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Dependence of Colorectal Cancer Risk on the Parent-of-Origin of Mutations in DNA Mismatch Repair Genes

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Abstract

Genetic diseases associated with dynamic mutations in microsatellite DNA often display parent-of-origin effects (POEs) in which the risk of disease depends on the sex of the parent from whom the disease allele was inherited. Carriers of germline mutations in mismatch repair (MMR) genes have high risks of colorectal carcinoma (CRC). We investigated whether these risks depend on the parent-of-origin of the mutation. We studied 422 subjects, including 89 MMR gene mutation carriers, from 17 population-based families who were each recruited via a CRC case diagnosed before age 45 years and found to carry a MMR gene mutation. The POE hazard ratio (HR_{POE}), defined to be the CRC incidence for carriers with maternally derived mutations divided by the corresponding paternal incidence, was estimated using a novel application of modified segregation analysis. HR_{POE} (95% confidence interval) was estimated to be 3.2 (1.1–9.8) for males ($P=0.03$) and 0.8 (0.2–2.8) for females ($P=0.5$) and the corresponding cumulative risks to age 80 years were 88% (54%–100%) for male carriers with maternally derived mutations and 38–48% for all other carriers. If confirmed by larger studies, these results will have important implications for the etiology of CRC and for the clinical management of MMR gene mutation carriers.

Keywords

Lynch syndrome; HNPCC; colorectal cancer; mismatch repair; MMR; parent-of-origin; POE; penetrance; segregation analysis; maximum likelihood

Introduction

DNA mismatch repair (MMR) proteins repair base–base mismatches that occur during cell replication [Umar et al., 2004] and play a role in meiotic chromosome pairing and recombination [Baker et al., 1996; Martin et al., 2000]. A person with a germline mutation in one of the MMR genes *MSH2*, *MLH1*, *MSH6* or *PMS2* (MIM#s 609309, 120436, 600678, 600259) is at increased risk of colorectal carcinoma (CRC), endometrial carcinoma (EC), and cancers of the stomach, ovary, ureter, renal pelvis, brain, small bowel, and hepatobiliary tract [Umar et al., 2004]. Carriers of MMR gene mutations who develop polyps or cancers with MMR deficiency are said to have Lynch syndrome, a condition also known as hereditary nonpolyposis colon cancer (HNPCC) [Jass, 2006].

Cells lacking both functioning copies of their MMR genes exhibit a mutator phenotype in which the spontaneous mutation rate is at least 100 times normal [Parsons et al., 1993]. This phenotype is characterised by genome-wide somatic expansion or contraction of repeat DNA sequence motifs (microsatellites), a phenomenon known as microsatellite instability (MSI). Recent studies have raised the possibility of haploinsufficiency in MMR carriers during gametogenesis. Martin et al. [2000] found that male *MSH2* gene mutation carriers have higher rates of aneuploidy and mutations in their sperm than noncarriers. In addition, a study of *MLH1* mutation carriers found increased variability and lengthening of the fragile-X repeat of carriers compared to controls, suggesting MMR deficiency during gametogenesis could increase the likelihood of transition from normal to a premutation range of repeats in genes with dynamic mutations [Fulchignoni-Lataud et al., 1997].

Neurodegenerative and neuromuscular disorders caused by dynamic germline mutations in DNA microsatellites, such as fragile-X syndrome and congenital myotonic dystrophy, display a parent-of-origin effect (POE) whereby the age-specific cumulative risk (penetrance) depends on the sex of the parent who transmitted the disease allele. For both these diseases, expansion of the repeat occurs during maternal meiosis. This results in an earlier age at onset and more severe disease in subsequent generations, a phenomenon known as genetic anticipation. The relationship between MMR gene mutations and DNA instability in neurodegenerative and neuromuscular conditions is complex [Goellner et al., 1997; Manley et al., 1999], however there have been numerous reports of genetic anticipation in Lynch syndrome [Menko et al., 1993; Rodriguez-Bigas et al., 1996; Vasen et al., 1994; Warthin, 1925; Westphalen et al., 2005], although these are controversial [Tsai et al., 1997; Voskuil et al., 1997].

Despite these findings, little research has been conducted into POEs for MMR gene mutation carriers. To date, only one study [Green et al., 2002] has investigated a POE in the context of MMR deficiency but it was subject to the limitations of traditional statistical methods to estimate POEs (see Discussion). We therefore developed an asymptotically efficient and unbiased statistical method to estimate POEs and applied this to CRC risks for MMR gene mutation carriers.

Materials and Methods

Subjects

Subjects were from the Victorian Colorectal Cancer Family Study (VCCFS), a multigenerational, population-based prospective study of probands with early-onset CRC and their relatives. The probands were 131 adult men and women living in the Melbourne metropolitan area (population 3.4 million) who were under the age of 45 years when diagnosed with a histopathologically confirmed first primary adenocarcinoma of the colon or rectum (International Classification of Diseases for Oncology C18, C19 and C20) [Fritz

et al., 2000]. Approval for the study was obtained from the Human Research Ethics Committees (HREC) of The University of Melbourne and The Cancer Council of Victoria (HREC No. 020702).

The subjects for the present study consisted of 17 probands, recruited independently of family history, who were found to carry germline mutations in MMR genes (nine in *MLH1*, four in *MSH2*, three in *MSH6*, and one in *PMS2*) as well as their adult relatives [Southey et al., 2005]. One of the 18 mutation-carrying families reported in Southey et al. [2005] was excluded from this study because of unreliable baseline data on family cancers.

Data Collection

As described in Jenkins et al. [2006], all probands were administered a risk factor questionnaire and asked about their family cancer history during an in-person interview at baseline (1993–1997). A blood sample was collected. In addition, each proband was requested to ask his or her adult first- and second-degree relatives for permission for the VCCFS to contact them. Relatives of the proband who consented were administered the same questionnaire and asked about their family's cancer history during a telephone interview. Blood samples were sought from all living relatives with colorectal and Lynch syndrome-associated cancers and relatives that linked such affecteds to the probands, as well as from other members of the family depending on funding available at the time. Verification of all reported family cancers was sought through cancer registries, hospital records, treating clinicians, and death certificates. All names of participants were also linked to the National Death Index (NDI) and the Victorian Cancer Registry (VCR) to confirm reported cancers and identify unreported cancers.

At follow-up in 2004, we attempted to reinterview all surviving probands and relatives who had participated at baseline, updating their personal and family cancer histories, and expanding the pedigrees where appropriate. In addition, information on colorectal surgery, hysterectomy, oophorectomy, and polypectomy was obtained. Family pedigrees were expanded using the Cannings-Thompson ascertainment scheme [Cannings and Thompson, 1977] whereby attempts were made to similarly study all first-degree relatives of family members affected with a cancer associated with Lynch syndrome. This process was repeated until no new affected subjects were obtained.

Mutation Detection

As previously described [Mead et al., 2007; Smith et al., 2006; Southey et al., 2005], extensive testing for MSI and loss of protein expression for the four MMR genes was conducted on all 131 proband tumors. Germline mutation testing of the MMR genes was conducted for the following probands: (1) those from the 12 families that at baseline fulfilled the Amsterdam Criteria II for Lynch syndrome; (2) a further 25 with tumors that demonstrated MSI or lacked expression of at least one MMR gene protein; and (3) a further 23 who were a random sample of the probands not fulfilling the previous criteria. Despite extensive mutation testing, we could not exclude the possibility that the apparent *PMS2* mutation actually occurred in the *PMS2* pseudogene.

Statistical Analysis

Descriptive analyses—Average ages at onset (if affected) or ages at the earliest of last contact, death, or surgery (if unaffected) of known and obligate carriers with unambiguous parent-of-origin of their mutations were compared using Student's *t*-tests implemented in R 2.11.1 [R Development Core Team, 2008].

A novel statistical technique to estimate POEs—Modified segregation analyses use a likelihood-based approach to analyze data from pedigrees of arbitrary size and structure and can be applied even if there is incomplete genotyping. For each family, the joint likelihood function, $L(\theta)$, is expressed as a sum

$$L(\theta) = \sum_g P(g) L(\theta|g)$$

where g is a vector specifying the phased genotypes of all family members; the sum is taken over all the phased genotypes g that are compatible with the observed genotype data; $P(g)$ is the joint probability of the set of genotypes, as determined by given genotype frequencies and known laws of genetic inheritance; θ is a vector of the model parameters to be estimated; and $L(\theta|g)$ is the likelihood of the family given the complete set of phased genotypes, g [Carayol and Bonaiti-Pellie, 2004; Elston and Stewart, 1971].

We have extended this established statistical method in a novel way to estimate POEs. By the above formula, to specify a likelihood for the family it is only necessary to specify the likelihood functions $L(\theta|g)$ in which all phased genotypes are known. So when specifying $L(\theta|g)$ for a genetic disease caused by a disease allele at a single locus, heterozygotes with maternally inherited disease alleles can be distinguished from those with paternally inherited ones. The ratio of the risks for these two types of heterozygotes, which quantifies the POE, can then be included in the model as a parameter and estimated by the method of maximum likelihood.

Main analyses—Modified segregation analyses were used to jointly estimate hazard ratios (HR: the incidence for carriers divided by that for noncarriers) for CRC, EC, and minor Lynch cancers combined (MC: cancers of the stomach, ovary, ureter, renal pelvis, brain, small bowel, and hepatobiliary tract) [Umar et al., 2004]. As in Quehenberger et al. [2005] and Senter et al. [2008], likelihood functions were used that implement parametric survival analyses with noncarrier hazards equal to the average age-specific and age-standardized incidences for the Australian population for CRC and MC (1988–2000), and for EC (1983–2000) (Australian Institute of Health and Welfare, <http://www.aihw.gov.au/cancer/databases/index>). For CRC, the hazard ratios $HR_{pa}(t)$ and $HR_{ma}(t)$ at age t years for mutation carriers with paternally and maternally inherited mutations (respectively) were assumed to have the parametric form

$HR_{pa}(t) = \exp[\theta_1 + \theta_2^*(t - 40)]$ and $HR_{ma}(t) = \exp(\theta_1 + \theta_2^*(t - 40) + \theta_3)$. Under this parameterization, $HR_{POE} = \exp(\theta_3)$ quantifies the POE and was assumed to be independent of age. Homozygous carriers, although absent from our data, arise as a theoretical possibility in the expression for $L(\theta)$ above and were assigned an HR at age t years equal to the maximum of $HR_{pa}(t)$ and $HR_{ma}(t)$ where necessary. For EC and MC, HRs were assumed to be independent of the parent-of-origin of the mutation and to be constant with age.

For each family, the joint likelihood was expressed as a function of the observed cancer status, carrier status (carrier, noncarrier, or unknown) and the ages at earliest of last contact, death, or cancer diagnosis of each family member. Individuals were censored at their first cancer or at polypectomy or hysterectomy. No cancer cases had missing ages except for three CRC cases who died at ages 29, 34, and 49 years. These ages at death were used in place of the ages at diagnosis because they are upper bounds for the missing ages and so will give either unbiased or slightly conservative risk estimates. A sensitivity analysis in which these three cases were instead censored at birth gave similar HR_{POE} estimates (results not shown). There were also 47 individuals without cancer who had missing ages, and they were censored at birth. All cancers (verified and unverified) were included in the analysis. Hardy-

Weinberg equilibrium and an allele frequency of 0.001 for all MMR gene mutations combined were assumed.

To adjust for ascertainment, the joint likelihood for each family was conditioned on the ascertainment criteria of the proband [Cannings and Thompson, 1977; Carayol and Bonaiti-Pellie, 2004], namely, that he or she was an MMR gene mutation carrier diagnosed with CRC before the age of 45 years. Models were then fitted by maximizing the sum of these conditional log-likelihoods over all carrier families, assuming independence of families.

Cumulative risk estimates were derived from the population incidences and the HR estimates as one minus the exponential of the appropriate cumulative incidences. Corresponding 95% confidence intervals (CI) were calculated with a parametric bootstrap based on 10,000 draws from the asymptotic distribution of the HR estimates. Specifically, the 10,000 random draws were used to produce a sample of 10,000 corresponding cumulative risks at each age, and the 2.5 and 97.5 percentiles of these samples were taken as the endpoints of the CI for that age.

The main analyses were performed with the pedigree analysis program MENDEL 3.2 [Lange et al., 1988] in product mode and supporting analyses were performed with R 2.11.1 [R Development Core Team, 2008]. Cumulative risk estimates were based on the most parsimonious model, as chosen by forward model selection. The asymptotic likelihood ratio test was used to compare the goodness of fit of nested models.

Results

The 17 families contained a total of 422 subjects including 53 CRC cases, 10 EC cases, and 15 other Lynch syndrome-associated cancer cases. Of the 53 first primary CRCs reported, 36 (68%) were verified, as were 5 (50%) of the EC cases and 11 (73%) of the other Lynch syndrome-associated cancer cases. There were 80 known MMR gene mutation carriers, 9 obligate carriers, 77 known noncarriers, 3 obligate noncarriers, and 253 relatives with unknown mutation status.

Table 1 presents descriptive analyses for the subset of the probands and their relatives who were known or obligate carriers with unambiguous parent-of-origin of their mutations. The average age at CRC diagnosis for male known or obligate carriers with maternally inherited mutations was 6 years earlier than for those with paternally inherited mutations ($P=0.05$). No other age differences were observed (all $P>0.2$).

Table 2 presents ascertainment-adjusted results based on all of the data. Male carriers who inherited their mutations from their mothers were estimated to have higher CRC incidences than males with paternally inherited mutations (HR_{POE} 5.3.2; 95% CI, 1.1–9.8; $P=0.03$). For female carriers, there was no evidence that their CRC risks depend on the parent of origin of their mutations ($HR_{POE} = 0.8$; 95% CI, 0.2–2.8; $P=0.5$).

After exclusion of the *PMS2* family (see Materials and Methods), the estimated HR_{POE} for male carriers was 3.0 (95% CI, 0.9–9.2; $P=0.06$), showing that the *PMS2* family data is contributing to but not driving this estimate. The observed POE is also unlikely to be caused by the genetic heterogeneity of our data because the HR_{POE} estimates for male carriers of mutations in individual MMR genes were similar to or higher than the combined estimates: 3.1 (95% CI 0.7–13.7; $P=0.1$) for *MLH1* mutation carriers; 7.5 (95% CI 1.2–46.9; $P=0.07$) for *MSH2* mutation carriers; 1.9 (95% CI 0.1–24.3; $P=0.6$) for *MSH6* mutation carriers; not able to be estimated for *PMS2* mutation carriers; and 2.9 (95% CI 0.5–17.0; $P=0.3$) for all non-*MLH1* MMR gene mutation carriers combined.

Table 3 and Figure 1 show the estimated CRC cumulative risks for MMR gene mutation carriers. For male carriers, the cumulative risk to age 80 years was 88% (95% CI, 54–100%) for those with maternally inherited mutations and 48% (95% CI, 24–82%) for those with paternally inherited mutations. Estimates of lifetime cumulative risks for female carriers did not vary greatly with the parent-of-origin of the mutation and were similar to those for male carriers with paternally inherited mutations.

Discussion

We used a novel, sophisticated, and rigorous method that gives unbiased estimates to investigate whether CRC risks for carriers of mutations in MMR genes depend on the parent-of-origin of their mutations. We estimated that male carriers who inherit their mutations from their mothers have on average a threefold increased incidence of CRC compared with those who inherit their mutations from their fathers. The corresponding cumulative risk to age 80 years was 88% (95% CI, 54–100%) for male carriers with maternally inherited mutations, almost double that for male carriers with paternally inherited mutations. We did not observe a POE for female carriers but we had limited statistical power so we cannot exclude this possibility. If confirmed by other studies, our findings have clear implications for genetic counselling, screening protocols, and counseling about prophylactic surgery for unaffected MMR gene mutation carriers.

Our findings suggest a maternally transmitted mechanism modifying CRC risk for MMR gene mutation carriers. Potential mechanisms include epimutations or the expansion of dynamic mutations in CRC susceptibility genes during maternal meiosis. Alternatively, as MMR proteins have been found to play a role in meiotic chromosome pairing and recombination [Baker et al., 1996; Martin et al., 2000] and sperm have a 50% lower recombination rate than ova [Tease and Hulten, 2004] and are exposed to strong selective pressures, potential MMR haploinsufficiency during gametogenesis could result in a higher prevalence of mutations in ova compared to sperm. This might result in higher cancer risks for the offspring of female carriers. If the mutations accumulate over subsequent generations, this might cause genetic anticipation.

To date, only one other study has been conducted into a POE for MMR gene mutation carriers. Green et al. [2002] used survival analysis to estimate a POE using 12 families who were recruited from a medical genetics clinic and segregated the same mutation in MSH2. Opposite to our findings, they did not observe a POE for male carriers and they estimated that female carriers who inherited their mutations from their fathers rather than their mothers were at higher risk of CRC (relative risk [RR] 2.5; $P=0.05$, CIs not given), although their estimated cumulative risks for female carriers to age 80 years were 76% regardless of the parent-of-origin of the mutation. As an aside, we note that this finding is similar to the POE reported in the recent study of familial CRC by Lindor et al. [2010], although that study is of low relevance here because it specifically excluded all known or likely Lynch syndrome cases.

Given the marginal significance of our findings and those of Green et al. [2002], the different genes studied and the fact that failure to reject the null hypothesis can occur simply because of a lack of statistical power, the results of our study and theirs are not necessarily inconsistent. However, there is some doubt about the validity of their results due to errors in their analytical methods. Green et al. [2002] did not adjust for the clinic-based ascertainment of their families, they imputed values of the exposure variable (mutation status) using the outcome variable (CRC affected status) and they preferentially excluded sibships with few cancers from their analysis. Each of these would tend to upwardly bias their risk estimates [Carayol et al., 2002] and indeed their cumulative risk estimates are much higher than

recent, more statistically rigorous estimates [Jenkins et al., 2006; Quehenberger et al., 2005]. Biases might have been introduced into their POE estimates by the sibship exclusion criterion described above coupled with the fact that they observed more female carriers transmitting their mutations to their offspring than male carriers.

Having said that, a strength of Green et al. [2002] was the genetic homogeneity of their study sample and a corresponding weakness of our study is that we combined families with mutations in all four MMR genes in order to increase statistical power. The genetic heterogeneity of our study sample could cause a spurious POE if carriers who inherit their mutations from their mothers are more likely to carry mutations in higher risk MMR genes than carriers who inherit their mutations from their fathers. This would happen, for instance, if mutations in a high-risk MMR gene reduce male fertility while mutations in lower risk MMR genes do not. However, if genetic heterogeneity were acting as a confounder then the HR_{POE} estimates for the individual MMR genes would all be close to 1. This is in striking contrast to the observed estimates that were all similar to or larger than the combined estimate. It therefore seems unlikely that the genetic heterogeneity of our families has caused the observed POE.

Strengths of our study include the population-based ascertainment of families, the large number and proportion of relatives typed for the family mutations, our attempts to verify cancer diagnoses, the consistency between our main results and descriptive analyses, and our rigorous and powerful statistical methods. Limitations include the genetic heterogeneity discussed above, the small number of carrier families, and the marginal statistical significance of our findings. Nevertheless, we have demonstrated the feasibility of our approach to investigate POEs.

Our method for estimating POEs is a novel application of modified segregation analysis so it inherits the following advantages from this established method: it is asymptotically unbiased and efficient, data from pedigrees of arbitrary size and structure can be combined, it maximizes power because all data are used (including data for relatives not typed for the family mutation), rigorous adjustment for ascertainment can be performed without difficulty, and it is readily implemented in the freely available pedigree analysis software MENDEL 3.2.

Our study provides the first rigorous estimate of a POE in this setting. We did not find evidence of a POE for female carriers but CRC incidences were higher for male carriers who had inherited their mutations from their mothers rather than their fathers. If confirmed, this finding will have important implications for the aetiology of CRC in Lynch syndrome and for the clinical management of MMR gene mutation carriers.

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Figure 1. Age-specific cumulative risks of colorectal carcinoma (bold lines) and corresponding 95% confidence intervals (shaded regions) for MMR gene mutation carriers with maternally inherited mutations (unbroken lines and horizontal shading), carriers with paternally inherited mutations (dashed lines and vertical shading) and noncarriers (dotted lines), separately for females (**A**) and males (**B**).

Table 1

Average Ages at Diagnosis (If Affected) or Ages at the Earliest of Last Contact, Death, or Surgery (If Unaffected), with 95% Confidence Intervals in Parentheses, of MMR Gene Mutation Carriers with Unambiguous Parents-of-Origin of Their Mutations by Colorectal Carcinoma (CRC) Status and Sex of Proband

Sex	CRC status	<u>Average ages in years (95% CI) number of subjects</u>		
		Maternal origin	Paternal origin	<i>P</i> -value
Female	Unaffected	44 (38–49) 19	35 (25–46) 9	0.2
	Affected	40 (29–50) 7	38 (34–42) 3	0.7
Male	Unaffected	37 (32–41) 8	41 (31–50) 6	0.4
	Affected	34 (31–37) 11	40 (35–46) 7	0.05

P-Values are for tests of the null hypothesis that there is no difference by parent-of-origin of the mutation.

Estimated Hazard Ratios (HRs) with 95% Confidence Intervals (CIs), Degrees of Freedom, and Log-Likelihoods for Three Models Used to Estimate a Parent-of-Origin Effect (POE) on Risk of Colorectal Carcinoma (CRC) for MMR Gene Mutation Carriers

Table 2

		Model		
		No POE	POE for male carriers only	Separate POEs for male and female carriers
Male carriers	HR _{POE} (95% CI)	1	3.2 (1.1–9.8)	3.1 (1.0–9.6)
	HR _{CRC} at age 40 years (95% CI) [paternal]	99 (56–173)	52 (23–117)	53 (23–120)
	Factor of increase in CRC HR for each year of age (95% CI)	0.92 (0.89–0.95)	0.92 (0.89–0.95)	0.92 (0.89–0.95)
	HR _{minor Lynch cancers} (95% CI)	20 (8.9–45)	19 (8.6–42)	19 (8.7–42)
Female carriers	HR _{POE} (95% CI)	1	1	0.8 (0.2–2.8)
	HR _{CRC} at age 40 years (95% CI) [paternal]	62 (35–111)	64 (36–113)	76 (29–199)
	Factor of increase in CRC HR for each year of age (95% CI)	0.91 (0.88–0.95)	0.92 (0.88–0.95)	0.92 (0.88–0.95)
	HR _{endometrial cancer} (95% CI)	53 (26–107)	56 (28–113)	56 (28–114)
	HR _{minor Lynch cancers} (95% CI)	9.0 (3.3–23)	9.0 (3.6–24)	9.0 (3.6–24)
Degrees-of-freedom		7	8	9
Log-likelihood		–723.236	–720.960	–720.871

Table 3

Estimated Age-Specific Cumulative Risks of Colorectal Carcinoma (as a Percentage), with 95% Confidence Intervals (CIs) in Parentheses, for MMR Gene Mutation Carriers by Parent-of-Origin of Their Mutation and Sex

Age (years)	Female carriers		Male carriers	
	Paternal origin	Maternal origin	Paternal origin	Maternal origin
30	5 (2–16)	4 (1–10)	3 (1–8)	9 (4–20)
40	12 (4–30)	9 (4–21)	7 (3–17)	22 (11–41)
50	22 (9–48)	17 (8–35)	16 (8–34)	44 (24–71)
60	34 (15–66)	27 (14–51)	29 (14–55)	67 (39–93)
70	42 (18–78)	34 (17–63)	41 (20–71)	81 (49–99)
80	47 (21–86)	38 (19–73)	48 (24–82)	88 (54–100)