

Penetrance of Colorectal Cancer among *MLH1/MSH2* Carriers Participating in the Colorectal Cancer Familial Registry in Ontario

Corresponding author: Dr. Laurent Briollais, Prosserman Centre for Health Research, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 60 Murray Street, room 5-218, Toronto, ON M5G 1X5. Email: laurent@lunenfeld.ca; Telephone: 416 586 8863; Fax: 416 586 8404

Affiliations of authors:

Yun-Hee Choi, Prosserman Centre for Health Research, Samuel Lunenfeld Research Institute, Toronto, Canada;

Michelle Cotterchio, Division of Preventive Oncology and Division of Population Studies and Surveillance, Cancer Care Ontario, Toronto, Canada;

Gail McKeown-Eyssen, Dalla Lana School of Public Health and Department of Nutritional Sciences, University of Toronto, Canada;

Monga Neerav, Division of Preventive Oncology, Cancer Care Ontario, Toronto, Canada;

Bharati Bapat, Fred A. Litwin Centre for Cancer Genetic, Samuel Lunenfeld Research Institute and Laboratory of Medicine and Pathobiology, Mount Sinai Hospital, Toronto, Canada;

Kevin Boyd, Division of Preventive Oncology, Cancer Care Ontario, Toronto, Canada;

Steven Gallinger, Fred A. Litwin Centre for Cancer Genetic, Samuel Lunenfeld Research Institute, Department of Surgery, University of Toronto and Ontario Familial Colon Cancer Registry, Ontario Cancer Genetics Network, Toronto, Ontario, Canada;

John McLaughlin, Dalla Lana School of Public Health, University of Toronto, Prosserman Centre for Health Research, Samuel Lunenfeld Research Institute and Division of Preventive Oncology, Cancer Care Ontario, Toronto, Canada;

Melyssa Aronson, Dr. Zane Cohen Digestive Disease Clinical Research Centre, Mount Sinai Hospital, Toronto, Canada

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Abstract

Background: Several DNA mismatch repair (MMR) genes, responsible for the majority of Lynch Syndrome cancers, have been identified, predominantly *MLH1* and *MSH2*, but the risk associated with these mutations is still not well established. The aim of this study is to provide population-based estimates of the risks of colorectal cancer (CRC) by gender and mutation type from the Ontario population. **Methods:** We analyzed 32 families segregating MMR mutations selected from the Ontario Familial Colorectal Cancer Registry and including 199 first-degree and 421 second-degree relatives. The cumulative risks were estimated using a modified segregation-based approach which allows correction for the ascertainment of the Lynch Syndrome families and permits account to be taken for missing genotype information. **Results:** The risks of developing CRC by age 70 were 60% and 47% among men and women carriers of any MMR mutation, respectively. Among *MLH1* mutation carriers, males had significantly higher risks than females at all ages (67% vs. 35% by age 70, p-value=0.02), while the risks were similar in *MSH2* carriers (about 54%). The risk associated with *MLH1* was almost constant with age (hazard ratio (HR) varied 5.5-3.3 over age 30-90), while *MSH2* decreased with age (HR varied 13.1-3.2). **Conclusions:** This study provides a unique population-based study of CRC risks among *MSH2/MLH1* mutation carriers in a Canadian population. The different patterns of risks found for *MLH1* and *MSH2* gene mutations could be help defining better clinical management and prevention of CRC among members of Lynch Syndrome families.

Introduction

Lynch Syndrome, also referred to as Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant condition which predisposes carriers to both colorectal and extra-colonic cancers.¹ Several DNA mismatch repair (MMR) genes responsible for the majority of Lynch Syndrome cancers have been identified, predominantly *MLH1* and *MSH2*.² An understanding of risk associated with MMR mutations is important to assist in decisions about prophylactic surgery, annual screening and chemoprevention and to help allay psychological distress related to the uncertainty of colorectal cancer predisposition among Lynch Syndrome family members.¹

Despite this importance, only a small number of studies provided penetrance estimates for these gene mutations. In addition, most of them studied populations from Finland, Scotland, the Netherlands and Nebraska³⁻⁶ and it is unlikely that they are generalizable to the Canadian population as genetic mutations, lifestyle and environmental factors may differ. Moreover, most previous studies focused on families with a very strong history of cancers associated with the Lynch Syndrome and the derived penetrance estimates are only valid for families with comparable family history. Our study provides population-based estimates of penetrance by age, sex and type of mutation for CRC cases from the population of all CRC cases identified through the Ontario Familial Colorectal Cancer Registry (OFCCR)⁷ and selected to be representative of families likely to harbour MMR mutations. We also used an ascertainment-corrected segregation approach to correct for the specific ascertainment and missing mutation information. Therefore, our analyses reduce the chance for biases and provide penetrance estimates that are applicable to a larger population of CRC cases harbouring MMR mutations.

Materials and Methods

Study Population

The OFCCR is one of six international registries established by the National Cancer Institute (NCI) as a resource for the study of CRC.⁷ The OFCCR used the population-based Ontario Cancer Registry (OCR) to identify incident CRC cases (probands), aged 20-74, diagnosed July 1997 to July 2000. With physician consent, colorectal cases were contacted by a letter describing the study and requesting completion of a family history questionnaire (FHQ) which ascertained birth, death and disease history for first- and second-degree relatives. This information was used by genetic counselors to produce family pedigrees and to classify families as either 1) Amsterdam 1, 2) Familial Risk which included early-onset CRC and/or extra-colonic cancer in proband or relative, or family history of multiple CRC and/or extra-colonic cancers, 3) Sporadic/low risk with no family history of CRC.⁷ Probands from Amsterdam 1 and Familial Risk families were interviewed by counselors to clarify information regarding all first-degree relatives, complete the pedigree to include all second-degree relatives, and ascertain full cancer histories. A randomized sample of 25% of the sporadic/low risk cases was also studied but because the prevalence of MMR germline mutations is very low in the general population, around 1:3139⁸, this group was not further considered in the penetrance study. Indeed, only one individual in this group was found mutation carrier after tumor analysis and germline testing.

Whenever possible, probands' tumours were first screened for microsatellite instability (MSI) and Immunohistochemistry (IHC). Germline mutation analysis of *MSH2*, *MLH1* and/or *MSH6* genes was initiated in probands based on MSI-high and/or IHC deficient tumours results. Germline screening of a specific MMR gene was guided by IHC deficiency for a specific MMR

protein. Germline mutations were also assessed among probands with MSI-low or microsatellite stable (MSS) tumours if their tumours exhibited MMR protein deficiency when assessed by IHC.⁹ Probands were also screened for germline mutations in *MLH1* and *MSH2* when the tumours were MSI-high and IHC intact, or where tumours were not available for probands in Amsterdam families. Testing for MMR genes involved sequence analysis (*MSH2*, *MLH1*, *MSH6*) and Multiplex Ligation-dependent Probe Amplification (MLPA) (*MSH2* and *MLH1*) of genomic DNA obtained from blood samples.⁹

For each proband found to carry an MMR mutation, all first- and second-degree relatives on the “at-risk side” of the proband’s pedigree were considered to be eligible for the present study. Blood samples from these relatives were obtained in two stages. First, the OFCCR had requested blood samples from all probands’ first-degree relatives, together with all second-degree relatives who had had cancer or who were a first-degree relative of a family member with cancer, and who resided in North America, most European countries, or the Caribbean. Second, to ensure as many kin as possible were ascertained for the present study, we requested blood from all probands’ second-degree relatives who had not previously been contacted by the OFCCR. Inclusion of these additional second-degree relatives was important to avoid over representation of relatives with a cancer diagnosis and resultant upwardly biased risk estimates.

Mutations in the MMR genes were ascertained from genomic DNA for all participants from whom blood samples could be obtained. For deceased relatives, obligate gene mutation carriers were defined as persons with a descendent with a MMR gene mutation. Therefore, the final retrospective study cohort included all living *MSH2*, *MLH1* and *MSH6* mutation carriers, and all deceased obligate gene mutation carriers. There were 446 probands eligible for our study (i.e., with blood sample available, with consent to contact their relatives, satisfying the

Amsterdam 1 or other familial risk criteria, see above). Of these, 358 were tested for IHC (80%), 346 for MSI (77%), and 379 for either IHC or MSI (85%). Our final cohort included 32 probands found to carry a MMR mutation and their relatives. There were 27 probands from Amsterdam 1 families and 5 from other familial risk families. The relatives consisted of 199 first-degree and 421 second-degree relatives, from whom 56 and 41, respectively, were CRC cases (see Table 1). The number of blood samples available was 71 and 56, respectively, in first- and second-degree relatives from whom 38 and 22 were tested mutation positive (see Table 1). Survival analyses with the classical Kaplan-Meier estimator¹⁰ were carried out on the sample of individuals with blood available. The modified segregation-based approach (see methods section) was able to use the full cohort of individuals (i.e. with or without blood available) but age at diagnosis or at examination was required for the analysis. This was available for 506 individuals of the 622 recruited.

The Ethic approval was obtained from Mount Sinai Hospital REB for this research project.

Verification of cancer diagnoses in relatives carrying a MMR gene mutation

The pathology and date of diagnosis of all subject-reported CRC in a first- or second-degree relative were verified where possible using pathology reports obtained from the OCR, other cancer registries outside of Ontario, hospital discharge data, death certificates, and reports from Regional Cancer Centres and Princess Margaret Hospital, the main cancer hospital in Toronto. If only a death certificate was available, it was reviewed. We attempted to verify cancer diagnoses reported among relatives living in other countries by requesting a pathology report from the relevant cancer registry or hospital. All probands had their colorectal cancer

diagnosis confirmed by pathology reports (100%), as required for study eligibility. Of the 622 first- and second-degree relatives identified in the 32 mutation positive families, 61% of CRCs identified were confirmed by medical records and 38% of relatives identified as deceased were confirmed by death records found in the mortality database.

Identification of probands/families carrying a MMR gene mutation

Microsatellite instability (MSI) analysis

Written informed consent was obtained from 99.6% of OFCCR participants for the collection of tumour tissue for use in cancer research. Colorectal tumour blocks were collected from all eligible patients. Tumour DNA was extracted from paraffin embedded matched normal and tumour tissue specimens and tested for MSI status, using a panel of 5-10 microsatellite markers recommended by NCI, National Institute of Health.¹¹ MSI was defined as the presence of altered/additional bands in the polymerase chain reaction (PCR) amplified product of tumour DNA in comparison with the matched normal DNA samples obtained from the adjacent normal colon. Tumours were designated MSI-high if > 40% of the markers show altered band patterns, MSI-low if there is < 40% instability, and MSS if there was no instability.¹¹

MMR gene mutation analysis

MMR (*MSH2*, *MLH1*, *MSH6*) mutational analysis was performed on DNA from peripheral blood lymphocytes or lymphoblastoid cell lines. Large genomic deletions/duplications in *MSH2* and *MLH1* genes were identified by MLPA¹² and, if absent, underlying germline mutations were further screened by genomic DNA sequencing.⁹ Briefly, the entire coding regions of the *MSH2* (16 exons) and *MLH1* (19 exons) genes were amplified by PCR and

screened for mutations using ABI 377 automated sequencer. Similar functional analysis was also performed for *MSH6* gene using exon-by-exon sequencing strategy. Functional mutations were confirmed by assessing published literature, as well as from the human genome mutation database and the International Collaborative Group-HNPCC database (InSIGHT). We used the computational programs Polymorphism Phenotyping to predict the pathogenicity of novel MMR alterations (<http://genetics.bwh.harvard.edu/pph/>)¹⁴ and Sorting Intolerant From Tolerant (<http://blocks.fhcrc.org/sift/SIFT.html>)¹⁵. The description of the mutations found in this study are given in Table 1.

Table 1. Description of MMR mutation-carrying participants—mutation information and counts of kin (family size), kin with blood, colorectal cancer (CRC) amongst kin, and mutation carriers amongst kin by relative degree (1st = first degree relatives, 2nd = second degree relatives)

Gene	Germline Mutation	Mutation Consequence	No. of kin reported		No. of kin with CRC reported		No. of kin with blood		No. of mutation carriers	
			1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
<i>MLH1</i>	c.1852_1853 AA>GC	p.Lys618Ala	4	16	2	0	2	5	0	0
	c.1689_1690insA	p.Ile563IlefsX4	3	12	1	2	2	0	0	0
	c.1732_1896del	Exon 16 del	6	11	2	0	2	4	1	3
	c.116+5 G>C	Splice-site defect	7	13	1	1	2	0	1	0
	c.793C>T	p.Arg265Cys	10	23	2	5	1	6	1	1
	c.793C>T	p.Arg265Cys	7	25	1	5	2	5	1	0
	c.346delA	p.Thr116GlnfsX20	2	5	1	2	0	0	0	0
	c.731G>A	p.Gly244Asp	6	5	2	0	5	0	5	0
	c.790+2T>C	Splice-site defect	9	19	3	1	6	7	3	3
	c.298C>T	p.Arg100X	7	6	2	0	1	0	0	0
	c.1975C>T	p.Arg659X	3	5	2	3	3	0	0	0
	c.2223_2231del	In-frame deletion	9	11	2	1	3	0	2	0
	c.793C>T	p.Arg265Cys	4	11	1	1	2	0	1	0
	c.350C>T	Thr117Met	9	10	1	0	4	0	3	0
	Total:			86	172	23	21	35	27	18

<i>MSH2</i>	c.1216C>T	p.Arg406X	5	5	2	0	1	1	0	0	
	c.1165C>T	p.Arg389X	3	7	1	2	1	1	1	1	
	c.1277_1386del	Exon 8 deletion	5	18	4	3	1	2	1	0	
	c.2075G>T	p.Gly692Val	5	10	1	3	0	0	0	0	
	c.136_164del	p.His46GlyfsX35	3	5	1	2	2	0	2	0	
	c.2135_2136insT	p.Val712ValfsX4	5	11	2	1	1	0	0	0	
	c.942+3A>T	Splice-site defect	13	33	3	1	1	3	1	0	
	c.1511-2A>G	Splice-site defect	9	3	1	0	2	1	2	1	
	c.363T>G	p.Tyr121X	10	23	3	1	7	1	1	0	
	c.1_1386del	Exons 1-8 deletion	9	21	3	0	4	6	3	3	
	c.1705_1706del	p.Glu569IlefsX2	3	7	1	2	1	4	1	3	
	c.965G>T	p.Gly322Asp	6	14	1	0	1	0	0	0	
	c.942+3A>T	Splice-site defect	5	9	2	1	2	1	2	1	
	c.645+1G>A	Splice-site defect	5	7	1	2	1	0	1	0	
	c.1705_1706del	p.Glu569IlefsX2	5	12	2	0	2	0	0	0	
	c.1165C>T	p.Arg389X	7	22	3	1	3	6	2	3	
	c.1165C>T	p.Arg389X	3	5	1	1	1	2	1	2	
	Total:			101	212	32	20	31	28	18	14
	<i>MSH6</i>	c.3335_3336insATGA	p.Asp1112GlufsX2	12	37	1	0	5	1	2	1
		Total:			12	37	1	0	5	1	2
Grand total:			199	421	56	41	71	56	38	22	

Statistical Methods

Kaplan-Meier survival analysis

For the cohort of proband's family members, Kaplan-Meier survival analysis¹⁰ was used to estimate the age-specific cumulative CRC incidence, with the corresponding 95% confidence interval (CI), where observation time was taken from birth to the earlier of age at diagnosis of CRC, or current age. The analysis was stratified by: (a) MMR mutation status (*MSH2* or *MLH1*); (b) gender among MMR mutation carriers and non-carriers.

Segregation-based analysis

To account for missing genotype data and the non-random ascertainment of the families, we fitted likelihood-based segregation analyses.^{16,17} This allowed the estimation of the cumulative risk (penetrance) associated with either any Lynch Syndrome mutation, or with specific *MLH1* or *MSH2* mutations, in males and females. Based on preliminary data analysis, we found that a logistic regression model for the hazard function (see Equation (1) in Appendix) fitted the data well, and we used this to estimate the cumulative risk of developing CRC by each decade of age, for each type of mutation. The logistic model had the added advantage that it allowed the hazard rate to follow a non-monotonic distribution, in which the risk initially increased with age and then decreased, as observed in Jenkins et al. .¹⁸

The analyses were adjusted for ascertainment by conditioning the likelihood of the family's observed genotypes on the probability of the observed diseases in the family members, given their ages at examination. Thus, we obtain the maximum likelihood estimates of the needed parameters by maximizing this ascertainment-corrected retrospective likelihood^{16,17} into the software Mendel¹⁹ and used the parameters to estimate the cumulative risk of CRC at age t , using the penetrance function in equation 2 of the Appendix. For this analysis, the ascertainment correction was based on the first degree relatives of probands (i.e., the proband and his/her sibs and their parents), where probands carried a MMR mutation. For all analyses, the 95% CI for the cumulative risk by age was estimated by simulation of 1,000 sets of parameters, assuming a multivariate normal distribution of the parameters being estimated.¹⁸

Results

Distribution of Missing Ages and Ages at Onset in the Families

We had information on age at CRC onset for all 32 probands. This information was missing for 6 out of 97 affected relatives but 3 of them had their age at examination available. Among the 493

unaffected individuals (255 males and 238 females), age at examination was missing for 113 (60 males and 53 females). Among affected individuals (probands and relatives), the mean age at onset was 45.1 (n=25, standard deviation (s.d.)=13.3) in *MSH2* carriers, 53.6 (n=21, s.d.=12.3) among *MLH1* carriers and 51.4 (n=76, s.d.=14.6) for individuals with unknown mutation status. Only one individual was negative for *MSH2* among the affected relatives.

Kaplan-Meier Analysis

Descriptive analyses with the Kaplan-Meier (KM) estimator were carried out on the sample of kin with blood and age information available. The kin included 60 individuals who were positive for any MMR mutation (16 affected and 44 unaffected individuals) and 62 who were negative (1 affected and 61 unaffected individuals). In addition, we also estimated separately the cumulative risk in 352 individuals with unknown mutation status (77 affected and 275 unaffected individuals). By age 90, about two thirds of male and female carriers of any MMR mutation had experienced CRC (Table 2). Importantly, the risk among those with unknown mutation status was considerably closer to that of carriers than non-carriers among females but was between the two estimates among males (Table 2). Thus, omitting the unknown group or combining their estimates with another genotype category could lead to serious biases.

Table 2. Estimates of cumulative risks (%) to ages 30, 50, 70 and 90 (and corresponding 95% confidence intervals) for colorectal cancer stratified by MMR mutation status and gender using Kaplan-Meier analysis (n represents the number of kin with blood available and age information available).

Mutation	Gender	Age 30	Age 50	Age 70	Age 90
Carrier of any MMR mutation	Male (n=27)	–	21 (0, 39)	67 (3, 89)	67 (3, 88)

Carrier	Female (n=33)	4 (0, 10)	35 (6, 55)	51 (16, 71)	63 (19, 83)
	Combined (n=60)	2 (0, 6)	29 (10, 44)	55 (28, 72)	64 (31, 81)
<i>MLH1</i> carrier	Male (n=13)	–	40 (0, 70)	70 (0, 94)	70 (0, 94)
	Female (n=12)	–	–	38 (0, 68)	58 (0, 85)
	Combined (n=25)	–	16 (0, 34)	50 (4, 73)	62 (11, 84)
<i>MSH2</i> carrier	Male (n=12)	–	–	–	–
	Female (n=23)	5 (0, 15)	61 (9, 83)	61 (9, 83)	61 (9, 83)
	Combined (n=35)	3 (0, 10)	36 (6, 56)	56 (15, 77)	56 (15, 77)
Non-carrier of all mutations	Male (n=22)	–	–	–	–
	Female (n=40)	–	7 (0, 19)	7 (0, 19)	7 (0, 19)
	Combined (n=62)	–	4 (0, 11)	4 (0, 11)	4 (0, 11)
Any mutation unknown	Male (n=195)	2 (0, 5)	23 (14, 31)	46 (34, 57)	53 (39, 64)
	Female (n=157)	4 (0, 7)	13 (6, 20)	41 (27, 52)	59 (30, 72)
	Combined (n=352)	3 (1, 5)	19 (13, 24)	44 (35, 52)	68 (45, 81)

Modified segregation-based analysis

The modified segregation-based approach was able to use the full cohort of probands and their kin, with or without blood available. After removing individuals without information on age at diagnosis or examination, the final sample included 506 individuals out of the 622 recruited. Therefore, the estimation of the cumulative risk with the segregation approach took into account 32 probands, 60 kin who tested positive for any MMR mutation, 62 kin who tested negative, and

352 kin with unknown mutation status. These 32 probands and 352 kin could not be included in the KM estimation of carriers and non-carriers.

As assessed by segregation analysis, 60% of male and 47% of female carriers of any MMR gene mutation had developed CRC, by age 70. These risks were 81% and 72% by age 90, respectively. The cumulative risk appeared higher for men than for women at all ages (Table 3 and Figure 1), although a formal test of difference was not significant (Wald Chi-square test; p-value=0.25). This arose because male carriers of *MLH1* gene mutations had a significantly higher risk of cancer than female carriers at all ages (67% vs. 35% by age 70, p-value=0.02) (Table 3 and Figure 1), while male and female carriers of *MSH2* mutations had similar cumulative risks of disease ($\approx 54\%$, p-value=0.89) (Table 3). Among men, the estimated risk was higher among carriers of *MLH1* mutations than *MSH2* mutations (e.g. 67% vs. 55% by age 70), while the opposite was observed among women (35% vs. 53%).

For *MLH1* mutations, the age-specific risk of cancer among male carriers (the hazard rate) increased to about age 70 but remained relatively constant thereafter (Figure 2), while female carriers continued to experience an increase in risk after age 70 (Figure 2). However, for *MSH2* mutation carriers of both sexes, risk increased only until about age 70 and stabilized afterwards. As observed in previous studies, male carriers were at higher risk than female carriers for *MLH1* but not for *MSH2* mutations.

Table 3. Estimates of cumulative risks (%) at ages 30, 50, 70 and 90 (and corresponding 95% confidence intervals) for colorectal cancer among mutation carriers and non-carriers (any MMR, *MLH1*, *MSH2*) based on a one-gene segregation model

Mutation	Gender	Age 30	Age 50	Age 70	Age 90
Carrier of any MMR mutation	Male	1 (0, 2)	23 (10, 33)	60 (35, 73)	81 (59, 89)
	Female	1 (0, 1)	15 (7, 23)	47(26.9, 60)	72(50, 82.7)
	Combined	1 (0, 1)	18 (11, 25)	53 (37, 64)	76 (61, 85)
<i>MLH1</i> Carrier	Male	1 (0, 2)	25 (7, 51)	67 (27, 89)	87 (53, 97)
	Female	0 (0, 1)	8 (2, 19)	35 (10, 59)	64 (25, 84)
	Combined	0 (0, 12)	13 (4, 30)	44 (19, 70)	71 (39, 89)
<i>MSH2</i> Carrier	Male	1 (0, 2)	21 (1, 36)	55 (2, 75)	77 (5, 91)
	Female	1 (0, 2)	20 (1, 32)	53 (2, 70)	75 (6, 88)
	Combined	1 (0, 2)	20 (1, 29)	54 (3, 69)	76 (9, 87)
Non-carrier of all mutations	Male	0 (0, 1)	2 (0, 7)	10 (1, 27)	24 (4, 51)
	Female	0 (0, 1)	1 (0, 6)	6 (1, 24)	16 (2, 47)
	Combined	0 (0, 1)	2 (0, 6)	9 (2, 24)	23 (5, 47)

Estimates of hazard ratios (HRs) for CRC by age group are presented in Table 4. Estimates for males and females were combined in the table due to their large confidence intervals. The effect of *MLH1* on CRC was almost constant with age (the HR varied between 5.5 at age 30 and 3.3 at age 90). *MSH2* had a stronger effect on CRC and exhibited a decreasing pattern with age (the HR fell from 13.1 at age 30 to 3.2 at age 90). The global effect of any MMR mutation on CRC was significant and showed a decreasing trend with age (the HR fell from 10.5 at age 30 to 3.3 at age 90).

Table 4. Estimated hazard ratios of colorectal cancer risk at ages 30, 50, 70 and 90 (and corresponding 95% confidence interval) in gene mutation carriers (any MMR, *MLH1*, *MSH2*) compared with that in the general population, based on a one-gene segregation model

Mutation	Age 30	Age 50	Age 70	Age 90
Any MMR	10.5 (2.5, 46.9)	8.8 (2.3, 40.1)	5.5 (1.8, 25.0)	3.3 (1.5, 13.5)
<i>MLH1</i>	5.5 (0.8, 53.6)	6.5 (1.4, 41.8)	5.1 (1.3, 26.9)	3.3 (1.2, 15.1)
<i>MSH2</i>	13.1 (0.3, 78.8)	9.3 (0.3, 44.4)	5.4 (0.3, 24.5)	3.2 (0.3, 12.7)

Discussion

To our knowledge, only three other studies^{6,18,20} have provided population-based estimate of CRC risk in Lynch Syndrome families but none of them was reported in North America. While our results confirmed a relatively high penetrance associated with MMR gene mutations, our risk estimates seem lower than many clinic-based estimates.^{3,5,21-26}

Previous studies have estimated the risk of developing CRC among MMR gene mutation carriers in Lynch Syndrome families to vary between 30 to 100%^{3-6,21-26}, where the lowest rates are generally reported in women from population-based studies. The excess of risk in males compared to females found in several studies^{3,4,6}, was also confirmed in our analyses. This could suggest that females are protected from CRC; perhaps due to environmental/reproductive factors unique to women or to a sex-linked modifier gene. We also found a gender specific mutation effect with a higher risk in male carriers of *MLH1* mutations (67%) vs. carriers of *MSH2* mutations (55%), while the opposite was observed among women carriers (35% for *MLH1* vs. 53% for *MSH2*), by age 70. Our study also showed that age-specific risk (hazard ratio) associated with *MLH1* was almost constant with age, while the risk associated with *MSH2* decreased with age. The same trends were also suggested in a recent study.¹⁸ If differences in

risks observed for *MLH1* and *MSH2* are confirmed, then the distribution of the types of MMR mutations could have a profound impact on the cancer risk.

Our cumulative risk estimates are close to the lower estimates previously published. They are slightly higher than another recent population-based study¹⁸, but this latter selected exclusively early-onset probands. Several factors might explain the discrepancies with the previous penetrance estimates. First, methods of kindred ascertainment varied between studies, and the distribution of factors which likely affect risk may also vary across studies conducted in different countries. Because MMR mutations are rare in the general population^{6,8}, most penetrance estimates are derived from high-risk Lynch Syndrome families, who usually satisfy the original or revised Amsterdam criteria²⁷. Because, these designs are enriched with mutation carriers, they could be more efficient for estimating penetrance than population-based designs¹⁷ but also more prone to biases.^{28,29} Extrapolation to the general population (i.e. all CRC cases) is not possible unless appropriate ascertainment correction is applied to account for the nonrandom sampling. This was performed in this study by the use of the modified segregation-based analysis. Our recent simulation studies¹⁷ confirmed the validity of our ascertainment-corrected approach. Second, it is likely that there exist other genetic and non-genetic contributors to HNPCC, other than a MMR mutation, that could also aggregate within families. Some of our additional analyses suggest the role of a second major gene within these families, but further work is still needed to distinguish its effect from a common environmental factor. Third, data quality was improved in the present investigation compared to previous studies in two ways. Previous studies, unlike the present study, were conducted before techniques were available to test for MMR gene mutations so could not determine accurately the status of the MMR gene.^{22,23} In addition, missing genotype is a common problem in family studies and the classical analysis approaches for time-to-onset

data such as Kaplan-Meier estimation or Cox regression model, in their original formulations, cannot solve this problem. In this study, the use of a modified segregation-based approach allows inferences on the missing genotypes by using the Mendelian transmission probabilities and genealogical relationships. As a consequence, the segregation-based analysis was able to use the information on 32 probands and 352 kin who were not included in the KM analyses, resulting in more precise and potentially less biased penetrance estimates.

In summary, many sources of bias have been reduced in this study, in part through the choice of study subjects and use of the modified segregation-based analyses. However, several limitations may still exist. First, the sample size is relatively small for a risk estimation study and the confidence intervals are large, especially for estimating mutation-specific and gender-specific penetrances. This problem was only partly overcome by using the modified segregation-based approach. Efficiency was also improved by selecting preferentially probands carrying the mutation and thus who are more informative than random probands.¹⁷ Second, inaccuracy of cancer history might introduce error. While we attempted to confirm with pathology reports all reported family members with a CRC diagnosis, this was not possible for all cases. However, recent research conducted in Ontario found that proband's reports of relatives' cancer diagnoses are fairly accurate; 93% of proband-reported CRC among first-degree relatives were verified by either hospital records, cancer registry or death certificates, though reporting was less accurate for second-degree relatives with only 72% of reported CRCs verified.³⁰ Third, some studies classified tumours as MSI-high if > 30% of the markers show altered band patterns. Because we identified MSI-high tumours as those with altered band patterns in >40% of markers, we may have missed some carriers. However, because this classification was not associated with the proband's family history, it is unlikely that this biased our penetrance estimates. Finally, we

assumed that the probands selected are representative of the entire population of CRC families in Ontario. Because of our relatively small sample size, this hypothesis is difficult to assess and deviation from this hypothesis could lead to a selection bias. Although unlikely, it is possible that the estimates of penetrance could be biased upwards if families carrying the gene participated differentially according to the prevalence of cancer in the family.

In conclusion, this study provides a unique population-based study of CRC risks among *MSH2/MLH1* mutation carriers in a Canadian population. The different patterns of risks found for *MLH1* and *MSH2* gene mutations could help defining better clinical management and prevention of CRC among members of Lynch Syndrome families.

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Appendix

The form of the hazard function, $h(t|G)$, is given by

$$(1) \quad h(t|G) = \frac{\lambda \rho \{\lambda(t-20)\}^{\rho-1} e^{\beta_s x_s + \beta_1 x_1}}{1 + \{\lambda(t-20)\}^\rho e^{\beta_s x_s + \beta_1 x_1}},$$

where x_1 indicates the mutation status for the MMR gene (either *MLH1*, *MSH2*, or any) and x_s distinguishes between males ($x_s = 1$) and females ($x_s = 2$).

We denote the disease status at age of examination by δ , where $\delta = 1$ if the disease occurred before age at examination a i.e., $T < a$, 0, otherwise, and the phenotype by $D = (T, \delta)$. Then, the penetrance is defined by the cumulative risk of a disease up to age t associated with genotype G , i.e.

$$(2) \quad F(t|G) = \frac{\{\lambda(t-20)\}^\rho e^{\beta_s x_s + \beta_1 x_1}}{1 + \{\lambda(t-20)\}^\rho e^{\beta_s x_s + \beta_1 x_1}}.$$

The survival function for the two gene model has the following form:

Figure 1. Age-specific cumulative risks of colorectal cancer (CRC) among carriers and non-carriers of MMR (any MMR, *MLH1*, *MSH2*) mutation specified by gender, based on the segregation analysis.

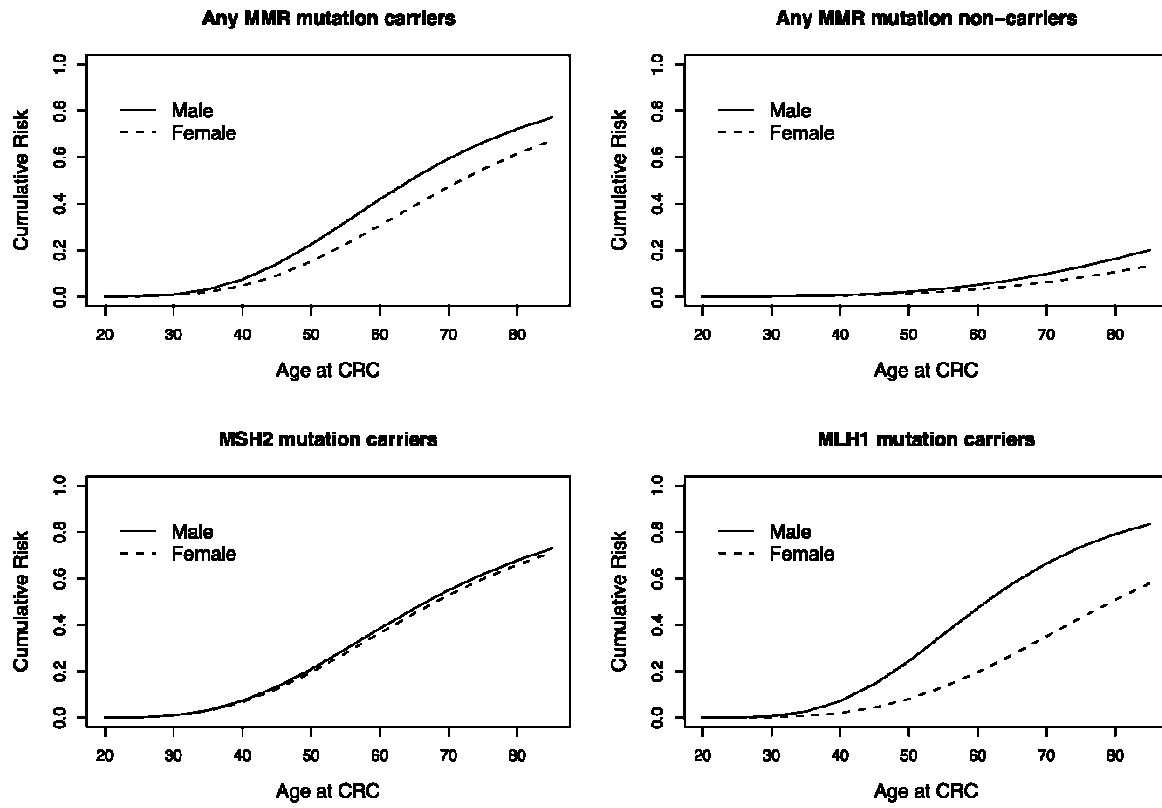
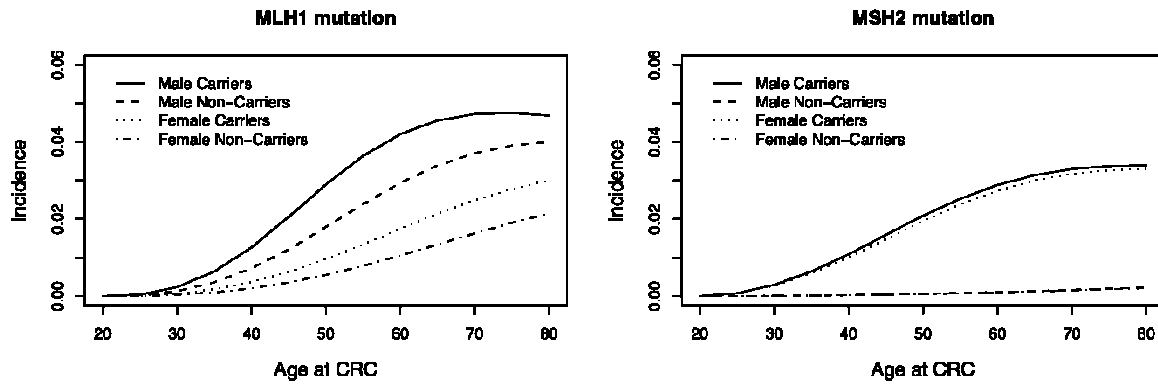


Figure 2. Gender-specific and/or mutation-specific hazard rate estimates of developing colorectal cancer (CRC) in *MLH1* gene (left) and *MSH2* gene (right) mutations, based on the segregation analysis.



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