Published in final edited form as:

Gut. 2015 January ; 64(1): 101–110. doi:10.1136/gutjnl-2013-306567.

Role of tumor molecular and pathology features to estimate colorectal cancer risk for first-degree relatives

Aung Ko Win¹, aniel D. Buchanan², Christophe Rosty^{2,3,4}, Robert J. MacInnis^{1,5}, James G. Dowty¹, Gillian S. Dite¹, Graham G. Giles^{1,5}, Melissa C. Southey⁶, Joanne P. Young², Mark Clendenning², Michael D. Walsh², Rhiannon J. Walters², Alex Boussioutas^{7,8,9}, Thomas C. Smyrk¹⁰, Stephen N. Thibodeau¹⁰, John A. Baron¹¹, John D. Potter^{12,13,14}, Polly A. Newcomb^{12,13}, Loïc Le Marchand¹⁵, Robert W. Haile¹⁶, Steven Gallinger^{17,18}, Noralane M. Lindor¹⁹, John L. Hopper¹, Dennis J. Ahnen²⁰, and Mark A. Jenkins¹

¹Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, Victoria, Australia. ²Cancer and Population Studies Group, Queensland Institute of Medical Research, Clive Berghofer Cancer Research Centre, Herston, Queensland, Australia. ³Department of Molecular and Cellular Pathology, University of Queensland, Herston, Queensland, Australia. ⁴Envoi Specialist Pathologists, Herston, Queensland, Australia. ⁵Cancer Epidemiology Centre, Cancer Council Victoria, Carlton, Victoria, Australia. ⁶Genetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, Parkville, Victoria, Australia. ⁷Department of Medicine, Royal Melbourne Hospital, The University of Melbourne, Parkville, Australia. ⁸Cancer Genomics and Predictive Medicine, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia. ⁹Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Victoria, Australia. ¹⁰Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA. ¹¹Department of Medicine, University of North Carolina, Chapel Hill, North Carolina, USA. ¹²Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. ¹³School of Public Health, University of Washington, Seattle, Washington, USA. ¹⁴Centre for Public Health Research, Massey University, Wellington, New Zealand. ¹⁵University of Hawaii Cancer Center, Honolulu, Hawaii, USA. ¹⁶Stanford Cancer Institute, Stanford University, San Francisco, California, USA ¹⁷Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada. ¹⁸Cancer Care Ontario, Toronto, Ontario, Canada. ¹⁹Department of Health Science Research, Mayo Clinic Arizona, Scottsdale, Arizona, USA. ²⁰Department of Veterans

Corresponding author Mark Jenkins, PhD Centre for Epidemiology and Biostatistics Melbourne School of Population and Global Health Level 3, 207 Bouverie Street The University of Melbourne VIC 3010 Australia Tel: +61 3 8344 0902 Fax: +61 3 9349 5815 m.jenkins@unimelb.edu.au.

DISCLAIMER The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the CFRs, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government or the CFR. Authors had full responsibility for the design of the study, the collection of the data, the analysis and interpretation of the data, the decision to submit the manuscript for publication, and the writing of the manuscript.

LICENCE FOR PUBLICATION The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in Gut editions and any other BMJ PGL products to exploit all subsidiary rights, as set out in our licence (<http://group.bmj.com/products/journals/instructions-for-authors/licence-forms>).

DISCLOSURE The authors have no conflict of interest to declare with respect to this manuscript.

Affairs, Eastern Colorado Health Care System, University of Colorado School of Medicine, Denver, Colorado, USA.

Abstract

Objective—To estimate risk of colorectal cancer (CRC) for first-degree relatives of CRC cases based on CRC molecular subtypes and tumor pathology features.

Design—We studied a cohort of 33,496 first-degree relatives of 4,853 incident invasive CRC cases (probands) who were recruited to the Colon Cancer Family Registry through population cancer registries in the US, Canada, and Australia. We categorized the first-degree relatives into four groups: 28,156 of 4,095 mismatch repair (MMR)-proficient probands, 2,302 of 301 MMR-deficient non-Lynch syndrome probands, 1,799 of 271 suspected Lynch syndrome probands, and 1,239 of 186 Lynch syndrome probands. We compared CRC risk for first-degree relatives stratified by the absence or presence of specific tumor molecular pathology features in probands across each of these four groups and for all groups combined.

Results—Compared with first-degree relatives of MMR-proficient CRC cases, a higher risk of CRC was estimated for first-degree relatives of CRC cases with suspected Lynch syndrome (hazard ratio [HR] 2.06, 95% confidence interval [CI] 1.59-2.67), and with Lynch syndrome (HR 5.37, 95% CI 4.16-6.94), but not with MMR-deficient non-Lynch syndrome (HR 1.04, 95% CI 0.82-1.31). A greater risk of CRC was estimated for first-degree relatives if CRC cases were diagnosed before age 50 years, had proximal colon cancer or if their tumors had any of the following: expanding tumor margin, peritumoral lymphocytes, tumor-infiltrating lymphocytes, or synchronous CRC.

Conclusion—Molecular pathology features are potentially useful to refine screening recommendations for first-degree relatives of CRC cases, and to identify which cases are more likely to be caused by genetic or other familial factors.

Keywords

colorectal cancer; molecular pathology, mismatch repair deficiency, relatives; risk prediction; Lynch syndrome, suspected Lynch syndrome

INTRODUCTION

Having a family history of colorectal cancer (CRC) is a well-established risk factor for the disease; people with one first-degree relative (parent, offspring, sibling) with CRC are, on average, twice as likely to be diagnosed with CRC than those without a family history.(1, 2) This has clinical implications for prevention as more intensive screening can be targeted to those who have a relative with CRC; many guidelines for CRC screening reflect this approach.(3-6) In addition, CRC diagnosed in a setting of a family history is more likely to be due to an inherited mutation and, when identified through genetic screening, has significant and quantifiable risks for CRC and other cancers.(7-9) However, there is substantial variation around this average risk of family history.(10) For example, the

younger the age at diagnosis of the affected relative and the more closely related the affected relative, the greater the risk.(11)

As for breast cancer, recent analyses of population-based family studies have shown that, at least and perhaps more so for cancers diagnosed before the age of 40 years, tumor morphology can be used to further sub-categorise familial risks. For example, trabecular growth pattern and high mitotic index are strong predictors for having a germline mutation in *BRCA1*,(12) and hence predict risk for first-degree relatives. After excluding known *BRCA1* and *BRCA2* mutation carriers, absence of extensive sclerosis, circumscribed growth, extensive intraductal carcinoma, and a lobular growth pattern of breast cancers were independent predictors associated with a two- to three-fold increased risk of breast cancer for first-degree relatives.(13) The first-degree relatives of the one-third of early-onset cases who had none of these morphology features showed no evidence for an increased (familial) risk of breast cancer.

For CRC, it is well established that tumor molecular features can predict germline mutation status with greater sensitivity and specificity and in a more cost-effective way than using family history alone, particularly in early-onset disease.(14) Tumor-infiltrating lymphocytes, proximal colonic location, mucinous histology, poor differentiation and Crohn's-like reaction are now well-recognized pathology predictors for mismatch repair (MMR)-deficiency resulting from a germline mutation in one of the MMR genes.(15, 16) In this study, we estimated CRC risk for first-degree relatives of CRC cases based on tumor molecular subtypes and tumor pathology features.

MATERIALS AND METHODS

Study Sample

We studied a cohort of the first-degree relatives of people with incident invasive CRC (probands) recruited by the Colon Cancer Family Registry between 1997 and 2007 through state or regional population cancer registries in the USA (Washington, California, Arizona, Minnesota, Colorado, New Hampshire, North Carolina, and Hawaii), Australia (Victoria) and Canada (Ontario). Study designs and recruitment for the Colon Cancer Family Registry can be found at <http://coloncfr.org/> and have been previously published in detail.(17) Written informed consent was obtained from all study participants, and the study protocol was approved by the institutional research ethics review board at each study center.

Data Collection

Information on demographics, personal characteristics, personal and family history of cancer, cancer-screening history, and history of polyps, polypectomy, and other surgeries was obtained by questionnaires from all probands and participating relatives. Participants were followed up approximately every 5 years after baseline to update information across all the study centers. The present study was based on all available baseline and follow-up data until 2012. Reported cancer diagnoses and age at diagnosis were confirmed, where possible, using pathology reports, medical records, cancer registry reports, and death certificates. The tumor anatomic location and histology were coded and stored using International

Classification of Diseases for Oncology, third edition (ICD-O-3).(18) Blood samples and permission to access tumor tissue were requested from all participants.

Colorectal Cancer Tumor Pathology Review

Probands' CRCs were reviewed by pathologists from each study center of the Colon Cancer Family Registry and assessed for the following features: histologic type (adenocarcinoma, mucinous, signet ring cell), histologic grade (low or high grade), tumor margin (expanding or infiltrative), and peritumoral lymphocytes, Crohn's-like reaction, tumor-infiltrating lymphocytes, venous invasion, and synchronous CRC (present or absent). Tumors were classified as mucinous carcinoma or signet-ring-cell carcinoma if at least 50% of the tumor demonstrated the specific morphology. Low-grade was defined as adenocarcinoma with 50% gland formation and high-grade as adenocarcinoma with <50% gland formation. Tumor margin describes the advanced edge of the tumor into the bowel wall and was categorized as expanding margin for well-circumscribed edge pushing into the deep portion of the wall and infiltrative margin for widespread dissection of the bowel wall by the cancer cells.(19) The presence of peritumoral lymphocytes was based on the finding of a cap of lymphocytes at the deepest part of the invasive tumor front.(20) Crohn's-like reaction was recorded as present for tumors demonstrating at least 4 nodular lymphoid aggregates within a single x4 field, deep to the advancing edge of the tumor.(21) Tumor-infiltrating lymphocytes were regarded as present when 5 intratumoral lymphocytes were identified within a single x40 field of a hematoxylin and eosin-stained section.(16) Colon cancer was defined as any diagnosis of cancer in the proximal colon (caecum to transverse colon; ICD-O-3 codes C18.0, C18.2–C18.4), distal colon (splenic flexure to sigmoid colon; C18.5–C18.7), or an unspecified location of the colon (C18.8, C18.9 and C26.0). Rectal cancer was defined as any diagnosis of cancer in the rectosigmoid junction (C19.9) and rectum (C20.9 and C21.8).

Molecular Characterization

Mismatch repair status—Probands' CRCs were characterized for MMR-deficiency by microsatellite instability (MSI) using a ten-marker panel and/or by immunohistochemistry (IHC) for the four MMR proteins.(22) Tumors were classified as MMR-deficient if they were MSI-high (30% or more of the markers show instability) and/or showed loss of expression of one or more of the MMR proteins by IHC; and MMR-proficient if they were microsatellite stable (no unstable markers) or MSI-low (<30% unstable markers) and/or showed normal expression of all four MMR proteins by IHC. Of the 4843 proband CRCs with a MMR IHC or MSI result, 3325 (68.7%) had both tests performed, 1508 (31.1%) had MMR IHC only and 10 (0.2%) had MSI only (Supplementary table 1). Of the 3325 proband CRCs that had both tests performed, 3202 were concordant between MSI and MMR IHC (96.3%, 95% confidence intervals (CIs) 95.6–96.9%). Probands with a MMR-deficient CRC underwent germline mutation testing (Sanger sequencing and MLPA) as previously described.(17, 23-25)

Molecular testing—A fluorescent allele-specific PCR assay was used to detect the somatic T>A mutation at nucleotide 1799 in exon 15 of the *BRAF* gene (*BRAF* V600E) as has been previously described.(26, 27) Methylation of the *MLH1* gene promoter was

measured in all MSI-high and MSI-low cases with sufficient tumor DNA and a random sample of microsatellite-stable cases. *MLH1* methylation was measured using the MethyLight *MLH1*-M2Methylight reaction using an *ALU* control reaction to normalize for bisulfite-converted input DNA,(28) with the modifications described in Poynter et al.(29) We classified samples with a proportion of methylated reference (PMR) greater than or equal to 10 as positive for *MLH1* methylation. An *ALU* control reaction cycle threshold [C(t)] value of <25 was used to retain the largest sample size possible for the analysis while minimizing the potential for false negatives.

Classification

We categorized the probands into four groups:

- “MMR-proficient”: probands with a mismatch repair (MMR)-proficient CRC;
- “MMR-deficient non-Lynch syndrome”: probands with a CRC that had loss of *MLH1* and *PMS2* protein expression with evidence of *MLH1* methylation and/or had the *BRAF* V600E mutation;
- “Suspected Lynch syndrome”: probands with a CRC that had *MLH1*/*PMS2* loss with no evidence of *MLH1* methylation and/or *BRAF* V600E mutation or had *MSH2*/*MSH6* loss or solitary loss of *PMS2* or *MSH6* or were MSI-H, for which no MMR germline mutation had been identified; and
- “Lynch syndrome”: probands known to carry a pathogenic MMR germline mutation.

Statistical Analysis

Observation time for each member of the cohort (a first-degree relative of a CRC-proband) started at birth and ended at: first diagnosis of invasive cancer; first polypectomy; last contact; or death, whichever occurred earliest. Of the 35,567 first-degree relatives, unaffected relatives with no information on age were censored at birth i.e., excluded from analysis (n=2071; 6%) and the remaining 33,496 entered into the analysis. For cancer-affected relatives with missing age at diagnosis (n=120; 0.4%), age at diagnosis was assumed to be one year prior to the last known age (n=102) or, if last known age was not available, the median age at diagnosis of the specific cancer in the general population (n=18). The sex-specific median age for each cancer was obtained from the SEER Cancer Statistics Review (1975– 2000).(30)

We estimated the standardized incidence ratio (SIR) of CRC for the first-degree relatives of CRC cases compared with the general population as a function of measured covariates by using the data on first-degree relatives of case-probands. Each SIR was calculated by dividing the observed number of CRCs first-degree relatives by the number expected based on the age-, sex- and country-specific incidence in the general population.(31) The Jackknife method was used to estimate 95% CIs to adjust for correlation of risk between relatives from the same family.(32)

In this study, exposure is the tumor pathology features of colorectal cancer of the proband and outcome is the incidence of CRC in the first-degree relatives. We estimated CRC hazard ratios (HRs) for first-degree relatives of CRC cases depending on the absence or presence of specific tumor pathology features. The HRs and robust estimates of corresponding 95% CIs were calculated from Cox proportional hazard regression models by taking into account clustering by family membership to allow for correlation of risk between relatives from the same family.(33) To investigate independent associations of the tumor pathology features with familial risk, we fitted variables that showed a nominally statistically significant association in the univariable analyses into a multivariable model. We estimated CRC risk for first-degree relatives associated with a total number of proband's features that were shown to be statistically significant in the multivariable model, as a categorical (≥ 3 vs <3) as well as continuous (per feature) factor.

To account for the missing values that occurred for some pathology features, we devised a multiple imputation model based on previous recommendations of Van Buuren et al.(34) and Ali et al.(35) The missing data were assumed to be 'missing at random' so that the reason for missingness can be explained by the observed data.(36) The model included all predictor variables, the outcome variable (diagnosis of CRC) and additional variables that we considered may increase the plausibility of the missing at random assumption in order to improve the imputation process. While some researchers claim that as few as 3–5 imputed datasets is sufficient for imputation,(37) we chose 10 sets based on recommendations that the number of sets should approximate the percentage of subjects with some missing data. (38) Location of CRC was imputed using polytomous logistic regression while the other pathology features were imputed using logistic regression. Missing values were sampled and replaced with a set of plausible values randomly drawn from their predicted distribution based on the other observed variables, thus creating 10 completed datasets. Cox proportional hazard regression models were run separately for each imputed data set and estimates of the predictor variables were combined using the programmes written by Carlin et al.(39) As a sensitivity analysis, we also compared the estimates of association using the imputed missing data with the estimates from a complete case analysis.

Some centers of the Colon Cancer Family Registry used stratified sampling based on family history for recruitment and we did take this into account when combining and analyzing the data. To adjust for this stratified sampling, we gave each relative a probability weight equal to the reciprocal of the family sampling fraction of their proband used by each study center. (17) All statistical tests were two-sided and $p < 0.05$ was regarded as nominally statistically significant. All statistical analyses were performed using Stata 12.1.(40)

RESULTS

Of the 4,853 probands with an invasive CRC from the population-based resources of the Colon Cancer Family Registry, 4,095 (84%) were MMR-proficient; 301 (6%) were MMR-deficient non-Lynch syndrome; 271 (6%) were suspected Lynch syndrome (104 MLH1/PMS2 loss, 45 MSH2/MSH6 loss, 17 PMS2 loss, 27 MSH6 loss, and 78 MSI-high), and 186 (4%) were Lynch syndrome with confirmed pathogenic mutations in MMR gene mutations (59 *MLH1*, 70 *MSH2*, 25 *MSH6*, 30 *PMS2*, 2 *EPCAM*). The probands were diagnosed with a

CRC at a mean age of 54.2 (standard deviation [SD] 11.6) years, with approximately one-third of tumors diagnosed in each of the proximal colon, distal colon, and rectum (Table 1). Compared with MMR-proficient probands, the mean age at diagnosis of CRC was 10.3 (95%CI 9.02–11.6) years older for MMR-deficient non-Lynch syndrome probands, 5.28 (95%CI 3.95–6.61) years younger for suspected Lynch syndrome probands, and 9.33 (95%CI 7.74–10.9) years younger for Lynch syndrome probands (Supplementary Table 2).

We identified 33,496 first-degree relatives of the participating probands: 28,156 (84%) relatives of MMR-proficient probands; 2,302 (7%) of MMR-deficient non-Lynch syndrome probands; 1,799 (5%) of suspected Lynch syndrome probands, and 1,239 (4%) of Lynch syndrome probands (Table 2). Of all first-degree relatives, 1,404 (4%) were diagnosed with a CRC at a mean age of 62.1 (SD 14.1) years. Compared with first-degree relatives of MMR-proficient probands, mean age at diagnosis of CRC was 3.10 (95%CI 0.57–5.63) years older for first-degree relatives of MMR-deficient non-Lynch syndrome probands, 6.01 (95%CI 3.54–8.47) years younger for first-degree relatives of suspected Lynch syndrome probands, and 14.8 (95%CI 12.5–17.0) years younger for first-degree relatives of Lynch syndrome probands (Supplementary Table 2). Verification was sought for all reported CRCs in the relatives. Of the 1404 CRCs reported in the first-degree relatives, 605 (43%) had been verified by pathology reviews, medical records, death certificates or cancer registries (Supplementary Table 3). For cancers that we could not obtain verification, we did not solely rely on the proband to report family history of cancer. Rather, we interviewed multiple family members to ascertain all diagnoses of cancer in family. For apparently conflicting reports within the family, we gave priority to the report provided by the closest family member.

Using all the data, we observed 1,404 CRCs in the 33,496 first-degree relatives of CRC cases over the 1,792,526 person-years. We estimated that first-degree relatives of CRC cases had an approximately 2-fold higher risk of CRC compared with the general population (SIR 1.79, 95%CI 1.66–1.94). Compared with first-degree relatives of MMR-proficient CRC cases, a higher risk of CRC was estimated for first-degree relatives of suspected Lynch syndrome CRC cases (HR 2.06, 95%CI 1.59–2.67), and for first-degree relatives of Lynch syndrome CRC cases (HR 5.37, 95%CI 4.16–6.94), but not for relatives of MMR-deficient non-Lynch syndrome CRC cases (HR 1.04, 95%CI 0.82–1.31) (Table 3).

A higher risk of CRC was estimated for first-degree relatives if the CRC case's tumor had: diagnosis before age 50 years (HR 1.64, 95%CI 1.43–1.88); a proximal colonic location (HR 1.33, 95%CI 1.16–1.53); an expanding tumor margin (HR 1.66, 95%CI 1.35–2.05); peritumoral lymphocytes (HR 1.26, 95%CI 1.06–1.49); tumor-infiltrating lymphocytes (HR 1.33, 95%CI 1.15–1.55); and a synchronous tumor (HR 1.69, 95%CI 1.23–2.32) (Table 4). There was marginal evidence that presence of Crohn's-like reaction in CRC case was associated with a higher CRC risk for first-degree relatives (HR 1.17, 95%CI 0.99–1.49). There was no evidence that CRC risks for first-degree relatives depend on the CRC case's sex ($p=0.42$), tumor histologic subtype ($p=0.68$), grade ($p=0.72$), and venous invasion ($p=0.91$) (See detail in Supplementary Table 4).

We therefore fitted proband's age at diagnosis, tumor location, tumor margin, peritumoral lymphocytes, tumor-infiltrating lymphocytes and synchronous CRC in the multivariable model (Table 5). When combined all groups, we found that the CRC case's age at diagnosis <50 years (HR 1.61, 95%CI 1.40–1.87), tumor proximal colonic location (HR 1.29, 95%CI 1.12–1.49), expanding tumor margin (HR 1.48, 95%CI 1.19–1.83), presence of peritumoral lymphocytes (HR 1.21, 95%CI 1.01–1.44), presence of tumor-infiltrating lymphocytes (HR 1.21, 95%CI 1.03–1.43), and presence of synchronous CRC (HR 1.61, 95%CI 1.19–2.18) were all independently associated with an increased CRC risk for first-degree relatives. When we stratified by MMR-deficiency status of the CRC case, we estimated that a higher risk of CRC for first-degree relatives was associated with: age at diagnosis <50 years ($p=0.008$) and an expanding tumor margin ($p<0.001$) when the CRC case was MMR-proficient; age at diagnosis <50 years ($p=0.006$) and presence of synchronous CRC ($p=0.004$) when the CRC case was MMR-deficient non-Lynch syndrome; and age at diagnosis <50 years ($p=0.001$) and presence of peritumoral lymphocytes ($p<0.001$) when the CRC case was Lynch syndrome (Table 5).

When we combined the proband's age at diagnosis and tumor pathology features that were independently associated with CRC risk for first-degree relatives, we estimated that there was an approximately two-fold increased risk of CRC for first-degree relatives (HR 1.91, 95%CI 1.65–2.22) if probands had three or more of the following six features: age at diagnosis <50 years, tumor's proximal colonic location, presence of expanding tumor margin, peritumoral lymphocytes, tumor-infiltrating lymphocytes and synchronous CRC. When we stratified by MMR-deficiency status of the CRC case, the approximate two-fold increase in risk was still present for first-degree relatives of CRC cases with three or more features compared with first-degree relatives of CRC cases with less than three features in each group (Table 6).

When we fitted these six features as a continuous factor, we estimated the HR per feature of the CRC case to be 1.33 (95%CI 1.26–1.42) for first-degree relatives when combined all groups, 1.21 (95%CI 1.13–1.30) for first-degree relatives of MMR-proficient CRC cases, 1.54 (95%CI 1.23–1.92) for first-degree relatives of MMR-deficient non-Lynch syndrome CRC cases, 1.28 (95%CI 1.06–1.55) for first-degree relatives of suspected Lynch syndrome CRC cases, and 1.33 (95%CI 1.11–1.58) for first-degree relatives of Lynch syndrome CRC cases.

In a complete case analysis without any missing data, we found that use of the imputed missing data had no considerable effect on estimates of association (details not shown). We also estimated CRC risk for first-degree relatives by fitting the specific tumor location of the proband as an ordinal (continuous) variable in the model. We found a hazard ratio of 1.05 (95%CI 1.02–1.07) showing that the more proximally located CRC of the proband is associated with the higher risk of CRC for first-degree relatives when combined all groups (Supplementary Table 5). When we investigated the association between *BRAF* V600E mutation of proband's CRC tumor and CRC risk for first-degree relatives, we found no evidence of association even after stratification by MMR status (Supplementary Table 6).

DISCUSSION

Using a large family cohort study, we estimated that first-degree relatives of suspected Lynch syndrome CRC cases had a higher risk of CRC compared with first-degree relatives of MMR-proficient CRC cases, and a lower risk of CRC compared with first-degree relatives of Lynch syndrome CRC cases. We found that first-degree relatives had a higher risk of CRC if the CRC case was diagnosed <50 years, had proximal colon cancer, an expanding tumor margin, peritumoral lymphocytes, tumor-infiltrating lymphocytes, or a synchronous CRC. These CRC features could be useful in identifying the first-degree relatives of CRC cases who are at increased CRC risk, even within a specific molecular subtype of CRC.

The clinical management of CRC cases with suspected but genetically unproven to have Lynch syndrome (i.e. MMR-deficient, no MMR germline mutation, and not *MHL1*-methylated or *BRAF*-mutated), and of their relatives, can be particularly challenging as it is not possible to distinguish which if any relatives are at increased risk because they carry a mutation in an MMR gene. Little is known about the cancer risks for members of these families and therefore screening and treatment guidelines are not well defined. The average age at CRC diagnosis for first-degree relatives of CRC cases with suspected Lynch syndrome was between that for first-degree relatives of MMR-proficient cases and that for first-degree relatives of confirmed Lynch syndrome cases (58 vs 49 and 64 years, respectively). We estimated that CRC risk for first-degree relatives of suspected Lynch syndrome CRC cases was between that for first-degree relatives of MMR-proficient cases and that for first-degree relatives of confirmed Lynch syndrome cases, and this is consistent with the observation by Rodriguez-Soler et al.(41)

It is possible that a proportion of these suspected Lynch syndrome CRC cases might be due to the existence of complex or cryptic mutations in MMR genes that are not readily identified by current Sanger sequencing and MLPA techniques.(42-44) It could also be argued that the suspected Lynch syndrome CRC cases carrying undetected mutations have associated with them a more moderate CRC penetrance compared with that for the exonic and splice site mutations that are more readily detected.(45) With the increasing use of next generation sequencing in clinical diagnostics, further investigation of more complex and cryptic mutational mechanisms will be possible. This will be accompanied by an increasing need to assess the pathogenicity of variants of uncertain clinical significance (VUS) in the MMR genes and quantify the risk of CRC for carriers of VUS and their relatives, and build upon the significant progress achieved to date.(46, 47) Alternatively, a proportion of suspected Lynch syndrome cases have been shown to result from somatic inactivation within the tumor through biallelic mutations.(48, 49) Though an intermediate screening recommendation has been recommended for suspected Lynch syndrome families,(41) optimal screening strategies are yet to be defined given this group is likely to be heterogeneous with regards to family history and to the mechanism of MMR inactivation.

First-degree relatives of CRC cases with confirmed Lynch syndrome have a greater CRC risk than first-degree relatives of MMR-proficient CRC cases. Current screening recommendations for untested first-degree relatives of Lynch syndrome CRC cases is

typically annual colonoscopy starting at age 20-25 years, or 10 years younger than the youngest diagnosed CRC in the family, whichever is earliest.(50) Our finding that the average age at CRC diagnosis for first-degree relatives of Lynch syndrome cases is approximately 15 years earlier than for first-degree relatives of MMR-proficient cases supports the rationale for starting CRC screening at an earlier age in first-degree relatives of Lynch syndrome cases until they have been genetically tested and shown not to be MMR gene mutation carriers. Similarly, our finding that younger age at diagnosis of CRC for Lynch syndrome cases is a risk factor for CRC in their first-degree relatives supports the rationale for varying the recommendations for age of starting CRC screening in first-degree relatives based on the age of the youngest CRC in the family.

We estimated that first-degree relatives of MMR-proficient CRC cases and MMR-deficient non-Lynch syndrome CRC cases had an approximately 1.6-fold higher risk of CRC compared with the general population. Current CRC screening recommendations for first-degree relatives of CRC cases vary but in general the recommendation is to start screening earlier (at age 40 rather than age 50 for those without a family history of CRC) and to use colonoscopy if the CRC occurs in a relative before the age of 60 years. Our data that a younger age of the proband's CRC was associated with an increased CRC risk for first-degree relatives of both the MMR-proficient and MMR-deficient non-Lynch syndrome CRC cases, is consistent with this type of age-dependent screening recommendations.

We found that first-degree relatives of MMR-deficient CRC cases as a result of *MLH1* methylation were at no greater risk of CRC compared with first-degree relatives of MMR-proficient CRC cases. However, we estimated that CRC risk for first-degree relatives of MMR-deficient non-Lynch syndrome cases is about 4.5-fold higher when the case's CRC was diagnosed before age 50 years and about 2-fold greater when the case had a synchronous CRC. It is possible that this increased CRC risk may be explained by shared lifestyle and environmental factors within families, such as smoking, which has been reported to be associated with MMR-deficient and *BRAF*-mutated CRC.(51) These features are also comparable with familial serrated neoplasia (Jass syndrome)(52) which has been linked to 2q33,(53) and characterised by early age at diagnosis, multiplicity of lesions, presence of *BRAF* V600E-mutated and *MLH1*-methylated MSI-H CRCs.(54)

We found no evidence of an association between *BRAF* V600E mutation status of proband's CRC tumor and an increased risk of CRC for their first-degree relatives, even after stratification by MMR status. A subset of this data has been previously reported highlighting an inverse association between *BRAF*-mutated CRC and family history of CRC.(27) In contrast, three other studies support an association between a family history of CRC and an increased risk of the *BRAF*-mutated CRC.(55-57) One potential explanation for the discrepancy in findings between this study and the previous reports might be the difference in the mean age at diagnosis of the CRC cases.

In this study, the molecular features analyzed were those commonly utilized for the identification of Lynch syndrome. Additional molecular features may provide greater insights for CRC risk prediction for relatives. Two studies recently reported that a family history of CRC is associated with a high risk of MMR-proficient CRC with low-levels of

tumor LINE-1 methylation (LINE-1 hypomethylation).(58, 59) Furthermore, the recent comprehensive characterization of CRCs from the The Cancer Genome Atlas project has identified many key genetic and epigenetic mutations in CRC.(60) Although these tumor molecular features were beyond the scope of this study they require further investigation in large resources for their effectiveness in CRC risk prediction for relatives.

Currently anatomic and pathology features of CRCs are not used to guide CRC screening intensity or to estimate CRC risk for relatives. We found that proximal colonic location, synchronous CRCs, expanding tumor margin and inflammatory infiltrate assessed by the presence of peritumoral lymphocytes, and tumor infiltrating lymphocytes in the proband's tumor are associated with an increased CRC risk for first-degree relatives. Many of these pathology features are strongly associated with MMR-deficiency secondary to Lynch syndrome or caused by somatic alterations in MMR genes.(15, 16) The mechanism accounting for the prominent inflammation in MMR-deficient CRC (peritumoral lymphocytes, tumor infiltrating lymphocytes and Crohn's-like reaction) is unclear and may be related to the host response to tumor cells demonstrating an altered phenotype. Interestingly, some of these pathology features were also found to be associated with a higher CRC risk for first-degree relatives of MMR-proficient CRC. This may be due to undetected MMR gene mutations within MMR-proficient CRC cases (61), or alternatively molecular alterations other than MMR-deficiency may trigger a similar immunological reaction in the tumor environment.

When analyzed for each of the molecular subtypes of CRCs, there was a complex relationship of these pathologic features to CRC risk for first-degree relatives. In an effort to simplify the pathology analysis, we investigated the role of all of the pathology factors that were related to CRC risk for first-degree relatives and found that having three or more features in CRC cases was associated with an increased CRC risk for first-degree relatives in the entire group as well as all of the molecular subtypes. When we considered the specific colorectal tumor location of the proband, we found an increased risk of CRC for first-degree relatives with a more proximal location of CRC in the proband. Further larger studies will be required to gain enough power to investigate the effect of specific colorectal tumor location in each specific molecular subtype of CRC. This site-specific study may be more informative to understand site-specific tumor molecular pathology feature differences beyond the simple proximal-distal divide.(62) Future studies with computer-assisted morphology analysis may identify new pathology features associated with an increased cancer risk for relatives.(63)

This is the first study to investigate the association of CRC tumor molecular pathology features with CRC risk for relatives. We used a large population-based cohort of first-degree relatives of incident CRC cases, and therefore inference can be readily made, at least to the populations from which these families were sampled, if not more generally. Family history of cancer was collected in a systematic manner across the Colon Cancer Family Registry. Our application of weights to each relative depending on the different sampling strategies used by some centers of the Colon Cancer Family Registry would minimize any selection bias due to sampling based on family history.

For CRC cases with suspected Lynch syndrome and confirmed Lynch syndrome, we estimated risk for all first-degree relatives, even though, on average only half will carry a mutation in an MMR gene. Therefore, if suspected Lynch syndrome is due to an autosomal dominantly inherited syndrome the associations for both these groups are an average over carrier and non-carrier relatives. Our study families were predominantly Caucasians and we therefore cannot infer that these results apply to other ethnic groups. Further, we did not report risks of cancers other than CRC for relatives given we emphasized only on associations between CRC tumor molecular pathology features and risk of CRC for their first-degree relatives in this study. 57% of colorectal cancer diagnosis in the first-degree relatives were based on the proband's or relative's self-report which, if a proportion are false positive, may lead to an underestimation of the true associations. However, the specificity of self-reported cancer diagnoses is generally more than 98% suggesting that there are few false positives in such data (64). It is possible that people who are likely to have a genetic predisposition give a more complete family history of cancer compared with those who are not. If this is the case, there will be more complete cancer diagnosis data reported in Lynch syndrome or suspected Lynch syndrome families rather than non-Lynch syndrome families.

In conclusion, findings from this study provide evidence that molecular and pathology characterization of CRCs can both help define the CRC risk for first-degree relatives, and therefore may be useful to refine screening recommendations for first-degree relatives of CRC cases and to identify which cases are more likely to be caused by genetic or other familial factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

The authors thank all study participants of the Colon Cancer Family Registry and staff for their contributions to this project.

FUNDING This work was supported by the National Cancer Institute, National Institutes of Health under RFA #CA-95-011 and through cooperative agreements with members of the Colon Cancer Family Registry and Principal Investigators. Collaborating centers include Australasian Colorectal Cancer Family Registry (U01 CA097735), Familial Colorectal Neoplasia Collaborative Group (U01 CA074799) [USC], Mayo Clinic Cooperative Family Registry for Colon Cancer Studies (U01 CA074800), Ontario Registry for Studies of Familial Colorectal Cancer (U01 CA074783), Seattle Colorectal Cancer Family Registry (U01 CA074794), and University of Hawaii Colorectal Cancer Family Registry (U01 CA074806). This work was also supported by a Centre for Research Excellence grant from the National Health and Medical Research Council (NHMRC), Australia (APP1042021). AKW is supported by the Picchi Brothers Foundation Cancer Council Victoria Cancer Research Scholarship, Australia. MAJ is an NHMRC Senior Research Fellow. JLH is a NHMRC Senior Principal Research Fellow. CR is a Jass Pathology Fellow.

Abbreviations

CRC	colorectal cancer
CI	confidence intervals
HR	hazards ratio

IHC	immunohistochemistry
MMR	mismatch repair
MSI	microsatellite instability
SD	standard deviation
SIR	standardized incidence ratio
SEER	Surveillance, Epidemiology, and End Results

REFERENCES

1. Taylor DP, Burt RW, Williams MS, et al. Population-Based Family History-Specific Risks for Colorectal Cancer: A Constellation Approach. *Gastroenterology*. 2010; 138:877–85. [PubMed: 19932107]
2. Johns LE, Houlston RS. A systematic review and meta-analysis of familial colorectal cancer risk. *Am J Gastroenterol*. 2001; 96:2992–3003. [PubMed: 11693338]
3. Cairns SR, Scholefield JH, Steele RJ, et al. Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). *Gut*. 2010; 59:666–89. [PubMed: 20427401]
4. Screening for colorectal cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med*. 2008; 149:627–37. [PubMed: 18838716]
5. Smith RA, Cokkinides V, Brooks D, et al. Cancer screening in the United States, 2011: A review of current American Cancer Society guidelines and issues in cancer screening. *CA Cancer J Clin*. 2011; 61:8–30. [PubMed: 21205832]
6. Australian Cancer Network Colorectal Cancer Guidelines Revision Committee. Clinical practice guidelines for the prevention, early detection and management of colorectal cancer. The Cancer Council Australia and Australian Cancer Network; Sydney: 2005.
7. Win AK, Young JP, Lindor NM, et al. Colorectal and other cancer risks for carriers and noncarriers from families with a DNA mismatch repair gene mutation: a prospective cohort study. *J Clin Oncol*. 2012; 30:958–64. [PubMed: 22331944]
8. Win AK, Lindor NM, Winship I, et al. Risks of Colorectal and Other Cancers After Endometrial Cancer for Women With Lynch Syndrome. *J Natl Cancer Inst*. 2013; 105:274–9. [PubMed: 23385444]
9. Win AK, Lindor NM, Young JP, et al. Risks of primary extracolonic cancers following colorectal cancer in Lynch syndrome. *J Natl Cancer Inst*. 2012; 104:1363–72. [PubMed: 22933731]
10. Win AK, Ait Ouakrim D, Jenkins MA. Risk profiling: familial colorectal cancer. *Cancer Forum*. 2014; 38:15–25.
11. Hopper JL. Disease-specific prospective family study cohorts enriched for familial risk. *Epidemiol Perspect Innov*. 2011; 8:2. [PubMed: 21352566]
12. Southey MC, Ramus SJ, Dowty JG, et al. Morphological predictors of BRCA1 germline mutations in young women with breast cancer. *Br J Cancer*. 2011; 104:903–9. [PubMed: 21343941]
13. Dite GS, Makalic E, Schmidt DF, et al. Tumour morphology of early-onset breast cancers predicts breast cancer risk for first-degree relatives: the Australian Breast Cancer Family Registry. *Breast Cancer Res*. 2012; 14:R122. [PubMed: 22929433]
14. Hopper JL, Jenkins MA, Dowty JG, et al. Using tumour pathology to identify people at high genetic risk of breast and colorectal cancers. *Pathology*. 2012; 44:89–98. [PubMed: 22198256]
15. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. *Histopathology*. 2007; 50:113–30. [PubMed: 17204026]
16. Jenkins MA, Hayashi S, O'Shea AM, et al. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. *Gastroenterology*. 2007; 133:48–56. [PubMed: 17631130]

17. 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; 16:2331–43. [PubMed: 17982118]
18. Fritz, A.; Percy, C.; Jack, A., et al., editors. *International Classification of Diseases for Oncology (ICD-O)*. 3rd ed. World Health Organization; Geneva: 2000.
19. Jass JR, Love SB, Northover JM. A new prognostic classification of rectal cancer. *Lancet.* 1987; 1:1303–6. [PubMed: 2884421]
20. Young J, Simms LA, Biden KG, et al. Features of colorectal cancers with high-level microsatellite instability occurring in familial and sporadic settings: parallel pathways of tumorigenesis. *Am J Pathol.* 2001; 159:2107–16. [PubMed: 11733361]
21. Graham DM, Appelman HD. Crohn's-like lymphoid reaction and colorectal carcinoma: a potential histologic prognosticator. *Mod Pathol.* 1990; 3:332–5. [PubMed: 2362940]
22. Lindor NM, Burgart LJ, Leontovich O, et al. Immunohistochemistry Versus Microsatellite Instability Testing in Phenotyping Colorectal Tumors. *J Clin Oncol.* 2002; 20:1043–8. [PubMed: 11844828]
23. Senter L, Clendenning M, Sotamaa K, et al. The Clinical Phenotype of Lynch Syndrome Due to Germ-Line PMS2 Mutations. *Gastroenterology.* 2008; 135:419–28. [PubMed: 18602922]
24. Southey MC, Jenkins MA, Mead L, et al. Use of molecular tumor characteristics to prioritize mismatch repair gene testing in early-onset colorectal cancer. *J Clin Oncol.* 2005; 23:6524–32. [PubMed: 16116158]
25. Rumilla K, Schowalter KV, Lindor NM, et al. Frequency of deletions of EPCAM (TACSTD1) in MSH2-associated Lynch syndrome cases. *J Mol Diagn.* 2011; 13:93–9. [PubMed: 21227399]
26. Buchanan DD, Sweet K, Drini M, et al. Risk Factors for Colorectal Cancer in Patients with Multiple Serrated Polyps: A Cross-Sectional Case Series from Genetics Clinics. *PLoS One.* 2010; 5:e11636. [PubMed: 20661287]
27. Buchanan DD, Win AK, Walsh MD, et al. Family History of Colorectal Cancer in BRAF p.V600E mutated Colorectal Cancer Cases. *Cancer Epidemiol Biomarkers Prev.* 2013; 22:917–26. [PubMed: 23462926]
28. Weisenberger DJ, Campan M, Long TI, et al. Analysis of repetitive element DNA methylation by MethyLight. *Nucleic Acids Res.* 2005; 33:6823–36. [PubMed: 16326863]
29. Poynter JN, Siegmund KD, Weisenberger DJ, et al. Molecular characterization of MSI-H colorectal cancer by MLH1 promoter methylation, immunohistochemistry, and mismatch repair germline mutation screening. *Cancer Epidemiol Biomarkers Prev.* 2008; 17:3208. [PubMed: 18990764]
30. Ries, L.; Eisner, M.; Kosary, C., et al., editors. *SEER Cancer Statistics Review, 1975-2000*. National Cancer Institute; Bethesda, MD: 2003.
31. Parkin, DM.; Whelan, SL.; Ferlay, J., et al. *Cancer Incidence in Five Continents, Vol. VII (1988-1992)*. IARC CancerBase No.7; Lyon: 2005.
32. Gould W. Jackknife estimation. *Stata Technical Bulletin.* 1995; 4:25–9.
33. Rogers WH. Regression standard errors in clustered samples. *Stata Technical Bulletin.* 1993; 3:19–23.
34. van Buuren S, Boshuizen HC, Knook DL. Multiple imputation of missing blood pressure covariates in survival analysis. *Stat Med.* 1999; 18:681–94. [PubMed: 10204197]
35. Ali AM, Dawson SJ, Blows FM, et al. Comparison of methods for handling missing data on immunohistochemical markers in survival analysis of breast cancer. *Br J Cancer.* 2011; 104:693–9. [PubMed: 21266980]
36. Schafer JL. Multiple imputation: a primer. *Stat Methods Med Res.* 1999; 8:3–15. [PubMed: 10347857]
37. Rubin, DB. *Multiple imputation for nonresponse in surveys*: Wiley. com. 2009.
38. Bodner TE. What improves with increased missing data imputations? *Struct Equation Model.* 2008; 15:651–75.
39. Carlin JB, Li N, Greenwood P, et al. Tools for analyzing multiple imputed datasets. *The Stata Journal.* 2003; 3:226–44.

40. StataCorp. Stata Statistical Software: Release 12. StataCorp LP; College Station, TX: 2011.
41. Rodriguez-Soler M, Perez-Carbonell L, Guarinos C, et al. Risk of Cancer in Cases of Suspected Lynch Syndrome Without Germline Mutation. *Gastroenterology*. 2013; 144:926–32. [PubMed: 23354017]
42. 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; 41:112–7. [PubMed: 19098912]
43. Clendenning M, Buchanan DD, Walsh MD, et al. Mutation deep within an intron of MSH2 causes Lynch syndrome. *Fam Cancer*. 2011; 10:297–301. [PubMed: 21360204]
44. Morak M, Koehler U, Schackert HK, et al. Biallelic MLH1 SNP cDNA expression or constitutional promoter methylation can hide genomic rearrangements causing Lynch syndrome. *J Med Genet*. 2011; 48:513–9. [PubMed: 21712435]
45. Dowty JG, Win AK, Buchanan DD, et al. Cancer risks for MLH1 and MSH2 mutation carriers. *Hum Mutat*. 2013; 34:490–7. [PubMed: 23255516]
46. Thompson BA, Goldgar DE, Paterson C, et al. A multifactorial likelihood model for MMR gene variant classification incorporating probabilities based on sequence bioinformatics and tumor characteristics: a report from the Colon Cancer Family Registry. *Hum Mutat*. 2013; 34:200–9. [PubMed: 22949379]
47. Thompson BA, Spurdle AB, Plazzer JP, et al. Application of a 5-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants in the InSiGHT locus-specific database. *Nature genetics*. 2013
48. Sourrouille I, Coulet F, Lefevre JH, et al. Somatic mosaicism and double somatic hits can lead to MSI colorectal tumors. *Fam Cancer*. 2013; 12:27–33. [PubMed: 22987205]
49. Mensenkamp AR, Vogelaar IP, van Zelst-Stams WA, et al. Somatic mutations in MLH1 and MSH2 are a Frequent Cause of Mismatch-repair Deficiency in Lynch Syndrome-like Tumors. *Gastroenterology*. 2014; 146:643–6. [PubMed: 24333619]
50. National Comprehensive Cancer Network. [cited 2013 June 24] NCCN Clinical Practice Guidelines in Oncology Version 1.2013. Colorectal Cancer Screening: National Comprehensive Cancer Network. 2013. Available from: http://www.nccn.org/professionals/physician_gls/pdf/colorectal_screening.pdf
51. Limsui D, Vierkant RA, Tillmans LS, et al. Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. *J Natl Cancer Inst*. 2010; 102:1012–22. [PubMed: 20587792]
52. Young J, Jass JR. The case for a genetic predisposition to serrated neoplasia in the colorectum: hypothesis and review of the literature. *Cancer Epidemiol Biomarkers Prev*. 2006; 15:1778–84. [PubMed: 17035382]
53. Roberts A, Nancarrow D, Clendenning M, et al. Linkage to chromosome 2q32.2-q33.3 in familial serrated neoplasia (Jass syndrome). *Fam Cancer*. 2011; 10:245–54. [PubMed: 21165777]
54. Snover, DC.; Ahnen, DJ.; Burt, RW., et al. Serrated polyps of the colon and rectum and serrated polyposis. In: Bosman, FT.; Carneiro, F.; Hruban, RH., et al., editors. *WHO Classification of Tumours of the Digestive System*. 4th ed. IARC; Lyon, France: 2010. p. 160-5.
55. Wish T, Hyde A, Parfrey P, et al. Increased Cancer Predisposition in Family Members of Colorectal Cancer Patients Harboring the p. V600E BRAF Mutation: a Population-Based Study. *Cancer Epidemiol Biomarkers Prev*. 2010; 19:1831–9. [PubMed: 20570909]
56. Samowitz WS, Sweeney C, Herrick J, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. *Cancer Res*. 2005; 65:6063–9. [PubMed: 16024606]
57. Vandrovcova J, Lagerstedt-Robinson K, Pahlman L, et al. Somatic BRAFV600E Mutations in Familial Colorectal Cancer. *Cancer Epidemiol Biomarkers Prev*. 2006; 15:2270–3. [PubMed: 17119056]
58. Goel A, Xicola RM, Nguyen TP, et al. Aberrant DNA methylation in hereditary nonpolyposis colorectal cancer without mismatch repair deficiency. *Gastroenterology*. 2010; 138:1854–62. [PubMed: 20102720]

59. Ogino S, Nishihara R, Lochhead P, et al. Prospective study of family history and colorectal cancer risk by tumor LINE-1 methylation level. *J Natl Cancer Inst.* 2013; 105:130–40. [PubMed: 23175808]
60. Weinstein JN, Collisson EA, Mills GB, et al. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet.* 2013; 45:1113–20. [PubMed: 24071849]
61. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch Syndrome (Hereditary Nonpolyposis Colorectal Cancer). *N Engl J Med.* 2005; 352:1851–60. [PubMed: 15872200]
62. Yamauchi M, Lochhead P, Morikawa T, et al. Colorectal cancer: a tale of two sides or a continuum? *Gut.* 2012; 61:794–7. [PubMed: 22490520]
63. Beck AH, Sangoi AR, Leung S, et al. Systematic analysis of breast cancer morphology uncovers stromal features associated with survival. *Sci Transl Med.* 2011; 3:108ra13.
64. Ziogas A, Anton-Culver H. Validation of family history data in cancer family registries. *Am J Prev Med.* 2003; 24:190–8. [PubMed: 12568826]

Summary Box

What is already known about this subject?

- Family history of colorectal cancer (CRC) is a well-established risk factor for the disease, but even for specific family histories there is a wide variation in CRC risk limiting the effectiveness of CRC screening based on family history alone.
- We hypothesized that molecular and pathology features of a colorectal cancer may be useful to estimate CRC risk for their relatives.

What are the new findings?

- Compared with first-degree relatives of MMR-proficient CRC cases, a higher risk of CRC was estimated for first-degree relatives of CRC cases with suspected Lynch syndrome (hazard ratio [HR] 2.06, 95% confidence interval [CI] 1.59-2.67), and with Lynch syndrome (HR 5.37, 95% CI 4.16-6.94), but not with MMR-deficient non-Lynch syndrome (HR 1.04, 95% CI 0.82-1.31).
- A greater risk of CRC was estimated for first-degree relatives if CRC cases were diagnosed before age 50 years, had proximal colon cancer or if their tumors had any of the following: expanding tumor margin, peritumoral lymphocytes, tumor-infiltrating lymphocytes, or synchronous CRC.

How might it impact on clinical practice in the foreseeable future?

- Molecular and pathology characterization of CRCs can help define the risk of CRC for first-degree relatives, and therefore may be useful to refine screening recommendations for first-degree relatives of CRC cases and also to identify which cases are more likely to be caused by genetic or other familial factors.

Table 1

Baseline characteristics and tumor pathology features of colorectal cancer case-probands

	Number (%)				
	All (n=4,853)	MMR- proficient (n=4,095)	MMR- deficient non-Lynch syndrome (n=301)	Suspected Lynch syndrome (n=271)	Lynch syndrome (n=186)
Sex					
female	2,368 (49)	1,945 (48)	221 (73)	118 (44)	83 (45)
male	2,485 (51)	2,149 (52)	80 (27)	153 (56)	103 (55)
Country					
Canada	1,610 (33)	1,366 (33)	99 (33)	79 (29)	66 (35)
USA	2,439 (50)	2,015 (49)	192 (64)	144 (53)	88 (47)
Australia	804 (17)	714 (17)	10 (3)	48 (18)	32 (17)
Age at diagnosis (years)					
mean (SD)	54.2 (11.6)	54.2 (11.2)	64.5 (8.31)	48.8 (12.3)	45.0 (11.3)
median (range)	53 (18-75)	53 (18-75)	66 (22-74)	47 (18-74)	45 (18-74)
Location					
rectum	1,647 (34)	1,568 (39)	10 (3)	42 (16)	27 (15)
distal colon	1,488 (31)	1,352 (33)	30 (10)	65 (24)	41 (22)
proximal colon	1,656 (35)	1,124 (28)	256 (86)	159 (60)	117 (63)
missing	62	51	5	5	1
Histologic type					
adenocarcinoma	3,928 (86)	3,423 (89)	200 (71)	187 (74)	118 (73)
mucinous or signet ring-cell carcinoma	631 (14)	441 (11)	80 (29)	67 (26)	43 (27)
missing	294	231	21	17	25
Histologic grade					
low grade	3,455 (81)	3,042 (83)	152 (60)	146 (67)	115 (78)
high grade	817 (19)	609 (17)	102 (40)	73 (33)	33 (22)
missing	581	444	47	52	38
Tumor margin					
infiltrating	1,046 (62)	926 (64)	53 (73)	38 (43)	29 (38)
expanding	646 (38)	527 (36)	20 (27)	51 (57)	48 (62)
missing	3,161	2,642	228	182	109
Peritumoral lymphocytes					
absent	1,195 (64)	1,056 (66)	41 (56)	55 (48)	43 (48)
present	675 (36)	537 (34)	32 (44)	59 (52)	47 (52)

	Number (%)				
	All (n=4,853)	MMR- proficient (n=4,095)	MMR- deficient non-Lynch syndrome (n=301)	Suspected Lynch syndrome (n=271)	Lynch syndrome (n=186)
missing	2,983	2,502	228	157	96
Crohn's-like reaction					
absent	1,358 (73)	1,228 (77)	32 (44)	57 (50)	41 (46)
present	510 (27)	363 (23)	41 (56)	57 (50)	49 (54)
missing	2,985	2,504	228	157	96
Tumor-infiltrating lymphocytes					
absent	1,414 (74)	1,339 (82)	16 (22)	38 (33)	21 (23)
present	495 (26)	289 (18)	57 (78)	77 (67)	72 (77)
missing	2,944	2,467	228	156	93
Venous invasion					
absent	2,749 (83)	2,330 (83)	157 (82)	157 (87)	105 (88)
present	544 (17)	472 (17)	34 (18)	24 (13)	14 (12)
missing	1,560	1,293	110	90	67
Synchronous tumor					
absent	4,137 (97)	3,520 (97)	248 (93)	229 (97)	140 (93)
present	130 (3)	93 (3)	19 (7)	8 (3)	10 (7)
missing	586	482	34	34	36

SD=standard deviation; MMR=mismatch repair

Table 2

Baseline characteristics of first-degree relatives of colorectal cancer case-probands

	Number (%)				
	All (n=33,496)	MMR- proficient (n=28,156)	MMR- deficient non-Lynch syndrome (n=2,302)	Suspected Lynch syndrome (n=1,799)	Lynch syndrome (n=1,239)
Sex					
female	16,770 (50)	14,088 (49)	1,139 (49)	910 (51)	633 (51)
male	16,726 (50)	14,068 (51)	1,163 (51)	889 (49)	606 (49)
Country					
Canada	11,645 (35)	9,842 (35)	807 (35)	562 (31)	434 (35)
USA	16,562 (50)	13,580 (48)	1,435 (62)	959 (53)	588 (47)
Australia	5,289 (16)	4,734 (17)	60 (3)	278 (15)	217 (18)
Colorectal cancer					
absent	32,092 (96)	27,145 (96)	2,184 (95)	1,675 (93)	1,088 (88)
present	1,404 (4)	1,011 (4)	118 (5)	124 (7)	151 (12)
Age at diagnosis* (years)					
mean (SD)	62.1 (14.1)	63.9 (13.1)	67.1 (12.8)	57.9 (14.8)	49.1 (13.1)
median (range)	64 (17-90)	65 (17-90)	68 (33-90)	58 (22-90)	46 (24-84)
Last known age^ (years)					
mean (SD)	53.1 (19.8)	53.1 (19.7)	58.5 (18.2)	51.0 (19.7)	46.0 (20.1)
median (range)	53 (1-90)	53 (1-90)	58 (1-90)	51 (1-90)	46 (1-90)

* Age at diagnosis at colorectal cancer for colorectal cancer-affected relatives

^ Last known age (age at diagnosis at any other cancer, age at first polypectomy, age at death or age at last contact, whichever occurred first) for colorectal cancer-unaffected relatives.

SD=standard deviation; MMR=mismatch repair

Table 3

Risk of colorectal cancer for first-degree relatives of colorectal cancer cases by different tumor molecular subtypes

	Person-years	Observed	Expected	SIR (95% CI)	HR (95% CI)	p-value
MMR-proficient	1,506,769	1,011	510.2	1.55 (1.42-1.69)	1.00	
MMR-deficient non-Lynch syndrome	135,600	118	54.5	1.62 (1.27-2.07)	1.04 (0.82-1.31)	0.75
Suspected Lynch syndrome	92,643	124	28.0	3.45 (2.62-4.57)	2.06 (1.59-2.67)	<0.001
Lynch syndrome	57,514	151	13.9	9.67 (7.10-13.1)	5.37 (4.16-6.94)	<0.001

HR=hazard ratio, SIR=standardized incidence ratio, CI=confidence interval, MMR=mismatch repair

Hazard ratios of colorectal cancer for first-degree relatives of colorectal cancer cases by personal and colorectal cancer tumor pathology features in univariable models

Table 4

Features	All			MMR-proficient			MMR-deficient non-Lynch syndrome			Suspected Lynch syndrome			Lynch syndrome		
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)
Age at diagnosis (<50 vs ≥ 50 years)	1.64 (1.43-1.88)	<0.001	1.30 (1.11-1.53)	0.001	3.95 (1.51-10.3)	0.005	1.66 (1.03-2.69)	0.04	2.10 (1.34-3.27)	0.001					
Sex (male vs female)	0.95 (0.83-1.08)	0.42	0.94 (0.80-1.10)	0.42	0.70 (0.41-1.18)	0.18	1.17 (0.72-1.90)	0.52	0.76 (0.50-1.17)	0.22					
Location (proximal colon vs distal colon/rectum)	1.33 (1.16-1.53)	<0.001	1.21 (1.02-1.45)	0.03	1.28 (0.61-2.67)	0.51	1.19 (0.69-2.07)	0.53	0.88 (0.56-1.36)	0.56					
Histologic subtype (mucinous or signet ring cell-carcinoma vs adenocarcinoma)	1.04 (0.86-1.26)	0.68	0.93 (0.72-1.20)	0.57	1.16 (0.73-1.85)	0.53	1.11 (0.62-1.96)	0.73	0.74 (0.40-1.37)	0.34					
Histologic grade (high vs low)	1.03 (0.86-1.24)	0.72	0.91 (0.72-1.15)	0.44	1.42 (0.89-2.27)	0.15	1.20 (0.73-1.99)	0.47	0.78 (0.43-1.42)	0.41					
Tumor margin (expanding vs infiltrating)	1.66 (1.35-2.05)	<0.001	1.58 (1.29-1.94)	0.01	2.02 (1.17-3.50)	0.01	1.62 (0.87-3.04)	0.13	1.08 (0.61-1.92)	0.79					
Peritumoral lymphocytes	1.26 (1.06-1.49)	0.008	1.13 (0.93-1.38)	0.21	1.64 (1.02-2.63)	0.04	1.32 (0.78-2.25)	0.30	2.32 (1.43-3.78)	0.001					
Crohn's-like reaction	1.17 (0.99-1.38)	0.06	1.09 (0.90-1.31)	0.39	1.35 (0.82-2.22)	0.23	0.89 (0.52-1.52)	0.66	1.43 (0.88-2.30)	0.14					
Tumor-infiltrating lymphocytes	1.33 (1.15-1.55)	<0.001	1.18 (0.96-1.44)	0.11	1.21 (0.76-1.90)	0.42	1.25 (0.76-2.07)	0.38	1.40 (0.90-2.18)	0.13					
Venous invasion	1.01 (0.80-1.28)	0.91	1.07 (0.82-1.39)	0.61	0.84 (0.37-1.95)	0.69	0.69 (0.24-1.95)	0.47	1.34 (0.78-2.31)	0.28					
Synchronous colorectal cancer	1.69 (1.23-2.32)	0.001	1.50 (0.97-2.33)	0.07	2.23 (1.38-3.60)	0.001	2.30 (0.67-7.94)	0.18	0.96 (0.33-2.77)	0.93					

Table 5

Hazard ratios of colorectal cancer for first-degree relatives of colorectal cancer cases by personal and colorectal cancer tumor pathology features in a multivariable* model

Features	All			MMR-proficient			MMR-deficient non-Lynch syndrome			Suspected Lynch syndrome			Lynch syndrome		
	HR (95% CI)	p-value		HR (95% CI)	p-value		HR (95% CI)	p-value		HR (95% CI)	p-value		HR (95% CI)	p-value	
<50 vs >50 years	1.61 (1.40-1.87)	<0.001		1.25 (1.06-1.47)	0.008		4.47 (1.53-13.1)	0.006		1.57 (0.95-2.59)	0.08		2.11 (1.35-3.31)	0.001	
Proximal colon vs distal colon/rectum	1.29 (1.12-1.49)	<0.001		1.17 (0.98-1.40)	0.08		1.46 (0.71-3.01)	0.31		1.28 (0.75-2.19)	0.37		0.94 (0.62-1.14)	0.77	
Expanding vs infiltrating margin	1.48 (1.19-1.83)	0.001		1.50 (1.22-1.85)	<0.001		1.68 (0.94-2.99)	0.08		1.36 (0.70-2.65)	0.35		0.94 (0.52-1.71)	0.84	
Peritumoral lymphocytes	1.21 (1.01-1.44)	0.04		1.10 (0.89-1.36)	0.36		1.59 (0.98-2.58)	0.06		1.25 (0.73-2.16)	0.41		2.31 (1.46-3.66)	<0.001	
Tumor-infiltrating lymphocytes	1.21 (1.03-1.43)	0.02		1.12 (0.91-1.39)	0.29		1.10 (0.69-1.75)	0.69		1.02 (0.57-1.81)	0.95		1.10 (0.69-1.75)	0.68	
Synchronous CRC	1.61 (1.19-2.18)	0.002		1.46 (0.96-2.23)	0.08		2.15 (1.28-3.61)	0.004		1.81 (0.50-6.52)	0.36		1.12 (0.48-2.62)	0.80	

* multivariable model included all features shown in the table.
HR=hazard ratio, CI=confidence interval, CRC=colorectal cancer

Table 6

Risk of colorectal cancer for first-degree relatives of colorectal cancer cases by the total number of features

	Total number of features*	SIR (95% CI)	HR (95% CI)	p-value
All	<3	1.48 (1.36-1.61)		
	3	2.56 (2.32-2.82)	1.91 (1.65-2.22)	<0.001
MMR-proficient	<3	1.39 (1.27-1.53)		
	3	2.00 (1.78-2.24)	1.57 (1.29-1.91)	<0.001
MMR-deficient non-Lynch syndrome	<3	1.13 (0.84-1.52)		
	3	2.17 (1.67-2.85)	2.08 (1.28-3.39)	0.004
Suspected Lynch syndrome	<3	2.66 (1.92-3.70)		
	3	4.63 (3.28-6.55)	1.81 (1.09-3.01)	0.02
Lynch syndrome	<3	6.42 (4.33-9.50)		
	3	12.0 (7.90-17.7)	1.84 (1.13-2.99)	0.01

* included the following features: age at diagnosis <50 years, proximal colonic location, presence of expanding tumor margin, peritumoral lymphocytes, tumor-infiltrating lymphocytes and synchronous CRC.

HR=hazard ratio, SIR=standardized incidence ratio, CI=confidence interval, MMR=mismatch repair