Cancer Risks For Mismatch Repair Gene Mutation Carriers: A Population-Based Early Onset Case-Family Study

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Background & Aims: **Cancer risks for mismatch repair gene mutation carriers have been derived almost exclusively using families ascertained owing to their strong cancer family history. These may be overestimates, due to analytic problems, and not generalizable. We estimated average cancer risks for mutations identified in population-based early onset colorectal cancer cases (probands) unselected for family history.** ***Methods:*** **Data were cancer histories and mutation status (carrier, noncarrier, or unknown) of 17 mismatch repair gene mutation carrier probands with colorectal cancer diagnosed before age 45 (8 *hMLH1*, 4 *hMSH2*, 4 *hMSH6*, 1 *hPMS2*) and their first- and second-degree relatives. We used modified segregation analysis theory, adjusting for the family being ascertained through the proband being an early onset mutation carrier.** ***Results:*** **Eleven carrier probands (64%) were from families meeting the Amsterdam II criteria for hereditary nonpolyposis colorectal cancer. The cumulative risk for colorectal cancer (95% confidence interval) to age 70 was 45% (29%–62%) for men and 38% (19%–51%) for women. Corresponding risks were 67% (47%–84%) and 72% (48%–85%) for any hereditary nonpolyposis colorectal cancer–related cancer. Compared with the general population, colorectal cancer incidence for men was approximately 180-fold higher before age 50, but about the same after age 50. For women, incidence was approximately 100-fold higher before age 50 and 7-fold higher thereafter.** ***Conclusions:*** **For carriers of the mutations in the mismatch repair genes that cause early onset colorectal cancer, colorectal cancer increases rapidly until age 50, and the incidence decreases to general population levels at older ages.**

Several studies have shown that carriers of germline mutations in the mismatch repair (MMR) genes *hMLH1*, *hMSH2*, and *hMSH6* are at greatly increased risk for colorectal cancer, and to a lesser extent cancer of other tissues, particularly the endometrium.[1,2] The majority of studies[3–10] have analyzed data from families in whom individuals were screened for MMR gene mutations specifically because they had a strong family history of cancers associated with hereditary nonpolyposis colorectal cancer (HNPCC) syndrome.[11] The estimates of age-specific cumulative risk (penetrance) obtained from those studies are problematic because: (1) no analyses were adjusted correctly for the fact that the families were ascertained because of a strong family history of cancer, possibly leading to overestimation[12]; and (2) they may apply only to the mutations segregating within families with a comparable cancer history. In contrast, a recent reanalysis of a clinic-based series of *hMLH1* and *hMSH2* carriers from 84 Dutch HNPCC families, using a statistical method that adjusted appropriately for clinic-based ascertainment, found a substantially lower risk for colorectal cancer in carriers.[13]

Microsatellite instability (MSI) and MMR gene protein expression in colorectal tumors are predictive of germline MMR gene mutation status, not only for cases with strong family history of colorectal and other cancers[14–18] but also in early onset colorectal cancer cases irrespective of their family history.[19] Therefore, routine immunohistochemistry and/or MSI testing of early onset cases may become standard practice,[20–22] resulting in identification of the cases, and subsequently their relatives, who are mutation carriers. The risks for colorectal and other cancers for carriers identified in the context of newly diagnosed early onset colorectal cancer need to be

Abbreviations used in this paper: CI, confidence interval; DHPLC, denaturing high-performance liquid chromatography; HNPCC, hereditary nonpolyposis colorectal cancer; MMR, mismatch repair; MSI, microsatellite instability.

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determined to provide appropriate genetic counseling and optimal management.

The only population-based studies of MMR mutation penetrance are the family studies of 6 Scottish mutation-carrying colorectal cancer cases diagnosed before age 30[23] and 7 US mutation-carrying endometrial cancer cases.[2] A recent review concluded that "there is a considerable need for estimates of penetrance based on systematically collected familial or population data."[1]

Methods

Subjects

Subjects were 17 early onset colorectal cases, unselected for family history, identified as being a carrier of germline mutation in a MMR gene (8 in *hMLH1*, 4 in *hMSH2*, 4 in *hMSH6*, and 1 in *hPMS2*),[19] and their adult first- and second-degree relatives. These were identified from the Victorian Colorectal Cancer Family Study, a population-based case-family study conducted between 1993 and 1997 of 131 adult men and women (probands) living in the Melbourne metropolitan area who were younger than age 45 when diagnosed with a histologically confirmed, first primary adenocarcinoma of the colon or rectum (International Classification of Disease-0 C18, C19, and C20).[24] Ethics approval was obtained from The University of Melbourne and The Cancer Council Victoria. Informed consent was obtained from subjects.

Data Collection

Probands were administered a questionnaire (median, 9 months after diagnosis) including items on family cancer history and were asked to donate a blood sample. Probands were asked to obtain permission from their adult first- and second-degree relatives for us to contact them to seek their participation.

To update cancer family history since baseline, and therefore improve estimates of cancer risks, we attempted to re-interview all surviving subjects in 2004. In addition, we asked about their histories of colorectal surgery, hysterectomy, oophorectomy, and polypectomy. Of the 97 first- and second-degree relatives of the mutation-carrying probands interviewed at baseline, we re-interviewed 62 (17 subjects were dead, 12 subjects refused, and 6 subjects were lost to follow-up evaluation), and for all but 1 family at least 1 relative per family was re-interviewed about all cancers occurring in the family.

Verification of reported cancers at baseline and follow-up evaluation was sought through personal interviews, cancer registries, hospital records, treating clinicians, and death certificates. All names of subjects were linked to the National Death Index and the Victorian Cancer Registry to confirm reported cancers and to identify unreported cancers.

Families were categorized as meeting the Amsterdam II Criteria for HNPCC if all of the following criteria were met: at least 3 relatives diagnosed with cancer of the colon, rectum, endometrium, small bowel, ureter, or renal pelvis; at least 1 of these relatives being a first-degree relative of at least 2 other cases; relevant cancers diagnosed in at least 2 successive generations; at least 1 of these cancers diagnosed before age 50; and familial adenomatous polyposis being excluded.[25] HNPCC-related cancers were defined as those of the colon, rectum, endometrium, small bowel, stomach, ovary, pancreas, ureter, renal pelvis, biliary tract, or brain.[11]

Mutation Detection

Testing for germline MMR mutations was conducted for all 36 of the 105 probands for which tumors were available and found to be MSI-high, MSI-low, or lacked expression of MLH1, MSH2, MSH6, or PMS2 protein, and for a random sample of 23 of the other probands.[19] All exonic and flanking intronic sequences of the *hMLH1*, *hMSH2*, *hMSH6*, and *hPMS2* were screened for mutations using sequencing approaches, except for exon 4 of *hMSH6*, which was screened in 8 overlapping fragments using denaturing high-performance liquid chromatography (DHPLC). Confirmation of putative mutations identified via DHPLC or sequencing was sought by independent polymerase chain reaction and sequencing. The Multiplex Ligation-dependent Probe Amplification assay[26] (MR-C-Holland, Amsterdam, Netherlands) to detect large genomic alterations in *hMLH1* and *hMSH2* was performed on samples from the probands whose tumors lacked at least 1 MMR protein expression and for which no mutation had been identified by sequencing.[19] Variants were defined as mutations if they were predicted or known to produce a shortened or truncated protein product, or were missense variants previously reported as deleterious.

A total of 18 mutations were identified in the probands (9 in *hMLH1*, 4 in *hMSH2*, 4 in *hMSH6*, and 1 in *hPMS2*). All were predicted to produce a truncated protein product previously reported to be deleterious and all had tumors that were either MSI-high or MSI-low or were absent for at least one MMR protein.[19] Sixteen (89%) were men, and the mean age at diagnosis of colorectal cancer was 36 years (range, 27–44 y). Because one of the *hMLH1* mutations was de novo (Southey et al, unpublished data), none of the relatives could be a carrier and were not used to estimate risk, leaving 17 families for analysis. The DNA samples from first- and second-degree relatives of carriers who provided a blood sample were tested for the mutation identified in the proband. Obligate carriers were defined as untested individuals who had an offspring who was a carrier, and the other biological parent of the offspring had tested negative for the mutation or a sibling of the parent of the untested individual had tested positive for the mutation.

Statistical Analysis

Age-specific incidence, penetrance, and the hazard ratio (ratio of the age-specific incidence in carriers of a MMR gene mutation to that in non-carriers) were estimated by a modified segregation analysis fitted under maximum likelihood theory using the statistical package MENDEL (http://www.genetics.ucla.edu/software).[27] Full details of the mutation status and cancer history for each proband and all first-