Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-3515

Research Article

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

Shelley S. Tworoger,[1,4] Margaret A. Gate,[1,4] I-Min Lee,[2,4] Julie E. Buring,[2,4] Linda Titus-Ernstoff,[5] Daniel Cramer,[3,4] and Susan E. Hankinson[1,4]

[1]Channing Laboratory and [2]Division of Preventive Medicine, Department of Medicine, [3]Department of Obstetrics and Gynecology, Brigham and Women's Hospital; [4]Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts; and [5]Departments of Community and Family Medicine and Pediatrics, Dartmouth Medical School and the Norris Cotton Cancer Center, Lebanon, New Hampshire

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 *Ff* 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 the Women's Health Study (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, ages 30 to 55 y, and the NHSII was established in 1989 among 116,609 female registered nurses, ages

Requests for reprints: Shelley S. Tworoger, Channing Laboratory, 181 Longwood Avenue, 3rd Floor, Boston, MA 02115. Phone: 617-525-2087; Fax: 617-525-2008; E-mail: nhsst@channing.harvard.edu.

©2009 American Association for Cancer Research.

doi:10.1158/0008-5472.CAN-08-3515

Downloaded from cancerres.aacrjournals.org on November 20, 2019. © 2009 American Association for Cancer Research.

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-3515

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 (24). 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 to 1999, 29,611 NHSII participants provided blood samples and completed a short questionnaire (25). 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 ($\pm$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 the 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 analytes 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 replicates, was 8% to 10% for 25-hydroxyvitamin D and 9-14% for 1,25-dihydroxyvitamin D.

Statistical analysis. We evaluated whether each genotype was in Hardy-Weinberg equilibrium using a χ^2 test. The distribution of the alleles was assessed separately in each study (NHS, NHSII, WHS, and NECC), and by sample type for the NHS (cheek versus WBC). Unconditional (NECC) and conditional logistic regression (NHS/NHSII/WHS) were used to estimate the odds ratios (OR) and 95% confidence intervals (CI) associated with the main effects of each gene variant. Data analyses were initially conducted separately for the cohort studies but were combined into one data set because the $P_{heterogeneity}$ 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, $\geq$55 y), menopausal status at diagnosis (premenopausal, postmenopausal), BMI (<25, $\geq$25 kg/m^2), 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 $P_{heterogeneity}$. 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 $\leq$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

Downloaded from cancerres.aacrjournals.org on November 20, 2019. © 2009 American Association for Cancer Research.

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-3515

Table 1. Characteristics of women in the Nurses' Health Studies (NHS and NHSII), the WHS, and the NECC

	NHS/NHSII		WHS		NECC	
	Cases	Controls	Cases	Controls	Cases	Controls
n	238	684	62	121	1,173	1,201
Age at diagnosis (y)*	59.3 (10.0)	59.2 (10.1)	55.8 (7.2)	55.6 (7.1)	51.3 (12.8)	50.8 (13.0)
Duration of OC use (mo)*	24.1 (37.8)	27.5 (43.9)	21.7 (19.3)	24.6 (19.0)	25.1 (45.7)	36.7 (52.2)
Parity*	2.7 (1.5)	3.0 (1.7)	2.1 (1.6)	2.6 (1.6)	1.7 (1.6)	2.2 (1.7)
BMI (kg/m^2)*	26.0 (5.5)	25.7 (4.7)	24.6 (3.9)	25.0 (4.4)	26.3 (6.3)	25.7 (5.5)
Tubal ligation †	36 (15.1)	145 (21.2)	12 (19.4)	32 (26.5)	166 (14.2)	220 (18.3)
Menopause †						
Premenopausal	48 (20.2)	157 (23.0)	15 (24.2)	36 (29.8)	552 (47.1)	585 (48.7)
Postmenopausal	175 (73.5)	487 (71.2)	35 (56.5)	69 (57.0)	563 (48.0)	562 (46.8)
Unknown	15 (6.3)	40 (5.9)	12 (19.4)	16 (13.2)	58 (4.9)	54 (4.5)
PMH use †						
Never	49 (28.0)	157 (32.2)	6 (17.1)	12 (17.4)	387 (68.7)	362 (64.4)
Past	27 (15.4)	101 (20.7)	5 (14.3)	5 (7.3)	53 (9.4)	61 (10.9)
Current	93 (53.1)	197 (40.5)	24 (68.6)	52 (75.4)	121 (21.5)	137 (24.4)
Unknown	6 (3.4)	32 (6.6)	0 (0.0)	0 (0.0)	2 (0.4)	2 (0.4)
Family history of ovarian cancer †	18 (7.6)	23 (3.4)	2 (3.2)	7 (5.8)	60 (5.1)	34 (2.8)
Morphology †‡						
Invasive	199 (83.6)	NA	62 (100)	NA	907 (77.3)	NA
Borderline	36 (15.1)		0 (0.0)		266 (22.7)	
Histology †‡						
Serous invasive	115 (48.3)	NA	50 (80.7)	NA	479 (40.8)	NA
Serous borderline	20 (8.4)		0 (0.0)		168 (14.3)	
Mucinous	30 (12.6)		4 (6.5)		153 (13.0)	
Endometrioid	31 (13.0)		4 (6.5)		171 (14.6)	
Clear cell	7 (2.9)		4 (6.5)		143 (12.2)	

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+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+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

Downloaded from cancerres.aacrjournals.org on November 20, 2019. © 2009 American Association for Cancer Research.

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-3515

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).

There were 210 incident cases and 522 matched controls with both genotype and plasma data in the three prospective cohort studies. We examined whether the association between plasma vitamin D concentrations differed by *VDR* genotype (Table 3). Overall, the risk of ovarian cancer comparing plasma 25-hydroxyvitamin D <32 ng/mL versus ≥32 ng/mL or 1,25-dihydroxyvitamin D above versus below study-specific medians was similar by *Fok1, Bsm1,* and *Cdx2* genotype. For example, among women with the *Fok1 FF* genotype, the OR comparing ≥32 versus <32 ng/mL of 25-hydroxyvitamin D was 0.66 (95% CI, 0.34–1.28) and among those with *Ff* or *ff* genotype the OR was 0.71 (95% CI, 0.43–1.18). Plasma vitamin D levels were not clearly associated with the *VDR* SNPs among controls (data not shown).

Discussion

To our knowledge, this is the largest ovarian cancer study to examine the association between SNPs in the *VDR* gene and risk, and it is the first study to examine whether these SNPs modify the association of plasma 25-hydroxy and 1,25-dihydroxyvitamin D levels with risk. Overall, our results suggest that the *Fok1* but not the *Bsm1* and *Cdx2,* polymorphism is associated with ovarian cancer risk. Furthermore, the combined *Fok1* and *Cdx2* genotype may be important as well, although additional studies are needed to confirm a potential interrelationship. In general, the association with plasma vitamin D levels did not vary by *VDR* genotype, suggesting that these two factors may act independently.

Our results are consistent with those observed in a case-control study of ovarian cancer from Hawaii (n = 72 Caucasian cases), which observed more than a 2-fold increase in risk for either the *Ff* or *ff* genotype versus the wild-type *FF* but no association for the *Bsm1* or *Cdx2* SNPs (22). Interestingly, no association with the *Fok1* genotype was observed for Japanese women (n = 94 cases), suggesting that this relationship may differ by race/ethnicity. A second study (21), using a nested case-control design among 2 cohort studies, did not observe any associations between the *Fok1* relative risk [(RR) *ff* versus *FF,* 1.23; 95% CI, 0.61–2.51] or *Bsm1* (RR *BB* versus *bb,* 1.08; 95% CI, 0.54–2.17) SNPs and ovarian cancer risk; however, the sample size was relatively small (n = 170 cases), limiting power to detect a modest association. In a meta-analysis of the current cohort and case-control studies plus the 2 prior studies, the summary OR comparing women with the *Fok1 ff* versus *FF* allele was 1.29 (95% CI, 1.05–1.58; Fig. 1), suggesting an overall positive association for this SNP.

We observed that the association of the *Bsm1 VDR* SNP with risk differed by oral contraceptive use history; however, the log-additive *Bsm1* associations were not statistically significant in either never or ever users. The two prior studies did not examine interactions by

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

Genotype	NECC*		NHS / NHSII / WHS*†		Pooled‡
	n, case/control	OR (95% CI)	*n*, case/control	OR (95% CI)	OR (95% CI)
Fok1					
FF	409 / 450	1.00 (ref.)	98 / 304	1.00 (ref.)	1.00 (ref.)
Ff	502 / 511	1.08 (0.89, 1.30)	141 / 340	1.28 (0.93, 1.76)	1.13 (0.96, 1.33)
ff	193 / 175	1.23 (0.95, 1.58)	49 / 113	1.37 (0.89, 2.10)	1.26 (1.01, 1.57)
P_{trend}		0.12		0.10	0.03
Ff + ff		1.12 (0.94–1.33)		1.30 (0.97, 1.75)	1.16 (1.00, 1.35)
Bsm1					
bb	409 / 430	1.00 (ref.)	94 / 267	1.00 (ref.)	1.00 (ref.)
bB	521 / 518	1.07 (0.89, 1.29)	143 / 353	1.20 (0.87, 1.66)	1.10 (0.94, 1.30)
BB	183 / 203	0.93 (0.73, 1.20)	41 / 114	1.00 (0.64, 1.58)	0.95 (0.76, 1.18)
P_{trend}		0.80		0.72	0.96
bB + BB		1.03 (0.87, 1.23)		1.15 (0.85, 1.57)	1.06 (0.91, 1.23)
Cdx2					
GG	670 / 746	1.00 (ref.)	179 / 496	1.00 (ref.)	1.00 (ref.)
GA	399 / 356	1.23 (1.02, 1.48)	92 / 220	1.09 (0.79, 1.49)	1.19 (1.02, 1.39)
AA	51 /56	1.01 (0.67, 1.51)	14 / 36	0.99 (0.51, 1.92)	1.00 (0.71, 1.42)
P_{trend}		0.11		0.74	0.13
GA + AA		1.20 (1.01, 1.43)		1.07 (0.79, 1.45)	1.16 (1.00, 1.36)

*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.

† $P_{heterogeneity}$ 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.

Downloaded from cancerres.aacrjournals.org on November 20, 2019. © 2009 American Association for Cancer Research.

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-3515

Table 3. RRs and 95% CIs for the association between prediagnostic 25-hydroxy and 1,25-dihydroxy vitamin D levels and ovarian cancer risk in the NHS, NHSII, and WHS, stratified by polymorphisms in the *VDR* gene

Polymorphism	25-hydroxyvitamin D*		1,25-dihydroxyvitamin D*	
	<32 ng/mL	≥32 ng/mL	<Median	≥Median
Fok1				
n, cases/controls	166/385	44/137	101/264	108/257
FF	1.0	0.66 (0.34, 1.28)	1.0	1.03 (0.59, 1.81)
Ff	1.0	0.80 (0.45, 1.43)	1.0	1.35 (0.83, 2.19)
ff	1.0	0.49 (0.17, 1.38)	1.0	0.57 (0.24, 1.32)
Ff + ff	1.0	0.71 (0.43, 1.18)	1.0	1.08 (0.71, 1.64)
Bsm1				
n, cases/controls	158/369	41/125	94/250	104/243
bb	1.0	0.61 (0.30, 1.27)	1.0	0.89 (0.50, 1.58)
bB	1.0	0.75 (0.41, 1.35)	1.0	1.27 (0.77, 2.08)
BB	1.0	0.70 (0.26, 1.92)	1.0	1.14 (0.47, 2.77)
bB+BB	1.0	0.73 (0.43, 1.22)	1.0	1.23 (0.80, 1.89)
Cdx2				
n, cases/controls	160/371	43/132	97/254	105/248
GG	1.0	0.59 (0.36, 0.98)	1.0	1.15 (0.75, 1.74)
GA+AA	1.0	0.91 (0.46, 1.81)	1.0	0.97 (0.55, 1.74)

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.

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 f* 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 transactivation 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

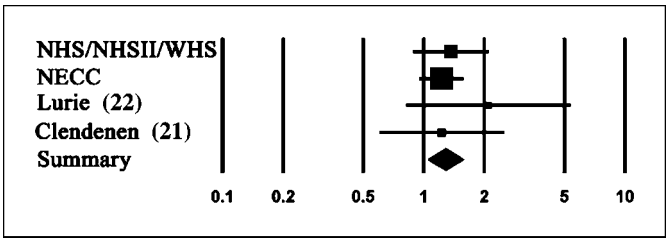

Figure 1. Forest plot of the ORs and 95% CIs comparing risk of ovarian cancer for women with the *Fok1 ff* versus *FF* allele in the *VDR* for the cohort studies (NHS, NHSII, and WHS) and the NECC study in the current analysis and two prior published reports. The summary OR is 1.29 (95% CI, 1.05–1.58; $P = 0.01$).

Downloaded from cancerres.aacrjournals.org on November 20, 2019. © 2009 American Association for Cancer Research.

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-3515

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-hydroxlase (1, 4, 11), the former of which can convert 25-hydroxyvitamin D to the more active 1,25-dihydroxyvitamin 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.

Acknowledgments

Received 9/9/2008; revised 11/18/2008; accepted 11/25/2028; published OnlineFirst 02/17/2009.

Grant support: NIH grants P01 CA87969, CA49449, CA67262, CA50385, P50 CA105009, HL-43851, HL-080467, and CA-47988. M.A. Gates was supported by the National Cancer Institute training grant R25 CA098566.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank the women in the NHS, NHSII, WHS, and NECC studies for their valuable participation and Marilyn Chown, Allison Vitonis, and Jeanne Sparrow for data management support.

References

1. Anderson MG, Nakane M, Ruan X, Kroeger PE, Wu-Wong JR. Expression of VDR and CYP24A1 mRNA in human tumors. Cancer Chemother Pharmacol 2006;57:234–40.
2. Saunders DE, Christensen C, Lawrence WD, et al. Receptors for 1,25-dihydroxyvitamin D3 in gynecologic neoplasms. Gynecol Oncol 1992;44:131–6.
3. Ahonen MH, Zhuang YH, Aine R, Ylikomi T, Tuohimaa P. Androgen receptor and vitamin D receptor in human ovarian cancer: growth stimulation and inhibition by ligands. Int J Cancer 2000;86:40–6.
4. Friedrich M, Rafi L, Mitschele T, Tilgen W, Schmidt W, Reichrath J. Analysis of the vitamin D system in cervical carcinomas, breast cancer and ovarian cancer. Recent Results Cancer Res 2003;164:239–46.
5. Villena-Heinsen C, Meyberg R, Axt-Fliedner R, Reitnauer K, Reichrath J, Friedrich M. Immunohistochemical analysis of 1,25-dihydroxyvitamin-D3-receptors, estrogen and progesterone receptors and Ki-67 in ovarian carcinoma. Anticancer Res 2002;22:2261–7.
6. Dokoh S, Donaldson CA, Marion SL, Pike JW, Haussler MR. The ovary: a target organ for 1,25-dihydroxyvitamin D3. Endocrinology 1983;112:200–6.
7. Saunders DE, Christensen C, Williams JR, et al. Inhibition of breast and ovarian carcinoma cell growth by 1,25-dihydroxyvitamin D3 combined with retinoic acid or dexamethasone. Anticancer Drugs 1995;6:562–9.
8. Jiang F, Bao J, Li P, Nicosia SV, Bai W. Induction of ovarian cancer cell apoptosis by 1,25-dihydroxyvitamin D3 through the down-regulation of telomerase. J Biol Chem 2004;279:53213–21.
9. Jiang F, Li P, Fornace AJ, Jr., Nicosia SV, Bai W. G2/M arrest by 1,25-dihydroxyvitamin D3 in ovarian cancer cells mediated through the induction of GADD45 via an exonic enhancer. J Biol Chem 2003;278:48030–40.
10. Li P, Li C, Zhao X, Zhang X, Nicosia SV, Bai W. p27(Kip1) stabilization and G(1) arrest by 1,25-dihydroxyvitamin D(3) in ovarian cancer cells mediated through down-regulation of cyclin E/cyclin-dependent kinase 2 and Skp1-Cullin-F-box protein/Skp2 ubiquitin ligase. J Biol Chem 2004;279:25260–7.
11. Miettinen S, Ahonen MH, Lou YR, et al. Role of 24-hydroxylase in vitamin D3 growth response of OVCAR-3 ovarian cancer cells. Int J Cancer 2004;108:367–73.
12. Zhang X, Jiang F, Li P, et al. Growth suppression of ovarian cancer xenografts in nude mice by vitamin D analogue EB1089. Clin Cancer Res 2005;11:323–8.
13. Freedman DM, Dosemeci M, McGlynn K. Sunlight and mortality from breast, ovarian, colon, prostate, and non-melanoma skin cancer: a composite death certificate based case-control study. Occup Environ Med 2002;59:257–62.
14. Garland CF, Garland FC, Gorham ED, et al. The role of vitamin D in cancer prevention. Am J Public Health 2006;96:252–61.
15. Grant WB. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. Cancer 2002;94:1867–75.
16. Grant WB. Ecologic studies of solar UV-B radiation and cancer mortality rates. Recent Results Cancer Res 2003;164:371–7.
17. Lefkowitz ES, Garland CF. Sunlight, vitamin D, and ovarian cancer mortality rates in US women. Int J Epidemiol 1994;23:1133–6.
18. Tworoger SS, Lee IM, Buring JE, Rosner B, Hollis BW, Hankinson SE. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of incident ovarian cancer. Cancer Epidemiol Biomarkers Prev 2007;16:783–8.
19. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr 2005;135:317–22.
20. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene 2004;338:143–56.
21. Clendenen TV, Arslan AA, Koenig KL, et al. Vitamin D receptor polymorphisms and risk of epithelial ovarian cancer. Cancer Lett 2008;260:209–15.
22. Lurie G, Wilkens LR, Thompson PJ, et al. Vitamin D receptor gene polymorphisms and epithelial ovarian cancer risk. Cancer Epidemiol Biomarkers Prev 2007;16:2566–71.
23. Terry KL, De Vivo I, Titus-Ernstoff L, Shih MC, Cramer DW. Androgen receptor cytosine, adenine, guanine repeats, and haplotypes in relation to ovarian cancer risk. Cancer Res 2005;65:5974–81.
24. Hankinson SE, Willett WC, Manson JE, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1998;90:1292–9.

Downloaded from cancerres.aacrjournals.org on November 20, 2019. © 2009 American Association for Cancer Research.

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-3515

25. Tworoger SS, Sluss P, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. Cancer Res 2006;66:2476–82.

26. Cook NR, Lee IM, Gaziano JM, et al. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. JAMA 2005; 294:47–55.

27. Lee IM, Cook NR, Gaziano JM, et al. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. JAMA 2005;294:56–65.

28. Ridker PM, Cook NR, Lee IM, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. N Engl J Med 2005; 352:1293–304.

29. Gates MA, Tworoger SS, Terry KL, et al. Talc use, variants of the GSTM1, GSTT1, and NAT2 genes, and risk of epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev 2008;17:2436–44.

30. Hollis BW. Quantitation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D by radioimmunoassay using radioiodinated tracers. Methods Enzymol 1997;282:174–86.

31. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.

32. Rosner B. Percentage points for a generalized ESD many-outier procedure. Technometrics 1983;25:165–72.

33. Harris SS, Dawson-Hughes B. The association of oral contraceptive use with plasma 25-hydroxyvitamin D levels. J Am Coll Nutr 1998;17:282–4.

34. Nesby-O'Dell S, Scanlon KS, Cogswell ME, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr 2002;76:187–92.

35. Pinter B, Kocijancic A, Marc J, Andolsek-Jeras L, Prezelj J. Vitamin D receptor gene polymorphism and bone metabolism during low-dose oral contraceptive use in young women. Contraception 2003;67:33–7.

36. Jurutka PW, Remus LS, Whitfield GK, et al. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Mol Endocrinol 2000;14:401–20.

37. Whitfield GK, Remus LS, Jurutka PW, et al. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Mol Cell Endocrinol 2001;177: 145–59.

38. Colin EM, Weel AE, Uitterlinden AG, et al. Consequences of vitamin D receptor gene polymorphisms for growth inhibition of cultured human peripheral blood mononuclear cells by 1, 25-dihydroxyvitamin D3. Clin Endocrinol (Oxf) 2000;52:211–6.

39. Arai H, Miyamoto KI, Yoshida M, et al. The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. J Bone Miner Res 2001;16:1256–64.

40. Yamamoto H, Miyamoto K, Li B, et al. The caudal-related homeodomain protein Cdx-2 regulates vitamin D receptor gene expression in the small intestine. J Bone Miner Res 1999;14:240–7.

41. Welsh J, Wietzke JA, Zinser GM, Byrne B, Smith K, Narvaez CJ. Vitamin D-3 receptor as a target for breast cancer prevention. J Nutr 2003;133:2425–33S.

42. Palmer-Toy DE, Szczepiorkowski ZM, Shih V, Van Cott EM. Compatibility of the Abbott IMx homocysteine assay with citrate-anticoagulated plasma and stability of homocysteine in citrated whole blood. Clin Chem 2001; 47:1704–7.

Downloaded from cancerres.aacrjournals.org on November 20, 2019. © 2009 American Association for Cancer Research.

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.

1. Tworoger SS, Gate MA, Lee I-M, Buring JE, Titus-Ernstoff L, Cramer D, Hankinson SE. Polymorphisms in the vitamin D receptor and risk of ovarian cancer in four studies. Cancer Res 2009;69:1885–91.

Published OnlineFirst 6/2/09.
©2009 American Association for Cancer Research.
doi:10.1158/0008-5472.CAN-69-12-COR1

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-3515

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

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

Shelley S. Tworoger, Margaret A. Gate, I-Min Lee, et al.

Cancer Res 2009;69:1885-1891. Published OnlineFirst February 17, 2009.

Updated version Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-08-3515

Cited articles This article cites 42 articles, 13 of which you can access for free at: http://cancerres.aacrjournals.org/content/69/5/1885.full#ref-list-1

Citing articles This article has been cited by 3 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/69/5/1885.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/69/5/1885. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.

Downloaded from cancerres.aacrjournals.org on November 20, 2019. © 2009 American Association for Cancer Research.