Published in final edited form as:

Clin Gastroenterol Hepatol. 2011 June ; 9(6): 497–502. doi:10.1016/j.cgh.2010.10.021.

DNA Mismatch Repair Gene Alterations in a Population-Based Sample of Young-Onset Colorectal Cancer Patients

Paul J. Limburg¹, William S. Harmsen¹, Helen H. Chen¹, Steven Gallinger², Robert W. Haile³, John A. Baron⁴, Graham Casey³, Michael O. Woods⁵, Stephen N. Thibodeau¹, and Noralane M. Lindor¹

¹Division of Gastroenterology & Hepatology (P.J.L.), Division of Biomedical Statistics and Informatics (W.S.H.), Department of Laboratory Medicine (S.N.T.) and Department of Medical Genetics (H.H.C., N.M.L.), Rochester, Minnesota

²Cancer Care Ontario, Mount Sinai Hospital, Samuel Lunenfeld Research Institute, University of Toronto, Toronto, Ontario, Canada

³Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA

⁴Department of Community and Family Medicine, Dartmouth Medical School, Hanover, New Hampshire

⁵Department of Genetics, Faculty of Medicine, Memorial University, St John's, Newfoundland, Canada

Abstract

Background & Aims—Direct germline analysis could be used to screen high-risk patients for DNA mismatch repair gene mutations associated with Lynch Syndrome. To further evaluate this potential strategy, we examined the prevalence of *MLH1*, *MSH2* and *MSH6* mutations in a population-based sample of young-onset (age < 50 years) CRC cases.

Methods—Young-onset CRC cases were randomly selected from three Colon Cancer Family Registry sites (Cancer Care Ontario, Mayo Clinic, University of Southern California). Extracted DNA from peripheral blood leukocytes was shipped to Myriad Genetic Laboratories (Salt Lake City, UT) for *MLH1*, *MSH2* and *MSH6* sequencing, and duplication/deletion analyses for *MLH1* and *MSH2*. Results were reported as: deleterious/suspected deleterious, likely neutral, variant of

© 2010 The American Gastroenterological Association. Published by Elsevier Inc. All rights reserved.

Corresponding Author: Paul J. Limburg, M.D., M.P.H., 200 First Street SW, Rochester, MN 55905. Tel (507) 266-4338. Fax (507) 266-0350. limburg.paul@mayo.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosures: Dr. Limburg served as a consultant for Genomic Health, Inc. from 8/12/08–4/19/10.

Author Roles: Study concept and design (SG, R.W.H., J.A.B., S.N.T., N.M.L.); acquisition of data (P.J.L., SG, R.W.H., J.A.B., S.N.T., N.M.L.); analysis and interpretation of data (P.J.L., W.S.H., H.H.C., SG, R.W.H., J.A.B., G.C., M.O.W., S.N.T., N.M.L.); drafting of the manuscript (P.J.L., W.S.H., H.H.C., SG, R.W.H., J.A.B., G.C., M.O.W., S.N.T., N.M.L.); critical revision of the manuscript for important intellectual content (P.J.L., W.S.H., H.H.C., SG, R.W.H., J.A.B., G.C., M.O.W., S.N.T., N.M.L.); statistical analysis (P.J.L., W.S.H., H.H.C.); obtained funding (SG, R.W.H., J.A.B., N.M.L.)

Transcript Profiling: Not applicable.

Writing Assistance: None.

uncertain significance, or no alteration detected. Germline data were compared to Amsterdam II criteria (ACII) and immunohistochemistry testing (IHC) in secondary analyses.

Results—In 195 subjects, 11 had deleterious/suspected deleterious mutations (5.6%; 95% CI, 2.8%–9.9%), 12 had likely neutral alterations (6.2%; 3.2%–10.5%), 14 had variants of uncertain significance (7.2%; 4.0%–11.8%), 2 had both a likely neutral alteration and a variant of uncertain significance (1.0%; 0.1%–3.7%) and 156 had no alteration detected (80.0%; 73.7%–85.4%). Sensitivity, specificity, positive and negative predictive values for detecting deleterious/suspected deleterious mutations by ACII were 36.4% (4/11), 96.7% (178/184), 40.0% (4/10), and 96.2% (178/185) and by IHC testing were 85.7% (6/7), 91.9% (136/148), 33.3% (6/18) and 99.3% (136/137).

Conclusion—In this population-based sample of young-onset CRC cases, germline *MLH1*, *MSH2* and/or *MSH6* mutations were more prevalent than previously reported for CRC patients overall. Yet, since only about 1 in 20 young-onset CRC cases had confirmed deleterious/suspected deleterious mutations, further comparative effectiveness research is needed to determine the preferred Lynch Syndrome screening strategy for this high-risk group.

Keywords

Lynch Syndrome screening; mutation prevalence; direct germline analysis; Colon Cancer Family Registry

INTRODUCTION

In the United States, approximately 6.5% of all incident colorectal cancers (CRCs) are diagnosed prior to age 50 years (1). Unfortunately, these “young-onset” CRC cases often present with advanced stage tumors (2) and contribute disproportionately to the estimated 800,000 person-years of life lost annually to CRC (3). Recent data from the Surveillance, Epidemiology and End Results (SEER) program demonstrate that CRC incidence rates are gradually increasing in younger adults (4). Improved clinical management strategies are promptly needed to address the effects of this discouraging trend.

Lynch Syndrome is caused by germline defects in one or more DNA mismatch repair (MMR) genes and characterized by young-onset cancer(s) arising in the colorectum and other target organs (5). Most Lynch Syndrome-associated CRCs are reportedly due to *MLH1*, *MSH2* or *MSH6* mutations (6–8). Several clinical algorithms, molecular tests and statistical models have been developed to predict the presence or absence of Lynch Syndrome (7,10–15). Yet, despite these readily accessible tools, Lynch Syndrome patients continue to be widely under-recognized in clinical practice (16–18).

Direct germline mutation analysis, i.e., without clinical or molecular triage, represents a straightforward, potentially applicable strategy for Lynch Syndrome screening in young-onset CRC patients. However, the feasibility of this approach depends, in part, on the prevalence of MMR gene mutations in this defined patient group. To our knowledge, no prior studies have examined the prevalence of *MLH1*, *MSH2*, and *MSH6* mutations using an unselected, population-based sample of young-onset CRC cases identified at multiple North American centers. In the present study, we utilized data and tissue resources from the Colon Cancer Family Registry (Colon CFR) (9) to address this knowledge gap.

MATERIALS AND METHODS

Subject Population

Subjects were recruited through the Colon CFR, an international collaboration of six participating centers (University of Hawaii, Honolulu, HI; Fred Hutchinson Cancer Research Center, Seattle, SA; Mayo Clinic, Rochester, MN; University of Southern California Consortium, Los Angeles, CA; Cancer Care Ontario, Toronto, Canada; and University of Melbourne, Melbourne, Australia) organized in 1997 to create a comprehensive resource for genetic epidemiology studies. As described elsewhere (9), recruitment strategies differed across centers with respect to family ascertainment (population-based versus clinic-based) and CRC subject enrollment (all incident cases versus over-sampling by family history or early age of onset). For the current investigation, a random sample of exclusively population-based, young-onset (diagnosed prior to age 50 years) CRC cases who (a) were recruited during phase I of the Colon CFR collaboration (1997–2002) through Cancer Care Ontario (n=67), Mayo Clinic Rochester (n=67) and University of Southern California (n=67) and (b) had extracted DNA available for germline *MLH1*, *MSH2* and *MSH6* mutation analyses was included. Specifically, there was no preselection based upon family history or availability/results of tumor testing. From the initial sample of 201 young-onset CRC cases, 6 subjects were excluded based on tumor location in the appendix (n=4) or anus (n=2), rather than the colorectum. Demographic data, venipuncture samples, tumor blocks and pathology reports were collected according to established protocols (available at http://epi.grants.cancer.gov/documents/CFR/CCFR_BiospecimenCoreSOP_040607.pdf). CRC anatomic subsites were ascertained from pathology reports. Proximal cancers were defined as tumors located in the cecum, ascending colon, hepatic flexure, transverse colon and splenic flexure; distal cancers were defined as tumors located in the descending colon, sigmoid colon, rectosigmoid colon, and rectum, respectively.

Gene Mutation Analyses

Extracted DNA samples (from peripheral blood leukocytes) were shipped to Myriad Genetic Laboratories (Salt Lake City, UT) for full mutation analyses of *MLH1*, *MSH2* and *MSH6*. The testing center was blinded to all clinical data associated with the specimens. Assay methods were identical to those used for clinical testing. Upon receipt, samples were assigned a unique barcode for robotic specimen tracking. DNA was amplified by polymerase chain reaction (PCR) for each subject. The amplified products were each directly sequenced in forward and reverse directions, using fluorescent dye-labeled sequencing primers: *MLH1* (approximately 2,300 base pairs comprising 19 exons and approximately 560 adjacent non-coding intronic base pairs), *MSH2* (approximately 2,800 base pairs comprising 16 exons and approximately 470 adjacent non-coding intronic base pairs) and *MSH6* (approximately 4,080 base pairs comprising 10 exons and approximately 290 adjacent non-coding intronic base pairs). The analyzed, non-coding intronic regions of *MLH1*, *MSH2* and *MSH6* do not extend more than 20 base pairs proximal to the 5' end and 10 base pairs distal to the 3' end of each exon. Chromatographic tracings of each amplicon were analyzed by a proprietary computer-based review, followed by visual inspection and confirmation. Genetic variants were detected by comparison with a consensus wild-type sequence constructed for each gene. Sequence information of the coding region was derived from RefSeq NM_000249.3 (*MLH1*), NM_000251.1 (*MSH2*) and NM_000179.2 (*MSH6*). Intronic nucleotide information was derived from genomic sequences from NCBI - AC011816.17 (*MLH1*), AC079775.6 (*MSH2*), AC006509.15 (*MSH6*). All potential genetic variants were independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above. Large rearrangement testing for *MLH1* and *MSH2* was performed by Southern Blot Analysis in conjunction with multiplex ligation-

dependent probe amplification (MLPA reagents from MRC Holland). For Southern analysis of *MLH1* and *MSH2*, three sets of restriction digests were performed for each gene using individual enzymes or a combination of enzymes to yield appropriate banding patterns. Digested DNA was electrophoresed on agarose gels, transferred to nylon membranes, and hybridized with a gene-specific probe radiolabeled with ³²P. Autoradiographs and phosphorimages were analyzed for the presence of novel bands and for fragment dosage, to assess the presence of deletions or duplications in exon regions. Southern blot and MLPA data were subjected to dual reviews involving technical personnel and at least one laboratory director for confirmation. Germline alterations were categorized as deleterious/suspected deleterious, likely neutral, or variant of uncertain significance, with specific sequence alterations recorded for each gene.

Immunohistochemistry Testing

Immunohistochemistry (IHC) for MLH1, MSH2, and MSH6 protein expression was performed as previously described (10,11) at Mayo Clinic Rochester (for MCR and USC cases) and at Cancer Care Ontario (for CCO cases), according to established protocols for clinical and research evaluation. In brief, 4–6 µm tissue sections were cut from formalin-fixed, paraffin-embedded tissue blocks and stained using the avidin-biotin complex method of Ventana Medical Systems (BioTek Solutions buffer kit and DAB detection kit) and the Tech Mate 500 (Ventana) automated immunohistochemical stainer. Antibodies to MLH1 (clone G168-728, 1/250; Pharmingen, San Diego, Calif), MSH2 (clone FE11, 1/50; Oncogene Research Products, Cambridge, Mass) and MSH6 (clone 44, 1/500; Transductions Laboratories, Lexington, Ky) were employed. MMR protein expression was reported as present, absent, or inconclusive for each immunostain. Since tumor phenotype was not considered a primary study endpoint, complete IHC data were only available for 155/195 (79%) subjects.

Statistical Analyses

All genotyping results were provided to the Colon CFR participating sites by Myriad Genetics. Data analyses were conducted by the Colon CFR investigators. Summary statistics were reported as mean (SD) or frequency (percent), as appropriate. Baseline demographic characteristics were compared across the three Colon CFR participating sites using the Student's t test or chi-square test, as appropriate. Prevalence estimates for germline MMR gene alterations, in aggregate (i.e., finding present in one or more genes) and for *MLH1*, *MSH2* and *MSH6* separately, were defined *a priori* as the primary study endpoint, with accompanying 95% exact binomial confidence intervals (CI) reported. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for detecting deleterious/suspected deleterious MMR gene mutations were also estimated based on the Amsterdam II criteria (ACII [12]; fulfilled vs. not fulfilled) and IHC testing for MLH1, MSH2 and MSH6 protein expression (any absent vs. all present), as secondary analyses in subjects for whom relevant data were available. All statistical tests were performed two-sided (alpha level 0.05) using SAS version 9.0 software.

RESULTS

One hundred ninety-five subjects were included in the final analysis cohort, with 91 (47%) men and 104 (53%) women. The mean (standard deviation) age at CRC diagnosis was 42.9 (+/- 6.1) years. Subject characteristics were similarly distributed across the three participating Colon CFR sites, with the exception of age at CRC diagnosis (Table 1).

Germline *MLH1*, *MSH2* and *MSH6* sequencing data were obtained for 195 (100%), 195 (100%) and 189 (97%) subjects, respectively. Specific MMR gene sequence alteration data

are provided in Table 2. Results were interpreted as deleterious/suspected deleterious mutations in 11 (5.6%; 95% CI, 2.8–9.9%) subjects, likely neutral alterations in 12 (6.2%; 95% CI, 3.2%–10.5%) subjects, variants of uncertain significance in 14 (7.2%; 95% CI, 4.0–11.8%) subjects, both likely neutral alteration and variant of uncertain significance in 2 (1.0%; 95% CI, 0.1%–3.7%) subjects, and no alteration detected in 156 (80.0%; 95% CI: 73.7%–85.4%) subjects. The prevalence of deleterious/suspected deleterious mutations was not significantly associated with Colon CFR participating site: 3/65 (4.6%; 95% CI, 1.0%–12.9%) subjects from CCO, 3/65 (4.6%; 95% CI, 1.0%–12.9%) subjects from MCR and 5/65 (7.7%; 95% CI, 2.5%–17.0%) subjects from USC ($p=0.79$). Subjects with deleterious/suspected deleterious mutations were of comparable age to subjects with other variant or normal sequencing results (mean [SD] of 38.8 [9.6] versus 43.2 [5.8] years, respectively; $p=0.19$). The prevalence of deleterious/suspected deleterious mutations was also similar in men (8.8% [8/91]; 95% CI, 3.9–16.6%) and women (2.9% [3/104]; 95% CI, 0.6%–8.2%) ($p=0.07$). Conversely, among subjects for whom CRC anatomic subsite was known, deleterious/suspected deleterious mutations were more commonly observed ($p<0.001$) in those with proximal tumors (18.2% [8/44]; 95% CI: 8.2%–32.7%) than distal tumors (2.3% [3/130]; 95% CI: 0.5–6.6%).

Self-reported family history data were available for all cases, and 10/195 (5.1%) fulfilled ACII. Sensitivity, specificity, PPV and NPV estimates for detecting subjects with deleterious/suspected deleterious mutations by ACII were 36.4% (4/11), 96.7% (178/184), 40.0% (4/10) and 96.2% (178/185). Complete IHC data were available for 155 (79.5%) cases, and absent expression of at least one MMR protein was detected in 18/155 (12%) subjects, distributed as: 10 MLH1 deficient only (6.5%), 5 MSH2 and MSH6 deficient (3.2%) and 3 MSH6 only deficient (1.9%). Sensitivity, specificity, PPV and NPV estimates for detecting subjects with deleterious/suspected deleterious mutations by IHC testing were 85.7% (6/7), 91.9% (136/148), 33.3% (6/18) and 99.3% (136/137).

DISCUSSION

In this multi-center study of young-onset CRC cases, direct germline analyses of *MLH1*, *MSH2* and *MSH6* yielded a prevalence of 5.6% for deleterious/suspected deleterious mutations. To our knowledge, this study represents the first description of non-triaged MMR gene testing in a North American, population-based sample of young-onset CRC cases. Encouragingly, our findings are consistent with results from a prior, population-based study in Scotland that reported an MMR gene mutation prevalence of 4.4% for CRC cases diagnosed prior to age 55 years (13). Conversely, the reported prevalence of MMR gene mutation carriers among CRC patients overall (i.e., irrespective of age) is 2.2%, even after molecular pre-screening (14). Together, these data emphasize the need for heightened awareness of possible Lynch Syndrome in the context of young-onset CRC.

Aggressive CRC surveillance programs appear to reduce morbidity and mortality for MMR gene mutation carriers (15–17). Yet, widespread application of existing Lynch Syndrome screening tools has been compromised by inadequate provider knowledge, inattention to family history, inaccessible laboratory expertise, and other factors. In addition, the screening performance characteristics of clinically-based algorithms, such as the Amsterdam criteria (I or II) or Bethesda Guidelines (original or revised) are suboptimal (18,19). Even within our relatively homogeneous cohort of young-onset CRC cases, the ACII failed to detect a majority of subjects (6/11; 54.5%) with deleterious/suspected deleterious MMR gene mutations. To overcome the shortfalls associated with these clinically-based algorithms, Hampel and colleagues have proposed that all CRC patients, regardless of age, undergo primary IHC testing for MMR protein expression, with subsequent gene mutation and/or methylation analyses determined by the IHC results (23). In our study, IHC testing yielded 1

false negative (IVS14+2T>C alteration in *MLH1*) and 13 false positive results in secondary analyses of 155 young-onset CRC cases for whom complete protein expression data were available. Possible contributing factors to the observed specificity estimate (91.9%) include epigenetic modification (i.e., *MLH1* promoter hypermethylation), recently described mutations not detectable by the applied methodology (e.g., deletion in TACSTD1 [20]) or mutations in other non-analyzed genes that interact with the MMR proteins tested. By extrapolation, IHC triage would have permitted detection of the large majority of MMR gene mutation carriers in our subject sample, with a marked reduction in the proportion of young-onset CRC cases (18/155; 11.6%) for whom targeted or complete germline analyses were indicated.

Several statistical models have also been developed to assist in selecting high-risk CRC patients for MMR gene mutation analyses. Notable examples include the Leiden Model, PREMM_{1,2}, MMRpredict and MMRpro (6,13,21–23). Using a consecutive series of 725 CRC cases (< 75 years of age at diagnosis; 18 confirmed MMR gene mutation carriers) identified through the Newfoundland Colorectal Cancer Registry, Green, et al. evaluated and directly compared the discriminatory capacity of the Leiden Model, PREMM_{1,2}, MMRpredict and MMRpro (24). At a defined sensitivity threshold of 94%, these predictive models exhibited greater specificity (range 61–88%) than the revised Bethesda Guidelines (51%), with the MMRpredict model demonstrating the most favorable performance characteristics. Interestingly, development of the MMRpredict model was based on data from CRC cases diagnosed at age < 55 years, implying that this model may be particularly beneficial in stratifying Lynch Syndrome risk for young-onset CRC cases. Unfortunately, limited availability of some key input parameters (i.e., family history of endometrial cancer) did not allow us to fully assess these statistical models in our study.

Major strengths of our study include the population-based, multi-center design with randomly identified, clearly defined young-onset CRC cases, reducing the possibility of referral or selection bias; *MLH1*, *MSH2* and *MSH6* germline analyses using well-established laboratory techniques, decreasing the potential for misclassification of mutation status; and universal gene testing, which permitted a more accurate description of the mutation prevalence than would have been obtained through triaged germline analyses. As noted above, the germline mutation prevalence reported from our study is consistent with limited existing data (13), lending credence to the external validity of our findings. One acknowledged weakness of our study is the absence of more comprehensive molecular marker data (such as microsatellite instability phenotype, *MLH1* promoter methylation status, *BRAF* mutation testing, etc.) to further complement the reported germline analyses. Practical and technological constraints at the time the present study was initiated did not permit routine availability of such data. Since our primary aim was to estimate the prevalence of *MLH1*, *MSH2*, and *MSH6* mutation carriers in an unselected, population-based case sample, we did not want to introduce possible selection bias by requiring ancillary laboratory data for study inclusion. Of note, further molecular characterization of Colon CFR case subjects is ongoing and will be reported in future studies.

Other MMR genes such as *PMS2* have been associated with Lynch Syndrome, but were not assessed in our study. Although not definitively established (5,14,25–29), *MLH1*, *MSH2* and *MSH6* reportedly account for around 95% of all mutations observed in Lynch Syndrome families, suggesting that additional germline analyses would have had limited effect on our mutation prevalence estimates. Duplication/deletion analyses for *MSH6* were not conducted in the small number of young-onset CRC cases for whom deficient protein expression was detected by IHC, which could have contributed to a slight underestimation of the observed prevalence estimates as well. Importantly, likely neutral alterations, variants of uncertain significance, or both were detected in 6.2%, 7.2% and 1.0% of our young-onset CRC cases,

respectively. Such sequence alterations can pose clinical and psychological challenges with respect to cancer risk assessment (30,31), particularly if additional protein expression and/or other molecular data are not available. These issues would need to be carefully considered for any screening strategies that apply germline mutation analyses as the primary testing modality.

In summary, data from our study and others (13) suggest that only about 1 in 20 young-onset CRC cases are attributable to germline mutations in *MLH1*, *MSH2*, or *MSH6*. Yet, a more systematic approach to Lynch Syndrome screening seems appropriate in this patient population, since the prevalence of MMR gene mutations is considerably higher than reported for CRC patients overall, and early detection of Lynch Syndrome kindreds has been shown to provide distinct clinical benefits. Nonetheless, given the relatively low proportion of MMR gene mutation carriers observed in our study, further comparative effectiveness research is needed to determine the most appropriate strategy for Lynch Syndrome screening in this high-risk patient group.

Acknowledgments

The authors gratefully acknowledge funding for and performance of the reported gene mutation analyses by Myriad Genetic Laboratories (Salt Lake City, UT).

Grant Support: The Colon Cancer Family Registry is supported by NIH National Cancer Institute grants CA074783 (Ontario Registry for Studies of Familial Colorectal Cancer), U01 CA074800 (Mayo Clinic Cooperative Family Registry for Colon Cancer Studies) and U01 CA074799 (USC Familial Colorectal Neoplasia Collaborative Group), as well as U01 CA097735 (Australian Colorectal Cancer Family Registry), U01 CA074794 (Seattle Colorectal Cancer Family Registry), and U01 CA074806 (University of Hawaii Colorectal Cancer Family Registry). The study sponsor had no role in the study design or collection, analysis, and interpretation of the data.

Funding for and conduct of the gene mutation analyses described in this report were provided by Myriad Genetic Laboratories, Salt Lake City, UT.

Abbreviations

ACII	Amsterdam II criteria
Colon-CFR	Colon Cancer Family Registry
CRC	colorectal cancer
IHC	immunohistochemistry
MMR	mismatch repair
NPV	negative predictive value
PPV	positive predictive value.

REFERENCES

1. Rim SH, Seeff L, Ahmed F, et al. Colorectal cancer incidence in the United States, 1999–2004 : an updated analysis of data from the National Program of Cancer Registries and the Surveillance, Epidemiology, and End Results Program. *Cancer*. 2009; 115:1967–1976. [PubMed: 19235249]
2. Dozois EJ, Boardman LA, Suwanthanma W, et al. Young-onset colorectal cancer in patients with no known genetic predisposition: can we increase early recognition and improve outcome? *Medicine (Baltimore)*. 2008; 87:259–263. [PubMed: 18794708]
3. Yabroff KR, Bradley CJ, Mariotto AB, et al. Estimates and projections of value of life lost from cancer deaths in the United States. *J Natl Cancer Inst*. 2008; 100:1755–1762. [PubMed: 19066267]

4. Siegel RL, Jemal A, Ward EM. Increase in incidence of colorectal cancer among young men and women in the United States. *Cancer Epidemiol Biomarkers Prev.* 2009; 18:1695–1698. [PubMed: 19505901]
5. Lindor NM, McMaster ML, Lindor CJ, et al. Concise handbook of familial cancer susceptibility syndromes - second edition. *J Natl Cancer Inst Monogr.* 2008:1–93. [PubMed: 18559331]
6. Wijnen JT, Vasen HF, Khan PM, et al. Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. *N Engl J Med.* 1998; 339:511–518. [PubMed: 9709044]
7. Abdel-Rahman WM, Mecklin JP, Peltomaki P. The genetics of HNPCC: application to diagnosis and screening. *Crit Rev Oncol Hematol.* 2006; 58:208–220. [PubMed: 16434208]
8. Woods MO, Williams P, Careen A, et al. A new variant database for mismatch repair genes associated with Lynch syndrome. *Hum Mutat.* 2007; 28:669–673. [PubMed: 17347989]
9. Newcomb PA, Baron J, Cotterchio M, et al. Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. *Cancer Epidemiol Biomarkers Prev.* 2007; 16:2331–2343. [PubMed: 17982118]
10. Lindor NM, Burgart LJ, Leontovich O, et al. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. *J Clin Oncol.* 2002; 20:1043–1048. [PubMed: 11844828]
11. Casey G, Lindor NM, Papadopoulos N, et al. Conversion analysis for mutation detection in MLH1 and MSH2 in patients with colorectal cancer. *JAMA.* 2005; 293:799–809. [PubMed: 15713769]
12. Vasen HF, Watson P, Mecklin JP, et al. 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]
13. Barnetson RA, Tenesa A, Farrington SM, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. *N Engl J Med.* 2006; 354:2751–2763. [PubMed: 16807412]
14. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med.* 2005; 352:1851–1860. [PubMed: 15872200]
15. Jarvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology.* 2000; 118:829–834. [PubMed: 10784581]
16. Wagner A, van Kessel I, Kriege MG, et al. Long term follow-up of HNPCC gene mutation carriers: compliance with screening and satisfaction with counseling and screening procedures. *Fam Cancer.* 2005; 4:295–300. [PubMed: 16341806]
17. de Jong AE, Hendriks YM, Kleibeuker JH, et al. Decrease in mortality in Lynch syndrome families because of surveillance. *Gastroenterology.* 2006; 130:665–671. [PubMed: 16530507]
18. Kievit W, de Bruin JH, Adang EM, et al. Current clinical selection strategies for identification of hereditary non-polyposis colorectal cancer families are inadequate: a meta-analysis. *Clin Genet.* 2004; 65:308–316. [PubMed: 15025724]
19. Julie C, Tresallet C, Brouquet A, et al. Identification in daily practice of patients with Lynch syndrome (hereditary nonpolyposis colorectal cancer): revised Bethesda guidelines-based approach versus molecular screening. *Am J Gastroenterol.* 2008; 103:2825–2835. quiz 2836. [PubMed: 18759827]
20. Ligtenberg MJ, Kuiper RP, Chan TL, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. *Nat Genet.* 2009; 41:112–117. [PubMed: 19098912]
21. Chen H, Sullivan G, Quon MJ. Assessing the predictive accuracy of QUICKI as a surrogate index for insulin sensitivity using a calibration model. *Diabetes.* 2005; 54:1914–1925. [PubMed: 15983190]
22. Balmana J, Stockwell DH, Steyerberg EW, et al. Prediction of MLH1 and MSH2 mutations in Lynch syndrome. *JAMA.* 2006; 296:1469–1478. [PubMed: 17003395]
23. Chen S, Euhus DM, Parmigiani G. Quantitative models for predicting mutations in Lynch Syndrome genes. *Curr Colorectal Cancer Reports.* 2007; 3:206–211.

24. Green RC, Parfrey PS, Woods MO, et al. Prediction of Lynch syndrome in consecutive patients with colorectal cancer. *J Natl Cancer Inst.* 2009; 101:331–340. [PubMed: 19244167]
25. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. *J Clin Oncol.* 2008; 26:5783–5788. [PubMed: 18809606]
26. Clendenning M, Senter L, Hampel H, et al. A frame-shift mutation of PMS2 is a widespread cause of Lynch syndrome. *J Med Genet.* 2008; 45:340–345. [PubMed: 18178629]
27. South CD, Yearsley M, Martin E, et al. Immunohistochemistry staining for the mismatch repair proteins in the clinical care of patients with colorectal cancer. *Genet Med.* 2009; 11:812–817. [PubMed: 19752738]
28. Mueller J, Gazzoli I, Bandipalliam P, et al. Comprehensive molecular analysis of mismatch repair gene defects in suspected Lynch syndrome (hereditary nonpolyposis colorectal cancer) cases. *Cancer Res.* 2009; 69:7053–7061. [PubMed: 19690142]
29. Niessen RC, Kleibeuker JH, Westers H, et al. PMS2 involvement in patients suspected of Lynch syndrome. *Genes Chromosomes Cancer.* 2009; 48:322–329. [PubMed: 19132747]
30. Vos J, Otten W, van Asperen C, et al. The counsellees' view of an unclassified variant in BRCA1/2: recall, interpretation, and impact on life. *Psychooncology.* 2008; 17:822–830. [PubMed: 18157792]
31. Grover S, Stoffel EM, Mercado RC, et al. Colorectal cancer risk perception on the basis of genetic test results in individuals at risk for Lynch syndrome. *J Clin Oncol.* 2009; 27:3981–3986. [PubMed: 19620492]

TABLE 1

Subject Characteristics, Overall and by Colon CFR Registry Site

	Overall (n=195)	CCO (n=65)	MCR (n=65)	USC (n=65)	p value
Age at CRC diagnosis, years ¹	42.9 (6.1)	44.9 (4.2)	42.2 (6.6)	41.5 (6.8)	0.004 [†]
Women, N (%)	104 (53.3)	38 (58.5)	32 (49.2)	34 (52.3)	0.56
Proximal:distal tumor site, N (%) ²	44 (25.3):130 (74.7)	15 (23.8):48 (76.2)	16 (25.8):46 (74.2)	13 (26.5):36 (73.5)	0.95 [‡]
Amsterdam II criteria met, N (%) ³	10 (5.1)	4 (6.2)	4 (6.2)	2 (3.1)	0.78
Deleterious/suspected deleterious mutation	11 (5.6)	3 (4.6)	3 (4.6)	5 (7.7)	0.79
MMR protein loss by IHC, N (%) ⁴					0.86
MLH1 deficient only	10 (6.5)	3 (5.6)	4 (6.3)	3 (8.1)	
MSH2 deficient only	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
MSH6 deficient only	3 (1.9)	2 (3.7)	0 (0.0)	1 (2.7)	
MLH1 and MSH2 deficient	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
MLH1 and MSH6 deficient	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
MSH2 and MSH6 deficient	5 (33.2)	2 (3.7)	2 (3.1)	1 (2.7)	
None deficient	137 (88.4)	47 (87.0)	58 (90.6)	32 (86.5)	

CCO = Cancer Care Ontario; MCR = Mayo Clinic Rochester; USC = University of Southern California consortium.

¹ mean (standard deviation);

² percentages based on subjects with known tumor site (n=174);

³ percentages based on subjects with complete family history data (n=195);

⁴ percentages based on subjects with complete IHC data (n=155).

[†] pair-wise comparisons between sites: CCO vs. MCR p=0.007; CCO vs. USC p=0.002; MCR vs. USC p=0.59.

[‡] excluding cases with tumor site not specified (CCO=2, MCR=3, USC=16).

TABLE 2
Clinical and Molecular Data for Young-onset CRC Cases with Germline Alterations in MMR Genes

Case	Gender	Age	CRC Site	Gene (Exon/Intron)	Alteration	Interpretation ¹	ACII Met	IHC Analysis		
								MLH1	MSH2	MSH6
1	M	34	Sigmoid	<i>MLH1</i> (8)	c.676C>T (p.R226X)	Deleterious	No	Absent	Present	Present
2	F	45	Splenic Flexure	<i>MLH1</i> (19)	c.2146G>A (p.V716M)	Likely neutral	No	Present	Present	Present
3	F	43	Rectum	<i>MLH1</i> (30)	c.307-19A>G	Likely neutral	No	N/A	N/A	N/A
4	F	30	Descending	<i>MLH1</i> (19)	c.2146G>A (p.V716M)	Likely neutral	No	Present	Present	Present
5	F	N/A	Ascending	<i>MLH1</i> (140)	c.1668-2A>G	Suspected Deleterious	No	Absent	Present	Present
6	M	39	Transverse	<i>MLH1</i> (151)	c.1732-19T>A	Uncertain	No	Present	Present	Present
7	F	41	Colon NOS	<i>MLH1</i> (150)	c.1732-19T>A	Uncertain	No	N/A	N/A	N/A
8	M	47	Sigmoid	<i>MLH1</i> (12)	c.1321G>A (p.A441T)	Uncertain	No	Present	Present	Present
9	M	47	Hepatic Flexure	<i>MLH1</i> (140)	c.1667+2T>C	Suspected Deleterious	Yes	Present	Present	Present
10	F	47	Ascending	<i>MLH1</i> (11)	c.911A>G (p.D304G)	Uncertain	Yes	Present	Present	Absent
11	M	34	Descending	<i>MSH1</i> (2)	c.199G>A (p.G67R)	Deleterious	No	Absent	Present	Present
12	M	39	Colon NOS	<i>MLH1</i> (17); <i>MSH2</i> (3)	c.1963A>G (p.1655V); c.380A>G (p.N127S)	Uncertain; Likely neutral	No	N/A	N/A	N/A
13	M	25	Ascending	<i>MSH2</i> (51)	c.942+3A>T (p.Val265_Gln314del)	Deleterious	Yes	Present	Absent	Absent
14	M	46	Sigmoid	<i>MSH2</i> (11)	c.1730T>C (p.I577T)	Uncertain	No	Present	Present	Present
15	F	36	Transverse	<i>MSH2</i> (121)	c.2006-4G>A	Uncertain	No	Present	Present	Present
16	F	49	Ascending	<i>MSH2</i> (12)	c.1786_1788delAAT (p.N596del)	Deleterious	No	N/A	N/A	N/A
17	M	47	Descending	<i>MSH2</i> (51)	c.942+3A>T (p.Val265_Gln314del)	Deleterious	No	N/A	N/A	N/A
18	M	49	Sigmoid	<i>MSH2</i> (3)	c.380A>G (p.N127S)	Likely neutral	No	Present	Present	Present
19	F	27	Sigmoid	<i>MSH2</i> (121)	c.2006-4G>A	Uncertain	No	Present	Present	N/A
20	F	47	Ascending	<i>MSH2</i> (101)	c.1662-18T>C	Likely neutral	No	Present	Present	Present
21	M	22	Ascending	<i>MSH2</i> (14)	c.2297delT (p.I766NfsX46)	Deleterious	Yes	N/A	N/A	N/A
22	F	37	Rectum	<i>MSH2</i> (7)	c.1168C>T (p.L390F)	Likely neutral	No	Present	Present	Present
23	F	38	Colon NOS	<i>MSH2</i> (3)	c.380A>G (p.N127S)	Likely neutral	No	N/A	N/A	N/A
24	M	45	Rectum	<i>MSH2</i> (5)	c.835C>G (p.L279V)	Uncertain	No	Present	Present	N/A
25	M	43	Rectum	<i>MSH2</i> (2)	c.214G>A (p.A72T)	Uncertain	No	Present	Present	Present

Case	Gender	Age	CRC Site	Gene (Exon/Intron)	Alteration	Interpretation ¹	ACII Met	IHC Analysis		
								MLH1	MSH2	MSH6
26	F	36	Ascending	MSH2 (10)	c.1576delA (p.T526PfsX17)	Deleterious	Yes	Present	Absent	Absent
27	M	46	Ascending	MSH2 (3); MSH6 (5)	c.380A>G (p.N127S); c.3226C>T (p.R1076C)	Likely neutral; Uncertain	No	Present	Present	Absent
28	M	48	Descending	MSH6 (9i)	c.4002-10T>A	Uncertain	No	Present	Present	Present
29	F	45	Rectum	MSH6 (4)	c.2992T>A (p.S998T)	Uncertain	No	Present	Present	Present
30	F	45	Rectum	MSH6 (4)	c.2633T>C (p.V878A)	Likely neutral	No	Present	Present	Present
31	M	45	Rectum	MSH6 (4)	c.1186C>G (p.L396V)	Likely neutral	No	Present	Present	Present
32	M	42	Ascending	MSH6 (9)	c.3991C>T (p.Alal268_Arg1334>GlyfsX6)	Suspected Deleterious	No	Present	Present	N/A
33	M	46	Sigmoid	MSH6 (6)	c.3488A>T (p.E1163V)	Uncertain	No	Present	Present	Present
34	F	48	Rectum	MSH6 (4)	c.2633T>C (p.V878A)	Likely neutral	No	Present	Present	Present
35	F	N/A	Colon NOS	MSH6 (4)	c.2633T>C (p.V878A)	Likely neutral	No	N/A	N/A	N/A
36	M	41	Colon NOS	MSH6 (9i)	c.4002-10T>A	Uncertain	No	N/A	N/A	N/A
37	F	40	Rectum	MSH6 (4)	c.1186C>G (p.L396V)	Likely neutral	No	Present	Present	Present
38	F	44	Rectum	MSH6 (4)	c.686A>G (p.E229G)	Uncertain	No	Present	Present	Present
39	M	46	Ascending	MSH6 (9)	3957ins19	Deleterious	No	N/A	N/A	N/A

N/A = data not available; NOS = not otherwise specified;

¹ based on results reported by Myriad Genetic Laboratories.