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Colorectal Cancer Risks in Relatives of Young-Onset Cases: Is Risk the Same Across All First Degree Relatives?

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Abstract

Background and Aims—In the last fifteen years, several single-gene Mendelian disorders have been discovered that may account for some of the familial aggregation detected in large population studies of colorectal cancer (CRC). Mutations in DNA mismatch repair (MMR) genes cause HNPCC-Lynch Syndrome, the most common of the recognized CRC-predisposition syndromes, in which one major feature is a young age for cancer onset. However, for young onset microsatellite stable(MSS) CRC, the familial risk for CRC is unknown.

Methods—Cases with CRC < 50 years old were identified through Minnesota Cancer Surveillance System (MCSS), and Mayo Clinic, Rochester, MN. CRC in which the DNA MMR function was deficient as evidenced by high level microsatellite instability and/or loss of expression of MMR gene product by immunostaining were excluded. A total of 278 probands (131 from MCSS; 147 from Mayo Clinic) were included. Data on 1862 relatives were collected, of whom 68 were found to have had CRC and an additional 165 had primary cancers of other types.

Results—Compared to SEER data, relatives of young onset CRC probands had increased risks for CRC. This relative risk was increased among first degree relatives (RR=1.65; 95% C.I.=1.29–2.07), and was greater for siblings (RR = 2.67; 95% C.I=1.50–4.41) than parents (RR= 1.5; 95% C.I.=1.14–1.94)

Conclusions—We studied 278 probands with young-onset microsatellite stable CRC. We determined that the relative risk for CRC was greatest in siblings, which is consistent with an autosomal recessive inheritance pattern.

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No conflicts of interest exist

Introduction

Large population studies have repeatedly confirmed that colorectal cancer (CRC) shows familial aggregation. Commonly cited relative risks for CRC are 1.7–1.8 for first degree relatives.[1,2] In the last fifteen years, several single-gene Mendelian disorders have been discovered that may account for some of the aggregation detected in population studies. Specifically, mutations in the DNA mismatch repair (MMR) genes cause HNPCC-Lynch Syndrome, the most common of the recognized CRC-predisposition syndromes. Other syndromes that predispose to CRC are due to mutations in the base-excision repair gene, *MYH*, and Familial Adenomatous Polyposis due to mutations in the *APC* gene.[4] It is unclear if the observed familial aggregation risks for CRC is explained just by these known conditions or if this hereditary risk exists also for MSS CRC not related to *MYH* or *APC* mutations. Familial cancer risk as assessed in the population-based Swedish Family-Cancer Database was found to be increased in siblings greater than in parents of CRC probands not classified by either DNA MMR status or by germline MYH mutation status. [3]

Typically CRC is a disease of aging, with the majority of cases arising after 65 years of age. Still, 15% of cases will occur in people ≤ 50 years old. In 17–30% of young onset cases of CRC, DNA MMR defects will account for the younger age of onset of CRC, but the remaining majority of young onset MSS CRC cases occur for unknown reasons. CRC patients originally qualified as having Lynch-HNPCC syndrome if they met Amsterdam criteria(AC), which included kindreds in which at least 3 family member had CRC, with one of them being the first degree relative of the other two and one of them having developed CRC at <50 years of age. Kindreds that fulfill AC but who do not have defective DNA MMR related CRC have also been recently classified as having Familial colorectal cancer syndrome X and relatives beyond the three members used to meet AC of Familial CRC syndrome X patients were found to have an increased risk; albeit lower than that for DNA MMR related cancer; for CRC but not the extracolonic cancers that can occur in Lynch- HNPCC Syndrome patients. However, the risk for family members of young onset MSS CRC patients who do not meet AC has not been examined. To further explore the cancer risk for first degree relatives of individuals with young onset MSS CRC, we studied a series of cases with CRC diagnosed under age 50 years, who had no evidence of DNA mismatch repair deficiency, and found that the risk for parents was increased and that is was greater for siblings, suggesting a possible autosomal recessive CRC predisposition not explained by currently known genes.

Methods

Population

Cases were identified through a contract with the Minnesota Cancer Surveillance System (MCSS), a population-based cancer registry, and the Mayo Clinic site of the Colon CFR. The Colon CFR is an NCI-supported consortium established in l997 to create a multinational comprehensive collaborative infrastructure for interdisciplinary studies in the genetic epidemiology of colorectal cancer. (Detailed information about the Colon CFR can be found at http://epi.grants.cancer.gov/CFR/.) The MCSS ascertains all cases of CRC in Minnesota and the Mayo CFR received 50% of the names of those diagnosed with CRC between 1997–1999. Of these, we invited 100% of cases that were diagnosed with CRC under age 50 years to enroll in the Colon CFR. In addition, we searched the Mayo Clinic-modified H-ICDA (Hospital Adaptation of the International Classification of Disease in the United States) diagnostic codes for all colorectal cancers diagnosed between 1996–1998 and invited all individuals with a diagnosis of colorectal cancer under age 50. Age at diagnosis was the sole criteria for eligibility. Specifically, family history was not a selection criterion, and was unknown to the researchers at the time the invitations were sent to prospective participants. Institutional Review Board-approved written informed consent was obtained from all participants for collection of family

history, risk factor exposure, medical records, blood specimens, histology slides, and tumor blocks. Additional affected and unaffected family members were also enrolled in the same manner after the proband was enrolled. Interviews done with the proband by phone and a detailed 4 generation pedigree was obtained. All first degree relatives with CRC and up to 4 sibs per proband without CRC were invited to enroll in the registry. For each who agreed to enroll, a family history was again obtained using a standardized written instrument [Epidemiology questionniare with family history used across the Colon CFR is available at http://epi.grants.cancer.gov/CFR/index.html. In this way discrepancies between the phone interview and other self reported diagnoses might be identified. For all enrolled individuals, medical records, blood specimens and tumors were collected if possible.

For all individuals in this study, tumor tissue was studied for DNA mismatch repair competency, using methods described previously.[5,6] Those with deficiency of MMR function (high level of microsatellite instability or loss of expression of MMR gene product) were excluded from this study. A total of 278 probands (131 from MCSS; 147 from Mayo Clinic) with mismatch repair-competent CRC diagnosed under age 50 years were included in this study. The mean age of diagnosis for males was 43.4 (n=131, range = 22–49); for females the mean age was 42.7 (n=147, range 22–49). From these probands, complete family histories of first-degree relations were obtained by interviews and questionnaires. Data on a total of 1862 relatives were collected, of whom 68 were found to have had CRC and an additional 165 had primary cancers of other types. The subject data so collected provided over 40,000 person-years of exposure data that was then compared with the age and year-adjusted rates found in data from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute (NCI). The SEER Program of the National Cancer Institute (NCI) is a source of information on cancer incidence and survival in the United States. SEER currently collects and publishes cancer incidence and survival data from population-based cancer registries covering approximately 26 percent of the US population.[7]

Laboratory Investigations

For all participants, tumors were characterized for MMR proficiency by conducting microsatellite instability testing (MSI) and immunohistochemistry for MMR gene product expression (*MLH1, MSH2, MSH6*). Probands were only included in this study is their tumor showed no microsatellite instability and/or their tumor expressed all three MMR gene products normally.

For MSI, DNA from microdissected frozen tissue sections (10 μm) was extracted by standard phenol/chloroform procedure. For tumor DNA, only those areas containing more than 70% tumor cells were used. For DNA extraction from paraffin-embedded tissues, the Qiamp tissue kit (Qiagen, Inc, Santa Clarita, CA) was used according to the manufacturer's instructions. The corresponding normal control DNA for each patient was derived from peripheral blood. For these specimens, DNA was extracted using the Puregene nucleic acid isolation kit (Gentra Systems, Minneapolis, MN). Paired normal and tumor DNA was analyzed for MSI with 10 markers: mononucleotide markers BAT25, BAT26, BAT40, BAT34C4; dinucleotide markers D5S346, D17S250, ACTC, D18S55, and D10S197; and penta-mono-tetra compound marker MYCL.PCR and gel electrophoresis were carried out as described by Thibodeau et al.[8] Tumors were classified as MSI-H if >= 30% markers demonstrated instability, MSI-L if < 30% demonstrated MSI, and MSS if no marker exhibited MSI.

For immunohistochemical (IHC) analysis, tissue sections were cut at 6 μm and mounted on Probe On charged slides (Fisher Scientific, Pittsburgh, PA). After deparaffinization, slides were steam pretreated in EDTA buffer, pH 8.0, in a Black & Decker Handy Steamer Plus (Black & Decker, Shelton, CT) for 30 minutes. After rinsing in cool water, slides were loaded onto the Tech Mate 500 (Ventana Medical Systems, Tuscon, AZ) automated immunohistochemical

stainer. Staining was performed using an avidin-biotin complex methodology, supplied in kit form from Ventana Medical Systems (Biotek Solutions buffer kit, Biotek Solutions DAB detection kit). This test uses a primary antibody against *hMLH1* (clone G168–728, 1/250; Pharmingen, San Diego, CA) and *hMSH2* (clone FE11, 1/50; Oncogene Research Products, Cambridge, MA) that has been titered on colon cancer sections and also tested on various normal and pathologic tissue specimens.

For *MYH* mutation analysis for the 11 siblings with CRC in this young-onset cohort, we analyzed for *MYH* gene mutations in the proband. Genomic DNA was screened for *MYH* Y165C and G382D mutations by using denaturing high-performance liquid chromatography (Transgenomic Wave 3500HT System; Transgenomic, Omaha, NE). Each genomic DNA sample was subjected to polymerase chain reaction (PCR) amplification using primers (*see* Supplemental Table available at: http://jncicancerspectrum.oupjournals.org/jnci/content/vol96/issue21) designed by the Transgenomics Mutation Discovery program (www.mutationdiscovery.com). The GenBank accession number is MYH: http://www.ncbi.nlm.nih.gov/Omim/. To ensure detection of Y165C and G382D homozygous mutations, the resulting test PCR products were mixed with PCR products amplified from a wild-type sample (25% of the test sample amount). The mixed samples were heated to 95 °C for 5 minutes and then cooled for 50 minutes at a rate of −1.5 °C per cycle for 46 cycles to enhance heteroduplex formation prior to analysis (8). Samples were passed through a SepHT Cartridge (Transgenomic) under partially denatured conditions; temperatures were calculated using Wavemaker version 4.1 software for each amplicon (see Supplemental Table. Samples showing variant mobility patterns were sequenced. We then used denaturing high-performance liquid chromatography to screen the entire *MYH* gene (16 exons) of all monoallelic and biallelic MYH Y165C and/or G382D mutation carriers for additional mutations.

Statistical analysis

Relative risk ratios were computed as the ratio of observed cased to the number expected. Expected rates were a function of both patient age (5-year increments) and year (1973–2002). To deal with missing years in the SEER data we ran the analyses only on the years covered by the data (1972–2002). We also investigated the results using a simple extrapolation of the 1973 rates to permit use of exposure years before 1973. Person-years were calculated from age 20 years until the earliest cancer diagnosis or death. All cancers were recorded. Using the SAS macro PERSONYRS, we compared the observed primary cancer rates within the subject population with the rates observed in the population as a whole (using the Iowa cohort-base SEER data). The PERSONYRS macro uses Poisson regression to estimate the relative risk ratio and to produce confidence intervals. The standardized incidence ratios (SIRs) of each primary cancer among members were calculated as the ratio of the observed to the expected numbers of cases. The latter was calculated as the sum of the products of the number of person-years for each 5-year age/sex group and the corresponding age/sex-specific incidence rates from the Surveillance, Epidemiology, and End Result (SEER) database. All analyses were stratified by gender. In addition, analyses were conducted separately in the population-and clinic-based groups. Statistical analyses were performed using SAS version 8 (SAS Institute Inc, Cary, NC) using PROC PERSONYRS.[9] Results where the 95% confidence intervals did not cover 1.0 were identified as statistically significant.

Results

Summary

Compared to SEER data, relatives of young onset CRC probands had increased risks for development of CRC; however, of the 165 other primary cancers, age-adjusted rates did not reveal increased risks for any other cancers for siblings or parents compared to SEER data.

Since no CRC were reported among the probands' children, no analysis was feasible. As shown in Table 1, the relative risks for CRC was increased among first degree relatives (RR=1.65; 95% C.I.=1.29–2.07), and this risk was greater for siblings (RR = 2.67; 95% C.I=1.50–4.41) than for parents (RR= 1.5; 95% C.I.=1.14–1.94). This trend was maintained in the population-based cases (as opposed to those ascertained via the clinic), though the statistical power was diminished by sample size. Regarding the 11 proband-sibling pairs from which the sibling relative risk was calculated, the cancers arose in the ascending or right colon in 2 cases, in the sigmoid colon or rectum in 2 cases, and in an unknown site in 7 cases. Because of the small sample size neither gender nor site specific differences were able to be assessed for statistical significance among between siblings or parents with the CRC probands. No mutations, neither monoallelic nor biallelic, were found in the *MYH* gene in the probands who had siblings diagnosed with CRC.

Discussion

We studied 278 probands selected solely on the basis of having developed MSS CRC at a young age of onset. Exclusion of cases with tumor microsatellite instability was aimed at excluding cases that had a high likelihood of having HNPCC-Lynch Syndrome, the most common cause of hereditary CRC. We determined that the relative risk for CRC in this cohort was greatest in siblings (RR 2.67; 95% C.I=1.50–4.41), which was nearly double the relative risk of CRC in parents (RR 1.5; 95% C.I.=1.14–1.94). However, because the confidence intervals overlap, we can only say that the data are suggestive of a trend for siblings of young onset CRC patients to have an increased RR for CRC over that of the parents. It is possible that environmental factors could contribute to the greater CRC risk observed in siblings rather than parents. In particular, increased CRC rates in the second half of the twentieth century may be attributable to the substantial decrease in physical activity coupled with excess energy intake and may now be manifesting in sibilings who shared these exposures during childhood and younger adulthood.[10] However, while we cannot rule out some shared environmental exposures as the explanation for CRC in siblings, these results may suggest an autosomal recessive mechanism for CRC predisposition. Biallelic mutations in *MYH*, a base excision repair gene, have recently been discovered to predispose to CRC, often, but not always, in association with an attenuated polyposis phenotype. To determine if MYH mutations accounted for this increased risk in siblings, we tested for *MYH* mutations in our 11 cases with affected siblings and did not find any mutations, either biallelic nor monoallelic, to explain our observations. This suggests the possibility of additional genes to be discovered as predisposing causes of CRC.

A paper recently published by the Swedish Family-Cancer Database found similar results[11] by studying 6,774 offspring diagnosed with CRC between 0–68 years. In this population-based study, 306 had only a parent affected, 100 had only a sibling affected, and 10 had both a parent and sibling affected. Standardized incidence ratios (SIRs) were presented for CRC in first degree relatives. The SIR for offspring of a parent with CRC was 2.13 and the SIR was 2.75 when a sib was affected. When looking only at right sided tumors, there was a 1.8 fold increased risks in siblings compared to offspring of an affected parent. They concluded that the most likely explanation for the high risk of right sided CRC among siblings was recessive inheritance predisposition, which they suggested could account for 0.75% of all CRC. DNA MMR status of the incident CRC cases was not known in the Swedish Family- Cancer Database, however, to minimize that the known hereditary condition of HNPCC might confound results, kindreds with three or more CRC affected relatives were excluded from their analysis. *MYH* gene testing was not able to be conducted in this epidemiologic study, but as the authors highlight, the 0.75% recessive inheritance rate detected in their population exceeds the rate attributable to MYH-related recessive inheritance reported in the literature. Our results corroborate this autosomal recessive inheritance predisposition and indicate that this autosomal recessive

inheritance pattern is present in a population known to have CRC not related to DNA MMR or *MYH* mutations.

In an editorial in the Journal of the National Cancer Institute discussing similar results in a Swedish study,[12] Zelen suggested that length-based bias and surveillance bias are important considerations in such studies.[13] Length-based bias is the consequence of the fact that large families are more likely to be found because there are simply more people available to become probands in large families. Thus, the distribution of family size is not representative of the population at large. Surveillance bias reflects the assumption that people who are relatives of the proband may, because of heightened awareness and/or concern, be more likely to detect disease. Length-based bias is not an issue for this work as the statistical test was done to see if the proband-relatives population has cancer rates that differ from the population as a whole (SEER-based rates). Thus, while the probands are more likely to come from large families, the rates within the probands were not analyzed, the incidences were not collapsed to family-based single measure. The proband-relatives are therefore suitable for relative risk analyses. Surveillance bias would only be relevant if the severity of the disease upon detection was being addressed; however, the presence of CRC was the only endpoint measured in this study. Even so, we compared rates in the first year following detection in the proband with rates in subsequent years and found no significant difference.

Though the methodology between our study and the Hemminki and Chen paper differed, our conclusions are strikingly similar. First degree relatives of MSS CRC patients do have an increased risk for the development of GI tract cancer in general and of CRC in particular. Siblings have a higher GI tract cancer risk than parents, indicating that MSS young onset CRC may confer an increased familial risk for CRC that follows an autosomal recessive pattern of inheritance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CRC, Colorectal cancer; MMR, mismatch repair; MSI, microsatellite instability.

Table 1

Relative risks (RR) for colorectal cancers in first degree relatives of 278 probands with young-onset CRC (diagnosed under age 50 years). All probands were selected for having tumor with normal DNA mismatch repair.

Subset of Relatives	All probands N=278	Population based N=131
All	**RR = 1.65** (1.29–2.07) 45612 personyrs	**RR = 1.77** (1.19–2.53) 21083 personyrs
Parents	**RR = 1.50** (1.14–1.94) 17455 personyrs	**RR = 1.64** (1.06–2.42) 8066 personyrs
Siblings	**RR = 2.67** (1.50–4.41) 24844 personyrs	**RR = 3.00** (0.97–6.98) 12413 personyrs