Polymorphisms in the Vitamin D Receptor and Risk of Ovarian Cancer in Four Studies

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Abstract

Prior studies have suggested that vitamin D may reduce ovarian cancer risk. Thus, we examined whether three single nucleotide polymorphisms (SNP) in the vitamin D receptor (VDR) gene (Fok1, Bsm1, Cdx2) were associated with risk of epithelial ovarian cancer in a retrospective case-control study (New England Case-Control study, NECC) and a nested case-control study of three prospective cohort studies: the Nurses’ Health Study (NHS), NHSII, and the Women’s Health Study. Data from the cohort studies were combined and analyzed using conditional logistic regression and pooled with the results from the NECC, which were analyzed using unconditional logistic regression, using a random effects model. We obtained genotype data for 1,473 cases and 2,006 controls. We observed a significant positive association between the number of Fok1 f alleles and ovarian cancer risk in the pooled analysis ($P_{trend} = 0.03$). The odds ratio (OR) for the ff versus FF genotype was 1.26 [95% confidence interval (CI) = 1.01–1.57]. Neither the Bsm1 ($P_{trend} = 0.96$) or Cdx2 ($P_{trend} = 0.13$) SNPs were significantly associated with ovarian cancer risk. Among the prospective studies, the risk of ovarian cancer by plasma vitamin D levels did not clearly vary by any of the genotypes. For example, among women with the Fok1 FF genotype, the OR comparing plasma 25-hydroxyvitamin D ≥32 ng/mL versus <32 ng/mL was 0.66 (95% CI, 0.34–1.28), and among women with the Ef or ff genotype the OR was 0.71 (95% CI, 0.43–1.18). Our results of an association with the Fok1 VDR polymorphism further support a role of the vitamin D pathway in ovarian carcinogenesis. [Cancer Res 2009;69(5):1885–91]

Introduction

Experimental and epidemiologic studies have suggested that vitamin D may be involved in the etiology of ovarian cancer. The vitamin D receptor (VDR) is weakly to moderately expressed in normal ovarian cells but is more strongly expressed in ovarian cancer cell lines and tumor tissue (1–5). In vitro studies have reported that vitamin D administration inhibited cell growth and induced apoptosis in a dose-dependent manner in both animal (6) and human ovarian cancer cell lines (2, 3, 7–12).

UV-B exposure, which initiates vitamin D production in the skin, has been inversely associated with ovarian cancer mortality in ecologic studies (13–17). Recently, we reported that plasma concentrations of 25-hydroxyvitamin D (a measure of overall vitamin D status) and 1,25-dihydroxyvitamin D (the biologically active but more tightly regulated form) were not associated with risk of epithelial ovarian cancer overall in a prospective study (18). However, 25-hydroxyvitamin D levels were significantly inversely associated with ovarian cancer risk among overweight and obese women, possibly because vitamin D is fat soluble. Furthermore, women with adequate (≥32 ng/mL) versus inadequate (<32 ng/mL) 25-hydroxyvitamin D levels (19) had a 36% decreased risk of serous ovarian cancer (18).

The VDR is a critical component of the vitamin D pathway and a number of common single nucleotide polymorphisms (SNP) have been identified in this gene (20). We focused on three SNPs that either have been associated with ovarian cancer risk in prior studies (21, 22) or have some known or hypothesized functional effect (20). Thus, we examined whether the Fok1 (rs10735810/ rs2228570), Bsm1 (rs1544410), and Cdx2 (rs11568820) VDR SNPs were associated with risk of epithelial ovarian cancer in a retrospective case-control study (New England Case-Control study, NECC) and a nested case-control study using data from three prospective cohort studies: the Nurses’ Health Study (NHS), NHSII, and WHS.

Materials and Methods

Study population. Four studies were included in the analysis, including a nested case-control study from the NHS, NHSII, and WHS, and a retrospective case-control study (NECC). The study populations and case-control selections are described below.

NECC. The NECC includes 1,231 population-based epithelial ovarian cancer cases and 1,244 controls from Massachusetts and New Hampshire. Participants were enrolled in the study in 2 phases, from 1992 to 1997 (563 cases, 523 controls) and from 1998 to 2003 (668 cases, 721 controls). Recruitment methods and eligibility criteria are described elsewhere (23). Briefly, trained interviewers asked participants about potential ovarian cancer risk factors that occurred >1 y before the date of diagnosis for cases or the interview date for controls. Of the 2,347 incident cases of ovarian cancer identified, 1,845 (79%) were eligible and 71% of the eligible cases were enrolled. Controls were identified using random digit dialing, license records, and town resident lists and were frequency matched to cases by age and state. Additional details of the control selection have been published previously (23). Over 95% of study participants provided a blood specimen at enrollment. DNA was available for 1,173 cases and 1,201 controls for this analysis. The institutional review boards of Brigham and Women’s Hospital and Dartmouth Medical School approved both phases of the study, and all participants provided written informed consent.

Cohort studies (NHS, NHSII, WHS). The NHS cohort was established in 1976 among 121,700 U.S. female registered nurses, aged 30 to 55 y, and the NHSII was established in 1989 among 116,609 female registered nurses, ages
25 to 42 y. Women in both cohorts completed an initial questionnaire and have been followed biennially by questionnaire to update exposure status and disease diagnoses. In 1989 to 1990, 32,826 NHS participants provided a blood sample and completed a short questionnaire. Briefly, women arranged to have their blood drawn and shipped with an icepack, via overnight courier, to our laboratory where it was processed. In 2001 to 2004, 33,040 additional women provided a buccal cell specimen using a mouthwash protocol. We extracted DNA from each specimen within 1 wk of receipt. Between 1996 and 1999, 29,611 NHSII participants provided blood samples and completed a short questionnaire. Collection methods were similar to those in the NHS. Cohort follow-up was 98% for the NHS blood study in 2004, 99% for the NHS cheek study in 2004, and 98% for the NHSII blood study in 2003. The WHS is a completed randomized trial examining low-dose aspirin and vitamin E supplementation for the primary prevention of cancer and cardiovascular disease that was initiated in 1992 (26–28). Citrate and EDTA blood samples were collected from 28,345 women before randomization. We included women from the treatment and placebo groups. Morbidity and mortality follow-up through 2004 were 97% and 99% complete, respectively.

Cases had no previous history of cancer, except nonmelanoma skin cancer, before specimen collection and were diagnosed with ovarian cancer before June 1, 2004 (NHS), June 1, 2003 (NHSII), or December 1, 2004 (WHS). We included incident cases after sample collection from each study plus prevalent cases from the NHS/NHSII who submitted a specimen within 4 years after diagnosis. Prevalent and incident cases were similar on stage, histology, and survival time (median survival, incident, 58 mo; prevalent, 80 mo; ref. 29). Overall, 300 cases (235 incident and 65 prevalent) with DNA were confirmed by medical record review (210 from NHS, 28 from NHSII, and 62 from WHS). Cases were matched to two (WHS) or three (NHS/NHSII) controls, who had intact ovaries at the time of the case diagnosis and no prior history of cancer (except nonmelanoma skin cancer), on menopausal status at diagnosis, age (≥ 1 y), and type of sample collection (cheek, blood). Additional details on the control selection have been published previously (18). All three cohort studies were approved by the Committee on Use of Human Subjects in Research at the Brigham and Women's Hospital.

**Laboratory assays.** DNA was extracted from the Buffy coat or cheek cells using Qiagen DNA extraction kits (Qiagen, Inc.). Genotyping for samples for all four studies was performed at the Dana-Farber/Harvard Cancer Center High Throughput Genotyping Core. All the samples were genotyped for the Fok1, Bsm1, and Cdx2 SNPs in the VDR gene. Whole genome amplified DNA was genotyped using the 5 nuclease assay (TaqMan) on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems), in 384-well format. TaqMan primers and probes were designed using the Primer Express Oligo Design software v2.0 (ABI PRISM). Laboratory personnel were blinded to case-control status, and each plate included blinded replicate samples for quality control purposes. Over 94% of the samples were successfully genotyped for each polymorphism, except for the Bsm1 polymorphism in the cohort studies (success, 92%) due to a lack of DNA for some women in the WHS study. Genotyping failures were considered missing data. The quality control replicate samples (cohort studies, 244 replicates from 43 individuals; NECC, 203 replicates from 52 individuals) were 100% concordant for all genotypes. Vitamin D analyses were assayed by RIA, as described previously (30), in the prospectively collected heparin (NHS/NHSII) or citrate (WHS) plasma samples (see ref. 18 for details). Case-control sets and samples from the same study were assayed together and labeled to mask case-control status. The intraassay coefficient of variation, based on blinded quality control samples (cohort studies but were combined into one data set because the \( \beta_{\text{ovarian}} \) by study was >0.05 for all analyses. Because the NECC is a case-control study, these analyses were conducted separately from the cohort studies. The two resulting estimates were then combined using a random effects model to obtain pooled effect estimates (31).

We considered multiple a priori potential confounders and included those that changed the risk estimates or were very strong ovarian cancer risk factors in the final model for genetic analyses: number of pregnancies (continuous), postmenopausal hormone use before diagnosis (never, past, current), oral contraceptive use duration (never, < 3 y; 3 to < 5 y; 5 y), and age at menarche (<12, 12, 13, 14, >14 y). Other potential confounders such as body mass index (BMI), tubal ligation, and smoking did not substantially change risk estimates and therefore were not included in the final model. We additionally adjusted for age and study center in the NECC, as these were frequency matching variables. We calculated the \( P \) for trend for each unit increase in the number of minor alleles (log-additive model) using the Wald test. For analyses including plasma vitamin D levels, we additionally adjusted for BMI at blood draw (continuous), season of blood draw [winter to early spring (January, February, March, April), summer to early fall (July, August, September, October), late-spring/late fall (May, June, November, December)] and interaction terms of study with duration of oral contraceptive use and BMI; this mimics the statistical model used in our prior vitamin D analysis (18).

Our primary analysis included both invasive and borderline cases. However, in secondary analyses, we evaluated genetic associations among histologic subtypes of cases (all invasive, serous invasive, serous borderline, endometrioid, and mucinous). We also stratified by season of diagnosis (summer, other), age at diagnosis (<55, ≥55 y), menopausal status at diagnosis (premenopausal, postmenopausal), BMI (<25, ≥25 kg/m²), and oral contraceptive use (never, ever). Multiplicative interaction terms between the above strata and genotypes (homozygous wild-type versus heterozygous and homozygous variant) were used to determine the \( P_{\text{ovarian}} \). We also examined statistical interactions between the SNPs. These analyses used unconditional logistic regression for both the cohort and case-control studies, additionally adjusting the cohort analysis for the matching factors and study.

Furthermore, we examined the relationship between plasma vitamin D levels and ovarian cancer risk in prospectively collected cases and their matched controls in the three cohort studies. Outliers (32) were identified separately by sample type and set to missing using methods described previously (18). We used a cutpoint of 32 ng/ml for 25-hydroxyvitamin D, which reflects vitamin D adequacy (19) and cohort-specific medians for 1,25-dihydroxyvitamin D. We used unconditional logistic regression, adjusting for matching factors and potential confounders, to estimate ORs and 95% CIs.

All tests of statistical significance were two-sided and considered significant if the \( P \) values were ≤0.05. SAS version 9.1 (SAS Institute, Inc.) was used for the analyses.

**Results**

Women ranged in age from ages 39 to 79 years (mean, 62 years) in the NHS, from ages 31 to 52 years (mean, 42 years) in the NHSII, and from ages 45 to 73 years (mean, 56 years) in the WHS at blood collection (Table 1; ref. 18). Women in the NECC ranged in age from 16 to 77 years (mean, 51 years) at study entry. The characteristics of the NECC population have been described previously (29). Overall, the risk factor distributions were as expected within each study; NHSII women were on average younger and had a longer duration of oral contraceptive use than women in the NHS. We obtained genotype data for 1,473 cases (NECC, 1,173; NHS/NHSII/WHS, 300) and 2,006 controls (NECC, 1,201; NHS/NHSII/WHS, 805).

We evaluated the genotype frequencies of the Fok1, Bsm1, and Cdx2 VDR SNPs in each study population separately. There were no statistically significant differences in the genotype distributions...
Table 1. Characteristics of women in the Nurses’ Health Studies (NHS and NHSII), the WHS, and the NECC

<table>
<thead>
<tr>
<th>Morphology</th>
<th>NHS/NHSII</th>
<th>WHS</th>
<th>NECC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive</td>
<td>199 (83.6)</td>
<td>62 (100)</td>
<td>907 (77.3)</td>
</tr>
<tr>
<td>Borderline</td>
<td>36 (15.1)</td>
<td>0 (0.0)</td>
<td>266 (22.7)</td>
</tr>
<tr>
<td>Serous invasive</td>
<td>115 (48.3)</td>
<td>50 (80.7)</td>
<td>479 (40.8)</td>
</tr>
<tr>
<td>Serous borderline</td>
<td>20 (8.4)</td>
<td>0 (0.0)</td>
<td>168 (14.3)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>30 (12.6)</td>
<td>4 (6.5)</td>
<td>153 (13.0)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>31 (13.0)</td>
<td>4 (6.5)</td>
<td>171 (14.6)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>7 (2.9)</td>
<td>4 (6.5)</td>
<td>143 (12.2)</td>
</tr>
</tbody>
</table>

Abbreviations: OC, oral contraceptives; PMH, postmenopausal hormones.
* The mean (SD) are presented.
† The n (%) are presented; for PMH use, among postmenopausal women only.
‡ May not add up to total number of cases due to unknown status and other histologic subtypes. Not applicable to controls.

of these SNPs between the cases and controls within each study, and genotype distributions were in Hardy-Weinberg Equilibrium among the controls, except for Bsm1 in the NECC controls (P = 0.03; data not shown). Review of the screen shots for these plates revealed appropriate clustering, thus this finding is likely due to chance. The minor allele frequencies across the four studies were 0.39 for Fok1 f allele, 0.40 for the Bsm1 B allele, and 0.21 for the Cdx2 A allele.

We observed a nonstatistically significant increased risk of ovarian cancer with increasing numbers of the Fok1 variant f allele in the NECC and the combined cohort studies separately that became statistically significant (P trend = 0.03) in the pooled analysis (Table 2). The pooled OR comparing women with the ff versus FF genotype was 1.26 (95% CI, 1.01–1.56), with an intermediate risk for the Ff genotype (pooled OR, 1.13; 95% CI, 0.96–1.56). Results were somewhat attenuated when only including invasive cases likely due to the smaller sample size (pooled OR Ff versus FF, 1.21; 95% CI, 0.96–1.53). The association between Fok1 and ovarian cancer risk seemed stronger for the serous borderline (pooled OR Ff versus FF, 1.66; 95% CI, 1.17–2.36) and endometrioid (pooled OR ff versus FF, 1.61; 95% CI, 1.02–2.51) histologic subtypes. The corresponding, pooled OR for serous invasive cases was 1.05 (95% CI, 0.87–1.28) and for mucinous tumors was 1.11 (95% CI, 0.79–1.55). The Fok1 association was similar by age, menopausal status, season at diagnosis, BMI, and oral contraceptive use history as well as when excluding prevalent cases from the NHS and NHSII (data not shown).

Neither the Bsm1 or Cdx2 polymorphisms were statistically significantly associated with overall ovarian cancer risk in study-specific or pooled analyses (Table 2). For example the pooled OR comparing the Bsm1 BB to the bb genotype was 0.95 (95% CI, 0.76–1.18) and for the Cdx2 GG versus AA genotype was 1.00 (95% CI, 0.71–1.42). Results were similar among invasive cases only and by histologic subtype for the Bsm1 polymorphism (data not shown). However, the association between the Cdx2 polymorphism was statistically significant for invasive ovarian cancer overall (pooled OR GA+AA versus GG, 1.20; 95% CI, 1.02–1.41; P trend = 0.05) and for serous invasive tumors (comparable OR, 1.37; 95% CI, 1.13–1.67; P trend = 0.004) but not for the other subtypes (P trend >0.56). In general, associations were similar by strata of BMI, age, menopausal status, and season at diagnosis (data not shown); however, there was a statistically significant interaction between the Bsm1 genotype and oral contraceptive use history (P heterogeneity = 0.02). There was a modest positive association between the number of B alleles and risk among never users (OR BB versus bb, 1.31; 95% CI, 0.94–1.82; P trend = 0.08) and a suggestive inverse association among ever users (OR, 0.77; 95% CI, 0.54–1.10; P trend = 0.13).

We further examined whether there was a combined effect of multiple SNPs on ovarian cancer risk. The Bsm1 SNP association
was similar across strata of the Fok1 and Cdx2 genotypes. However, there was a nearly statistically significant interaction (P = 0.07) between the Fok1 and Cdx2 SNPs. Compared with the reference group of FF and GG, respectively, women with any other genotype were at a statistically significantly increased risk of ovarian cancer in the pooled analysis. Specifically, the OR for the women with the Ff-ff and GG genotype was 1.32 (OR, 1.37 for invasive cases) and for women with the GA-AA genotype regardless of Fok1 genotype was 1.36 (ORs, 1.28 for invasive cases).

Table 2. ORs and 95% CIs for the association between VDR SNPs and ovarian cancer risk in the NECC, the NHS, NHSII, and the WHS

<table>
<thead>
<tr>
<th>Genotype</th>
<th>NECC*</th>
<th>NHS / NHSII / WHS†</th>
<th>Pooled‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n, case/control</td>
<td>OR (95% CI)</td>
<td>n, case/control</td>
</tr>
<tr>
<td>Fok1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>409 / 450</td>
<td>1.00 (ref.)</td>
<td>98 / 304</td>
</tr>
<tr>
<td>Ff</td>
<td>502 / 511</td>
<td>1.08 (0.89, 1.30)</td>
<td>141 / 340</td>
</tr>
<tr>
<td>ff</td>
<td>193 / 175</td>
<td>1.23 (0.95, 1.58)</td>
<td>49 / 113</td>
</tr>
<tr>
<td>P&lt;sub&gt;trend&lt;/sub&gt;</td>
<td>0.12</td>
<td></td>
<td>0.10</td>
</tr>
<tr>
<td>Ff + ff</td>
<td>1.12 (0.94–1.33)</td>
<td></td>
<td>1.30 (0.97, 1.75)</td>
</tr>
<tr>
<td>Bsm1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bb</td>
<td>409 / 430</td>
<td>1.00 (ref.)</td>
<td>94 / 267</td>
</tr>
<tr>
<td>Bb</td>
<td>521 / 518</td>
<td>1.07 (0.89, 1.29)</td>
<td>143 / 353</td>
</tr>
<tr>
<td>BB</td>
<td>183 / 203</td>
<td>0.93 (0.73, 1.20)</td>
<td>41 / 114</td>
</tr>
<tr>
<td>P&lt;sub&gt;trend&lt;/sub&gt;</td>
<td>0.80</td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td>Bb + BB</td>
<td>1.03 (0.87, 1.23)</td>
<td></td>
<td>1.15 (0.85, 1.57)</td>
</tr>
<tr>
<td>Cdx2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>670 / 746</td>
<td>1.00 (ref.)</td>
<td>179 / 496</td>
</tr>
<tr>
<td>Gc</td>
<td>399 / 356</td>
<td>1.23 (1.02, 1.48)</td>
<td>92 / 220</td>
</tr>
<tr>
<td>Ac</td>
<td>51 / 56</td>
<td>1.01 (0.67, 1.51)</td>
<td>14 / 36</td>
</tr>
<tr>
<td>P&lt;sub&gt;trend&lt;/sub&gt;</td>
<td>0.11</td>
<td></td>
<td>0.74</td>
</tr>
<tr>
<td>Ga + Gg</td>
<td>1.20 (1.01, 1.43)</td>
<td></td>
<td>1.07 (0.79, 1.45)</td>
</tr>
</tbody>
</table>

*NECC, unconditional logistic regression; NHS/NHSII/WHs, conditional logistic regression. Adjusted for number of pregnancies, postmenopausal hormone use, oral contraceptive use duration, and age at menarche. Additionally adjusted for age and study center for the NECC analysis.
† P<sub> heterogeneity</sub> between NHS/NHSII and WHS was 0.70 for Fok1, 0.36 for Bsm1, and 0.72 for Cdx2.
‡ P values for tests for heterogeneity comparing the NECC and cohort results were all >0.36.
oral contraceptive use (21, 22). There are some data supporting a potential biological relationship between the vitamin D pathway and oral contraceptive use. Among premenopausal women, current oral contraceptive use is associated with higher vitamin D levels in Caucasians and African-Americans, and levels decline after women stop using oral contraceptives (33, 34). In addition, a small randomized trial of oral contraceptive use in premenopausal women observed changes in bone metabolism after 3 months only among the intervention group who had the Bsm1 BB and Bb genotypes (35).

However, further research is needed to replicate this association and elucidate the underlying biological mechanisms.

We also observed a nearly statistically significant interaction between the Fok1 and Cdx2 genotypes, such that women with the FF and GG genotypes, respectively, had the lowest risk of cancer. Women with any other genotype combination had over a 30% increased risk. Prior studies have not examined this combination of genotypes (21); thus, it will be important to examine this potential interaction in larger studies.

Our finding that the Fok1 ff allele is associated with an increased risk of ovarian cancer is consistent with functional data on the Fok1 SNP. The variant f allele has an earlier start codon, leading to a protein with three extra amino acids that is less transcriptionally active and has a lower transcriptional activation of VDR target genes than the F allele (20, 36, 37). Furthermore, the variant protein may have a decreased capacity to inhibit cellular growth after administration of vitamin D (38). This suggests that women with the f allele have a less active VDR, which may increase their ovarian cancer risk. The Bsm1 SNP is in the 3-prime untranslated region of the VDR gene and may alter mRNA stability; the B allele has been associated with increased osteopontin, calcitrol, and 1,25-dihydroxyvitamin D in serum (20). However, this potential functional effect may not be important in ovarian cancer carcinogenesis, given that we did not observe an association between this SNP and either risk of ovarian cancer or circulating vitamin D levels. Finally, the Cdx2 G->A SNP alters the binding site of a CDX transcription factor, with the G allele having a lower binding affinity (20, 39, 40). These studies also observed that the A allele was associated with increased VDR expression in intestinal cells and enhanced calcium absorption. To our knowledge, no functional studies have examined a potential biological interplay between the Fok1 and Cdx2 SNPs; however, such data could lend support to the interaction we observed with ovarian cancer risk.

Experimental data also support a role of the vitamin D pathway specifically in ovarian carcinogenesis. A number of studies have observed that 1,25-dihydroxyvitamin D inhibits ovarian cancer cell growth (2, 3, 7, 9–12) and increases apoptosis (8). High 1,25-dihydroxyvitamin D levels also can increase VDR expression in

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>25-hydroxyvitamin D*</th>
<th>1,25-dihydroxyvitamin D*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;32 ng/mL</td>
<td>≥32 ng/mL</td>
</tr>
<tr>
<td>Fok1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n, cases/controls</td>
<td>166/385</td>
<td>44/137</td>
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<tr>
<td>FF</td>
<td>1.0</td>
<td>0.66 (0.34, 1.28)</td>
</tr>
<tr>
<td>Ff</td>
<td>1.0</td>
<td>0.80 (0.45, 1.43)</td>
</tr>
<tr>
<td>ff</td>
<td>1.0</td>
<td>0.49 (0.17, 1.38)</td>
</tr>
<tr>
<td>Ff + ff</td>
<td>1.0</td>
<td>0.71 (0.43, 1.18)</td>
</tr>
<tr>
<td>Bsm1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n, cases/controls</td>
<td>158/369</td>
<td>41/125</td>
</tr>
<tr>
<td>bb</td>
<td>1.0</td>
<td>0.61 (0.30, 1.27)</td>
</tr>
<tr>
<td>Bb</td>
<td>1.0</td>
<td>0.75 (0.41, 1.35)</td>
</tr>
<tr>
<td>BB</td>
<td>1.0</td>
<td>0.70 (0.26, 1.92)</td>
</tr>
<tr>
<td>Bb+BB</td>
<td>1.0</td>
<td>0.73 (0.43, 1.22)</td>
</tr>
<tr>
<td>Cdx2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n, cases/controls</td>
<td>160/371</td>
<td>43/132</td>
</tr>
<tr>
<td>GG</td>
<td>1.0</td>
<td>0.59 (0.36, 0.98)</td>
</tr>
<tr>
<td>GA+AA</td>
<td>1.0</td>
<td>0.91 (0.46, 1.81)</td>
</tr>
</tbody>
</table>

NOTE: Used unconditional logistic regression adjusting for number of pregnancies, postmenopausal hormone use, oral contraceptive use duration, age at menarche, age, menopausal status at diagnosis, cohort, season of blood collection, BMI at blood collection, and the interaction of study with oral contraceptive use duration and BMI.*P heterogeneity across Fok1, Bsm1, and Cdx2 genotypes for 25-hydroxyvitamin D were 0.81, 0.81, and 0.32, respectively, and for 1,25-dihydroxyvitamin D were 0.47, 0.53, and 0.65, respectively.
ovarian cancer cell lines (8). One study reported that 25-
hydroxyvitamin D slightly increased cell growth of ovarian cancer cells; however, this effect was reduced at higher concentrations of 25-hydroxyvitamin D exposure (11). Recent data suggest that ovarian cancer cells and tissue contain measurable levels of 1α-
hydroxylase and 24-25-hydroxylase (1, 4, 11), the former of which can
convert 25-hydroxyvitamin D to the more active 1,25-dihydroxy-
vitamin D (3, 4). It is possible that 1,25-dihydroxyvitamin D
formed through this process acts intracellularly or as an autocrine/
paracrine factor (41). Because ovarian tumor tissue expresses the
VDR (1–5), these data suggest in total that some ovarian tumors may
have a functional vitamin D pathway that could potentially be
a target for prevention or treatment.

Interestingly, we did not observe that the association of plasma
vitamin D levels with ovarian cancer risk differed by VDR genotype;
however, the sample size precluded detecting small to modest
effects or comparing extreme ends of the vitamin D spectrum.
Examining a potential interrelationship between VDR genotype
and plasma vitamin D levels could be important, as it is possible
that women with genotypes associated with a higher risk of ovarian
cancer may benefit more from high plasma vitamin D levels.
Biological data support this hypothesis. Colin, and colleagues (38)
reported that the Fok1 polymorphism in cultured peripheral blood
mononuclear cells from postmenopausal women was associated
with growth inhibition only at low, physiologic doses. They
suggested that the polymorphism may be clinically relevant only
among those with insufficient vitamin D levels. Further
experimental and epidemiologic research is needed to elucidate these
relationships.

Our study has several limitations and strengths. One limitation is
that the NHS/NHSII and WHS collected different sample types
(heparin and citrate plasma, respectively) for measuring plasma
vitamin D levels. Citrate plasma can dilute specimens, thus
lowering the measured concentrations (42), although the levels
were similar between the two studies when adjusting for the
dilution factor (18). Furthermore, the study population was
primarily of European ancestry; thus, we were not able to examine
these associations in other races/ethnicities. The strength of this
study was the ability to combine four studies, one retrospective
case-control study and three prospective cohort studies. The
similar associations across studies and the statistically significant
results in both the pooled analysis and meta-analysis with prior
studies, lend support that our findings are not spurious; however,
given that we made a number of comparisons the results could be
due to chance. The ability to pool the results also substantially
increased our sample size to ~1,500 cases and 2,000 controls.

In conclusion, we observed that increasing copies of the Fok1 f
allele in the VDR gene were positively associated with ovarian
cancer risk. We did not observe a strong interrelationship between
25-hydroxyvitamin D and Fok1 genotype. Additional research is
needed to further evaluate possible relationships between multiple
vitamin D pathway genes and circulating levels of vitamin D.
Overall, these results provide further support that the vitamin D
pathway may play a role in the etiology of ovarian cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Correction: Article on VDR Polymorphisms and Ovarian Cancer

In the article on VDR polymorphisms and ovarian cancer in the March 1, 2009 issue of *Cancer Research* (1), the correct name of the second author is Margaret A. Gates.

Polymorphisms in the Vitamin D Receptor and Risk of Ovarian Cancer in Four Studies

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