Research Article

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Vitamin D-Related Genetic Variants, Interactions with Vitamin D Exposure, and Breast Cancer Risk among Caucasian Women in Ontario

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Abstract

Background: Vitamin D, from diet and sunlight exposure, may be associated with reduced breast-cancer risk. This study investigated if candidate gene variants in vitamin D pathways are associated with breast cancer risk, or modify the associations between breast cancer and vitamin D exposure.

Methods: Breast cancer cases aged 25 to 74 years were identified from the Ontario Cancer Registry (histopathologically confirmed and diagnosed 2002–2003) and population-based controls were identified through random digit dialing of Ontario households. Saliva (DNA) was available for 1,777 cases and 1,839 controls. Multivariate logistic regression was used to evaluate associations between 19 single nucleotide polymorphisms (SNP) in vitamin D related genes, including vitamin D binding protein (*GC*), vitamin D receptor (*VDR*), and cytochrome P450 type 24A1 (*CYP24A1*). Statistical interactions were assessed using the likelihood ratio test.

Results: Some SNPs were found to be significantly associated with breast cancer risk. For example, breast cancer risk was associated with the *GC* rs7041 TT genotype (age-adjusted odds ratio (OR) = 1.23; 95% CI: 1.01, 1.51) and inversely with the *VDR Fok*1 (rs2228570) *ff* genotype (OR = 0.71; 95% CI: 0.57, 0.88). Few significant gene-environment interactions were observed between dietary vitamin D and genetic variants.

Conclusion: Our study suggests certain vitamin D related genetic variants may influence breast-cancer risk and we found limited evidence that genetic variants modify the associations between vitamin D exposure and breast cancer risk.

Impact: Variation in vitamin D-related genotypes may help to explain inconsistent results from previous epidemiologic studies and may lead to targeted prevention strategies. *Cancer Epidemiol Biomarkers Prev; 20(8); 1708–17. ©2011 AACR.*

Introduction

Evidence from laboratory and epidemiologic studies suggests that vitamin D may reduce the risk of breast cancer, but data from prospective studies and randomized controlled trials are limited (1–4). Vitamin D is produced in the skin following sufficient sunlight [ultraviolet (UV) B] exposure and is found in a limited number of foods (e.g., fortified milk and fatty fish), vitamin supplements and cod liver oil. Vitamin D from all sources undergoes hydroxylation in the liver to 25-hydroxyvitamin D [25(OH)D], the circulating form of vitamin D, which is the preferred vitamin D biomarker (5). Meta-analyses of 25(OH)D levels and breast-cancer risk have reported some inverse associations but not when restricted to prospective studies only (6, 7). A second hydroxylation is necessary to synthesize the active form of vitamin D 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], which has been shown in animal studies to promote cell differentiation and inhibit proliferation, potentially modifying cancer risk (8–10). Vitamin D and calcium metabolism are closely related (11), thus studies of vitamin D often also evaluate calcium.

Several genes are involved in vitamin D metabolism and variants in these genes may modify cancer risk (12, 13). 1,25$(OH)_2$D actions are mediated through the vitamin D receptor (*VDR*), a nuclear transcription factor which has been found in most cells in the body including breast cells (14). *VDR* gene variants are frequently evaluated in studies of vitamin D and *VDR* null mice are more likely than wild-type mice to show undifferentiated breast tissue and develop cancer when exposed to carcinogens (14). Another key gene in vitamin D metabolism is the

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vitamin D binding protein gene known as the group specific component (*GC*) gene. Both 25(OH)D and 1,25 $(OH)_2D$ circulate bound to the vitamin D binding protein and *GC* single nucleotide polymorphisms (SNP) have been associated with serum 25(OH)D levels in both candidate gene studies (15) and recent genome wide association studies (GWAS) (16, 17). Other genes with established roles in the vitamin D pathway include cytochrome P450 type 27B1 (*CYP27B1*), involved in activation of vitamin D through the hydroxylation of 25(OH)D to $1,25(OH)_2D$, and *CYP24A1*, which is involved in the degradation of 25(OH)D and $1,25(OH)_2D$ (12, 15). Fewer studies have evaluated SNPs in these genes in relation to vitamin D biomarkers (15). Emerging evidence from animal studies suggests that megalin and cubilin, cell surface receptors for the vitamin D binding protein, are involved in the uptake of 25(OH)D bound to vitamin D–binding protein by mammary cells (18, 19). Associations between SNPs in the megalin [lipoprotein2 (*LRP2*)] and cubilin (*CUBN*) genes have not been evaluated in relation to vitamin D biomarkers.

A recent comprehensive review identified 18 studies of vitamin D gene pathway polymorphisms and breast-cancer risk and found limited evidence of any consistent associations (12). Most previous studies have investigated *VDR* variants only and although the *Fok*I (rs2228570, previously rs10735810) and *Bsm*I (rs1544410) SNPs have been associated with breast cancer risk, the associations are relatively weak and many studies have been limited by small sample size (12, 13, 20). Few studies have evaluated other genes related to vitamin D metabolism; only two breast cancer studies have evaluated *GC* SNPs (rs7041 and rs4588) with conflicting results (21, 22). To the best of our knowledge, only one *CYP24A1* SNP has been investigated (22) and no studies have evaluated other *CYP* genes or variants in any genes involved with vitamin D–uptake by mammary cells (e.g., *LRP2* and *CUBN*). Beyond the independent gene effects, few studies have evaluated interactions with vitamin D or calcium intake from foods and supplements and no studies have included person-specific measures of sunlight exposure (12). The purpose of this study was to investigate the associations between variants in 6 vitamin D–related genes, possible gene-environment interactions with vitamin D and calcium exposures, and breast cancer risk, in a population-based case-control study of Ontario women.

Materials and Methods

Study design and subject recruitment

Data for this study were from the Ontario Women's Diet and Health Study (OWDHS), a large population-based case-control study that has been described previously (23, 24). Cases were women aged 25–74 years with a first primary pathologically confirmed invasive breast cancer (*in situ* carcinoma are excluded) identified through the Ontario Cancer Registry from 2002–2003. Consent and contact information was obtained from physicians to contact 4,109 eligible cases (96%) and questionnaires were completed by 3,101 cases (75% response rate). Controls were women with no personal history of breast cancer, frequency matched on 5-year age group (1:1) to cases, and identified through random digit dialing of Ontario households. More than 25,000 households were telephoned, 4,352 households with an eligible woman were identified, and questionnaires were completed by 3,471 controls (80% response rate). The University of Toronto Research Ethics Board reviewed and approved this study.

Data collection

Self-administered questionnaires were mailed to all identified cases and controls between 2003 and 2004. Study participants completed a detailed risk factor questionnaire and a modified Block 1998 Food Frequency Questionnaire. The risk factor questionnaire collected information on a range of known breast-cancer risk factors and other potential covariates. Data were also collected on variables that influence cutaneous vitamin D production (i.e., ethnicity, time spent outdoors, location of residence, and sun protection practices) and we have previously developed an algorithm to derive a "solar vitamin D–score" using ultraviolet radiation data and the aforementioned person-specific sun exposure variables (25). Information on vitamin D and calcium intake from both diet and supplements was obtained from a modified Block Food Frequency Questionnaire (26). Reliability of the FFQ was high for both vitamin D ($r = 0.76$; 95% CI: 0.66, 0.83), and calcium ($r = 0.80$; 95% CI: 0.71, 0.86). Validity was also moderately high for vitamin D ($r = 0.54$; 95% CI: 0.29, 0.79) and calcium ($r = 0.71$; 95% CI: 0.35, 1.00) (26). The nutrient analysis specific to vitamin D was modified to account for additional items (e.g., fatty fish, vitamin D supplements/cod liver oil) and Canadian-specific food fortification (27). We have previously reported on the associations between breast-cancer risk and vitamin D from food and supplements (23) and time spent outdoors/UV exposure (25) among all women in the OWDHS.

The risk-factor questionnaire also asked participants about their willingness to provide a biologic sample for DNA in the future. In 2007, cases and controls who agreed to provide DNA were mailed an Oragene kit for saliva (DNA) collection along with instructions, a consent form, and a prepaid reply envelope. The saliva collected in the Oragene kit was sent for DNA extraction at the Biospecimen Repository at Mount Sinai Hospital. Blood samples were not collected.

Response rate and sample size

The OWDHS enrolled 3,101 breast cancer cases and 3,471 controls; among these women 2,563 (83%) cases and 2,567 (74%) controls indicated they were willing to provide a DNA sample in the future. Study packages were mailed to 2,466 cases and 2,515 controls; 50 women requested no further contact, 39 were no longer alive,

95 had moved (addresses unavailable). Saliva samples were obtained from 1,776 cases (71%) and 1,839 controls (73%). DNA was isolated and banked for a total of 3,503 participants and genotyping results obtained on 3,451 women. The vast majority of women, 3,193 (93%) self-reported their ethnic or racial background as Caucasian. Due to the small sample size of non-Caucasians, and diverse ethnic mix (4% South or South East Asian, 1% Black and 2% other), the analysis for this study was restricted to Caucasians only; 130 cases and 114 controls were non-Caucasian and excluded from this analysis.

DNA preparation, single nucleotide polymorphisms selection, and genotyping

DNA was isolated from saliva samples using Buccal Amp DNA Extraction kits and stored at −80°C. Based on the literature 23 SNPs in 6 vitamin D related genes were identified for investigation. 18 of these genotyped candidate SNPs were in 4 well-established vitamin D related genes: *GC* (rs7041, rs4588), *VDR* (rs731236, rs739837, rs1544410, rs1989969, rs2228570, rs7975232, rs11568820, rs2107301, rs2238135), *CYP24A1* (rs2181874, rs2296241, rs4809958, rs6013905) and *CYP27B1* (rs703842, rs4646536, rs10877012). Other candidate SNPs in the megalin gene, *LRP2*, were selected based on one previous study that evaluated megalin in relation to prostate cancer; four *LRP2* SNPs showed some associations with prostate cancer risk (rs831003, rs2239598, 2268373, rs3944004) (28). We also included one SNP (rs1907362) in the *CUBN* gene that has been shown to have some functional importance in another pathway (29). Genotyping was conducted at the University Health Network's Analytical Genetics Technology Centre using the MassARRAY iPLEX Gold Sequenom Platform. Negative controls were included to ensure that genotype calls were not the result of nonspecific extension products. Cluster plots were inspected to ensure that the samples fell into 3 separate, distinct call types on the plots. The success rate was 98%; of the 3509 samples only 70 samples failed in the genotyping. Several *VDR* SNPs are reported here using restriction fragment length polymorphism (RFLP) nomenclature for the major and minor alleles, to stay consistent with previous literature, as follows: *Fok*I (rs2238135) alleles C = *F* and T = *f*; *Taq*I (rs731236) alleles T = *T* and C = *t*; *Bsm*I (rs1544410) G = *b* and A = *B*; and *Apa*I (rs7975232) A = *A* and C = *a*.

Statistical data analysis

The associations between each vitamin D or calcium exposure variable, all genetic polymorphisms, and breast cancer risk were evaluated using multivariate unconditional logistic regression analyses to estimate age-adjusted odds ratios (AORs) and 95% confidence intervals (95% CI). Rare homozygous genotypes with frequencies below 5% were combined with the heterozygotes if the ORs were in the same direction. For comparability with previous literature, a combined *GC* genotype was derived from rs4588 and rs7041 using previously described methods (21). The multiplicative interaction between each vitamin D or calcium exposure variable and each genetic polymorphism was evaluated by the statistical significance ($P < 0.05$) of the likelihood ratio test comparing the models with and without the product term. We also evaluated the interactions between each SNP and both menopausal status and family history of breast cancer in a first degree relative.

Confounding was evaluated by examining whether the AOR for any SNPs or vitamin D exposure variables changed by more than 10% between models with and without each covariate (30). Potential confounders included age at menarche, age at first live birth, parity, age at menopause, oral contraceptive use, hormone/estrogen replacement, benign breast disease, family history of breast cancer, education, mammography, body mass index (BMI), dietary fat, alcohol intake, and physical activity (daily, moderate, and strenuous activity during multiple age periods). None of the variables evaluated met our definition of a confounder for any models, thus we present age-adjusted models only.

All analyses were carried out using SAS version 9.1 (SAS Institute) with the exception that departures from Hardy–Weinberg equilibrium were evaluated using the R package "Genetics" (31). Among the control group, significant departure from Hardy–Weinberg equilibrium was detected for the 3 *CYP27B1* SNPs (rs4646536, rs10877012, rs703842; $P < 0.01$), which were in complete linkage disequilibrium (LD) ($r^2 = 1.0$), and *LRP2* rs2239598 ($P = 0.008$). Therefore, these 4 SNPs were excluded. A borderline significant *P*-value of 0.04 was also detected for *VDR Fok*I (rs2228570). However, because the frequency of the minor allele *f* was 0.40, similar to other European populations (32), this SNP was still included.

Results

Vitamin D genotyping data were available for a total of 1560 cases and 1633 controls. The mean age was 56 years of age, and most women were postmenopausal (70% of cases and 66% of controls; Table 1). Women who provided DNA were less likely to be current smokers, and more likely to have a higher income than women who did not provide DNA; age and body mass index were similar between the two groups (data not shown). In this study population, we previously reported significant inverse associations between breast-cancer risk and vitamin D supplements (23) as well as time spent outdoors and our solar vitamin D score at all periods of life (25). Among the smaller subset of women in this study (i.e., Caucasian women who provided DNA) only time spent outdoors was significantly associated with reduced breast cancer risk (>21 versus ≤6 hours per week OR = 0.63; 95% CI: 0.49–0.82) all other AORs were <1.0 as reported previously, but not statistically significant possibly due to insufficient power (Table 1).

The association between each vitamin-D related SNP and breast cancer risk is shown in Table 2. Odds ratios are

Table 1. Distribution of breast cancer cases and controls, and age-adjusted odds ratio (AOR) estimates for selected characteristics among only Caucasian women who provided DNA in the Ontario Women's Diet and Health Study

	Cases ($n = 1560$) *n* (%)	Controls ($n = 1633$) *n* (%)	AOR[a] (95% CI)
Age (years)			
25–49	647 (41)	774 (47)	
50–59	527 (34)	474 (29)	
60–74	386 (25)	385 (24)	
Menopausal status			
Premenopausal	470 (30)	565 (36)	1.00
Postmenopausal	1090 (70)	1068 (66)	1.00 (0.80–1.27)
Breast cancer in a first degree relative			
No	1090 (70)	1382 (86)	1.00
Yes	470 (30)	225 (14)	1.76 (1.46–2.12)
Parity			
Nulliparous	261 (17)	185 (11)	1.00
1	187 (12)	170 (11)	0.77 (0.58–1.02)
2–3	910 (59)	1005 (62)	0.61 (0.50–0.76)
≥4	187 (12)	250 (16)	0.48 (0.36–0.63)
Calcium from supplements			
0 mg/day	670 (43)	678 (42)	1.00
1–1000 mg/day	637 (41)	691 (43)	0.90 (0.78–1.06)
>1000 mg/day	244 (16)	256 (16)	0.89 (0.72–1.10)
Total Calcium intake (food and supplements)			
<1000 mg/day	679 (44)	683 (42)	1.00
1000–1499 mg/day	358 (23)	399 (25)	0.90 (0.75–1.08)
≥1500 mg/day	514 (33)	543 (33)	0.90 (0.76–1.06)
Vitamin D from supplements[b]			
0 IU/day	810 (52)	815 (50)	1.00
1–400 IU/day	650 (42)	702 (43)	0.91 (0.79–1.05)
>400 IU/day	91 (6)	108 (7)	0.80 (0.60–1.08)
Total vitamin D intake (food and supplements)			
<200 IU/day	460 (30)	463 (28)	1.00
200–599 IU/day	744 (48)	773 (48)	0.95 (0.81–1.12)
≥600 IU/day	347 (22)	389 (24)	0.87 (0.71–1.06)
Time spent outdoors during adolescence			
≤6 hours	167 (11)	165 (10)	1.00
7–12 hours	237 (16)	254 (16)	0.91 (0.69–1.20)
13–21 hours	595 (40)	604 (38)	0.99 (0.77–1.26)
>21 hours	506 (34)	565 (36)	0.90 (0.70–1.15)
Time spent outdoors during current age period[c]			
≤6 hours	405 (27)	408 (26)	1.00
7–12 hours	440 (29)	423 (27)	1.05 (0.87–1.27)
13–21 hours	522 (35)	530 (34)	1.01 (0.84–1.21)
>21 hours	132 (9)	213 (14)	0.63 (0.49–0.82)
Solar vitamin D score (lifetime)[d]			
Low	337 (22)	343 (22)	1.00
Medium	703 (47)	700 (45)	1.02 (0.85–1.23)
High	460 (31)	522 (33)	0.84 (0.69–1.03)

[a]All models were adjusted for age at diagnosis for cases and referent date (November 14, 2002) for controls, all other variables evaluated as potential confounders did not change the odds ratio by >10% when added to the models.

[b]Single product vitamin D supplements, cod liver oil, and multiple vitamins.

[c]Average number of hours spent outdoors from April to October during weekdays and weekends. Time spent outdoors was collected for 4 age periods of exposure (teenage years, 20–39, 40–59, and 60–74). Data shown are for current age period for each study participant.

[d]Solar vitamin D score was derived from time spent outdoors, skin color, sun protection practices, and erythemal ultraviolet radiation of residence in June 2004.

Table 2. Distribution of breast cancer cases and controls recruited by the Ontario Women's Diet and Health Study, Ontario, Canada, and age-adjusted odds ratio (AOR) estimates and 95% CIs for selected polymorphisms in vitamin D-related genes

Vitamin D-related SNPs	Cases $n = 1560$ n (%)[b]	Controls $n = 1633$ n (%)[b]	AOR[a] (95% CI)
GC c.1307C > A (rs4588)			
CC	792 (52)	846 (53)	1.00
CA	608 (40)	642 (40)	1.01 (0.87–1.17)
AA	135 (9)	120 (7)	1.20 (0.92–1.57)
GC c.1296 T > G (rs7041)			
GG	486 (31)	288 (18)	1.00
GT	760 (49)	782 (48)	1.11 (0.95–1.30)
TT	309 (19)	558 (34)	1.23 (1.01–1.51)[c]
Combined *GC* genotype[d]			
GC 1–1	790 (52)	845 (53)	1.00
GC 2–1	605 (40)	640 (40)	1.01 (0.89–1.17)
GC 2–2	134 (9)	118 (7)	1.22 (0.93–1.59)
*CYP*24A1c.640+1653C > T (rs2181874)			
GG	869 (56)	959 (59)	1.00
GA	584 (38)	575 (35)	1.11 (0.96–1.29)
AA	98 (6)	93 (6)	1.16 (0.86–1.56)
*CYP*24A1c.552C > T (rs2296241)			
AA	449 (29)	468 (29)	1.00
GA	777 (50)	791 (49)	1.03 (0.87–1.21)
GG	330 (21)	371 (23)	0.93 (0.76–1.13)
*CYP*24A1 c.641–66A > C (rs4809958)[e]			
TT	1098 (71)	1111 (69)	1.00
GT	403 (26)	465 (29)	0.88 (0.75–1.03)
GG	47 (3)	40 (2)	1.20 (0.78–1.85)
CYP24A1 c.733–162A > G (rs6013905)[e]			
TT	1103 (71)	1115 (68)	1.00
TC	405 (26)	472 (29)	0.87 (0.74–1.02)
CC	48 (3)	42 (3)	1.17 (0.76–1.78)
VDR c.1056T > A (rs731236, *Taq*I)[e]			
TT	552 (35)	594 (36)	1.00
Tt	744 (48)	763 (47)	1.01 (0.82–1.24)
tt	260 (17)	274 (17)	1.04 (0.89–1.22)
*VDR*c.1024+283G > A (rs1544410, *Bsm*I)[e]			
bb	538 (35)	592 (36)	1.00
Bb	746 (48)	749 (46)	1.09 (0.93–1.27)
BB	269 (17)	288 (18)	1.02 (0.83–1.25)
VDR c.2T > C (rs2228570, *Fok*I)			
FF	602 (39)	606 (37)	1.00
Ff	747 (48)	741 (46)	1.01 (0.86–1.17)
ff	197 (13)	280 (17)	0.71 (0.57–0.88)
VDR c.1025–49G > T (rs7975232, *Apa*I)[e]			
AA	438 (28)	455 (28)	1.00
Aa	766 (50)	803 (50)	1.00 (0.86–1.18)
aa	340 (22)	364 (22)	0.98 (0.80–1.20)
VDR g.1270G > A (rs11568820, *Cdx2*)			
GG	969 (64)	983 (62)	1.00
AG	456 (30)	550 (35)	0.83 (0.72–0.97)
AA	84 (6)	57 (4)	1.49 (1.05–2.11)
VDR c.*308C > A (rs739837, *Bgl*I)[e]			
TT	438 (28)	449 (28)	1.00
GT	760 (48)	807 (50)	0.99 (0.82–1.21)
GG	357 (23)	372 (23)	0.97 (0.82–1.15)
VDR c.-83–1453T > C (rs1989969)			
CC	583 (38)	615 (38)	1.00

(Continued on the following page)

Table 2. Distribution of breast cancer cases and controls recruited by the Ontario Women's Diet and Health Study, Ontario, Canada, and age-adjusted odds ratio (AOR) estimates and 95 CIs for selected polymorphisms in vitamin D-related genes (Cont'd)

Vitamin D-related SNPs	Cases $n = 1560$ n (%)[b]	Controls $n = 1633$ n (%)[b]	AOR[a] (95% CI)
CT	706 (46)	760 (47)	0.97 (0.83–1.13)
TT	253 (16)	238 (15)	1.12 (0.90–1.38)
VDR c.277+3260C > T (rs2107301)			
CC	782 (50)	865 (53)	1.00
CT	638 (41)	631 (39)	1.11 (0.96–1.28)
TT	130 (8)	123 (8)	1.18 (0.90–1.54)
VDR c.-83–1633G > C (rs2238135)			
GG	914 (59)	938 (58)	1.00
GC	546 (35)	609 (37)	0.92 (0.79–1.06)
CC	90 (6)	81 (5)	1.15 (0.84–1.58)
CUBN c.3829+233C > T (rs1907362)			
GG	1369 (91)	1478 (93)	1.00
Pooled GA/AA	141 (9)	112 (7)	1.36 (1.05–1.78)
LRP2 c.1772+64C > G (rs831003)			
CC	916 (59)	1008 (62)	1.00
GC	554 (36)	533 (33)	1.15 (0.99–1.34)
GG	75 (5)	76 (5)	1.09 (0.78–1.52)
LRP2 c.6470–739G > C (rs2268373)			
GG	833 (55)	879 (55)	1.00
CG	573 (38)	604 (38)	0.99 (0.85–1.15)
CC	104 (7)	104 (7)	1.07 (0.80–1.42)
LRP2 c.12462–686T > G (rs3944004)			
TT	897 (58)	947 (59)	1.00
GT	565 (37)	566 (35)	1.06 (0.91–1.22)
GG	76 (5)	94 (6)	0.85 (0.62–1.17)

[a]All models were adjusted for age at diagnosis for cases and referent date (November 14, 2002) for controls (all other variables evaluated as potential confounders did not change the odds ratio by >10% when added to the models).

[b]Numbers may not add to total due to missing values.

[c]Linear dose-response trend, $P < 0.05$

[d]Combined GC genotypes derived as follows: *GC1*–1 = rs7041-GG, TG, or TT and rs4588-CC; GC2–1 = rs7041-TG or TT and rs4588-CA; GC2–2 = rs704-TT and rs4588-AA.

[e]The following SNPs are known to be in high linkage disequilibrium: rs731236 and rs1544410 ($r^2 = 1.0$); rs7975232 and rs739837 ($r^2 = 1.0$); rs4809958 and rs6013905 ($r^2 = 1.0$). All other combinations of SNPs within genes are not in high linkage disequilibrium ($r^2 < 0.6$) or unknown if data are not available in Hapmap.

adjusted for age only. *GC* rs7041 TT genotype was associated with increased breast-cancer risk (OR = 1.23; 95% CI: 1.01–1.51); no significant associations were observed for rs4588 or the combined *GC* genotype. Two of the ten *VDR* SNPs evaluated were significantly associated with breast-cancer risk. The *Fok*I (rs2228570) *ff* genotype was associated with reduced breast cancer risk (OR = 0.71; 95% CI: 0.57–0.88). In contrast, the *Cdx*2 (rs11568820) AG genotype was associated with reduced breast-cancer risk (OR = 0.83; 95% CI: 0.72–0.97), whereas the AA genotype was associated with increased risk (OR = 1.49; 95% CI: 1.05–2.11). The *CUBN* GA/AA genotype was also associated with increased risk (OR = 1.36; 95% CI: 1.05–1.75). No significant associations were observed between any of the *CYP24A1* or *LRP2* polymorphisms and breast-cancer risk.

The associations between vitamin D or calcium and breast-cancer risk stratified by genotype for all statistically significant interactions ($P < 0.05$) are shown in Table 3. All other interactions were not statistically significant. After stratification by genotype, significant inverse associations between vitamin D intake (from foods and supplements combined or supplements only) and breast-cancer risk were observed among women homozygous for the wild-type alleles of *VDR* rs2238135 and *CUBN* rs1907362. For calcium intake, significant inverse associations were observed among women with the *VDR Cdx*2 (rs11568820) GG and *CYP24A1* rs2181874 GG genotypes. No significant interactions were observed for any of the sun-exposure variables (time spent outdoors or solar vitamin D score).

Table 3. AOR estimates and 95% CI for vitamin D variables (cases versus controls) stratified by genotypes among Caucasian women only for all interactions with $P < 0.05$ between any vitamin D or calcium exposure variables and the 23 vitamin D related SNPs

Vitamin D or calcium variable	N cases/N controls	AOR[a] (95% CI)	N cases/N controls	AOR (95% CI)	N cases/N controls	AOR (95% CI)	P[b]
			VDR g.1270G > A (rs11568820) Cdx2				
	GG		**GA**		**AA**		
Total calcium intake[c]							
<1000 mg/day	414/396	1.00	204/247	1.00	33/22	1.00	0.03
1000–1499 mg/day	216/267	0.77 (0.61–0.97)	115/110	1.27 (0.92–1.75)	17/11	0.77 (0.27–2.14)	
≥1500 mg/day	332/314	0.95 (0.77–1.18)	135/191	0.80 (0.60–1.08)	34/24	0.71 (0.32–1.62)	
			VDR c.-83–1633G > C (rs2238135)				
	GG		**GC**		**CC**		
Total vitamin D intake[c]							
<200 IU/day	287/243	1.00	143/199	1.00	28/21	1.00	0.0006
200–599 IU/day	413/469	0.73 (0.58–0.90)	284/257	1.54 (1.17–2.03)	41/44	0.67 (0.32–1.38)	
≥600 IU/day	209/221	0.76 (0.59–0.98)	115/150	1.06 (0.77–1.48)	21/16	0.98 (0.40–2.38)	
			CYP24A1c.640+1653C > T (rs2181874)				
	GG		**GA**		**AA**		
Total Calcium intake[c]							
<1000 mg/day	390/379	1.00	238/253	1.00	481/49	1.00	0.003
1000–1499 mg/day	216/234	0.90 (0.71–1.14)	119/143	0.88 (0.65–1.19)	21/21	0.97 (0.46–2.07)	
≥1500 mg/day	260/343	0.70 (0.56—0.87)	221/174	1.26 (0.96–1.66)	29/23	1.23 (0.61–2.48)	
Calcium from supplements							
0 mg/day	392/388	1.00	224/246	1.00	48/42	1.00	0.02
1–1000 mg/day	353/407	0.84 (0.69–1.03)	249/244	1.09 (0.84–1.41)	33/38	0.68 (0.35–1.34)	
>1000 mg/day	121/161	0.69 (0.52–0.92)	105/80	1.33 (0.93–1.89)	17/13	1.13 (0.47–2.72)	
			CUBN c.3829+233C > T (rs1907362)				
	GG		**pooled GA/AA**				
Total vitamin D intake[c]							
<200 IU/day	404/411	1.00	37/40	1.00			0.02
200–599 IU/day	661/702	0.94 (0.79–1.12)	66/51	1.32 (0.73–2.39)			
≥600 IU/day	295/358	0.81 (0.66–0.99)	38/20	2.03 (0.98–4.22)			
Vitamin D from supplements							
0 IU/day	710/734	1.00	71/61	1.00			0.04
1–400 IU/day	576/635	0.92 (0.79–1.07)	56/46	0.96 (0.56–1.64)			
>400 IU/day	74/102	0.71 (0.51–0.97)	14/4	3.18 (0.96–10.46)			

[a]All models were adjusted for age at diagnosis for cases and referent date (November 14, 2002) for controls.
[b]*P* value for interaction assessed using the likelihood ratio statistic after the addition of the product term (exposure x genotype) to the model.
[c]Total intake from food and supplements combined.

No statistically significant interactions were observed between any polymorphisms and menopausal status or family history of breast cancer. However, among postmenopausal women only, *CYP24A1* rs2181874 GA genotype was associated with increased breast-cancer risk (OR = 1.21; 95% CI: 1.01–1.45). Whereas, among premenopausal women only, significant associations were observed for the following genotypes: *VDR Taq*I (rs731236) *Tt* (OR = 1.33; 95% CI: 1.01–1.74); *VDR Bsm*I (rs1544410) *Bb* (OR = 1.37; 95% CI: 1.04–1.80); and *LRP2* rs3944004 GG (OR = 0.48; 95% CI: 0.26–0.88). When the data are stratified by family history of breast cancer, several associations appear stronger among women with a family history only: *VDR Fok*I *ff* (OR = 0.56; 0.32–0.98), *VDR Cdx*2 AG (OR = 0.58; 95% CI: 0.40–0.85), and *CUBN* GA (OR = 2.17; 95% CI: 1.04–4.57).

Discussion

This population-based case-control study found that SNPs in some genes involved in the vitamin D pathway were associated with breast-cancer risk. Breast-cancer risk was significantly associated with the following 4 polymorphisms: *GC* rs7041, *VDR Fok*I (rs2228570), *VDR Cdx*2 (rs11568820), and *CUBN* rs1907362. We report that some genotypes modified the associations between vitamin D or calcium intake and breast-cancer risk, but not sun-exposure measures. Furthermore, no significant

interactions were observed between any genotype and either menopausal status or family history of breast cancer.

Most previous studies of vitamin D–related gene variants have included *VDR* variants only, in particular the *Bsm*I and *Fok*I polymorphisms (12). Our finding of an inverse association between the *Fok*1 *ff* genotype and breast-cancer risk is not consistent with recent meta-analyses (13, 32), or a pooled analysis of 6 cohorts (33), that suggest the *Fok*I *ff* genotype is associated with a 14% to 16% increase in breast-cancer risk. However, only two original studies (34, 35) and one additional cohort within the pooled analysis (33) reported significant positive associations; among most other Caucasian populations no associations were observed (22, 33, 36–40). It is unclear why our study results would differ from the previous studies. The *Fok*I minor allele *f* is associated with the production of a longer *VDR* protein that is less transcriptionally active (41). This *f* allele has also been associated with higher 25(OH)D concentrations (15); however, the mechanism for an association between *VDR* SNPs and 25(OH)D levels is not well understood. Among controls in our study population of Caucasians only, the *Fok*1 genotype showed significant departure from HWE, but the *P*-value ($P = 0.04$) was only borderline significant and the distribution of genotypes is similar to other studies. Future studies are needed to clarify the association between the *Fok*1 genotype and breast cancer risk.

With regards to other *VDR* SNPs, our results are consistent with previous meta-analyses of more than 12 studies that suggest no significant associations between *Bsm*I (rs1544410) and breast-cancer risk (13, 32). *Bsm*I *BB* genotype has been associated with lower risk of advanced breast cancer (33), but we are unable to evaluate cancer stage within our study. Previous studies of *Apa*I, and *Taq*I have been inconclusive (12) and, consistent with our results, a meta-analysis found no evidence of an association (32). Other *VDR* SNPs have been evaluated less frequently; the significant association that we observed between the *Cdx*2 genotype and breast-cancer risk was not observed in one previous study of this SNP (36). Elsewhere, *Bgl*I was associated with reduced risk among a subgroup of women with advanced breast cancer and medium skin pigmentation (39); we found no association among our study of Caucasian women only.

Several studies, including two recent GWAS (16, 17), have shown lower 25(OH)D levels are associated with rs7041 TT and rs4588 AA genotypes, or other *GC* SNPs in LD (15). Despite the known functionality of *GC*, only two studies have investigated *GC* SNPs in relation to breast cancer. The first study found no significant associations between either rs7041 or rs4588 and breast-cancer risk; however, this was among a relatively small sample of only 500 postmenopausal cases and 500 controls from the United States (22). The second study found the *GC*2 genotype (rs7041 TT and rs4588 AA combined genotypes) was significantly associated with reduced breast-cancer risk among 1,391 postmenopausal cases and 1,365 postmenopausal controls in Germany (21). Our results of a positive association between the rs7041 TT genotype and breast-cancer risk are not consistent with Abbas and colleagues (21), but they are in the expected direction if vitamin D is inversely associated with breast-cancer risk because this genotype is associated with lower 25(OH)D.

We are not aware of any previous breast-cancer studies evaluating any SNPs in *LRP2*, *CUBN*, or any other genes involved in the uptake of *GC* bound 25(OH)D by breast cells. Future studies are needed to confirm or refute our findings that *CUBN* is associated with breast-cancer risk and the interaction with vitamin D intake. One previous study of only one *CYP24A1* SNP (rs2296241) reported no association with breast-cancer risk among postmenopausal women (22). We also found no association between this SNP or another three *CYP*24A1 SNPs and breast cancer risk; although significant interactions were observed between rs2181874 and calcium intake.

Few previous studies have evaluated gene-environment interactions between *VDR* or *GC* polymorphisms and either dietary vitamin D (22, 34), serum 25(OH)D (21, 34, 36, 42), or calcium intakes (22, 33), and there is no consistent evidence of effect modification. It is a strength of our study that we had detailed measures of vitamin D exposure, from both sun and diet (including food and supplements), and calcium intake. Sun exposure measures, however, are only weakly correlated with 25(OH)D (43), which was not available in our study, introducing the potential for misclassification. 25(OH)D is the preferred biomarker for vitamin D, reflecting intake from both sunlight and diet, but it may not adequately represent long-term vitamin D–levels or the period prior to cancer diagnosis of interest in case-control studies (44). Although measurement error is a concern for our measures of vitamin D from sunlight and diet, our observed levels of vitamin D–intake were similar to several other North American studies and population-based surveys (27). Sun exposure is an important source of vitamin D, and there have been no previous studies of genetic interactions with person-specific sun exposure; no significant interactions have been observed for proxy measures such as skin pigment (39) and ultraviolet radiation of residence (22). We report some significant interactions for dietary vitamin D or calcium intakes but not for any of the sun-exposure measures. There is no reason to suspect different metabolic pathways for vitamin D from dietary intake versus sun exposure and the lack of significant interactions for sun exposure measures may be due to residual confounding or measurement error. We also cannot rule out the possibility that our gene-environment interaction findings may be due to chance. Only 6 of the 133 (<5%) potential interactions identified as important *a priori* were statistically significant and given the 5% significance level we would expect this many significant findings due to chance alone.

There is inconsistent evidence suggesting the associations between vitamin D intake and breast-cancer risk may (45, 46) or may not vary by menopausal status

(47, 48), and studies that have evaluated vitamin D related genotypes by menopausal status have also been inconsistent (33, 34, 49, 50). In our study, we did not find any significant interactions between any SNP and menopausal status, although stratified analyses suggested some differences that should be investigated in future studies with larger sample size. Elsewhere, family history of breast cancer has been found to modify the associations between *VDR* polymorphisms and breast-cancer risk (35, 51). Although we did not find any significant interactions, we cannot rule out the possibility of effect modification by family history of breast cancer.

There are many challenges associated with the measurement of vitamin D in observational studies (44) and measurement error may explain the somewhat inconsistent findings from previous studies of vitamin D and breast cancer. We comprehensively evaluated a range of potential confounders that may be associated with healthy lifestyle (e.g., physical activity, smoking, multivitamin) and none met our definition of a confounder. As with all observational studies we cannot rule out the possibility that our findings may be due to chance or recall bias. However, the potential for recall bias is likely minimal as there is no obvious reason why cases and controls would differentially report their vitamin D exposures and genotypes are likely independent of study participation. Although we restricted to Caucasian ethnicity only, the potential for confounding by population stratification remains as some admixture is expected among women of self-reported Caucasian ethnicity. It is well-established that *VDR* genotypes vary widely by ethnicity (41) and future studies are needed to evaluate these associations among other racial or ethnic groups. Despite our relatively large sample size, it is possible that we did not have the power to detect some associations or interactions especially among subgroups. Furthermore, genotyping quality did not seem to be of concern in our study but duplicate samples were not available to formally assess concordance for each SNP. Lastly, there is the possibility of survival bias because DNA was collected 3 years after study participants were originally recruited; however, breast cancer survival is very high with a five-year relative survival ratio of 87% (52).

We present one of the most comprehensive studies of vitamin D related genes and breast-cancer risk to date and, in addition to contributing to the literature on *VDR* and *GC* SNPs, our study provides novel results for several other SNPs. The results of our study suggest certain genetic variants that may influence vitamin D status are associated with breast-cancer risk. However, few polymorphisms were found to modify the associations between vitamin D intake and breast-cancer risk. Two GWAS, published after genotyping was complete for our study, identified other SNPs associated with 25(OH)D that were not included in our study (16, 17). Future studies are needed to confirm our findings and should consider these newly identified SNPs and a more comprehensive tag SNP selection approach. Because vitamin D intake is potentially modifiable through diet, supplements or limited sun exposure, understanding these associations may be of importance to breast-cancer prevention and may ultimately lead to the identification of genetic subgroups for targeted prevention strategies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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