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MDM2 SNP309 Is Associated with Endometrial Cancer Risk

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Abstract

Mouse double-minute 2 homologue (MDM2) is a key negative regulator of *p53*, a tumor suppressor gene that initiates cell cycle arrest and apoptosis in response to DNA damage and other cellular stresses. A $T > G$ polymorphism found in the promoter region of *MDM2* (SNP309) increases MDM2 expression and thereby attenuates p53 activity. We genotyped the *MDM2* polymorphism SNP309 in endometrial cancer case-control studies nested within the Nurses' Health Study (454 cases and 1,132 controls) and the Women's Health Study (137 cases and 411 controls). Due to a significant difference in genotype distribution by ethnicity, we restricted our analyses to Caucasians. We calculated odds ratios and 95% confidence intervals using conditional and unconditional logistic regression adjusted for age at menarche, parity and age at first birth, postmenopausal hormone use at diagnosis, age at menopause and menopausal status at diagnosis, first-degree family history of colon cancer, body mass index at diagnosis, and cigarette smoking status at diagnosis. Women with a heterozygous genotype had no greater risk whereas those with a homozygous variant genotype had a greater risk than women with a wild-type genotype for the *MDM2* SNP309 (covariate-adjusted odds ratio, 1.87; 95% confidence interval, 1.29-2.73) for endometrial cancer. We observed no association between age at diagnosis and genotype. Women carrying two copies of the *MDM2* SNP309 variant may be at greater risk of endometrial cancer.

Introduction

Mouse double-minute 2 homologue (MDM2) is a key negative regulator of *p53*, a tumor suppressor gene that initiates cell cycle arrest and apoptosis in response to DNA damage and other cellular stresses (1). MDM2 suppresses p53 activity by ubiquitination and degradation (1). Overexpression of MDM2 is associated with accelerated cancer progression and lack of response to radiation or other DNA-damaging therapies (2).

In endometrial cancer tissue, p53 and MDM2 levels are correlated, suggesting that p53 is inactivated by MDM2 in endometrial cancer (3). Furthermore, Stewart and colleagues

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sequenced the *TP53* gene in a series of endometrial cancer cases overexpressing p53 and found no mutations, suggesting that overexpression was due to another source such as MDM2 abnormalities (4).

A *T* > *G* polymorphism found in the promoter region of *MDM2* (SNP309) increases MDM2 expression and thereby attenuates p53 activity (5). The variant allele of SNP309 has been associated with an earlier age at cancer diagnosis (5-8), and increased risk of both sporadic and hereditary cancers (reviewed in ref. 9). Bond and colleagues have suggested that the SNP309 polymorphism accelerates tumor formation preferentially in women because it increases the affinity for Sp1, a cotranscriptional activator of the estrogen receptor (6). Women with the *GG* genotype were diagnosed with non–Hodgkin's lymphoma or soft tissue sarcoma 13 to 14 years earlier on average than those with a *TT* genotype, although there was no appreciable difference in age at diagnosis for men (6). Similarly, among women homozygous for the SNP309 variant (*GG*), those with estrogensensitive breast cancers were diagnosed 7 years earlier than those with estrogen receptor–negative tumors (6). Because the endometrium is estrogen sensitive, SNP309 is a logical candidate for endometrial cancer risk.

Walsh and colleagues recently evaluated the association between SNP309 and endometrial cancer risk among 73 cases and 79 controls (10), and reported a nearly 3-fold greater risk of endometrial cancer for women with a homozygous variant genotype. In an effort to validate this finding, we evaluated the association between SNP309 and endometrial cancer risk in 591 cases and 1,543 matched controls.

Materials and Methods

Nurses' Health Study

Study Population—The Nurses' Health Study (NHS) began in 1976 when 121,701 female registered nurses between the ages of 30 and 55 completed a self-administered questionnaire. Information regarding endometrial cancer risk factors was obtained from questionnaires completed every 2 years and at the time of blood collection. During 1989 and 1990, blood samples were collected from 32,826 women. Between 2001 and 2004, cheek cell samples were collected using the gswish-and-spith method (11) from 29,684 women who had not provided a blood sample.

Case-control Study—We included cases from the NHS blood cohort with pathologically confirmed invasive endometrial cancer and no previously diagnosed cancer except for nonmelanoma skin cancer. Controls were randomly selected participants with blood or cheek cell samples and neither a hysterectomy nor diagnosed cancer (except nonmelanoma skin cancer). Cases diagnosed before June 1, 1998 who gave blood were matched to three controls, whereas cases diagnosed after June 1, 1998 who gave blood and all cases who gave cheek cells were matched to two controls. Matching factors included year of birth, menopausal status at specimen collection and at the cycle prior to diagnosis, and postmenopausal hormone use at the time of specimen collection (current versus not current users). For cases that gave blood, controls were also matched by time of day of blood collection, month of blood return, and fasting status at blood draw. For cases that gave cheek cells, controls were also matched on month and year of cheek cell collection. This case-control study consists of 454 endometrial cancer cases and 1,132 matched controls. The study protocol was approved by the Committee on Use of Human Subjects of the Brigham and Women's Hospital, Boston, MA.

Women's Health Study

Study Population—Begun in April 1993, the Women's Health Study (WHS) is a completed randomized, double-blind, placebo-controlled trial investigating the benefits and risks of

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aspirin and vitamin E in the primary prevention of cancer and cardiovascular disease among 39,876 female health professionals, ages 45 years or older without a history of cancer (except nonmelanoma skin cancer), coronary heart disease, or cerebrovascular disease (12,13). Written informed consent was obtained. A detailed description of the participants has been published previously (14). At the time of randomization, blood samples were obtained from 28,345 women (71%).

Upon enrollment, all participants completed a detailed questionnaire including known or potential risk factors for endometrial cancer. Every 6 months for the first year, and annually thereafter, participants were sent follow-up questionnaires. Women were asked to report new diagnoses of major illnesses on their follow-up questionnaires; some women also provided this information through letters, or telephone calls. An Endpoints Committee of physicians blinded to treatment reviewed records to confirm diagnoses. The trial was approved by the Institutional Review Board of Brigham and Women's Hospital.

Case-control Study—Eligible WHS incident cases were women with pathologically confirmed invasive endometrial cancer diagnosed after blood collection (1993-1995) and before June 1, 2002, with no previously diagnosed cancer except for nonmelanoma skin cancer. Controls were randomly selected participants with a blood sample but with neither hysterectomy nor diagnosed cancer (except nonmelanoma skin cancer) at the time of blood draw. Controls were matched 3:1 to cases according to age at randomization, menopausal status, and postmenopausal hormone use at blood draw (current versus not current users). Controls were also matched to cases by date of blood return and fasting status at blood draw. This case-control study consisted of 137 incident endometrial cancer cases and 411 matched controls.

Genotyping: DNA was extracted from the buffy coat and cheek cell samples with the QIAGEN QIAmp Blood Kit (QIAGEN, Inc.). DNA extracted from NHS blood samples was whole genome–amplified with GE Healthcare Genomiphi (GE Healthcare Bio-Sciences Corp.). We have previously shown that this method is robust for SNP genotyping (15).

MDM2 SNP309 (rs2279744) was genotyped by PCR amplification followed by restriction enzyme digestion with *Msp*A1. Primers and conditions are available upon request. Replicate samples (5% of sample size) were included for quality control. Laboratory personnel were blinded to the location of quality control replicates and case/control status. Concordance of quality control replicates was 100%.

Statistical Analysis: Genotype frequencies were tested for Hardy-Weinberg equilibrium. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using conditional logistic regression to assess the association between *MDM2* genotypes and endometrial cancer risk. We evaluated a codominant model, a dominant model, and a trend in risk. Multivariate models were adjusted for age at menarche, parity and age at first birth, first-degree family history of colon cancer, smoking status at diagnosis, body mass index at diagnosis, age at menopause, and menopausal status at diagnosis, in addition to the matching factors. We used the DerSimonian and Laird random effects model to combine results from the two cohorts after testing for heterogeneity.

We assessed the association between *MDM2* SNP309 and endometrial cancer by factors hypothesized to modify this association, including cigarette smoking and postmenopausal hormone use. All stratified analyses used unconditional logistic regression adjusted for the matching factors. To test for interactions, we compared a model with all the cross-classified terms to a model with only the main effects using a likelihood ratio test. We evaluated whether

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age at diagnosis varied by genotype using the Kruskal-Wallis test. All analyses were done using SAS v 9.1 (SAS Institute).

Results

Our study population included 591 cases and 1,543 controls (454 cases and 1,132 controls from NHS; 137 cases and 411 controls from WHS). Details of the population characteristics have been reported previously (16). Briefly, the distribution of the matching factors was similar among cases and controls, as expected. In both cohorts, cases had a higher body mass index and were less likely to smoke than controls (16).

MDM2 genotype distributions met Hardy-Weinberg equilibrium criteria. Sixty-five cases and 194 controls were excluded due to missing genotypes. Because *MDM2* SNP309 genotype distribution differed significantly between Caucasians (45% *TT*, 44% *TG*, 11% *GG*) and non-Caucasians (42% *TT*, 27% *TG*, 30% *GG*) among controls ($P = 0.002$), we excluded all non-Caucasians ($n = 43$). Genotype distributions did not differ between prevalent (42% *TT*, 41% *TG*, 16% *GG*) and incident (44% *TT*, 41% *TG*, 15% *GG*) cases in NHS. All cases were incident in WHS. We observed no differences in the association between genotype and endometrial cancer by specimen type (blood versus cheek cell) or cohort (NHS versus WHS; $P_{heterogeneity} > 0.05$); therefore, we combined results within and across cohorts.

Women with a heterozygous genotype did not have a greater risk of endometrial cancer, but women with a homozygous variant genotype had a greater risk than women with a wild-type SNP309 genotype (covariate-adjusted OR, 1.87; 95% CI, 1.29-2.73; Table 1). Mean age at diagnosis for women with the wild-type variant (59.6 years), heterozygous variant (60.1 years), or homozygous variant genotypes (61.4 years) did not differ significantly ($P = 0.21$). In addition, the association between *MDM2* SNP309 and endometrial cancer was not modified by cigarette smoking or postmenopausal hormone use.

Discussion

We evaluated dominant and additive models because a previous study showed a 2-fold increase in the MDM2 protein for cell lines with the heterozygous (*TG*) genotype and a 4-fold increase for cell lines with the homozygous variant (*GG*) genotype (5). Although the WHS results showed increasing risk with the number of variant alleles, the associations were not significant, most likely due to the small sample size. The NHS and pooled results showed no increase in risk for heterozygotes but a significantly increased risk for homozygous variants.

Walsh and colleagues found an increased risk of endometrial cancer with the homozygous variant genotype (OR, 2.76; 95% CI, 1.06-7.20) based on a small number of cases ($n = 73$) and controls ($n = 79$; ref. 10). Our observations are consistent with these results, although attenuated. Potential explanations for the differences in the strength of the association include sample size and population differences. The large CIs in the Walsh study (10) indicate unstable effect estimates due to small sample size. Twenty percent of their cases and 15% of their controls were non-Caucasian. Because the frequency of the SNP309 variant varies according to race (9), the association may have been overestimated due to confounding.

The study strengths include our homogenous population (which minimizes confounding by race), nested case-control design, and comprehensive disease follow-up. Additional studies in non-Caucasians are needed to evaluate the role of SNP309 in endometrial cancer risk for minority populations. To date, this is the largest study of the association between the SNP309 polymorphism in *MDM2* and endometrial cancer risk.

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If SNP309 imparts a survival advantage, there would be an overrepresentation of this polymorphism among prevalent cases, leading to a spurious association with endometrial cancer. However, we observed no difference in the genotype distribution or association with disease when prevalent and incident cases were considered separately. In conclusion, the functional polymorphism in the *MDM2* promoter, SNP309, may increase endometrial cancer risk.

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Table 1

Association between categorical *MDM2* and endometrial cancer risk, NHS and WHS

***MDM2* SNP309**	**NHS**				**WHS**				**Pooled**			
	Cases	**Controls**	**OR (95% CI)**	**Adjusted***	**Cases**	**Controls**	**OR (95% CI)**	**Adjusted***	**Cases**	**Controls**	**OR (95% CI)**	**Adjusted***
	***n* (%)**	***n* (%)**		**OR (95% CI)**	***n* (%)**	***n* (%)**		**OR (95% CI)**	***n* (%)**	***n* (%)**		**OR (95% CI)**
TT	169 (43)	433 (46)	1.00	1.00	47 (39)	163 (44)	1.00	1.00	216 (42)	596 (45)	1.00	1.00
TG	162 (41)	420 (44)	0.98 (0.75-1.27)	0.95 (0.72-1.26)	54 (44)	155 (42)	1.22 (0.76-1.97)	1.42 (0.81-2.51)	216 (42)	575 (44)	1.03 (0.82-1.29)	1.07 (0.74-1.55)
GG	63 (16)	95 (10)	1.85 (1.26-2.72)	1.94 (1.26-3.00)	21 (17)	50 (14)	1.35 (0.74-2.47)	1.69 (0.81-3.56)	84 (16)	145 (11)	1.69 (1.22-2.34)	1.87 (1.29-2.73)
Test for trend			0.02	0.04			0.28	0.12			0.01	0.01

* Adjusted for the matching factors and age at menarche (<12, 12, 13, >13 y), parity and age at first birth (nulliparous, parous with age at first birth <24 years, parous with age at first birth >24), first degree family history of colon (because endometrial cancer is part of the hereditary nonpolyposis colon cancer syndrome) cancer (yes, no), smoking status (never, former, current), body mass index at (<25, 25-29, >30), age at menopause and menopausal status at diagnosis (premenopausal, postmenopausal with age at menopause <49, postmenopausal with age at menopause 49-51, postmenopausal with age at menopause >51, menopausal status unknown).