Endometrial Cancer Risk Is Associated with Variants of the Mismatch Repair Genes *MLH1* and *MSH2*

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Abstract

Women with germ-line mutations in the mismatch repair genes (responsible for hereditary nonpolyposis colorectal cancer) face an increased risk of colonic and endometrial cancer. However, these germ-line mutations are rare and are responsible for fewer than 1% of endometrial cancers. Therefore, we examined whether or not common variants of the hereditary nonpolyposis colorectal cancer–associated genes might also be associated with an increased risk of endometrial cancer. Three single-nucleotide polymorphisms were selected in the *MLH1* and *MSH2* mismatch repair genes. All the various 672 women with endometrial cancer and 880 controls were genotyped. Each of these three single-nucleotide polymorphisms was associated with an increased risk of endometrial cancer. Carriers of the *MLH1 nt-93 A* allele were at a 1.5-fold increased risk of developing endometrial cancer compared with controls [95% confidence interval (95% CI), 1.2-2.0; $P = 0.001$]. The risk was higher for homozygote carriers [odds ratio (OR), 1.9; 95% CI, 1.2-3.2; $P = 0.009$]. For carriers of the *MSH2 rs2303428 C* allele, the OR was 1.4 (95% CI, 1.0-1.9; $P = 0.05$), and for carriers of the *MSH2 rs2059520 G* allele, the OR was 1.3 (95% CI, 1.0-1.7; $P = 0.03$). More than 9% of endometrial cancer cases carried a variant allele in both *MLH1* and *MSH2*. For these women, the risk of endometrial cancer was particularly high (OR, 2.1; 95% CI, 1.2-3.6; $P = 0.005$). For patients younger than 50 years at diagnosis who carried both variants, the risk was even higher (OR, 3.4; 95% CI, 1.7-6.6; $P = 0.0005$). In summary, two common variant alleles of the *MLH1* and *MSH2* genes make a substantial contribution to endometrial cancer incidence in Ontario. (Cancer Epidemiol Biomarkers Prev 2006;15(9):1636–40)

Introduction

Endometrial cancer is the most common gynecologic cancer in Canada, with an estimated 3,900 new cases annually. The lifetime risk of endometrial cancer is 2.3% (Canadian Cancer Statistics 2005, Canadian Cancer Society/National Cancer Institute of Canada). Population-based studies have shown that a history of endometrial cancer in a first-degree female relative is associated with an increased risk of endometrial cancer of nearly 3-fold (1). Although the genetic basis of this disease is not well understood, to some extent, familial clustering may be explained by the fact that endometrial cancer is the most common extracolonic tumor in the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome (2). The HNPCC syndrome is an autosomal dominant inherited predisposition to colorectal, endometrial, ovarian, small intestinal, and renal cancers, and is due to germ-line mutations in the mismatch repair genes (2). The lifetime risk estimates for endometrial cancer in female carriers of mutations in HNPCC associated genes range from 40% to 60% (2). Of the five mismatch repair genes identified to date, mutations in two (*MLH1* gene on chromosome 3 and *MSH2* gene on chromosome 2) account for 90% of known HNPCC families. However, mutations in these two highly penetrant genes account for only 0.5% of endometrial cancers (2). The remaining familial risk could be due to high-penetrance mutations in as yet unidentified genes or to common low-penetrance alleles of these same genes. Recently, we found that three single-nucleotide polymorphisms (SNP) in the mismatch repair genes *MLH1* (*nt-93*) and *MSH2* (*rs2303428* and *rs2059520*) were associated with a significantly increased risk for ovarian cancer.[7] Based on these findings, we conducted an association study to assess the relationship between these three common polymorphic variants and the risk of endometrial cancer.

Materials and Methods

Study Population. We studied a hospital-based series of patients diagnosed with endometrial carcinoma between 1963 and 2002. Patients with a diagnosis of invasive endometrial cancer were approached when they attended an appointment (initial or follow-up) in the gynecology oncology clinic of the University Health Network. They were invited to participate in a study looking at genetic and hormonal factors in endometrial cancer. More than 80% of those who were invited agreed to participate and provided a blood sample for DNA studies and completed a risk factor questionnaire. Information about family history and ethnic group was also collected. Because we were particularly interested in women with early-onset endometrial cancer (<50 years at diagnosis) or with endometrial cancer and a history of colon or breast cancer, these study groups were enriched by reviewing the patient data files of the University Health Network and the Ontario Cancer Registry. Women with any of these conditions were invited to participate in the study through a mailed invitation or by telephone. The patients with endometrial and colon cancers were subjects of earlier studies (3). There were 672 patients with endometrial cancer who provided a blood sample and

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clinical information. Of these, 155 had cancer diagnosed before age 50 years, 39 had a history of colon cancer, and 139 of breast cancer. Eight hundred-eighty controls were selected from a database of women who attended a screening clinic for well women at the Women's College Hospital, Toronto, between 1996 and 2001. All controls provided a blood sample. Information on ethnic group, but not family history, was available for the controls. Among the 880 controls, 28 had a prior hysterectomy (3.2%).

Identification of SNPs from Mismatch Repair Pathway. DNA was extracted from lymphocytes of cases and controls. SNP genotyping was done using the MassARRAY system (Sequenom, Inc., San Diego, CA). Assays were designed using Assay Design 2.0 (Sequenom). SNPs were divided into two assay groups (Table 1), consisting of a 4-plexed assay (*MLH1 nt-93* and *rs2303428*) and a 3-plexed assay (*rs2059520*). Reaction conditions were the same in both assays except for different termination nucleotide mix (3-plex and 4-plex termination mix ddACT and ddACG, respectively). Following PCR amplification, shrimp alkaline phosphatase treatment and extension reaction products were analyzed by Chip-based matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry. Extension primers were designed to extend beyond the SNP site by one or two bases. The choice of the reverse or forward extension primer was based on GC content, primer dimerization, hairpin structures, and the presence of any additional SNP site.

The extension reaction mixture contained the following nucleotides: in the 4-plexed assay, dideoxy ACG and deoxy T, and in the 3-plexed assay, dideoxy ACT and deoxy G. Primer extension and PCR were done according to the standard protocol of the manufacturer (homogeneous MassEXTEND, Sequenom). PCR reaction volume was 5 μL, including deoxynucleotide triphosphates, primers, and HotStar Taq Polymerase (Qiagen, Hilden, Germany). The total amount of genomic DNA was 4.0 ng per reaction. After denaturation at 95°C for 15 minutes, samples were subjected to 45 cycles of denaturation at 95°C (20 seconds), annealing at 56°C (30 seconds), and elongation at 72°C (60 seconds) with the final cycle at 75°C for 5 minutes.

Following PCR, the residual unincorporated deoxynucleotide triphosphates were removed by incubating the samples with shrimp alkaline phosphatase at 37°C (20 minutes). Allele-specific primer extension reaction was conducted by adding to each sample appropriate deoxy and dideoxy nucleotide (4.5 nmol), extension primer (10 nmol), thermosequence (0.64 units) with the final volume of 9 μL per reaction. Allele-specific extension products were obtained under the following thermocycling conditions: 94°C for 30 seconds, followed by 20 cycles of 94°C for 5 seconds, 52°C for 5 seconds, and 80°C for 5 seconds. After desalting of the samples with SpectroCLEAN (Sequenom), analysis was done in fully automated mode with the matrix-assisted laser desorption/ionization-time-of-flight MassARRAY system (Bruker-Sequenom, San Diego, CA). Genotype calling was completed in the automated mode by SpectroCALLER (Sequenom). Overall, genotype calling was successful for 86% of attempts. The individual success rates for the three SNPs ranged from 83% to 91%.

Microsatellite Instability Analysis. Normal and tumor DNA samples from 62 cases were examined using the National Cancer Institute/Bethesda panel of microsatellite markers (*BAT25, BAT26, D2S123, D5S346*, and *D17S250*; ref. 4). Electrophoresis was done for normal and tumor-derived DNA using an ABI 3700 capillary sequence detection system. Peaks were analyzed using Genescan software. Cancers were considered to be microsatellite instability (MSI)–high when differences in the allele size in the tumor DNA as compared with the patient's matched normal DNA control were apparent with at least two markers. They were considered to be MSI-low when only one of five markers exhibited a difference and were considered to be microsatellite-stable when no marker exhibited a change.

Statistical Analyses. Cases and controls were stratified by ethnic group: (*a*) White non-French Canadian (subsequently called "other white"), (*b*) French Canadian, (*c*) Jewish, (*d*) Asian, (*e*) African black/American natives, (*f*) other/mixed, and (*g*) unknown. Mean values between cases and controls were compared by *t* test and frequencies were compared by χ^2 test. Odds ratios (OR) were used to estimate the relative risk of endometrial cancer for genotypes of each SNP and their combinations. The entire control group was used to estimate the allele frequencies for the purpose of generating the ORs for the subgroups, based on age (≤50 or >50 years), body mass index (≤median or >median), and family history. All ORs were estimated by unconditional logistic regression. Statistical analyses were done by SAS 9.1.3 (SAS Institute, Inc., Cary, NC).

Results

A total of 672 endometrial cancer cases and 880 controls were genotyped for the three mismatch repair SNPs. The characteristics of the endometrial cancer patients and controls are shown in Table 2. For each of the three SNPs, we observed a statistically significant higher frequency ($P < 0.05$) of the variant allele in the endometrial cancer group compared with the controls, with ORs ranging from 1.3 to 1.6 (Table 3). The ORs were not altered when the analysis was adjusted for year of birth and ethnicity.

Carriers of one or more *MLH1 nt-93 A* alleles were at an increased risk of developing endometrial cancer compared with controls [OR, 1.5; 95% confidence interval (95% CI), 1.2-2.0; $P = 0.001$]. The risk was higher for the homozygote carriers (OR, 1.9; 95% CI, 1.2-3.2; $P = 0.009$; Table 3). Carriers of the *MSH2 rs2303428 C* allele were also at increased risk for developing endometrial cancer (OR, 1.4; 95% CI, 1.0-1.9; $P = 0.05$). The risk increase was similar for carriers of the *MSH2 rs2059520 G* allele (OR, 1.3; 95% CI, 1.0-1.7; $P = 0.03$; Table 3). The two *MSH2* SNPs were found to be in linkage disequilibrium. On each (control) chromosome where *rs2303428 C* allele was present, the *rs2059520 G* allele was also present, and the *rs2059520* did not add an independent predictive value.

We studied the effect of these three SNPs on the risk of endometrial cancer for the subgroup of patients <50 years of age at diagnosis. For the *MLH1 nt-93 A* allele and for the *MSH2 rs2303428 C* allele, the ORs for endometrial cancer were higher than for women diagnosed at >50 years of age (Table 4).

Table 1. Primers for PCR and MassEXTEND reaction for genotyped SNPs

Polymorphism	Forward primer*	Reverse primer*	Extension primer
MLH1 nt-93	ATCAATAGCTGCCGCTGAAG	AAGTGCCTTCAGCCAATCAC	TGCTCACGTTCTTCCTT
MSH2 rs2303428	CAGTTTAGGACTAACAATCC	ATTTACCTCCCATATTGGGG	CCATATTGGGGCCTACA
MSH2 rs2059520	CTGTGAGCCATGGGGAAAATG	GCCTTTCATCAAGAGAAGCC	TCTTTCTAGGCCACAGTC

*Forward/reverse primer Tag sequence ACGTTGGATG.

Table 2. Characteristics of endometrial cancer patients and controls

Variables	Cases (%) N = 672	Controls (%) N = 880
Mean year of birth (range)	1,933 (1,906-1,965)	1,944 (1,906-1,965)
Mean age at diagnosis (range), y	60.4 (18-36)	—
Mean age at interview (range), y	—	56.7 (34-94)
Ethnicity		
Other white	560 (83.3%)	772 (87.3%)
French Canadian	8 (1.2%)	64 (7.2%)
Jewish	19 (2.8%)	4 (0.5%)
Asian	31 (4.6%)	10 (1.1%)
Black/native	16 (2.4%)	3 (0.3%)
Other/mix	18 (2.7%)	5 (0.6%)
Unknown	20 (3.0%)	26 (2.9%)

Of the cases and controls, 9.7% and 5.4%, respectively, carried both the *MLH1 nt-93 A* allele and the *MSH2 rs2303428 C* allele. These women were at particularly high risk for endometrial cancer (OR, 2.1; 95% CI, 1.2-3.6; P = 0.005; Table 5). The risk was greater for young patients who carried both variants (OR, 3.4; 95% CI, 1.7-6.6; P = 0.0005; Table 5).

We then studied the association after stratifying for body mass index, given that body mass index is a strong risk factor for endometrial cancer. Women were divided into a subgroup of patients of normal weight (body mass index ≤25) and a subgroup of patients who were overweight (body mass index >25). The three variant alleles had similar effects on endometrial cancer risk in both subgroups (data not shown).

We were also interested in whether or not the presence of a variant allele predisposed patients to develop a second malignancy, other than endometrial cancer. Two hundred-twenty patients had a second primary cancer, either before or following the diagnosis of endometrial cancer (139 subjects had breast cancer, 39 had colon cancer, 15 had ovarian cancer, and 36 had cancers of other sites). We did not find the frequencies of the variant alleles to be higher in women with second cancers compared with women who had endometrial cancer alone.

Only seven of the cases of endometrial cancer were from families that met the Amsterdam criteria for HNPCC. However, 65 women had one or more first-degree relatives with colon cancer. The effect of the three genes was similar in women with and without a family history of colon cancer (Table 6).

In classic HNPCC families, tumors among women with germ-line mutations in *MLH1* or *MSH2* usually show MSI (5). This phenomenon is believed to be a marker of the high rate of somatic mutation, which is the basis of carcinogenesis. We were interested to assess whether the presence of one or more variant alleles was associated with MSI in the tumor. We examined a sample of 62 endometrial cancers (and their matching normal DNAs) using a panel of five microsatellite markers (see Materials and Methods). We found that 19 of 62 (31%) cancers showed classic microsatellite instability (MSI-high) and three others exhibited the MSI-low phenotype. The remaining 40 cancers did not show evidence of MSI. The frequency of MSI-high was 2.3-fold greater than expected in tumors from women who carried both *MLH1 nt-93 A* and *MSH2 rs2303428 C* alleles, but because of the small sample size, this difference did not reach statistical significance (P = 0.23).

Discussion

Somatic cellular mutations may be induced by external factors, such as radiation and chemical mutagens, or by intrinsic errors that occur during DNA replication. These mutations may accumulate and lead to carcinogenesis. The DNA repair system provides a mechanism for the removal of these mutations. One component of the system is mismatch repair (6). The principal function of the DNA mismatch repair is to maintain genomic stability by correcting mismatches generated during DNA replication. Malfunction of mismatch repair results in an increased frequency of somatic mutations.

Defects in several of the key genes responsible for the mismatch repair mechanism have been found to be the cause of the HNPCC syndrome (6). The main clinical features of the syndrome are early-onset colon and endometrial cancer and the occurrence of multiple primary tumors. Recent data estimate the lifetime risk for endometrial cancer in mutation-positive women from HNPCC families to be from 40% to 60%, compared with a 2.3% lifetime risk in the general population (2). Germ-line mutations in these genes are rare and, in total, are responsible for 0.5% of all endometrial cancer cases (however, they account for ~5% of cases in women <55 years of age; refs. 1, 2).

Approximately 5% of young patients with endometrial cancer have a family history of the disease. Most familial and sporadic cases of endometrial cancer cannot be attributed to mutations in known genes. There are several alternate hypotheses to explain the familial burden of endometrial cancer not attributable to these mutations. Under the polygenic mechanism, one or more polymorphic alleles, each conferring a small genotypic risk, combine to confer susceptibility to endometrial cancer (7, 8). It is likely that these susceptibility alleles act in combination with hormonal factors as well. Based on this theory, we were interested to analyze

Table 3. Association between endometrial cancer risk and SNP polymorphism in mismatch repair genes

SNP/genotype	Cases (%), N = 672	Controls (%), N = 880	Univariate OR (95% CI), P	Multivariate* OR (95% CI), P
MLH1 nt-93				
GG	377 (57.7)	524 (68.6)	1	1
GA	220 (33.6)	202 (26.4)	1.5 (1.2-1.9), 0.0005	1.4 (1.1-1.9), 0.008
AA	57 (8.7)	38 (5.0)	2.1 (1.3-3.2), 0.0008	1.9 (1.2-3.2), 0.009
GA or AA	277 (42.3)	240 (31.4)	1.6 (1.3-2.0), $<10^{-4}$	1.5 (1.2-2.0), 0.001
MSH2 rs2303428				
TT	518 (77.9)	538 (82.3)	1	1
TC	131 (19.7)	100 (15.3)	1.4 (1.0-1.8), 0.04	1.4 (1.0-1.9), 0.05
CC	16 (2.4)	16 (2.4)	1.0 (0.5-2.1), 0.9	1.2 (0.6-2.7), 0.62
TC or CC	147 (22.1)	116 (17.7)	1.3 (1.0-1.7), 0.05	1.4 (1.0-1.9), 0.05
MSH2 rs2059520				
AA	251 (38.5)	296 (46.2)	1	1
AG	302 (46.3)	259 (40.4)	1.4 (1.1-1.7), 0.008	1.3 (1.0-1.7), 0.05
GG	99 (15.2)	86 (13.4)	1.4 (1.0-1.9), 0.07	1.4 (0.9-2.1), 0.11
AG or GG	401 (61.5)	345 (53.8)	1.4 (1.1-1.7), 0.005	1.3 (1.0-1.7), 0.03

*Adjusted for year of birth and ethnicity.

Table 4. Association between endometrial cancer and SNP polymorphism by age

Age at diagnosis (y)	SNP/genotype	Cases (%)	Controls (%)	Univariate OR (95% CI), *P*	Multivariate* OR (95% CI), *P*
MLH1 nt-93					
≤50	GG	77 (50.7)	524 (68.6)	1	1
	GA	58 (38.2)	202 (26.4)	1.9 (1.3-2.8), 0.0005	1.8 (1.2-2.7), 0.003
	AA	17 (11.1)	38 (5.0)	3.0 (1.6-5.8), 0.0004	2.1 (1.1-4.2), 0.03
	GA or AA	75 (49.3)	240 (31.4)	2.1 (1.5-3.0), $<10^{-4}$	1.9 (1.3-2.8), 0.0009
>50	GG	300 (59.9)	524 (68.6)	1	1
	GA	162 (32.3)	202 (20.6)	1.4 (1.1-1.8), 0.008	1.2 (0.9-1.7), 0.21
	AA	39 (7.8)	38 (5.0)	1.8 (1.1-2.9), 0.01	1.5 (0.8-2.7), 0.21
	GA or AA	201 (40.1)	240 (31.4)	1.5 (1.2-1.8), 0.002	1.3 (0.9-1.7), 0.12
MSH2 rs2303428					
≤50	TT	108 (71.1)	538 (82.3)	1	1
	TC	36 (23.7)	100 (15.3)	1.8 (1.2-2.8), 0.008	1.5 (1.0-2.5), 0.07
	CC	8 (5.2)	16 (2.4)	2.5 (1.0-6.0), 0.04	2.1 (0.8-5.4), 0.13
	TC or CC	41 (28.9)	116 (17.7)	1.9 (1.3-2.8), 0.002	1.6 (1.0-2.5), 0.03
>50	TT	409 (79.9)	538 (82.3)	1	1
	TC	95 (18.6)	100 (15.3)	1.2 (0.9-1.7), 0.16	1.3 (0.8-1.9), 0.26
	CC	8 (1.5)	16 (2.4)	0.7 (0.3-1.5), 0.34	0.9 (0.3-2.4), 0.78
	TC or CC	103 (20.1)	116 (17.7)	1.2 (0.9-1.5), 0.30	1.2 (0.8-1.8), 0.34
MSH2 rs2059520					
≤50	AA	55 (36.4)	296 (46.2)	1	1
	AG	69 (45.7)	259 (40.4)	1.4 (1.0-2.1), 0.07	1.3 (0.9-2.0), 0.18
	GG	27 (17.9)	86 (13.4)	1.7 (1.0-2.8), 0.05	1.5 (0.9-2.7), 0.13
	AG or GG	96 (63.6)	345 (53.8)	1.5 (1.0-2.1), 0.03	1.4 (0.9-2.0), 0.10
>50	AA	196 (39.2)	296 (46.2)	1	1
	AG	232 (46.4)	259 (40.4)	1.3 (1.0-1.7), 0.02	1.3 (1.0-1.9), 0.07
	GG	72 (14.4)	86 (13.4)	1.3 (0.9-1.8), 0.20	1.4 (0.9-2.2), 0.17
	AG or GG	304 (60.8)	345 (53.8)	1.3 (1.0-1.7), 0.02	1.5 (1.0-1.8), 0.05

*Adjusted for year of birth and ethnicity.

if common polymorphisms of the mismatch repair genes influence the risk for endometrial cancer. We have previously analyzed the association of seven relatively common SNPs in three mismatch repair genes (*MLH1, MSH2*, and *MSH6*) and ovarian cancer risk.[7] Women who carried the *MSH2 rs2303428 C* allele were at 1.4-fold increased risk for ovarian cancer and women who carried the *MLH1 nt-93 A* allele were at 1.5-fold risk. For women carrying the variant allele for both SNPs, the relative risk of ovarian cancer was 2.4 (95% CI, 1.4-4.0; $P = 0.0009$).

Based on these results, we asked whether or not these three SNPs might also increase the risk of endometrial cancer. The results of our study showed that each of these three relatively common polymorphisms of the *MLH1* and *MSH2* genes increases the risk of endometrial cancer to a similar degree (ORs between 1.3 and 1.5). Patients carrying both the *MLH1 nt-93 A* allele and the *MSH2 rs2303428 C* allele had a 2-fold risk for endometrial cancer. An even greater elevation in risk was found for patients younger than 50 years. By comparison, this risk increase exceeds the estimated 2-fold increased risk of developing endometrial cancer in women using Tamoxifen for >2 years or in women using unopposed estrogen replacement therapy for >5 years (9, 10).

The *MSH2 rs2303428* SNP is located near the splice acceptor site at the exon-intron border and is associated with an increased risk of sporadic colorectal cancer. It is believed to induce aberrant splicing of mRNA (11). The *MLH1 nt-93* SNP is located within the promoter region of this gene, 93 bases before the adenine residue of the start codon. It is overrepresented in HNPCC-associated cases of colorectal cancer. The function of this SNP is not clearly understood but it has been suggested that it promotes hypermethylation of the promoter sequence, and thereby influences the expression of the *MLH1* gene (12).

In many cases, inherited defects in the mismatch repair genes are revealed by the presence of MSI in the tumor DNA. Thirty-one percent of tumors in our population of endometrial cancer patients showed MSI. This rate is similar to that described by others (13). We also found a trend of a higher incidence of MSI in tumors of women who carry both high-risk variant alleles, but this was not statistically significant. It is possible that the effects of the low-penetrance alleles of these genes are through another mechanism of carcinogenesis than MSI. Alternatively, mismatch repair might be impaired but might not necessarily lead to the MSI phenotype. If so, then MSI may be a marker of genetic instability but may not be necessary for the clinical expression of MMR genes. Although

Table 5. Association between endometrial cancer risk and *MLH1 nt93* and *MSH2 rs2303428* variants in combination

MLH1 nt-93	*MSH2 rs2303428*	Cases (%)	Controls (%)	Univariate OR (95% CI), *P*	Multivariate* OR (95% CI), *P*
For all cases					
GG	TT	293 (45.2)	325 (57.8)	1.0	1.0
GG	TC/CC	79 (12.2)	68 (12.1)	1.3 (0.9-1.8), 0.17	1.5 (1.0-2.2), 0.07
GA/AA	TT	213 (32.9)	139 (24.7)	1.7 (1.3-2.2), $<10^{-4}$	1.7 (1.2-2.3), 0.0006
GA/AA	TC/CC	63 (9.7)	30 (5.4)	2.3 (1.5-3.7), 0.0003	2.1 (1.2-3.6), 0.005
Total		648	562		
For cases diagnosed ≤50 y					
GG	TT	54 (36.2)	325 (57.8)	1.0	1.0
GG	TC/CC	20 (13.4)	68 (12.1)	1.8 (1.0-3.1), 0.05	1.7 (0.9-3.2), 0.08
GA/AA	TT	52 (34.9)	139 (24.7)	2.2 (1.5-3.5), 0.0002	2.2 (1.4-3.5), 0.0007
GA/AA	TC/CC	23 (15.5)	30 (5.4)	4.6 (2.5-8.5), $<10^{-4}$	3.4 (1.7-6.6), 0.0005
Total		149	562		

*Adjusted for year of birth and ethnicity.

Table 6. Association between endometrial cancer and SNP by family history of colon cancer

Age at diagnosis	SNP/genotype	Cases (%)	Controls (%)	Univariate OR (95% CI), *P*	Multivariate* OR (95% CI), *P*
MLH1 nt-93					
No family history of colon cancer	GG	306 (59.4)	524 (68.6)	1	1
	GA	167 (32.4)	202 (26.4)	1.42 (1.10-1.82), 0.006	1.41 (1.05-1.88), 0.02
	AA	42 (8.2)	38 (5.0)	1.89 (1.19-3.00), 0.007	1.96 (1.14-3.35), 0.01
	GA or AA	209 (40.6)	240 (31.4)	1.49 (1.18-1.88), 0.0008	1.49 (1.14-1.96), 0.004
Family history of colon cancer	GG	33 (51.6)	524 (68.6)	1	1
	GA	25 (39.1)	202 (20.6)	1.97 (1.14-3.39), 0.02	1.81 (1.01-3.25), 0.05
	AA	6 (9.6)	38 (5.0)	2.51 (0.99-6.36), 0.05	1.78 (0.62-5.11), 0.29
	GA or AA	31 (48.7)	240 (31.4)	2.05 (1.23-3.43), 0.006	1.80 (1.04-3.14), 0.04
MSH2 rs2303428					
No family history of colon cancer	TT	408 (77.9)	538 (82.3)	1	1
	TC	102 (19.5)	100 (15.3)	1.35 (0.99-1.82), 0.06	1.45 (1.02-2.06), 0.04
	CC	14 (2.7)	16 (2.4)	1.15 (0.56-2.39), 0.70	1.28 (0.56-2.90), 0.56
	TC or CC	116 (22.2)	116 (17.7)	1.32 (0.99-1.76), 0.006	1.43 (1.02-1.99), 0.04
Family history of colon cancer	TT	48 (75.0)	538 (82.3)	1	1
	TC	14 (21.9)	100 (15.3)	1.57 (0.83-2.95), 0.16	1.68 (0.84-3.34), 0.14
	CC	2 (3.1)	16 (2.4)	1.40 (0.31-6.28), 0.66	2.11 (0.44-10.1), 0.35
	TC or CC	17 (25.0)	116 (17.7)	1.55 (0.85-2.82), 0.15	1.73 (0.90-3.31), 0.10
MSH2 rs2059520					
No family history of colon cancer	AA	197 (38.5)	296 (46.2)	1	1
	AG	234 (45.7)	259 (40.4)	1.36 (1.06-1.75), 0.02	1.32 (0.98-1.77), 0.06
	GG	81 (15.8)	86 (13.4)	1.42 (0.99-2.01), 0.05	1.43 (0.95-2.16), 0.08
	AG or GG	315 (61.5)	345 (53.8)	1.37 (1.08-1.74), 0.009	1.35 (1.02-1.77), 0.03
Family history of colon cancer	AA	27 (41.5)	296 (46.2)	1	1
	AG	29 (44.6)	259 (40.4)	1.23 (0.71-2.13), 0.47	1.45 (0.80-2.64), 0.22
	GG	9 (13.9)	86 (13.4)	1.15 (0.52-2.53), 0.73	1.47 (0.63-3.46), 0.37
	AG or GG	38 (58.5)	345 (53.8)	1.21 (0.72-2.03), 0.47	1.46 (0.83-2.57), 0.20

*Adjusted for year of birth and ethnicity.

there were cases identified with MSI and variant alleles, it is possible that tumor DNA from these patients also has an additional deleterious mutation in a mismatch repair gene.

Approximately 20% to 30% of patients with endometrial cancer have defects in mismatch repair (MSI). As a group, these patients exhibit clinical characteristics that distinguish them from patients without MSI. In particular, patients with MSI seem to have a better prognosis than women with endometrial cancer without MSI. Black et al. (5) estimated the hazard ratio to be 0.3 (95% CI, 0.1-0.8) despite the fact that the women with MSI-positive tumors were more likely to present with an advanced-stage tumor and/or myometrial invasion. It will be of interest to see if the presence of the polymorphic variants of the mismatch repair genes *MSH2* and *MLH1* also affects patient survival.

Our study has several limitations. Overall, genotype identification was successful for only 86% of attempts, although our current success rate using this method exceeds 90%. Patients were clinic-based cases of endometrial cancer and therefore may not be representative of all cases diagnosed in Ontario. We included prevalent cases; if there is a relationship between the presence of one of these alleles and survivorship, then the ORs may be biased. The histologic types of the endometrial cancers were not available for our analysis, although we assume that 80% will have endometrioid histology. Cases and controls were not matched on age and the controls were, on average, 9 years younger than the cases. However, the prevalence of the susceptibility alleles studied here did not vary by age and, therefore, this discrepancy should not introduce bias into our results. We included controls with a history of hysterectomy, who in theory are not at risk for endometrial cancer, but these were a small number ($n = 28$) and there was no difference in the allele frequencies for women who had or did not have a hysterectomy.

Nearly 10% of our case and >5% of our controls carried two high-risk alleles. These women are expected to experience roughly an 8% lifetime risk of endometrial cancer. Although this risk is much lower than that described for the germ-line mutations in *MLH1* and *MSH2* (40-60%), this allelic combination is much more common. We estimate that this combination is responsible for 6% of unselected endometrial cancer cases in Ontario—this is much greater than the 0.5% contribution of the germ-line mutations in the mismatch repair system. This level of risk may warrant intensified surveillance or other preventive measures (e.g., surgery and oral contraceptives). However, it is important that these studies be replicated before introducing clinical recommendations. These SNPs may also play an important role in elevating the risk for other cancers related to the mismatch repair system. The contribution of these alleles to colon and other cancers remains to be determined.

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