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Research Article

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Single-Nucleotide Polymorphisms in Vitamin D–Related Genes May Modify Vitamin D–Breast Cancer Associations

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Abstract

Background: We previously observed that high serum 25-hydroxyvitamin D (25(OH)D; >38.0 ng/mL) was inversely associated with breast cancer. Here, we examined effect modification by SNPs in vitamin D–related genes.

Methods: The Sister Study enrolled 50,884 U.S. women who had a sister with breast cancer, but who had never had breast cancer themselves. Using a case–cohort design, we compared 1,524 women who developed breast cancer within 5 years to 1,810 randomly selected participants. We estimated ratios of HRs (RHRs) for the 25(OH)D–breast cancer association per copy of the minor allele using Cox proportional hazards models. We considered 82 SNPs in 7 vitamin D–related genes (*CYP24A1*, *CYP27B1*, *CYP2R1*, *GC*, *DHCR7*/*NADSYN1*, *RXRA*, and *VDR*). We also tested gene-based interactions with 25(OH)D.

Results: The SNP with the smallest interaction *P* value was rs4328262 in *VDR* ($P = 0.0008$); the 25(OH)D HR was 0.92 [95% confidence interval (CI), 0.68–1.24] among those homozygous for the common allele, and the minor allele was estimated to decrease the HR by 33% per copy (RHR = 0.67; 95% CI, 0.53–0.85). Five other *VDR* SNPs showed evidence of interaction at $P < 0.05$, as did one SNP in *CYP2R1* and one in *RXRA*. As a group, the 82 SNPs showed evidence of multiplicative interaction with 25(OH)D ($P = 0.04$). In gene-based tests, only *VDR* showed strong evidence of interaction ($P = 0.04$).

Conclusions: SNPs in vitamin D–related genes may modify the association between serum 25(OH)D and breast cancer.

Impact: This work strengthens the evidence for protective effects of vitamin D. *Cancer Epidemiol Biomarkers Prev; 26(12); 1761–71. ©2017 AACR.*

Introduction

Vitamin D is a prohormone with known anticarcinogenic properties, including the ability to regulate cell growth and proliferation, stimulate apoptosis, and bolster immune response. Both vitamin D_2 and D_3 can be acquired through dietary sources and supplements, but most vitamin D_3 is produced by a reaction between ultraviolet B radiation and cutaneous 7-dehydrocholesterol (1). D_2 and D_3 are subsequently metabolized into 25-hydroxyvitamin D (25(OH)D) by the liver. 25(OH)D is then converted to the biologically active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), by the kidney (1). This conversion also occurs in other tissues, including the breast. Excess 25(OH)D and $1,25(OH)_2D$ are catabolized into a biologically inactive form, calcitroic acid ($24,25(OH)_2D$), and excreted (2).

Genes directly involved in this metabolic process include *DHCR7*, *CYP24A1*, *CYP27B1*, *CYP2R1*, *GC*, *VDR*, and *RXRA*. *DHCR7* encodes 7-dehydrocholesterol reductase, an enzyme that converts 7-dehydrocholesterol to cholesterol. Cytochrome P450 enzymes facilitate each of the three metabolic conversions: D_2 or D_3 to 25(OH)D (*CYP2R1*), 25(OH)D to $1,25(OH)_2D$ (*CYP27B1*), and 25(OH)D or $1,25(OH)_2D$ into $24,25(OH)_2D$ (*CYP24A1*).

While circulating in serum, 25(OH)D and $1,25(OH)_2D$ are usually bound to vitamin D–binding proteins or, to a lesser degree, albumin (3). Vitamin D–binding proteins are transcribed from the *GC* gene. Unbound $1,25(OH)_2D$ is considered "bioavailable" and is free to attach to a vitamin D receptor (VDR), encoded by the *VDR* gene. VDR plays a vital role in the regulation of many genes with diverse functions, as it can activate transcription at binding sites throughout the genome (4, 5). VDR can bind to these vitamin D response elements as a homodimer, but in the presence of $1,25(OH)_2D$, it preferentially forms heterodimers with retinoid X receptors (the gene product of *RXRA*; ref. 6), which may affect its affinity for certain sites.

Most previous studies of gene-by-environment interaction for vitamin D and breast cancer have focused on SNPs in *VDR* with putative functional roles. Primary candidates have included rs2228570 (also known as *FokI*), rs731236 (*TaqI*), and rs1544410 (*BsmI*). Some of these studies observed evidence

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of multiplicative interactions between these SNPs and vitamin D (measured as 25(OH)D, dietary intake, or sunlight exposure) on breast cancer risk (7–11), but others observed no noteworthy interactions for these or other vitamin D–related gene variants (12–18).

In a recent prospective observational study of vitamin D and incident breast cancer, we reported that women with 25(OH)D levels in the highest quartile (>38.0 ng/mL) had a reduced risk of breast cancer over the subsequent 5 years, relative to women with 25(OH)D levels in the lowest quartile [≤24.6 ng/mL; adjusted HR = 0.79; confidence interval (CI), 0.63–0.98; ref. 19]. The association may be limited to postmenopausal women. Our study was unique in that we examined prospectively measured, recent vitamin D levels and used liquid chromatography-mass spectrometry to measure total 25(OH)D ($25(OH)D_3 + 25(OH)D_2 + 3\text{-epi-}25(OH)D_3$). Although many long-term prospective cohort studies have reported null findings (8, 20–29), some have reported modestly protective (although often not statistically significant) associations (30–35), and a recent meta-analysis of prospective studies reported a protective association between plasma vitamin D levels and breast cancer in analyses limited to postmenopausal women (36). Furthermore, many previous case–control studies have reported strong and statistically significant inverse associations of similar magnitude to our reported results (37–41). This epidemiologic evidence suggests that recent 25(OH)D levels are associated with reduced risk of postmenopausal breast cancer.

Here, we explore how inherited genetic variants may affect individual responses to vitamin D and modify the association between 25(OH)D and breast cancer risk. Identification of such factors would help us understand the biological mechanisms behind vitamin D's protective effects and identify individuals with altered susceptibility to those effects. We are particularly interested in the influence of SNPs in genes involved in vitamin D metabolism. For our main analysis, we considered the vitamin D metabolism genes selected *a priori* and discussed here, *DHCR7* (and the adjacent gene, *NADSYN1*), *CYP24A1*, *CYP27B1*, *CYP2R1*, *GC*, *VDR*, and *RXRA*, but in secondary exploratory analyses, we also examined 1,439 SNPs in 89 other potentially related genes, including those selected from previously published breast cancer gene-by-environment interaction or vitamin D genome-wide association studies (13, 42, 43), those involved in other vitamin D–related pathways (44–46), and genes regulated through vitamin D response elements (47).

Materials and Methods

To explore how SNPs in vitamin D–related genes might modify the association between total 25(OH)D and incident breast cancer, we used data from the Sister Study (data release 4.1, updated 7/2014), a prospective cohort of 50,884 women who had a sister with breast cancer, but had never had breast cancer themselves at enrollment (2003–2009). To be eligible, participants had to be between 35 and 74 years old and reside in the United States or Puerto Rico. The Sister Study was approved by the institutional review boards of the National Institute of Environmental Health Sciences and the Copernicus Group.

All Sister Study participants completed a computer-assisted telephone interview at baseline and were visited by trained examiners who obtained written informed consent and took measurements and blood samples. Participants are regularly asked to complete annual follow-up questionnaires, and those who go on to develop breast cancer are then asked to provide detailed information about their diagnosis. We obtained information about breast cancers from medical records, whenever possible, but otherwise relied on patient self-report. To date, we have retrieved medical records for 82% of self-reported breast cancer cases, 99% of whom were confirmed as true cases. Because the false self-report rate is so low, we consider all self-reported cases to be true cases.

We used a case–cohort design to investigate the association between serum 25(OH)D and incident breast cancer. Cases were Sister Study participants diagnosed with invasive breast cancer or ductal carcinoma *in situ* within 5 years of their baseline interview ($n = 1611$). The comparison group was 1,843 women randomly selected from the Sister Study cohort (68 of whom were included among the 1,611 because they had developed breast cancer within 5 years of baseline).

Serum 25(OH)D assessment

As described previously (19), we used liquid chromatography-mass spectrometry to measure each of three vitamin D metabolites, $25(OH)D_3$, $25(OH)D_2$, and $3\text{-epi-}25(OH)D_3$. The concentration sum of these metabolites was used to estimate overall available serum vitamin D. $25(OH)D_3$ was the most prevalent metabolite, making up approximately 83% of the total.

We adjusted the total 25(OH)D values for time of year of blood collection using LOESS regression (48), allowing seasonal effects to vary by race/ethnicity, supplement use, and latitude. We also adjusted for assay batch effects using a random effects model. On the basis of our previous findings that 38.0 ng/mL was a risk-related cutoff point for breast cancer, we categorized women based on whether their season and batch-adjusted total 25(OH)D levels were above or below this threshold.

Genotype analysis

The vitamin D substudy was nested within a slightly larger case–cohort sample that was originally selected for genotype analysis. Genotyping of a subset of Sister Study participants was conducted using Illumina's Infinium OncoArray-500K beadchip platform via participation in the GAME-ON consortium (49). The OncoArray panel includes a genome-wide backbone of 230,000 tag SNPs. The remaining SNPs were selected because they were shown to be associated with breast, colorectal, lung, prostate, or ovarian cancer; located in fine-mapped regions around those variants; used to assess ancestry or quantitative traits; or because they had a putative functional role or association with important functions, such as DNA repair.

Data cleaning and quality control filtering were conducted by the GAME-ON consortium, with further details described elsewhere (49). After these exclusions, 3,363 Sister Study participants [1,829 in the subcohort (including 67 cases) and 1,534 additional cases] provided both genotype and 25(OH)D data. The final analyses sample consisted of 1,810 random subcohort members (including 66 cases) and 1,524 additional cases who had complete data for the key covariates, listed below.

For this specific candidate gene study, we identified SNPs located within 2,000 base pairs of the gene transcription start and termination sites, as defined by University of California Santa Cruz Genome Browser (GRCh37/hg19; RefSeq notation; ref. 50). We then excluded SNPs with minor allele frequencies less than 2% and one of each pair of SNPs that were in very high

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linkage disequilibrium ($r^2 > 0.95$) with one another. No exclusions were made based on Hardy–Weinberg equilibrium. In total, we evaluated the gene-by-environment interaction effect estimates of 82 SNPs in 7 genes (35 in *VDR*, 16 in *RXRA*, 4 in *CYP2R1*, 14 in *CYP24A1*, 8 in *GC*, 4 in *DHCR7*/*NADSYN1*, and 1 in *CYP27B1*).

Ancestry proportions for all Sister Study participants were defined using 2,294 ancestry informative markers (AIM) selected by the GAME-ON consortium. Genotypes for these markers were compared with those from three HapMap populations (CEU, YRI, and CHB) using Bayesian clustering methods, allowing for admixture. Using STRUCTURE (v2.3.4), we ran the clustering analysis for 35,000 iterations, discarding the first 10,000 as burn-in. We included proportion CEU ancestry and proportion YRI ancestry as covariates in all models, as well as self-reported race/ethnicity. As a sensitivity analysis, we adjusted the multivariate models for the first 10 principal components, defined using the selected AIMs, rather than ancestry. The results were nearly identical, and we have not included them here.

Statistical analysis

We used Cox proportional hazards models, with age as the primary time scale, to evaluate the joint association between total 25(OH)D and each candidate SNP on the risk of breast cancer. The model included terms for the main effect of vitamin D exposure (dichotomous: total 25(OH)D levels >38.0 ng/mL vs. ≤38.0 ng/mL), the main effects of each genotype (assuming a codominant model, so that the main SNP effects are saturated), and a 25(OH)D-by-SNP interaction term (with genotype coded as number of copies of the minor allele). The primary effect estimate of interest was the exponentiated beta coefficient for the multiplicative interaction term, which can be interpreted as the ratio of HRs (RHR), or the relative increase in the HR for the 25(OH)D–breast cancer association per copy of the minor allele. We calculated 95% CIs for this parameter using robust variance estimators to account for the case–cohort design (51, 52).

Models were adjusted for the following covariates, as determined at baseline and selected *a priori*: ancestry (proportion CEU and YRI), self-reported race/ethnicity (categorical), education (categorical), menopausal status (pre- or postmenopausal), physical activity during the preceding year (categorical), current hormone therapy use (none, estrogen plus progestin, or unopposed estrogen), current hormonal birth control use (yes/no), history of osteoporosis (yes/no), body mass index (BMI; continuous), alcohol consumption in the previous year (never/former drinker, current drinker <1 drink/day, current drinker ≥1 drink/day), parity (0, 1, 2, or ≥3 births) and a BMI-by-menopausal-status interaction term. Women were considered postmenopausal if they had experienced natural menopause and not had a menstrual period within the last year (63% of postmenopausal women), had previously had both ovaries removed (22%), or had had a hysterectomy with retained ovaries but were older than 55 (15%). As we previously found that the 25(OH)D–breast cancer association was stronger in postmenopausal women, we conducted analyses within this subgroup. We also examined modification by first-degree family history of breast cancer (1 first-degree relative vs. >1 first-degree relatives). All statistical analyses were carried out in SAS (v9.3, Cary, NC) or R (v3.2.1).

For each selected candidate SNP, we estimated HRs for SNP–breast cancer associations. These associations were assessed using a Cox proportional hazards model, adjusting for self-reported race/ethnicity and ancestry only, with genotype coded log-additively ($n = 1{,}829$ from the subcohort and 1,600 cases, after exclusions for missing covariates). We additionally conducted multi-SNP, gene-based gene-by-environment tests using the gene–environment set association test (GESAT; ref. 53). GESAT utilizes a variance component score test within a generalized linear model framework to test the association of a vector of SNP-by-environment interaction terms. Because this test was developed for case–control studies rather than a case–cohort design, we modified our analysis to compare genotypes and 25(OH)D levels of women who developed breast cancer within five years of enrollment ($n = 1{,}590$ cases with complete covariate information) to those who did not develop breast cancer during that time period ($n = 1{,}744$ noncases/controls with complete covariate information). We included the same covariates as in the single SNP interaction analyses, as well as age at blood draw.

Our primary analysis was a candidate gene study of 82 nonindependent SNPs from 7 genes known to be involved in vitamin D metabolism. We did not correct our main analysis for multiple comparisons, but note that based on the correlation structure of the included SNPs, the number of effective tests was 56 (54). As we considered our secondary analysis of the remaining 1,439 SNPs to be exploratory, we present both uncorrected *P* values and FDR q-values (55). Here, a q-value <0.05 was considered noteworthy.

Results

As expected, cases were slightly older than those in the randomly sampled subcohort (mean of 57.4 years vs. 55.3 years) and had lower average 25(OH)D levels (31.0 ng/mL vs. 31.8 ng/mL; Table 1). Compared with the subcohort, cases were more highly educated, more likely to be postmenopausal, more likely to be obese, more likely to be current hormone therapy users, and more likely to have two or more first-degree relatives with breast cancer. Cases were less likely to have a history of osteoporosis.

Eight of the 82 SNPs in the 7 candidate vitamin D metabolism genes had a statistically significant interaction with 25(OH)D in relation to breast cancer (based on an uncorrected $P < 0.05$; Table 2). The SNP with the lowest *P* value for interaction was rs4328262 in *VDR* ($P = 0.0008$). Here, the HR for the 25(OH)D effect in noncarriers of the variant allele was 0.92 (95% CI, 0.68–1.24), and each copy of the minor allele was associated with a 33% decrease in the HR between 25(OH)D and breast cancer (RHR = 0.67; 95% CI, 0.53–0.85).

Five other *VDR* SNPs also showed evidence of gene-by-vitamin D interaction (rs11168287, rs4237855, rs17883984, rs4516035, and rs2239182). For half of the six significant *VDR* SNPs, the RHR was below 1.00, indicating that 25(OH)D had a stronger inverse association with breast cancer among carriers of the variant allele. The other three SNPs had RHRs that exceeded 1.00, meaning that 25(OH)D had a stronger inverse association with breast cancer among noncarriers. These 6 SNPs were not in high linkage disequilibrium with one another within our subcohort sample ($r^2 < 0.80$; as seen in Supplementary Table S1), although some showed moderate

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Table 1. Characteristics of participants included in vitamin D and genetic substudies (Sister Study, 2003–2009)

Characteristics	Random subcohort ($n = 1{,}829$); *N* (%)	Breast cancer cases[a] ($n = 1{,}601$); *N* (%)
Age at blood draw; mean (SD)	55.3 (8.9)	57.4 (8.9)
Total 25(OH)D level; mean (SD)	31.8 (10.5)	31.0 (10.1)
Total 25(OH)D >38 ng/mL	460 (25)	330 (21)
Race/ethnicity		
Non-Hispanic white	1,576 (86)	1,365 (85)
Non-Hispanic black	134 (7)	122 (8)
Hispanic	81 (4)	63 (4)
Other	38 (2)	50 (3)
Education level		
High school or less	292 (16)	245 (15)
Some college	639 (35)	514 (32)
Bachelor's degree	468 (26)	421 (26)
Graduate degree	430 (24)	420 (26)
Menopausal status		
Premenopausal	613 (34)	461 (29)
Postmenopausal	1,216 (66)	1,140 (71)
Current BMI		
<25.0 kg/m^2	702 (38)	582 (36)
25–29.9 kg/m^2	581 (32)	506 (32)
≥30 kg/m^2	543 (30)	513 (32)
Current hormonal birth control use		
Yes	75 (4)	66 (4)
No	1,750 (96)	1,531 (96)
Current hormone therapy use		
Current, estrogen plus progestin	65 (4)	84 (5)
Current, unopposed estrogen	125 (7)	133 (8)
No current use	1,634 (90)	1,379 (86)
Physical activity (in last year)		
0–1 hours/week	635 (35)	539 (34)
1.1–3 hours/week	557 (30)	516 (32)
>3 hours/week	637 (35)	546 (34)
Alcohol consumption in last year		
Never/former drinker	341 (19)	300 (19)
Current drinker, <1 drink/day	1,230 (67)	1,069 (67)
Current drinker, ≥1 drink/day	253 (14)	231 (14)
Parity		
0 births	339 (19)	295 (18)
1 birth	276 (15)	235 (15)
2 births	664 (36)	576 (36)
≥3 births	549 (30)	495 (31)
History of osteoporosis		
Yes	431 (24)	343 (21)
No	1,397 (76)	1,258 (79)
Regular vitamin D supplement use (≥4 times/week)		
None	840 (47)	731 (46)
Multivitamin, no extra vitamin D	732 (41)	657 (42)
Multivitamin and vitamin D	131 (7)	122 (8)
Vitamin D and calcium	76 (4)	54 (3)
Vitamin D only	10 (1)	9 (1)
Family history of breast cancer		
Affected sister or half-sister only	1,359 (74)	1,047 (65)
>1 first-degree relative	470 (26)	554 (35)

NOTE: Missing values: Race (1 case), education (1 case), current BMI (3 in subcohort), current hormonal birth control use (4 in subcohort, 4 cases), current hormone therapy use (5 in subcohort, 5 cases), alcohol (5 in subcohort, 1 case), parity (1 in subcohort), osteoporosis (1 in subcohort), supplement use (40 in subcohort, 28 cases).

[a]Includes 67 women also selected as part of subcohort.

correlations with one another ($0.50 \leq r^2 < 0.80$), including rs4328262 and rs4237855 ($r^2 = 0.61$) and rs17883984 and rs4516035 ($r^2 = 0.64$).

Other SNPs with statistically significant interactions were rs117913124 (*CYP2R1*; $P = 0.005$) and rs3132301 (*RXRA*; $P = 0.04$). The RHRs exceeded 1.00 for both of these SNPs. Overall, the observed *P* values were smaller than would have been expected by chance under the null hypothesis of no association, as shown by the quantile–quantile plot in Fig. 1A. We formally tested the combined results for all 82 candidate SNPs relative to the global no-interaction null hypothesis. We did so by using Fisher method to combine *P* values and then comparing the observed test statistic (a sum of the $-2 \log P$ values) to those from each of 1,000 datasets with permuted case statuses. The estimated overall *P* value was 0.04.

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Table 2. Interacting effects of 25(OH)D and SNPs in vitamin D metabolism genes on the 5-year risk of breast cancer: Ratio of HRs and 95% CI [$N = 3{,}334$[a]; 1,810 in subcohort (66 cases) and 1,524 additional cases]

Rank	SNP	Location	Gene	Minor allele frequency[b]	25(OH)D-breast cancer HR in noncarriers	Ratio of HR (95% CI)[c]	Interaction *P*
1	rs4328262	12: 48285648	VDR	0.41	0.92 (0.68–1.24)	0.67 (0.53–0.85)	0.0008
2	rs11168287	12: 48285414	VDR	0.47	0.92 (0.64–1.31)	0.71 (0.56–0.90)	0.004
3	rs117913124	11: 14900931	CYP2R1	0.02	0.71 (0.59–0.84)	5.96 (1.71–20.8)	0.005
4	rs4237855	12: 48287203	VDR	0.37	0.83 (0.63–1.10)	0.73 (0.58–0.92)	0.007
5	rs17883984	12: 48293716	VDR	0.32	0.60 (0.46–0.78)	1.34 (1.05–1.71)	0.02
6	rs4516035	12: 48299826	VDR	0.4	0.56 (0.42–0.76)	1.30 (1.04–1.64)	0.02
7	rs2239182	12: 48255411	VDR	0.5	0.53 (0.37–0.76)	1.27 (1.01–1.58)	0.04
8	rs3132301	9: 137294030	RXRA	0.19	0.65 (0.52–0.81)	1.36 (1.01–1.84)	0.04
9	rs3132296	9: 137302631	RXRA	0.34	0.68 (0.52–0.89)	1.28 (1.00–1.65)	0.05
10	rs3118536	9: 137308462	RXRA	0.17	0.66 (0.53–0.81)	1.35 (0.98–1.84)	0.06
11	rs1989969	12: 48278010	VDR	0.4	0.69 (0.52–0.91)	1.25 (0.98–1.60)	0.07
12	rs35079168	9: 137280939	RXRA	0.39	0.62 (0.47–0.83)	1.25 (0.98–1.59)	0.07
13	rs11102986	9: 137285503	RXRA	0.18	0.66 (0.53–0.81)	1.32 (0.97–1.78)	0.07
14	rs7129781	11: 14912417	CYP2R1	0.08	0.70 (0.58–0.85)	1.44 (0.93–2.23)	0.10
15	rs12794714	11: 14913575	CYP2R1	0.42	0.98 (0.74–1.30)	0.81 (0.62–1.06)	0.12
16	rs886441	12: 48262964	VDR	0.21	0.62 (0.50–0.77)	1.25 (0.94–1.65)	0.12
17	rs2853564	12: 48278487	VDR	0.37	0.69 (0.53–0.91)	1.20 (0.95–1.53)	0.13
18	rs2228572	12: 48272840	VDR	0.02	0.76 (0.64–0.90)	0.52 (0.22–1.22)	0.13
19	rs7967152	12: 48244184	VDR	0.46	0.59 (0.42–0.82)	1.19 (0.95–1.51)	0.14
20	rs2239179	12: 48257766	VDR	0.42	0.76 (0.56–1.04)	0.84 (0.67–1.06)	0.14
21	rs4917354	9: 137237661	RXRA	0.31	0.64 (0.50–0.82)	1.20 (0.93–1.55)	0.16
22	rs1155563	4: 72643488	GC	0.27	0.65 (0.52–0.82)	1.22 (0.92–1.64)	0.17
23	rs1805343	9: 137328286	RXRA	0.38	0.66 (0.50–0.87)	1.18 (0.93–1.49)	0.18
24	rs4588	4: 72618323	GC	0.27	0.64 (0.51–0.80)	1.22 (0.91–1.65)	0.19
25	rs2181874	20: 52784478	CYP24A1	0.25	0.66 (0.52–0.85)	1.18 (0.91–1.54)	0.21
26	rs11574143	12: 48234917	VDR	0.11	0.78 (0.64–0.94)	0.80 (0.55–1.16)	0.24
27	rs2239186	12: 48269410	VDR	0.18	0.68 (0.55–0.85)	1.19 (0.89–1.60)	0.24
28	rs2248098	12: 48253356	VDR	0.48	0.62 (0.44–0.88)	1.15 (0.91–1.45)	0.24
29	rs2296241	20: 52786219	CYP24A1	0.48	0.84 (0.62–1.15)	0.87 (0.69–1.10)	0.24
30	rs2762934	20: 52771261	CYP24A1	0.18	0.68 (0.56–0.85)	1.19 (0.89–1.61)	0.25
31	rs9729	12: 48236623	VDR	0.46	0.61 (0.44–0.85)	1.14 (0.90–1.43)	0.28
32	rs757343	12: 48239675	VDR	0.12	0.77 (0.63–0.94)	0.82 (0.58–1.17)	0.28
33	rs2228570	12: 48272895	VDR	0.36	0.70 (0.53–0.92)	0.89 (0.71–1.11)	0.30
34	rs2238136	12: 48277713	VDR	0.24	0.79 (0.62–1.00)	0.87 (0.66–1.14)	0.31
35	rs16999116	20: 52777467	CYP24A1	0.14	0.77 (0.63–0.94)	0.85 (0.61–1.17)	0.31
36	rs35873579	20: 52788190	CYP24A1	0.03	0.75 (0.63–0.90)	0.68 (0.31–1.47)	0.33
37	rs10898193	11: 71197083	NADSYN1	0.17	0.69 (0.56–0.85)	1.17 (0.85–1.61)	0.35
38	rs1044535	11: 71145778	DHCR7	0.08	0.70 (0.58–0.84)	1.25 (0.78–1.99)	0.35
39	rs4809958	20: 52782438	CYP24A1	0.16	0.78 (0.64–0.96)	0.86 (0.62–1.19)	0.37
40	rs35603635	9: 137323202	RXRA	0.03	0.75 (0.63–0.90)	0.73 (0.37–1.45)	0.37
41	rs7975128	12: 48245828	VDR	0.39	0.76 (0.57–1.02)	0.90 (0.71–1.14)	0.38
42	rs3819545	12: 48265006	VDR	0.37	0.77 (0.58–1.02)	0.90 (0.71–1.14)	0.39
43	rs2238135	12: 48278190	VDR	0.25	0.78 (0.62–0.98)	0.88 (0.67–1.17)	0.39
44	rs61749689	20: 52790005	CYP24A1	0.02	0.75 (0.63–0.90)	0.73 (0.33–1.58)	0.42
45	rs1570669	20: 52774427	CYP24A1	0.37	0.85 (0.65–1.11)	0.91 (0.72–1.16)	0.45
46	rs11168275	12: 48272275	VDR	0.23	0.75 (0.60–0.95)	0.90 (0.68–1.18)	0.45
47	rs1045570	9: 137332311	RXRA	0.16	0.71 (0.58–0.88)	1.13 (0.83–1.53)	0.45
48	rs2254210	12: 48273714	VDR	0.36	0.73 (0.56–0.96)	1.10 (0.86–1.40)	0.46
49	rs1352844	4: 72647749	GC	0.12	0.77 (0.63–0.94)	0.89 (0.64–1.24)	0.48
50	rs12717991	12: 48259126	VDR	0.37	0.69 (0.52–0.91)	1.08 (0.85–1.37)	0.54
51	rs731236	12: 48238757	VDR	0.39	0.76 (0.57–1.01)	0.93 (0.74–1.17)	0.54
52	rs11185660	9: 137259992	RXRA	0.25	0.70 (0.56–0.88)	1.09 (0.83–1.43)	0.56
53	rs705120	4: 72614140	GC	0.42	0.66 (0.50–0.88)	1.08 (0.84–1.38)	0.57
54	rs12785878	11: 71167449	NADSYN1	0.3	0.71 (0.56–0.89)	1.08 (0.83–1.41)	0.57
55	rs6537998	9: 137292505	RXRA	0.03	0.73 (0.61–0.87)	1.22 (0.60–2.50)	0.58
56	rs222020	4: 72636272	GC	0.19	0.80 (0.65–0.99)	0.92 (0.67–1.26)	0.59
57	rs1540339	12: 48257326	VDR	0.36	0.67 (0.51–0.89)	1.06 (0.84–1.34)	0.61
58	rs67816242	9: 137268441	RXRA	0.24	0.71 (0.57–0.88)	1.07 (0.81–1.42)	0.62
59	rs2248359	20: 52791518	CYP24A1	0.41	0.62 (0.46–0.84)	1.05 (0.84–1.32)	0.68
60	rs7038018	9: 137309959	RXRA	0.14	0.74 (0.60–0.90)	1.07 (0.77–1.48)	0.68
61	rs3733359	4: 72649774	GC	0.09	0.75 (0.62–0.90)	0.92 (0.58–1.44)	0.71
62	rs2239180	12: 48256046	VDR	0.12	0.71 (0.59–0.87)	1.07 (0.75–1.51)	0.72
63	rs143304420	11: 71144815	DHCR7	0.03	0.73 (0.61–0.88)	1.13 (0.56–2.28)	0.73
64	rs2248461	20: 52792202	CYP24A1	0.39	0.63 (0.47–0.84)	1.04 (0.83–1.30)	0.76
65	rs222009	4: 72631735	GC	0.02	0.74 (0.62–0.88)	0.82 (0.21–3.22)	0.78
66	rs4364228	4: 72623347	GC	0.1	0.75 (0.62–0.90)	0.95 (0.63–1.41)	0.78

(Continued on the following page)

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Table 2. Interacting effects of 25(OH)D and SNPs in vitamin D metabolism genes on the 5-year risk of breast cancer: Ratio of HRs and 95% CI [$N = 3{,}334$[a]; 1,810 in subcohort (66 cases) and 1,524 additional cases] (Cont'd)

Rank	SNP	Location	Gene	Minor allele frequency[b]	25(OH)D-breast cancer HR in noncarriers	Ratio of HR (95% CI)[c]	Interaction *P*
67	rs10877012	12: 58162085	CYP27B1	0.3	0.68 (0.52–0.88)	0.97 (0.76–1.23)	0.79
68	rs2283342	12: 48255859	VDR	0.14	0.75 (0.61–0.92)	0.96 (0.69–1.33)	0.81
69	rs7136534	12: 48294626	VDR	0.25	0.73 (0.58–0.93)	1.03 (0.79–1.35)	0.82
70	rs11907350	20: 52770439	CYP24A1	0.04	0.73 (0.61–0.87)	1.07 (0.57–2.02)	0.82
71	rs11574026	12: 48288246	VDR	0.11	0.74 (0.61–0.90)	1.04 (0.70–1.55)	0.85
72	rs34312136	9: 137269456	RXRA	0.4	0.70 (0.52–0.95)	1.02 (0.81–1.29)	0.86
73	rs10741657	11: 14914878	CYP2R1	0.37	0.71 (0.52–0.97)	1.02 (0.81–1.29)	0.86
74	rs9615	9: 137331525	RXRA	0.04	0.73 (0.61–0.88)	0.96 (0.50–1.82)	0.90
75	rs2296239	20: 52775528	CYP24A1	0.24	0.79 (0.63–0.98)	0.99 (0.75–1.30)	0.92
76	rs11168267	12: 48251542	VDR	0.09	0.72 (0.60–0.87)	1.02 (0.68–1.53)	0.92
77	rs3782905	12: 48266167	VDR	0.32	0.72 (0.55–0.94)	0.99 (0.78–1.26)	0.93
78	rs6022987	20: 52770596	CYP24A1	0.29	0.75 (0.58–0.97)	1.01 (0.78–1.31)	0.94
79	rs10875693	12: 48269650	VDR	0.33	0.75 (0.57–0.98)	0.99 (0.78–1.26)	0.94
80	rs927650	20: 52772741	CYP24A1	0.46	0.73 (0.53–1.00)	1.00 (0.80–1.26)	0.97
81	rs78077736	9: 137257165	RXRA	0.05	0.74 (0.62–0.88)	1.00 (0.57–1.73)	0.99
82	rs2107301	12: 48255570	VDR	0.27	0.72 (0.56–0.91)	1.00 (0.78–1.29)	0.99

[a]After excluding those with missing covariate information.
[b]Among subcohort members.
[c]The minor allele is the index allele, so a value >1.00 indicates that 25(OH)D has a stronger protective association with breast cancer among those with no copies of the minor allele than those with 1 or 2, assuming a log-additive genetic model. Similarly, an RHR <1.00 indicates that 25(OH)D has a stronger inverse association with breast cancer among those 1 or 2 copies of the minor allele than those none.

Results from analyses restricted to postmenopausal person-time were generally similar to those from the main analyses, with the same top SNP (rs4328262; RHR = 0.65; 95% CI, 0.50–0.84; Supplementary Table S2). Four of the 8 statistically significant SNPs from the overall analysis still showed significant associations among postmenopausal women as well as two other SNPs (rs7967152 in *VDR* and rs3118536 in *RXRA*).

In an exploratory analysis, we examined whether the number of first-degree relatives with breast cancer modified

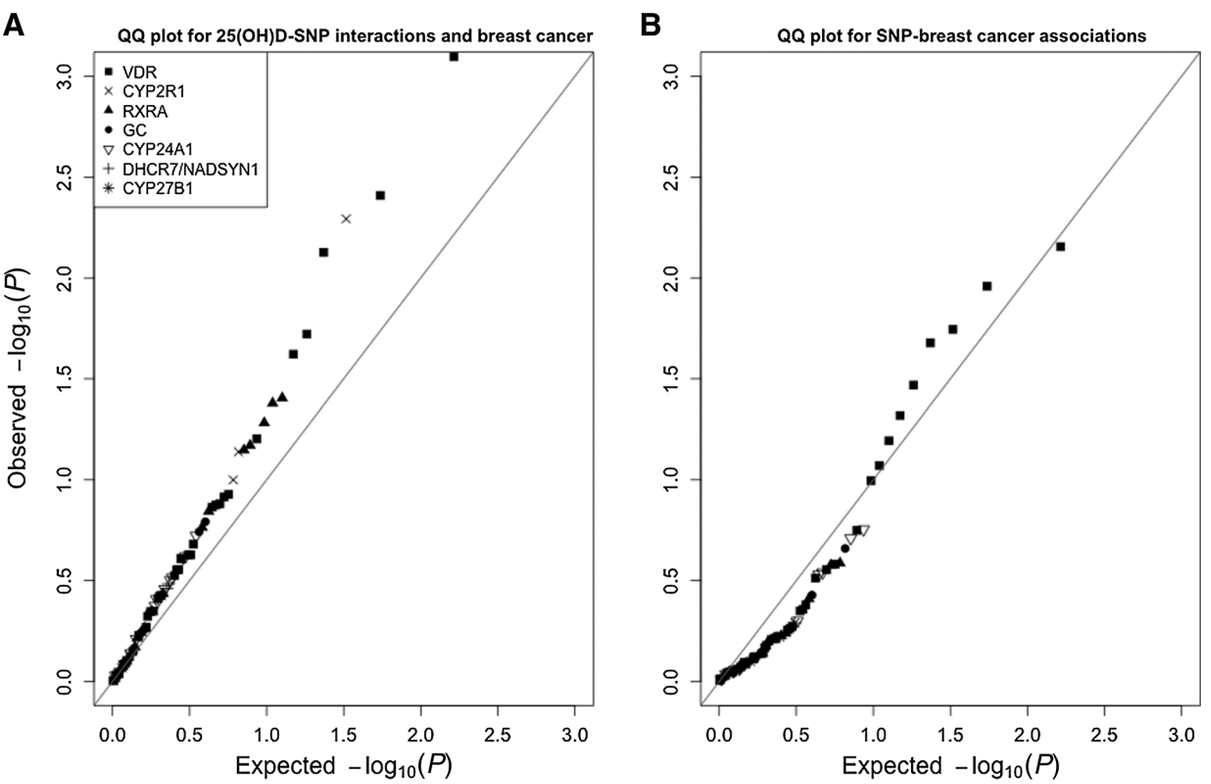

Figure 1.
Quantile–quantile plots. Figure 1 shows the observed versus expected *P* values for the associations between each 25(OH)D–SNP interaction and incident breast cancer (**A**) and between each SNP and incident breast cancer (**B**).

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Table 3. SNP–breast cancer associations for SNPs in selected candidate genes [$N = 3,334$; 1,810 in subcohort (66 cases) and 1,524 additional cases]

SNP–Breast cancer association rank	Overall rank for GxE	SNP	Location	Gene	HR (95% CI)	*P*
1	43	rs2238135	12: 48278190	VDR	1.17 (1.04–1.30)	0.007
2	2	rs11168287	12: 48285414	VDR	1.13 (1.03–1.25)	0.01
3	11	rs1989969	12: 48278010	VDR	0.89 (0.81–0.98)	0.02
4	34	rs2238136	12: 48277713	VDR	1.14 (1.02–1.28)	0.02
5	4	rs4237855	12: 48287203	VDR	1.11 (1.01–1.23)	0.03
6	6	rs4516035	12: 48299826	VDR	0.90 (0.82–1.00)	0.05
7	17	rs2853564	12: 48278487	VDR	0.91 (0.83–1.01)	0.06
8	69	rs7136534	12: 48294626	VDR	0.91 (0.81–1.01)	0.09
9	1	rs4328262	12: 48285648	VDR	1.09 (0.98–1.20)	0.10
10	64	rs2248461	20: 52792202	CYP24A1	1.07 (0.97–1.17)	0.18
11	48	rs2254210	12: 48273714	VDR	0.93 (0.85–1.03)	0.18
12	59	rs2248359	20: 52791518	CYP24A1	1.06 (0.97–1.17)	0.20
13	49	rs1352844	4: 72647749	GC	1.09 (0.95–1.25)	0.22
14	47	rs1045570	9: 137332311	RXRA	1.08 (0.95–1.23)	0.26
15	26	rs11574143	12: 48234917	VDR	0.91 (0.78–1.07)	0.26
16	81	rs78077736	9: 137257165	RXRA	1.13 (0.91–1.40)	0.27
17	5	rs17883984	12: 48293716	VDR	0.94 (0.85–1.05)	0.28
18	25	rs2181874	20: 52784478	CYP24A1	1.06 (0.95–1.18)	0.29
19	29	rs2296241	20: 52786219	CYP24A1	0.95 (0.86–1.05)	0.29
20	62	rs2239180	12: 48256046	VDR	0.92 (0.79–1.08)	0.31
21	61	rs3733359	4: 72649774	GC	0.92 (0.78–1.10)	0.37
22	74	rs9615	9: 137331525	RXRA	0.90 (0.70–1.15)	0.39
23	32	rs757343	12: 48239675	VDR	0.94 (0.81–1.09)	0.42
24	28	rs2248098	12: 48253356	VDR	1.04 (0.94–1.14)	0.44
25	46	rs11168275	12: 48272275	VDR	1.04 (0.93–1.17)	0.45
26	30	rs2762934	20: 52771261	CYP24A1	1.04 (0.92–1.18)	0.50
27	67	rs10877012	12: 58162085	CYP27B1	1.04 (0.93–1.15)	0.51
28	19	rs7967152	12: 48244184	VDR	1.03 (0.94–1.13)	0.53
29	18	rs2228572	12: 48272840	VDR	0.91 (0.67–1.24)	0.55
30	76	rs11168267	12: 48251542	VDR	0.95 (0.80–1.13)	0.56
31	21	rs4917354	9: 137237661	RXRA	0.97 (0.88–1.08)	0.58
32	37	rs10898193	11: 71197083	NADSYN1	0.97 (0.85–1.09)	0.59
33	40	rs35603635	9: 137323202	RXRA	0.93 (0.71–1.21)	0.59
34	44	rs61749689	20: 52790005	CYP24A1	0.92 (0.66–1.26)	0.60
35	65	rs222009	4: 72631735	GC	1.11 (0.76–1.62)	0.60
36	71	rs11574026	12: 48288246	VDR	0.96 (0.82–1.12)	0.61
37	7	rs2239182	12: 48255411	VDR	0.98 (0.89–1.07)	0.61
38	3	rs117913124	11: 14900931	CYP2R1	0.92 (0.67–1.27)	0.62
39	66	rs4364228	4: 72623347	GC	1.04 (0.89–1.22)	0.63
40	10	rs3118536	9: 137308462	RXRA	1.03 (0.91–1.17)	0.64
41	50	rs12717991	12: 48259126	VDR	0.98 (0.89–1.08)	0.66
42	31	rs9729	12: 48236623	VDR	1.02 (0.93–1.12)	0.68
43	57	rs1540339	12: 48257326	VDR	0.98 (0.89–1.09)	0.72
44	60	rs7038018	9: 137309959	RXRA	0.98 (0.85–1.12)	0.72
45	8	rs3132301	9: 137294030	RXRA	0.98 (0.87–1.11)	0.74
46	9	rs3132296	9: 137302631	RXRA	0.98 (0.89–1.09)	0.74
47	45	rs1570669	20: 52774427	CYP24A1	0.98 (0.89–1.09)	0.75
48	78	rs6022987	20: 52770596	CYP24A1	1.02 (0.92–1.12)	0.75
49	77	rs3782905	12: 48266167	VDR	1.02 (0.92–1.13)	0.76
50	20	rs2239179	12: 48257766	VDR	1.01 (0.92–1.12)	0.77
51	13	rs11102986	9: 137285503	RXRA	0.98 (0.87–1.11)	0.78
52	14	rs7129781	11: 14912417	CYP2R1	1.02 (0.86–1.21)	0.80
53	80	rs927650	20: 52772741	CYP24A1	0.99 (0.90–1.08)	0.80
54	53	rs705120	4: 72614140	GC	1.01 (0.92–1.11)	0.80
55	22	rs1155563	4: 72643488	GC	0.99 (0.89–1.10)	0.80
56	68	rs2283342	12: 48255859	VDR	0.98 (0.86–1.13)	0.81
57	27	rs2239186	12: 48269410	VDR	1.02 (0.90–1.15)	0.81
58	16	rs886441	12: 48262964	VDR	1.01 (0.90–1.14)	0.83
59	38	rs1044535	11: 71145778	DHCR7	0.98 (0.83–1.17)	0.84
60	33	rs2228570	12: 48272895	VDR	0.99 (0.90–1.09)	0.85
61	35	rs16999116	20: 52777467	CYP24A1	0.99 (0.86–1.13)	0.87
62	63	rs143304420	11: 71144815	DHCR7	0.98 (0.74–1.29)	0.87
63	72	rs34312136	9: 137269456	RXRA	1.01 (0.91–1.11)	0.87
64	73	rs10741657	11: 14914878	CYP2R1	0.99 (0.90–1.09)	0.88
65	36	rs35873579	20: 52788190	CYP24A1	0.98 (0.72–1.32)	0.88
66	54	rs12785878	11: 71167449	NADSYN1	1.01 (0.91–1.12)	0.89

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Table 3. SNP–breast cancer associations for SNPs in selected candidate genes [$N = 3,334$; 1,810 in subcohort (66 cases) and 1,524 additional cases] (Cont'd)

SNP–Breast cancer association rank	Overall rank for GxE	SNP	Location	Gene	HR (95% CI)	*P*
67	70	rs11907350	20: 52770439	CYP24A1	0.98 (0.77–1.26)	0.89
68	79	rs10875693	12: 48269650	VDR	1.01 (0.91–1.12)	0.89
69	42	rs3819545	12: 48265006	VDR	0.99 (0.90–1.10)	0.90
70	58	rs67816242	9: 137268441	RXRA	1.01 (0.90–1.13)	0.90
71	55	rs6537998	9: 137292505	RXRA	1.02 (0.74–1.40)	0.90
72	41	rs7975128	12: 48245828	VDR	0.99 (0.90–1.10)	0.90
73	56	rs222020	4: 72636272	GC	0.99 (0.88–1.12)	0.91
74	15	rs12794714	11: 14913575	CYP2R1	0.99 (0.90–1.09)	0.91
75	75	rs2296239	20: 52775528	CYP24A1	1.01 (0.90–1.12)	0.92
76	23	rs1805343	9: 137328286	RXRA	1.00 (0.90–1.10)	0.94
77	12	rs35079168	9: 137280939	RXRA	1.00 (0.91–1.11)	0.94
78	52	rs11185660	9: 137259992	RXRA	1.00 (0.90–1.12)	0.95
79	24	rs4588	4: 72618323	GC	1.00 (0.90–1.11)	0.97
80	39	rs4809958	20: 52782438	CYP24A1	1.00 (0.88–1.14)	0.97
81	51	rs731236	12: 48238757	VDR	1.00 (0.91–1.10)	0.97
82	82	rs2107301	12: 48255570	VDR	1.00 (0.90–1.11)	0.99

the gene-by-vitamin-D interaction. Despite having similar minor allele frequencies in both groups, some of the top 8 SNPs showed evidence of RHR modification (Supplementary Table S3). For example, rs17883984 in *VDR* (the fifth ranked SNP in the overall analysis) had an RHR of 1.69 (95% CI, 1.24–2.29) in those with only one affected sister, but an RHR of 0.80 (95% CI, 0.52–1.24) in those with >1 first-degree relatives with breast cancer (*P* value for three-way interaction = 0.008). In general, however, the observed associations for these 8 SNPs tended to be in the same direction for the two groups.

Only six of the 82 candidate SNPs were independently associated with breast cancer risk at $P < 0.05$ (Table 3). All of these were located in *VDR* (rs2238135, rs11168287, rs1989969, rs2238136, rs4237855, rs4516035). Of these six, only rs2238135 and rs2238136 were moderately correlated with one another ($r^2 = 0.73$; Supplementary Table S1). Three of the six also showed evidence of statistically significant gene-by-environment interaction with 25(OH)D (rs11168287, rs4237855, and rs4516035). Overall, the *P* values observed for the SNP–breast cancer association analysis were consistent with what would be expected by chance under the null hypothesis (Fig. 1B).

Of the 1,439 additional candidate SNPs with potential links to vitamin D, 77 showed evidence of a statistically significant interaction at an uncorrected *P* value of 0.05 (Supplementary Table S4), and none met the FDR threshold ($q \leq 0.05$).

In gene-based analyses, the only primary candidate gene to show strong evidence of multiplicative interaction was *VDR* (35 SNPs, $P = 0.04$; Table 4). Six genes from the secondary list of 89 genes had gene-by-environment interaction *P* values less than 0.05 (Supplementary Table S5), including *AMZ1* (8 SNPs, $P = 0.001$), *GPR114* (5 SNPs, $P = 0.01$), *EGFR* (85 SNPs, $P = 0.01$), *TRIM24* (9 SNPs, $P = 0.02$), *CYP3A4* (10 SNPs, $P = 0.03$), and *ITGB3* (15 SNPs, $P = 0.04$).

Table 4. GESAT for 25(OH)D and vitamin D–related genes on the 5-year risk of breast cancer ($N = 3,334$; 1,590 cases, 1,744 controls/noncases)

Gene	Number of included SNPs	GESAT *P*
VDR	35	0.04
RXRA	16	0.19
CYP2R1	4	0.22
GC	8	0.38
DHCR7/NADSYN1	4	0.53
CYP24A1	14	0.65

NOTE: Gene-level effects were not calculated for *CYP27B1* (1 SNP).

Discussion

Using a prospective, case–cohort design, we examined gene-by-25(OH)D interaction in relation to breast cancer risk over 5 years of follow-up. We examined 82 SNPs in 7 candidate vitamin D metabolism genes (*CYP24A1*, *CYP27B1*, *CYP2R1*, *GC*, *DHCR7/NADSYN1*, *RXRA*, and *VDR*), and 8 SNPs showed evidence of multiplicative interactions. Most of these were in *VDR*, with other hits appearing in *CYP2R1* and *RXRA*. As a group, the candidate SNPs were stronger modifiers of the 25(OH)D–breast cancer association than would have been expected by chance. In gene-level tests, 25(OH)D and *VDR* showed evidence of multiplicative interaction with breast cancer incidence over 5 years of follow-up. These findings were generally similar for analyses restricted to postmenopausal women.

Six SNPs in *VDR* were nominally associated with breast cancer risk, including several of the SNPs that modified the 25(OH)D–breast cancer association. For a few of the top SNPs in the gene-by-environment interaction analysis, the observed RHR was in the opposite direction for those who had one first-degree relative with breast cancer versus more than one first-degree relative with breast cancer. There was negligible evidence that any of the secondary candidate SNPs modified the 25(OH)D–breast cancer association.

Most of the eight SNPs that significantly modified the 25(OH)D–breast cancer association are intronic with no known function (56, 57). Exceptions included the SNP in *CYP2R1* (rs117913124), which is a synonymous substitution that does not result in an amino acid change, and one SNP upstream of *VDR* (rs4516035), which could affect transcription-binding sites in the gene's promotor region. Similarly, aside from rs4516035, all of the *VDR* SNPs associated with breast cancer risk were in intronic regions (56, 57). SNPs in introns can have important regulatory roles, such as affecting protein splicing (58) or encoding miRNA-binding sites (50, 59).

The two SNPs with the smallest gene-by-environment interaction *P* values (rs4328262 and rs11168287 in *VDR*) were

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previously shown to be associated with breast cancer risk in premenopausal European women and postmenopausal Asian women, respectively (60), although Engel and colleagues found no evidence of an interaction between rs11168287 and sunlight exposure in relation to breast cancer risk (15). Another of the statistically significant *VDR* SNPs, rs4516035, was not associated with breast cancer in a previous case–control study (61). We did not corroborate any of the previously observed gene-by-environment interaction effects for the putative functional SNPs in *VDR* [$P = 0.30$, $P = 0.54$, and $P = 0.38$, for rs2228570/*FokI*, rs731236/*TaqI*, and rs7975128 respectively, where rs7975128 is highly correlated with rs1544410/*BsmI* ($r^2 = 0.97$)].

Although difficult to study because of its rarity (2% minor allele frequency), the *CYP2R1* SNP identified here (rs117913124) was shown to be associated with 25(OH)D levels in a recent genome-wide meta-analysis (62). To the best of our knowledge, rs3132301 (*RXRA*) has not been previously assessed as a possible effect modifier. Clendenen and colleagues (14) previously examined the association between various *RXRA* SNPs (including several also included our analysis) and breast cancer risk, but identified no significant associations. Replication of our findings in an independent study is needed.

The *VDR* SNP with the lowest *P* value for the breast cancer risk analysis, rs2238135, has not been previously reported to be associated with breast cancer risk, but has been linked to esophageal (63), oral cavity (64), and prostate cancer (65). The second lowest *P* value was seen for rs11168287, which also showed evidence of modifying the 25(OH)D–breast cancer association and is discussed above. None of the remaining four *VDR* SNPs associated with breast cancer in our sample has been previously linked to the disease.

With the exception of rs117913124, few of the SNPs in the vitamin D metabolism genes (*CYP24A1*, *CYP2R1*, or *CYP27B1*) showed evidence of interacting with 25(OH)D to affect breast cancer risk. However, these genes may still play a key role in breast cancer prevention by mediating how individuals process and make use of UV-B radiation and dietary vitamin D intake. These relationships should be better explored in future analyses.

All of the women in our study have a first-degree family history of breast cancer. Although this does not affect the validity of our hypothesis tests, it may limit the generalizability of our estimates (66). More specifically, the genetic background could matter: the RHRs measured in our sample could be larger than those assessed in a population-based sample if the SNP of interest interacted with or was in linkage disequilibrium with one or more breast cancer risk variants. This could explain the RHR modification we observed for some SNPs when we stratified by the number of first-degree family members with a history of breast cancer. Of note, minor allele frequencies were very similar across those two strata, and 25(OH)D levels were slightly lower in women with >1 affected first-degree relative (23% of subcohort in top quartile vs. 26% of subcohort restricted to women with only 1 first-degree relative). We are interested in assessing possible interactions between *BRCA1/2* and vitamin D–related genes, but we currently only have self-reported data on *BRCA1/2* mutation status, and we estimate that despite overselecting those with a strong family history, only a small fraction of our participants carry deleterious mutations (66).

Our generalizability could also be limited by the fact that the majority of our participants are white and non-Hispanic. On the other hand, our unique study design of including sisters of women diagnosed with breast cancer presumably ensured that we had some enrichment for both risk alleles and factors associated with a healthier lifestyle, such as increased supplement use. This enrichment for both genetic and environmental risk factors enhances power for this prospective study to detect gene-by-environment interactions (66).

Although this is one of the largest gene-by-environment interaction studies of vitamin D and breast cancer to date, the sample size is limited, and some of our effect estimates are imprecise. Results for SNPs with low minor allele frequencies and for subgroup-specific analyses should be interpreted with caution. Because we selected specific candidate genes instead of conducting an agnostic search, we did not adjust for multiple comparisons. Consequently, some of the reported statistically significant interactions may be false positives rather than true associations. That being said, we observed more significant associations than would be expected under the null hypothesis. We calculated FDR q-values when conducting more exploratory analyses among the secondary candidate SNPs, finding none that met the criteria for statistical significance.

The gene-based tests are both a strength and limitation of this study. Although we believe it can be useful to consider the group-level effects of multiple SNPs within a gene, we were not able to identify any gene-based tests that could accommodate our case–cohort design. We instead chose to adapt our data to approximate a case–control study. Although the results should be roughly the same, some discrepancies may exist.

As discussed previously, the main strengths of our study include prospective collection of serum specimens, detailed covariate information, use of LC/MS to measure 25(OH)D levels (including 3-epi-25(OH)D_3), our use of a cohort with higher than average breast cancer risk, and data from a highly motivated and committed cohort of women (19). Most other studies of this topic have relied on either 25(OH)D levels in samples collected after cases' diagnoses, which may have been altered by the disease or disease-related behavioral changes, or on prospectively collected 25(OH)D levels that might have reflected levels from a time period not relevant to breast cancer risk.

We have conducted a large and comprehensive analysis of gene-by-environment interactions between recent serum 25(OH)D levels, genetic variants in vitamin D–related genes, and breast cancer risk. We found evidence of interactions for SNPs in *VDR*, *CYP2R1*, and *RXRA*. This research aims to advance our understanding of the biologic mechanisms involved in breast cancer etiology and the anticarcinogenic properties of vitamin D. If our findings are replicated in other prospective studies, they may ultimately help to personalize prevention by identifying individuals who would most benefit from interventions to modify vitamin D levels.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K.M. O'Brien, D.P. Sandler, J.A. Taylor, C.R. Weinberg
Development of methodology: K.M. O'Brien
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.M. O'Brien, D.P. Sandler, J.A. Taylor, C.R. Weinberg

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.M. O'Brien, C.R. Weinberg
Writing, review, and/or revision of the manuscript: K.M. O'Brien, D.P. Sandler, H.K. Kinyamu, J.A. Taylor, C.R. Weinberg
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.M. O'Brien
Study supervision: D.P. Sandler, C.R. Weinberg
Other (data interpretation): H.K. Kinyamu

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