

# Cancer Risks for *MLH1* and *MSH2* Mutation Carriers

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**ABSTRACT:** We studied 17,576 members of 166 *MLH1* and 224 *MSH2* mutation-carrying families from the Colon Cancer Family Registry. Average cumulative risks of colorectal cancer (CRC), endometrial cancer (EC), and other cancers for carriers were estimated using modified segregation analysis conditioned on ascertainment criteria. Heterogeneity in risks was investigated using a polygenic risk modifier. Average CRC cumulative risks at the age of 70 years (95% confidence intervals) for *MLH1* and *MSH2* mutation carriers, respectively, were estimated to be 34% (25%–50%) and 47% (36%–60%) for male carriers and 36% (25%–51%) and 37% (27%–50%) for female carriers. Corresponding EC risks were 18% (9.1%–34%) and

30% (18%–45%). A high level of CRC risk heterogeneity was observed ( $P < 0.001$ ), with cumulative risks at the age of 70 years estimated to follow U-shaped distributions. For example, 17% of male *MSH2* mutation carriers have estimated lifetime risks of 0%–10% and 18% have risks of 90%–100%. Therefore, average risks are similar for the two genes but there is so much individual variation about the average that large proportions of carriers have either very low or very high lifetime cancer risks. Our estimates of CRC and EC cumulative risks for *MLH1* and *MSH2* mutation carriers are the most precise currently available. Hum Mutat 34:490–497, 2013. © 2012 Wiley Periodicals, Inc.

**KEY WORDS:** Lynch syndrome; *MLH1*; *MSH2*; risk; penetrance; cancer; colorectal cancer; endometrial cancer; segregation analysis; Colon CFR

Additional Supporting Information may be found in the online version of this article.

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## Introduction

DNA mismatch repair (MMR) genes encode proteins that detect and repair DNA mismatches that can occur during cell replication [Aaltonen et al., 1994]. A person with a germline mutation in any of the MMR genes *MSH2*, *MLH1*, *MSH6*, or *PMS2* (MIM #120436, #609309, #600678, and #600259) has an increased risk of colorectal carcinoma (CRC), endometrial carcinoma (EC), and cancers of the stomach, ovary, ureter, renal pelvis, brain, small bowel, hepatobiliary tract [Umar et al., 2004], and possibly the breast

[Jensen et al., 2010; Lynch et al., 1988; Walsh et al., 2010; Win et al., 2012], prostate [Grindedal et al., 2009; Soravia et al., 2003], and pancreas [Kastrinos et al., 2009]. Carriers of deleterious MMR gene mutations who develop cancers with MMR deficiency are said to have Lynch syndrome (MIM #120435), formerly known as hereditary nonpolyposis colorectal cancer [Jass, 2006]. Lynch syndrome is the most common genetic CRC syndrome [Southey et al., 2005; Wagner et al., 2003; Wijnen et al., 1997] and 70%–85% of Lynch syndrome is caused by mutations in *MLH1* or *MSH2* [Barnetson et al., 2006; Hampel et al., 2005; Southey et al., 2005].

Although it is known that carriers of germline mutations in *MLH1* and *MSH2* have high lifetime cumulative risks (penetrance) of CRC, EC, and other cancers, estimates of these risks are imprecise (see Supp. Tables S1 and S2). It is also not known whether the estimates derived from carriers identified from familial cancer clinics are applicable to carriers sampled without regard to their family histories of cancer. If heritable modifiers of risk exist then carriers with strong family histories of cancer would be expected to carry, on average, more familial disease-causing factors, and hence to have greater cancer risks. However, the amount of variability in cancer risks between carriers has not been previously estimated.

Precise estimates of the average age-specific cumulative risks for *MLH1* and *MSH2* mutation carriers are needed for sound genetic counseling of carriers and their relatives, for choosing optimal surveillance strategies for known carriers and for the efficient identification of carriers based on their family histories of cancer using risk prediction models. The extent of variability in these risks is also of biological, epidemiological, and clinical interest. We therefore estimated cancer risks for *MLH1* and *MSH2* mutation carriers as well as the variability in these risks using one of the largest series of MMR gene mutation-carrying families in the world.

## Materials and Methods

### Subjects

Subjects were from all families recruited between 1997 and 2010 by the Colon Cancer Family Registry (Colon CFR; see [Newcomb et al., 2007] for a detailed description) in which a deleterious mutation in *MLH1* and *MSH2* has been identified. All participants who donated blood samples or completed questionnaires gave written, informed, consent for their data and biospecimens to be used in approved Colon CFR projects. The present study has been approved by the Colon CFR Steering Committee (project number C-CP-0606–03) and by the institutional human ethics committees of all participating centers.

Families were recruited via probands who were either recently diagnosed CRC cases ascertained through population-complete cancer registries in the USA (Puget Sound, Washington State; the State of Minnesota; Los Angeles, California; Arizona; Colorado; New Hampshire; North Carolina; and Hawaii), Australia (Victoria), and Canada (Ontario) (population-based recruitment) or were persons from multiple-case families referred to family cancer clinics in Australia (Melbourne, Adelaide, Perth, Brisbane, and Sydney), New Zealand (Auckland), and the USA (Mayo Clinic, Rochester, Minnesota and Cleveland) (clinic-based recruitment). Probands were asked for permission to contact their relatives to seek their enrollment in the Colon CFR. For population-based families, first-degree relatives of probands were recruited at all centers and recruitment was extended to more distant relatives at some centers. For clinic-based families, there were prespecified rules, consistent across centers, governing

which family members were to be approached for recruitment. Written informed consent was obtained from all study participants and the study protocol was approved at each Colon CFR site.

Standardized questionnaires were used to collect data on each participant and his or her first- and second-degree relatives, including their sexes, cancer sites and dates of birth, death (if applicable), onset of any cancer, and any prophylactic surgery to the colon, rectum, endometrium, or cervix. Validation was sought for all reported diagnoses of CRC and, at some centers, for all invasive cancers. In this article, hepatobiliary cancer means cancer of the liver, gall bladder, and biliary tract; urinary tract cancer means cancer of the bladder, ureter, renal pelvis, and kidney. Because of a lack of access to pathology reports, we could not differentiate cancers of the renal pelvis from other kidney cancers nor adenocarcinomas of the cervix from squamous cell carcinomas.

For population-based families, subjects were restricted to probands and their first- and second-degree relatives. For clinic-based families, all available family members were included in the analysis. Five probands with *de novo* mutations [Win et al., 2011c] (two in *MLH1* and three in *MSH2*) and their families could not be easily included in the segregation analyses so were excluded from all analyses. Families who were recruited through separate probands but found to contain members in common were combined, giving nine families which each had two probands, and these families were treated as clinic-based in the segregation analyses. All ages were truncated at the age of 80 years and subjects for whom sex was unknown were censored at birth. Affected subjects with missing ages at diagnosis (comprising 14% of CRC diagnoses, 8% of EC diagnoses, and 22% of noncolorectal, nonendometrial [NCNE] Lynch cancer diagnoses) were included in the analysis by marginalizing over the missing ages (see section *Statistical Methods*). Unaffected subjects with missing ages were censored at birth, effectively removing them from the analysis, as their affected statuses were considered to be unreliable.

### Mutation Detection

Standardized protocols were used to obtain and prepare biospecimens and to conduct all laboratory analyses. Sequence variants in *MLH1* and *MSH2* were termed “mutations” if they encoded stop codons, large duplications or deletions, frameshift mutations, or one of the missense mutations previously reported in the scientific literature as being pathogenic. Screening for germline mutations in *MLH1* and *MSH2* (and, at some centers, in *MSH6* and *PMS2*) was performed for all clinic-based probands and for those population-based probands whose colorectal tumors displayed impaired MMR function, as evidenced by either microsatellite instability or the absence of MMR protein expression in immunohistochemical assays. Mutation testing was performed by Sanger sequencing or denaturing high-pressure liquid chromatography, followed by confirmatory DNA sequencing [Newcomb et al., 2007; Southey et al., 2005]. Large duplications and deletions in MMR genes were detected by multiplex ligation-dependent probe amplification according to the manufacturer’s instructions (MRC Holland, Amsterdam, The Netherlands). Each participant who donated a blood sample and was related to a mutation-carrying proband underwent genetic testing for the proband’s mutation.

### Statistical Methods

Mean ages at diagnosis and the corresponding sample standard deviations were calculated for various cancer sites using R version

2.11.1 [R Development Core Team, 2010]. Proband and cases with missing or imputed ages at diagnosis were excluded from this descriptive analysis.

Age-specific hazard ratios (HRs), that is, the age-specific cancer incidence for carriers divided by that for the population, were estimated using modified segregation analysis [Antoniou et al., 2001; Lange, 2002] (as described in detail in Supp. Statistical Methods). This analytical method is not subject to population stratification, can be rigorously adjusted for many methods of ascertainment, and uses data on all study participants, whether genotyped or not, thereby maximizing statistical power. Models were fitted by the method of maximum likelihood using MENDEL [Lange et al., 1988] version 3.2 and were appropriately adjusted for the clinic- and population-based ascertainment of study participants using a combination of retrospective likelihood and ascertainment-corrected joint likelihood [Antoniou et al., 2001; Gong et al., 2010; Kraft and Thomas, 2000]. More specifically, a conditional likelihood was maximized where each pedigree's data was conditioned on the proband's genotype, cancer status, and age of onset (for population-based families) or on the proband's genotype and the affected statuses and ages of onset of all family members at the time the proband was found to be a MMR gene mutation carrier (for clinic-based families). HRs and measures of risk heterogeneity were estimated and the corresponding cumulative risks, risk distributions, and 10-year risks were then derived from these estimates (see Supp. Statistical Methods). Individuals were censored at the earliest of death, last known age alive, prophylactic surgery (polypectomy, bowel surgery, or hysterectomy), or the first diagnosis of cancer (as applicable), so the resulting estimates describe risks of first primary cancers for people who have not undergone prophylactic surgery, regardless of whether or not they are undergoing surveillance. Censoring for surgery was site-specific, for example, individuals were still considered to be at risk of CRC following hysterectomy.

Two different genetic models were used in the modified segregation analyses: a major gene model, in which only genotypes at the relevant MMR gene for each family were modeled, and a mixed model, which incorporated an unmeasured polygene in addition to the major gene [Antoniou et al., 2001; Cannings et al., 1978]. All estimates presented in this article for cumulative risks, 10-year risks, estimates of risk heterogeneity, and HRs for CRC and EC were based on the mixed model. HR estimates for separate NCNE Lynch cancers and non-Lynch syndrome associated cancers were based on the major gene model, as were hypothesis tests for the dependence of cancer risks on sex, gene, country, setting (clinic versus population), mutation category, proband's age at onset, and modeling assumptions. The use of different genetic models for different purposes was necessary because simulation has shown that major gene models are likely to give biased estimates of risk [Gong et al., 2010] but analyses using the mixed model were prohibitively slow to run so could not be used to individually estimate a large number of parameters or to test a large number of hypotheses. Therefore, a major gene model was used for model-building, whereas the final estimates of risk were based on a mixed model.

HRs for CRC, EC, and NCNE Lynch cancers were estimated simultaneously to allow proper adjustment for CRC-based ascertainment schemes when estimating the risks of non-CRC cancers and to increase power (by helping the model identify likely carriers from the placement of Lynch syndrome-associated cancers within each family). For each site, the age at cancer diagnosis was modeled as a random variable whose hazard was the relevant population incidence multiplied by a site-specific HR. Except when testing for differences in risk by country, HRs were assumed to be independent of country of residence, as would be expected if different population

incidences only occur because of variation in the prevalence of risk factors between countries and if these risk factors and MMR gene mutations all act multiplicatively on the HR. The ages at diagnosis of different family members were assumed to be conditionally independent given genotype, however residual correlation of cancer within families was incorporated in most analyses by including an unmeasured polygene (in addition to the major gene) in the definition of genotype (see Supp. Statistical Methods). As in Ref. Quehenberger et al. (2005), the ages at diagnosis for different cancer sites within the same individual were assumed to be conditionally independent, given genotype, until diagnosis of the first cancer. Missing ages at diagnosis were treated using the standard method for missing data in likelihood-based estimation, namely marginalization (integration) over all missing values before maximizing the likelihood function [Little and Rubin, 2002].

## Results

Our analyses were based on 17,576 people from 166 families segregating *MLH1* mutations and 224 families segregating *MSH2* mutations. Forty-eight (29%) of the *MLH1* and 64 (29%) of the *MSH2* mutation-carrying families were population based. Of all families, 253 (65%) carried a protein-truncating mutation, whereas 137 (35%) carried another type of pathogenic mutation. There were 160 (41%) families from Australasia (Australia and New Zealand combined), 104 (27%) from Canada, and 126 (32%) from USA.

Mutation-specific testing of those relatives of the probands who had provided blood samples identified an additional 768 carriers and 1128 noncarriers. The number and average ages at diagnosis of the first primary cancers of all cases, other than those of probands and cases with unknown ages at diagnosis, are listed in Table 1. There were almost twice as many extraintestinal Lynch cancers (i.e., Lynch cancers other than those of the colon, rectum, or stomach) in *MSH2* mutation-carrying families than in *MLH1* mutation-carrying families.

The estimated average HRs for various cancers are given in Table 2.

## Gene and Sex

The estimated age-dependent CRC HRs for *MSH2* mutation carriers were similar to those for *MLH1*, with any differences consistent with chance ( $P = 0.5$  for females and  $P = 0.9$  for males). Similarly, no statistically significant difference in EC risks between *MLH1* and *MSH2* mutation carriers was observed ( $P = 0.3$ ), although the point estimates for *MSH2* were higher (see Table 2). CRC HRs for male *MLH1* mutation carriers were higher than those for female carriers ( $P = 0.01$ ) but no such difference by sex was detected for *MSH2* mutation carriers ( $P = 0.8$ ).

## Mutation Type and Proband's Age at Diagnosis

Female *MLH1* mutation carriers with protein-truncating mutations were estimated to have CRC incidences 0.40 (95% confidence interval [CI] 0.16–0.98;  $P = 0.03$ ) times those for female *MLH1* mutation carriers with other types of mutations. No other differences in risk between families according to the type of mutation were observed for the other five combinations of sex, gene, and site (CRC, EC), raising the possibility that this marginally significant result is spurious.

For female *MLH1* mutation carriers, the estimated CRC incidence varied between families according to the age of diagnosis of the family's proband (a proxy for heritable risk modifiers), being lower

**Table 1. The Number and Average Ages at Diagnosis of First Cancers by Sex, Site, and Gene of the Family's Mutation**

Site	Sex	<i>MLH1</i>		<i>MSH2</i>	
		Number	Mean age of diagnosis (standard deviation)	Number	Mean age of diagnosis (standard deviation)
Colon or rectum	F	249	49.6 (15.9)	269	47.5 (15.1)
	M	345	44.6 (13.3)	385	47.3 (13.0)
Endometrium	F	90	48.1 (11.1)	131	46.8 (8.9)
Brain	F	3	18.7 (10.0)	15	48.5 (22.6)
	M	3	8.3 (10.2)	25	36.0 (23.1)
Hepatobiliary system <sup>a</sup>	F	9	59.3 (14.9)	7	54.6 (19.7)
	M	7	60.1 (14.8)	13	53.2 (12.3)
Ovary	F	26	48.2 (13.1)	46	48.6 (12.6)
Small intestine	F	2	60.0 (9.9)	2	66.5 (10.6)
	M	6	50.2 (15.4)	5	50.6 (11.5)
Stomach	F	18	57.1 (20.8)	25	53.9 (17.9)
	M	22	47.5 (14.0)	28	53.0 (12.6)
Urinary tract <sup>b</sup>	F	8	56.8 (10.8)	17	58.4 (12.7)
	M	10	45.5 (18.2)	21	56.3 (11.4)
Breast	F	53	55.4 (13.8)	102	55.4 (14.5)
	M	0	-	1	78
Cervix	F	20	38.7 (13.7)	42	40.9 (12.0)
Lung	F	17	63.9 (10.5)	16	62.3 (12.1)
	M	18	60.4 (12.6)	46	62.2 (11.4)
Pancreas	F	4	49.2 (13.8)	12	59.4 (19.1)
	M	8	61.4 (16.5)	22	56.9 (13.7)
Prostate	M	45	69.6 (10.4)	60	68.6 (11.5)
All sites	M&F	963	49.3 (15.6)	1290	50.2 (15.0)

<sup>a</sup>Hepatobiliary system means the liver, gall bladder, and biliary tract.

<sup>b</sup>Urinary tract means the bladder, ureter, renal pelvis, and (due to the limitations of the data) kidney other than renal pelvis.

This table includes all cases other than probands and cases with unknown ages at diagnosis.

Abbreviations: F, female; M, male; hyphen (-), not estimable.

by a factor of 0.94 (95% CI 0.90–0.98;  $P < 0.001$ ) for every year of increase in the proband's age at diagnosis. No differences in risk by proband's age were observed for any other combinations of sex, gene, and site.

## Setting and Country

No differences were observed between clinic- and population-based families for either CRC or EC risks except that female *MLH1* mutation carriers were estimated to have incidences of CRC, which are 2.2 times (95% CI: 1.2–4.0;  $P = 0.03$ ) higher in the clinic-based setting.

No differences between countries were observed for CRC or EC risks except for the CRC risks of female carriers. Female carriers from Canada and USA, respectively, were estimated to have CRC incidences equal to those of carriers from Australasia multiplied by factors (95% CI) of 0.44 (0.22–0.87) and 0.31 (0.14–0.71) for *MLH1* ( $P = 0.01$ ) and 2.2 (0.81–5.8) and 0.85 (0.28–2.6) for *MSH2* ( $P = 0.02$ ).

## Cancer Sites not Currently Included in the Lynch Syndrome Tumor Spectrum

There was no evidence that male carriers had higher than population levels of cancer risks at any of the non-Lynch syndrome-associated cancer sites considered (listed in Table 2) except that male *MSH2* mutation carriers had incidences of pancreatic cancer estimated to be 18.1 times (95% CI: 8.4–39.0;  $P < 0.001$ ) the population rates. For female *MLH1* and *MSH2* mutation carriers,

**Table 2. Estimated Average Hazard Ratios for First Cancers at Various Sites for *MLH1* and *MSH2* Mutation Carriers<sup>a</sup>**

	Hazard ratios, with 95% CIs in parentheses, for carriers of mutations in	
	<i>MLH1</i>	<i>MSH2</i>
Male carriers		
CRC at the age of 40 years and younger	183 (102–328)	139 (82.3–236)
CRC at the age of 50 years	84.3 (30.9–230)	134 (66.1–274)
CRC at the age of 60 years and older	7.8 (1.5–41.5)	34.6 (11.7–103)
Brain	-	15.7 (7.5–33.1)
Hepatobiliary system <sup>b</sup>	3.1 (0.14–67.0)	9.9 (3.7–26.2)
Small intestine	42.5 (10.5–172)	12.3 (1.3–114)
Stomach	36.0 (18.1–71.8)	3.6 (0.64–20.5)
Urinary tract <sup>c</sup>	1.1 (0.13–9.9)	7.9 (3.1–19.8)
Lung	0.25 (0.01–4.4)	0.39 (0.06–2.7)
Prostate	0.79 (0.25–2.5)	1.0 (0.47–2.3)
Pancreas	2.5 (0.07–85.2)	18.1 (8.4–39.0)
Female carriers		
CRC at the age of 40 years and younger	45.4 (19.4–106)	120 (64.3–223)
CRC at the age of 50 years	74.1 (29.3–187)	152 (67.5–344)
CRC at the age of 60 years and older	37.0 (12.7–108)	18.3 (5.6–59.6)
EC at the age of 40 years and younger	43.6 (14.9–128)	66.6 (30.2–146)
EC at the age of 50 years	11.3 (2.4–52.6)	70.0 (21.7–226)
EC at the age of 60 years and older	11.3 (2.4–52.6) <sup>d</sup>	11.8 (2.0–69.5)
Brain	-	11.1 (3.6–34.8)
Hepatobiliary system <sup>b</sup>	-	-
Ovary	14.5 (6.6–31.8)	10.5 (4.5–24.5)
Small intestine	4.1 (0.18–92.3)	-
Stomach	27.7 (9.4–81.4)	34.9 (14.8–82.4)
Urinary tract <sup>c</sup>	5.9 (1.3–27.2)	21.1 (8.5–52.1)
Breast	1.1 (0.47–2.6)	1.5 (0.71–3.3)
Cervix	5.5 (1.7–17.7)	9.7 (3.8–24.8)
Lung	-	-
Pancreas	5.9 (1.0–34.0)	4.7 (1.1–19.6)

<sup>a</sup>For example, the incidence of CRC for male *MLH1* mutation carriers was estimated to be 183 times the population incidence at ages 40 years and younger.

<sup>b</sup>Hepatobiliary system means the liver, gall bladder, and biliary tract

<sup>c</sup>Urinary tract means the bladder, ureter, renal pelvis, and (due to the limitations of the data) kidney other than renal pelvis.

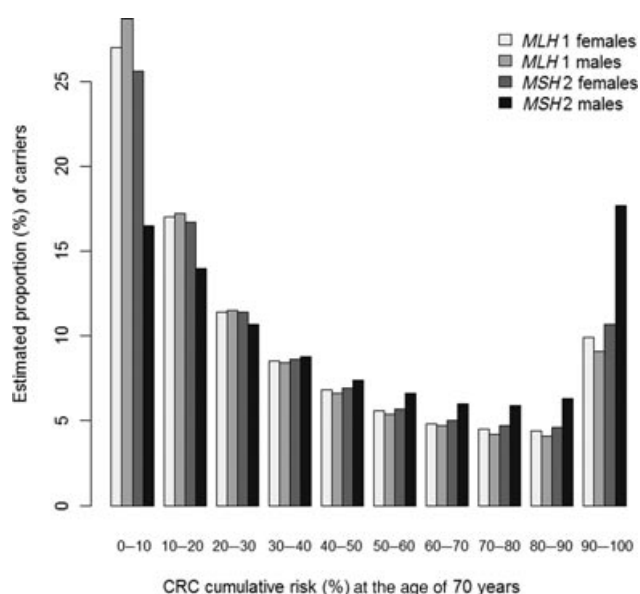
<sup>d</sup>This HR was not separately estimable so it was constrained to equal the corresponding HR at the age of 50 years.

Abbreviations: CRC, colorectal cancer; EC, endometrial cancer; CI, confidence interval; hyphen (-), not estimable due to the low numbers of cancers.

cervical cancer incidences were estimated to be 5.5 (95% CI: 1.7–17.7;  $P = 0.01$ ) and 9.7 (95% CI: 3.8–24.8;  $P < 0.001$ ) times the population incidences, respectively. No increased risks were observed for *MLH1* and *MSH2* mutation carriers at any other sites, notably not for breast cancer ( $P = 0.8$  and  $0.3$ , respectively) or prostate cancer ( $P = 0.7$  and  $0.9$ , respectively). The CIs for the HR estimates give likely upper bounds for the true breast and prostate HRs (respectively) of 2.6 and 2.5 for *MLH1*, and 3.3 and 2.3 for *MSH2*.

## Risk Heterogeneity

There was strong evidence for large heterogeneity in the CRC and EC risks about the average risks ( $P < 0.001$ ), with the standard deviation of the polygenic component of risk estimated to be 1.6 (95% CI: 1.1–2.1) for CRC and 1.2 (95% CI: 0.1–2.2) for EC. Figure 1 illustrates the predicted U-shaped distribution of lifetime risks of CRC. The standard deviation of the polygenic component of CRC risk was also estimated separately for population- and clinic-based families and found to be 1.5 (95% CI: 1.2–1.8) and 2.0 (95% CI: 1.4–2.7) respectively, consistent with no difference between the two settings ( $P = 0.1$ ).



**Figure 1.** The estimated distribution of colorectal cancer (CRC) cumulative risk at the age of 70 years for each combination of gene and sex. For example, from the leftmost black bar, 17% of male *MSH2* mutation carriers are estimated to have a less than 10% chance of developing CRC by age 70 years.

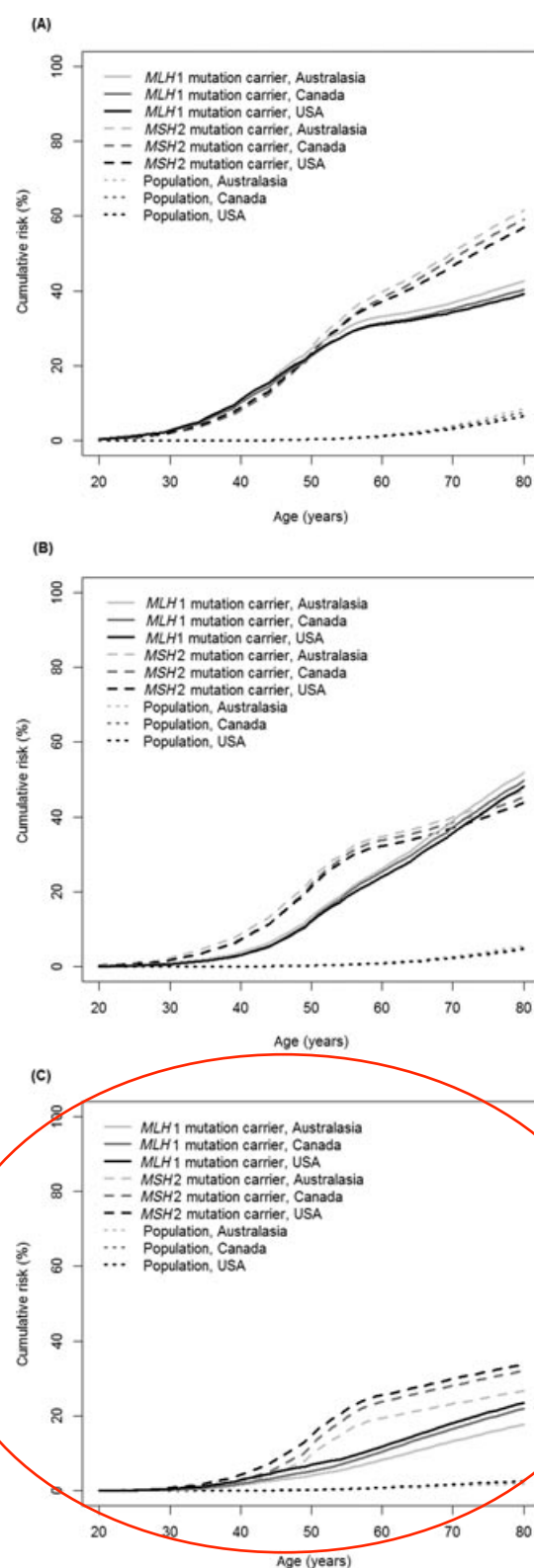
### Cumulative Risks and 10-Year Risks

The estimated average age-specific cumulative risks of CRC, EC, and other cancers for carriers from USA are given in Table 3 and illustrated in Figure 2. It is estimated that 34% (95% CI: 25–50) and 47% (95% CI: 36–60) of male *MLH1* and *MSH2* mutation carriers (respectively) will be diagnosed with CRC by the age of 70 years. Of all female *MLH1* and *MSH2* mutation carriers, an estimated 36% (95% CI: 25–51) and 37% (95% CI: 27–50), respectively, will be diagnosed with CRC by the age of 70 years, whereas 18% (95% CI: 9.1–34) and 30% (95% CI: 18–45), respectively, will develop EC. Ten-year risks of cancer for unaffected carriers from USA at various ages are given in Table 4. Corresponding results for carriers from Australasia and Canada are given in Supp. Tables S3–S6 and Figure 2.

### Discussion

Our estimates of the average risks of CRC at the age of 70 years are broadly consistent with the estimates of previous studies that have correctly adjusted for ascertainment (see Supp. Table S1). We observed no differences between the CRC HRs for *MLH1* and *MSH2* mutation carriers, though male carriers had higher CRC HRs than female carriers for *MLH1* but not *MSH2* (see Table 2). Evidence that CRC and EC risks vary with setting (clinic versus population) was weak or absent, consistent with no difference, perhaps because family cancer clinics tend to genetically test families with histories of cancer which are less severe now than they were in the past. The estimated 10-year risks of CRC for unaffected carriers at various ages were roughly constant from the fifth decade of life onward (see Table 4), suggesting that aggressive surveillance is as important for older mutation carriers as it is for younger ones.

Despite our study participants being drawn from countries with low population incidences of stomach cancer, we found relatively



**Figure 2.** Colorectal cancer cumulative risks for males (A) and females (B) and endometrial cancer cumulative risks for females (C). Cumulative risks are for *MLH1* mutation carriers (unbroken lines), *MSH2* mutation carriers (dashed lines) and the general population (dotted lines) living in Australia and New Zealand (light grey), Canada (dark grey), and USA (black).



**Table 3. Estimated Average Cumulative Risks (%) to Various Ages for *MLH1* and *MSH2* Mutation Carriers Living in the USA<sup>a</sup>**

Sex	Gene	Age (years)	Average cumulative risks (%), with 95% CIs in parentheses, for cancers of the				
			Colon and rectum (combined)	Endometrium	Ovary	Stomach	Urinary tract <sup>b</sup>
Males	<i>MLH1</i>	30	2.8 (1.6–4.7)	-	-	0.2 (0.08–0.3)	0.009 (0.001–0.08)
		40	11 (6.6–17)	-	-	0.7 (0.4–1.5)	0.04 (0.005–0.4)
		50	23 (16–33)	-	-	2.7 (1.3–5.2)	0.2 (0.02–1.5)
		60	31 (22–44)	-	-	8.0 (4.1–15)	0.5 (0.06–4.5)
		70	34 (25–50)	-	-	20 (10–35)	1.2 (0.1–9.8)
		80	39 (28–58)	-	-	37 (21–60)	2.0 (0.2–16)
	<i>MSH2</i>	30	2.2 (1.3–3.6)	-	-	0.02 (0.003–0.09)	0.06 (0.03–0.2)
		40	8.9 (5.6–14)	-	-	0.08 (0.01–0.4)	0.3 (0.1–0.7)
		50	23 (17–32)	-	-	0.3 (0.05–1.5)	1.2 (0.5–3.0)
		60	37 (28–49)	-	-	0.8 (0.1–4.6)	3.6 (1.4–8.8)
		70	47 (36–60)	-	-	2.2 (0.4–12)	7.8 (3.2–19)
		80	57 (44–72)	-	-	4.6 (0.8–23)	13 (5.5–30)
Females	<i>MLH1</i>	30	0.7 (0.3–1.6)	0.5 (0.2–1.4)	0.6 (0.3–1.2)	0.1 (0.04–0.4)	0.04 (0.009–0.2)
		40	3.2 (1.4–6.8)	2.8 (1.0–7.9)	1.2 (0.6–2.7)	0.5 (0.2–1.4)	0.2 (0.04–0.7)
		50	12 (7.1–21)	6.9 (3.5–16)	3.3 (1.5–7.0)	1.3 (0.4–3.8)	0.5 (0.1–2.4)
		60	24 (16–37)	12 (6.3–23)	7.2 (3.4–15)	3.1 (1.1–9.0)	1.4 (0.3–6.1)
		70	36 (25–51)	18 (9.1–34)	13 (6.1–26)	7.5 (2.6–20)	2.9 (0.7–13)
		80	48 (34–65)	23 (11–43)	20 (9.5–38)	15 (5.5–39)	5.0 (1.1–21)
	<i>MSH2</i>	30	1.8 (1.0–3.3)	0.8 (0.3–1.7)	0.4 (0.2–0.9)	0.2 (0.07–0.4)	0.1 (0.06–0.3)
		40	7.3 (4.3–12)	4.2 (2.0–8.6)	0.9 (0.4–2.1)	0.6 (0.3–1.4)	0.6 (0.2–1.4)
		50	21 (15–30)	15 (8.7–24)	2.4 (1.0–5.4)	1.6 (0.7–3.8)	1.8 (0.7–4.5)
		60	32 (23–44)	26 (15–39)	5.3 (2.3–12)	3.9 (1.7–9.1)	4.8 (2.0–11)
		70	37 (27–50)	30 (18–45)	9.5 (4.2–21)	9.3 (4.1–21)	10 (4.2–23)
		80	44 (32–60)	34 (20–52)	15 (6.5–31)	19 (8.5–39)	17 (7.1–36)

<sup>a</sup>See Supp. Tables S3 and S4 for cumulative risks for carriers living in Australia and Canada.

<sup>b</sup>Urinary tract means the bladder, ureter, renal pelvis, and (due to the limitations of the data) kidney other than renal pelvis.

Abbreviations: CI, confidence interval; hyphen (-), not applicable.

**Table 4. Estimated Average 10-year Risks (%) at Various Ages for *MLH1* and *MSH2* Mutation Carriers Living in USA<sup>a,b</sup>**

Sex	Gene	Age (years)	Average 10-year risks (%), with 95% CIs in parentheses, for cancers of the				
			Colon and rectum (combined)	Endometrium	Ovary	Stomach	Urinary tract <sup>c</sup>
Males	<i>MLH1</i>	30	8.4 (5.1–13)	-	-	0.6 (0.3–1.2)	0.03 (0.004–0.3)
		40	14 (9.2–21)	-	-	1.9 (1.0–3.8)	0.1 (0.02–1.2)
		50	10 (5.8–19)	-	-	5.5 (2.7–11)	0.4 (0.04–3.1)
		60	4.7 (1.0–16)	-	-	13 (6.6–24)	0.7 (0.07–5.6)
		70	7.2 (1.8–21)	-	-	22 (11–39)	0.9 (0.1–7.4)
		80	19 (11–30)	-	-	2.4 (0.5–13)	5.8 (2.4–14)
	<i>MSH2</i>	30	6.8 (4.3–11)	-	-	0.06 (0.01–0.3)	0.2 (0.09–0.6)
		40	16 (11–23)	-	-	0.2 (0.04–1.1)	0.9 (0.4–2.2)
		50	18 (13–26)	-	-	0.6 (0.1–3.1)	2.4 (1.0–5.8)
		60	15 (7.7–26)	-	-	1.4 (0.3–7.3)	4.4 (1.8–10)
		70	19 (11–30)	-	-	2.4 (0.5–13)	5.8 (2.4–14)
		80	19 (11–30)	-	-	2.4 (0.5–13)	5.8 (2.4–14)
Females	<i>MLH1</i>	30	2.5 (1.1–5.2)	2.3 (0.8–6.4)	0.7 (0.3–1.5)	0.3 (0.1–1.0)	0.1 (0.03–0.5)
		40	9.2 (5.3–17)	4.1 (2.3–9.1)	2.0 (0.9–4.4)	0.8 (0.3–2.5)	0.4 (0.08–1.5)
		50	14 (8.3–22)	5.3 (1.3–14)	4.1 (1.9–8.8)	1.9 (0.6–5.5)	0.8 (0.2–3.5)
		60	16 (7.7–27)	7.1 (1.9–16)	6.1 (2.8–13)	4.5 (1.5–13)	1.6 (0.4–6.6)
		70	19 (11–30)	6.6 (2.0–14)	7.6 (3.5–16)	8.4 (2.8–23)	2.2 (0.5–8.7)
		80	19 (11–30)	6.6 (2.0–14)	7.6 (3.5–16)	8.4 (2.8–23)	2.2 (0.5–8.7)
	<i>MSH2</i>	30	5.6 (3.2–9.3)	3.5 (1.6–6.9)	0.5 (0.2–1.2)	0.4 (0.2–1.0)	0.4 (0.2–1.1)
		40	15 (10–23)	11 (6.3–18)	1.5 (0.6–3.4)	1.0 (0.4–2.5)	1.3 (0.5–3.1)
		50	14 (8.8–21)	13 (5.9–22)	3.0 (1.3–6.8)	2.4 (1.0–5.5)	3.0 (1.2–7.2)
		60	7.1 (2.7–16)	5.8 (1.4–16)	4.5 (1.9–10)	5.6 (2.4–13)	5.6 (2.3–13)
		70	11 (4.5–21)	5.6 (1.4–14)	5.6 (2.4–13)	10 (4.6–23)	7.4 (3.0–17)
		80	11 (4.5–21)	5.6 (1.4–14)	5.6 (2.4–13)	10 (4.6–23)	7.4 (3.0–17)

<sup>a</sup>For example, male *MSH2* mutation carriers who are unaffected at the age of 70 years are estimated to have a 19% (95% CI: 11–30) chance of developing CRC before the age of 80 years.

<sup>b</sup>See Supp. Tables S5 and S6 for 10-year risks for carriers living in Australia and Canada.

<sup>c</sup>Urinary tract means the bladder, ureter, renal pelvis, and (due to the limitations of the data) kidney other than renal pelvis.

Abbreviations: CI, confidence interval; hyphen (-), not applicable.

high risks of stomach cancer in *MLH1* and, to a lesser extent, *MSH2* mutation carriers. Imprecision in these estimates warrants caution but they highlight potential benefits of gastroscopy screening, as suggested by [Capelle et al., 2010]. Differences in the estimated stomach cancer risks between male *MLH1* and *MSH2* mutation carriers could be due to the differences in the true risks, imprecision in the HR estimates, younger ages at onset for *MLH1* mutation carriers,

a higher proportion of *MLH1* mutation carriers among the stomach cancer cases than *MSH2* mutation carriers or a combination of these.

We assessed risks at cancer sites not currently part of the Lynch syndrome tumor spectrum and found strong evidence for higher incidences of pancreatic cancer, in agreement with a previous study [Kastrinos et al., 2009], but no evidence for higher incidences of

breast or prostate cancer (all  $P > 0.3$ ), although we cannot rule out twofold or threefold increased risks, consistent with [Win et al., 2012] (though this study partly overlaps with the present one). Despite these null results, a number of studies have shown that breast and prostate cancers in carrier families often show signs of MMR deficiency [Grindedal et al., 2009; Jensen et al., 2010; Lynch et al., 1988; Soravia et al., 2003; Walsh et al., 2010], suggesting some increase in risk and a possible role for molecular characteristics of these tumors in predicting carrier status. We also found a higher risk for cervical cancer, although this could be due to the misclassification of adenocarcinomas of the lower uterine segment.

Using a polygenic model of risk heterogeneity, we found very strong evidence that CRC and EC risks are highly heterogeneous, as has been observed anecdotally by a leading clinician [Henry Lynch, personal communication]. Our estimates of the size of this risk heterogeneity imply that a substantial proportion of mutation carriers are at population-level risk, whereas a significant minority is almost certain to develop CRC. The U-shapes of the histograms of Figure 1 are not likely to be caused by the mixture of countries, settings (clinic versus population), or mutation types (protein-truncating versus all other types) of our study participants because direct tests for differences in CRC risks by these characteristics found weak or no effects, whereas the estimated risk heterogeneity was quite large. Similarly, the risk heterogeneity is not likely to be caused by differences in screening practices between families because the effect of this must be weaker than that of polypectomy which roughly halves the risk [Jarvinen et al., 2000]. The estimated standard deviation for the polygenic component of CRC risk was 1.6, which corresponds to a relative risk of 3.6 associated with having an affected first-degree relative with early-onset disease [Pharoah et al., 2002]. The cause of this risk heterogeneity is unknown but its size is consistent with the existence of approximately 100 modifier SNPs, each with an HR of 1.05 and a minor allele frequency of 50%, acting independently and multiplicatively to alter the CRC risks for carriers. However, it could also be caused by environmental factors that are correlated within families or by mutation-specific penetrances. To date, only a few genetic variants and environmental risk factors have been found to modify the cancer risks of MMR gene mutation carriers [Botma et al., 2010; Campbell et al., 2007; Diergaarde et al., 2007; Felix et al., 2006; Frazier et al., 2001; Kruger et al., 2007; Pande et al., 2008; Pande et al., 2010; Reeves et al., 2008; Talseth-Palmer et al., 2011; Wijnen et al., 2009; Win et al., 2011a; Win et al., 2011b] and these reports have not been replicated by large studies. Large genome-wide association studies of mutation carriers, similar to those carried out for *BRCA1* and *BRCA2* by the CIMBA consortium [Couch and Wang, 2009], are needed.

The largest study of cancer risks for *MLH1* and *MSH2* mutation carriers to date [Bonadona et al., 2011] provided estimates that either agree with our results or are consistent with them. However, our estimates are far more precise, with CIs half as wide, probably because the families of Bonadona et al. were all clinic based whereas our study also included population-based families (which are more informative in penetrance analyses because they require a less stringent adjustment for ascertainment). We also note that the study of Bonadona et al. did not allow for any risk heterogeneity so their estimates are probably downwardly biased [Gong et al., 2010]. For the same reason, the cumulative risks of [Quehenberger et al., 2005] are also likely to be underestimates.

Our study has several notable strengths. It is one of the largest studies so far to estimate the penetrance of *MLH1* and *MSH2* mutations and it included population-based families. Standardized questionnaires and protocols were used by the six different study sites

comprising the Colon CFR, ensuring a high degree of homogeneity across sites. Systematic attempts were made to verify all reports of CRC and (at some Colon CFR sites) all cancers, using pathology reports, medical records, corroboration by relatives, cancer registry reports and/or death certificates (where available). Lastly, sophisticated statistical techniques were used which properly adjusted for ascertainment, accounted for residual familial aggregation of disease (thereby avoiding bias) and made use of data on all family members, whether genotyped or not (thereby maximizing statistical power). The main weaknesses of our study are its incomplete validation of cancers and that we could not adequately differentiate between cancers within the same organ. Another weakness, shared by all Lynch syndrome penetrance studies, was the need to either assume noninformative censoring at polypectomy (the approach of our study and most others) or to make somewhat speculative assumptions about CRC risks after polypectomy.

We have obtained unbiased estimates of CRC and EC penetrance for *MLH1* and *MSH2* mutation carriers that are more precise than any currently available. These estimates will be useful for genetic counseling, designing optimal surveillance strategies for carriers and as the key ingredient for risk prediction models, which identify likely carriers from their cancer family histories. We have also shown that penetrance varies greatly between carriers, perhaps because of genetic or environmental risk factors, with some mutation carriers at population levels of risk and others almost certain to develop CRC.

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