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Does risk of endometrial cancer for women without a germline mutation in a DNA mismatch repair gene depend on family history of endometrial cancer or colorectal cancer?

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HIGHLIGHTS

- It was unclear whether endometrial cancer risk for women without mismatch repair gene mutations depends on family history of cancer.
- We found that having a family history of endometrial cancer or early-onset colorectal cancer increases their risk of endometrial cancer.
- This indicates existence of genetic and environmental factors shared by colorectal and endometrial cancers other than mismatch repair gene mutations.

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ABSTRACT

Objective. To determine whether risk of endometrial cancer for women without a germline mutation in a DNA mismatch repair (MMR) gene depends on family history of endometrial or colorectal cancer.

Methods. We retrospectively followed a cohort of 79,166 women who were recruited to the Colon Cancer Family Registry, after exclusion of women who were relatives of a carrier of a MMR gene mutation. The Kaplan–Meier failure method was used to estimate the cumulative risk of endometrial cancer. Cox regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for association between family history of endometrial or colorectal cancer and risk of endometrial cancer.

Results. A total of 628 endometrial cancer cases were observed, with mean age at diagnosis of 54.4 (standard deviation: 15.7) years. The cumulative risk of endometrial cancer to age 70 years was estimated to be 0.94% (95% CI 0.83–1.05) for women with no family history of endometrial cancer, and 3.80% (95% CI 2.75–4.98) for women with at least one first- or second-degree relative with endometrial cancer. Compared with women without family history, we found an increased risk of endometrial cancer for women with at least one first- or second-degree relative with endometrial cancer (HR 3.66, 95% CI 2.63–5.08), and for women with one first-degree relative with colorectal cancer diagnosed at age <50 years (HR 1.48, 95% CI 1.15–1.91).

Conclusion. An increased risk of endometrial cancer is associated with a family history of endometrial cancer or early-onset colorectal cancer for women without a MMR gene mutation, indicating for potential underlying genetic and environmental factors shared by colorectal and endometrial cancers other than caused by MMR gene mutations.

Introduction

Endometrial cancer is the most common gynecological cancer and the fourth most common cancer in women in the United States [1]. It is estimated that 49,560 women will be newly diagnosed with and 8190 will die of endometrial cancer in the United States in 2013 [2]. Endometrial cancer is diagnosed in women at a median age of 62 years with the highest incidence in post-menopausal women aged 55 to 74 years. The

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overall 5-year relative survival is estimated to be 81.5% but varies by stage at diagnosis, 95.3% for localized cancer and 16.9% for distant metastases. Hence, the early diagnosis of endometrial cancer is important to reduce cancer-related morbidity and mortality [2].

Several personal and lifestyle factors have been identified to be associated with an increased risk of the disease, including increasing age, obesity, use of estrogen only, early menarche, late menopause, nulliparity, polycystic ovarian syndrome, metabolic syndrome, anti-estrogen use, tamoxifen, diabetes, alcohol drinking, and red meat consumption. In contrast, cigarette smoking, use of estrogen and progesterone, intrauterine device, aspirin, increased age at last birth, physical activity, and consumption of dietary fiber, fruit and vegetables are associated with a decreased risk of endometrial cancer (Supplementary Table 1). Apart from these lifestyle factors, one major genetic predisposition to endometrial cancer is a germline mutation in one of the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6* and *PMS2* or *EPCAM*. Women who carry a MMR gene mutation are at substantially elevated risk of colorectal, endometrial, and several other cancers (Lynch syndrome) [3,4].

There has been inconsistency on reporting a positive association between family history of endometrial cancer and risk of endometrial cancer [5–14], or between family history of colorectal cancer and risk of endometrial cancer [8,11–14], or between family history of endometrial cancer and risk of colorectal cancer [6,11,15,16]. All of these studies except two [12,14] did not exclude Lynch syndrome families in which colorectal and endometrial cancers occurred in multiple family members. Further, these studies were limited as they only considered for first-degree relatives [5–13,15,16], or only included women aged <55 years [8,10] or women aged 55 years and above [5,9]. The aim of this study was to investigate whether risk of endometrial cancer for women without a MMR gene mutation depends on a family history of endometrial cancer or colorectal cancer.

Materials and methods

Study cohort

This study comprised women from families who were recruited into the Colon Cancer Family Registry between 1997 and 2007 via people with a newly diagnosed colorectal cancer through population cancer registries (case-probands) in Australia (Victoria), Canada (Ontario), and the USA (Washington, California, Arizona, Minnesota, Colorado, New Hampshire, and North Carolina), or via people without any personal history of cancer (control-probands) through electoral rolls (Victoria, Australia), Medicare and Driver's License files (Fred Hutchinson Cancer Research Centre, Seattle, USA), and telephone subscribers lists (Cancer Care Ontario, Canada) [17]. Written informed consent was obtained from all study participants, and the study protocol was approved by the institutional human ethics committee at each study center.

Data collection

At recruitment, baseline information on demographics, personal characteristics, personal and family history of cancer, cancer screening history, hysterectomy, and other surgeries was obtained via questionnaires from all participants. Participants were given follow-up questionnaires at approximately every 5 years after baseline to update this information. The baseline and follow-up questionnaires are available at http://coloncfr.org. Reported cancer diagnoses and ages at which these occurred were confirmed, where possible, using pathology reports, medical records, cancer registry reports, and/or death certificates. Blood samples and permission to access tumor tissue were requested from all participants.

Molecular characterization and genetic testing

All population-based case-probands' colorectal cancer tumors were characterized for MMR-deficiency by microsatellite instability (MSI) using a ten-marker panel and/or by immunohistochemistry (IHC) for the four MMR proteins.[18] Tumors were classified as MMR-deficient if they were MSI-high (≥30% or more of the markers show instability) and/or showed loss of expression of one or more of the MMR proteins by IHC; and MMR-proficient if they were microsatellite stable (no unstable markers) or MSI-low (<30% unstable markers) and/or showed normal expression of all four MMR proteins by IHC. Probands with CRC that demonstrated MMR-deficiency underwent germline mutation testing.

Mutation testing for the *MLH1*, *MSH2* and *MSH6* genes was performed by Sanger sequencing or denaturing high performance liquid chromatography (dHPLC), followed by confirmatory DNA sequencing. Large duplication and deletion mutations including those involving *EPCAM*, which lead to *MSH2* methylation, were detected by Multiplex Ligation Dependent Probe Amplification (MLPA) according to the manufacturer's instructions (MRC Holland, Amsterdam, The Netherlands) [17,19,20]. *PMS2* mutation testing involved a modified protocol from Senter et al. [21] where exons 1–5, 9 and 11–15 were amplified in three long range PCRs followed by nested exon specific PCR/sequencing while the remaining exons (6, 7, 8 and 10) were amplified and sequenced directly from genomic DNA. Large-scale deletions in *PMS2* were detected using the P008-A1 MLPA kit (MRC Holland, Amsterdam, The Netherlands). The relatives of probands with pathogenic MMR germline mutation [3], who provided a blood sample, underwent testing for the specific mutation identified in the proband.

A fluorescent allele-specific PCR assay was used to detect the somatic T>A mutation at nucleotide 1799 in exon 15 of the *BRAF* gene (*BRAF* V600E) as has been previously described [22,23]. Methylation of the *MLH1* gene promoter was measured in all MSI-high and MSI-low cases with sufficient tumor DNA and a random sample of microsatellite stable cases. *MLH1* methylation was measured using the MethyLight MLH1-M2Methylight reaction using an ALU control reaction to normalize for bisulfite-converted input DNA, [24] with the modifications described in Poynter et al. [25]. We classified samples with a proportion of methylated reference (PMR) greater than or equal to 10 as positive for *MLH1* methylation. An *ALU* control reaction cycle threshold [C(t)] value of <25 was used to retain the largest sample size possible for the analysis while minimizing the potential for false negatives.

In this study, we included first- and second-degree female relatives and female spouses of case-probands and control-probands. We excluded all female relatives of case-probands who were known to have a MMR gene mutation (confirmed Lynch syndrome), and all female relatives of case-probands with a colorectal cancer that had MLH1/PMS2 loss with no evidence of *MLH1* methylation and/or *BRAF* V600E mutation or had MSH2/MSH6 loss or solitary loss of PMS2 or MSH6 or MSI-high, for which no MMR germline mutation had been identified (suspected Lynch syndrome). This analysis was therefore of women unlikely to be a carrier of a MMR gene mutation because: they were relatives of case-probands with no evidence of MMR-deficiency; or they were identified as being a relative of an unaffected woman (i.e., control-proband). We estimate the probability of being a carrier of a MMR gene mutation in this cohort to be less than 1 in 3000 (being the estimated prevalence of MMR gene mutations in the general population [26]).

Statistical analysis

History of endometrial cancer in the first- and/or second-degree relative(s) and history of colorectal cancer in the first- and/or second-degree relative(s) were considered as the main exposures, while diagnosis of endometrial cancer was the main outcome. Observation time for a woman began at birth and ended at: first diagnosis of cancer; hysterectomy; last contact; or death, whichever occurred earliest.

Kaplan–Meier failure method was used to estimate cumulative risks of endometrial cancer to age 50 and 70 years. For 95% confidence intervals (CIs) of the cumulative risks, we used the 2.5th and 97.5th percentiles from 10,000 bootstrap samples, using the family as the resampling unit to allow for clustering within families.