73)	10 (21)	38 (79)	

* χ^2 test was used to compare all variables.

** A total of 15 cases are missing information.

*** KRAS mutant vs wild-type, both genes by χ^2 test

Relationship between MMR-deficiency and various clinical and molecular factors in young patients with non-familial colorectal cancers

Table 2

Patient No.	Age (yrs)	Gender	MMR Deficiency	No. of MSI Markers mutated	KRAS Mutation Status	Amsterdam Criteria II	Family History (FDRs with CRC)	Tumor Location	T	TNM Stage*	
										T	N
001	34	Male	hMLH1	1	Mutant	Incompatible	None	Proximal	T2		N0
020	28	Male	hMLH1	3	Wild-type	Incompatible	None	Distal	T3		N1
067	24	Female	hMLH1	5	Mutant	Incompatible	None	Distal	T3		N1
031	33	Male	hMSH2	4	Wild-type	Incompatible	None	Distal	T3		N0
046	32	Female	hMSH2	5	Mutant	Incompatible	None	Distal	T3		N2
048	36	Female	hMSH2	5	Wild-type	Incompatible	None	Distal	missing		missing
072	39	Male	hMSH6	4	Wild-type	Incompatible	One (father)	Proximal	T3		N0
077	37	Male	hMSH6	3	Wild-type	Incompatible	One (brother)	Distal	T4		N0
004	39	Female	hMSH6	0	Wild-type	Incompatible	None	Distal	T3		N2
045	30	Female	hMSH6	3	Mutant	Incompatible	One (father)	Distal	T1		N0
082	34	Female	hMSH6	0	Wild-type	Incompatible	None	Distal	T1		N1
068	34	Male	hPMS2	5	Wild-type	Incompatible	One (sister)	Proximal	T1		N0
133	40	Female	hPMS2	4	N.D.	Incompatible	None	Distal	T1		missing
152	39	Female	hPMS2	5	N.D.	Incompatible	None	Distal	missing		missing
026	27	Male	hPMS2	4	Wild-type	Incompatible	None	Distal	T3		N1
034	20	Male	hPMS2	4	Wild-type	Incompatible	None	Proximal	T3		N1

* T, status of the depth of invasion of tumor; N, status of regional lymph node metastasis; M, status of distant metastasis; FDR, first degree relative.