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Vitamin-D associated genetic variation and risk of breast cancer in the Breast and Prostate Cancer Cohort Consortium (BPC3)

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Running Title: Vitamin D-related genes and breast cancer

Conflict of Interest: The authors have no conflicts to disclose.

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Abstract

Background: Two recent genome-wide association studies (GWAS) identified SNPs related to circulating 25-hydroxyvitamin D [25(OH)D] concentration in or near four genes. To examine the hypothesized inverse relationship between vitamin D status and breast cancer, we studied the associations between SNPs in these genes and breast cancer risk in a large pooled study of 9,456 cases and 10,816 controls from six cohorts.

Methods: SNP markers localized to each of four genes (*GC, CYP24A1, CYP2R1,* and *DHCR7*) previously associated with 25(OH)D were genotyped and examined both individually and as a 4-SNP polygenic score. Logistic regression was used to estimate the associations between the genetic variants and risk of breast cancer.

Results: We found no association between any of the four SNPs or their polygenic score and breast cancer risk.

Conclusions: Our findings do not support an association between vitamin D status, as reflected by 25(OH)D-related genotypes, and breast cancer risk.

Impact: These findings may contribute to future meta-analyses and scientific review articles, and provide new data about the association between vitamin D-related genes and breast cancer.

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Introduction

A meta-analysis of prospective studies showed an inverse association between breast cancer and circulating 25(OH)D, an indicator of vitamin D status (1). Genome-wide association studies (GWAS) identified SNPs related to circulating 25(OH)D in or near four genes (2, 3): *GC*, encoding vitamin D binding protein, the major transporter of circulating vitamin D compounds; *CYP24A1*, encoding the cytochrome p450 24-hydroxylase that initiates intracellular catabolism of 25(OH)D and 1,25-dihydroxyvitamin D; *CYP2R1*, encoding the 25-hydroxylase which converts vitamin D to 25(OH)D in the liver; and *DHCR7*, encoding the enzyme that converts 7-dehydrocholesterol to cholesterol rather than vitamin D_3 (2, 3). These four SNPs explain more variation in circulating 25(OH)D (5.2%) than a polygenic score including 9,000 SNPs (0.16%) (4).

The present study examines the four SNPs and their composite genetic score in relation to breast cancer in a large pooled analysis.

Materials and Methods

Details of the Breast and Prostate Cancer Cohort Consortium (BPC3) have been reported (5). Briefly, it is a consortium of breast cancer case-control sets nested in seven cohorts: the American Cancer Society Cancer Prevention Study II (CPS-II), the European Prospective Investigation into Cancer and Nutrition Cohort (EPIC), the Multiethnic Cohort (MEC), the Nurses' Health Study I and II (NHS and NHSII), the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), and the Women's Health Study (WHS). This analysis was restricted to women of European ancestry (9,456 cases,10,816 controls).

We chose SNPs identified in GWAS as associated with plasma/serum 25(OH)D concentrations (2, 3): rs2282679 (*GC*), rs10741657 (*CYP2R1*), rs12785878 (*DHCR7*), and

rs6013897 (*CYP24A1*). Genotyping was conducted by Taqman or genome-wide scans as previously described (6).

Logistic regression was used to estimate odds ratios and 95% confidence intervals for breast cancer risk by individual SNPs and an unweighted polygenic score summing the number of lower 25(OH)D-associated alleles (i.e. "low vitamin D alleles") (6). Models were adjusted for cohort and baseline age (continuous). Further adjustment for additional baseline factors did not alter the associations: body mass index (BMI), height, history of diabetes, smoking status, alcohol consumption, age at menarche, menopausal status, age at menopause, use of menopausal hormone therapy or oral contraceptives, age at first live birth, and family history of breast cancer. Details on collection and harmonization of these data have been published (5, 6). Analyses stratifying by estrogen (ER) and progesterone (PR) receptor status, cancer stage, menopausal status at diagnosis (postmenopausal at baseline or age 55+ at diagnosis vs. premenopausal or perimenopausal at baseline and age ≤51 at diagnosis), BMI, height, and cohort were conducted. SAS v9.3 was used.

Results

Characteristics of the cohorts have been published (5). Examination of the four SNPs individually revealed no association with breast cancer and no heterogeneity across cohorts for any SNP (Table). We observed no differences in the association for any SNP across subgroups of stage, menopausal status at diagnosis, or progesterone receptor (PR) status (data not shown), although women with two low vitamin D alleles for rs12785878 (*DHCR7)* appeared to be at lower risk of ER- breast cancer, consistent with a recessive genetic association (GG vs. TT: OR=0.56, 95% CI: 0.32–0.97; *p=0.04*). Consideration of multiple comparisons, however, rendered this finding not statistically significant (Bonferroni-corrected p-value threshold=*0.00125*). There was no statistically significant interaction with BMI or height after

consideration of multiple comparisons. The polygenic score was also not associated with breast cancer risk (Figure), and this did not vary by height, BMI, ER or PR status, stage, or menopausal status at diagnosis (data not shown).

Discussion

In this large, pooled analysis, we found no association between four SNPs related to vitamin D status (or the 4-SNP polygenic score) and breast cancer risk. Given that these SNPs explain ~5% of the 25(OH)D variation, if the OR of breast cancer per standard deviation increase of 25(OH)D were 0.83 or stronger we should have 80% power to detect it [6]. We observed no association with any of the breast cancer subtypes examined, with the possible exception of lower risk of ER- breast cancer among women with 2 low vitamin D alleles for rs12785878 near *DHCR7*, which encodes an enzyme that converts 7-dehydrocholesterol to cholesterol rather than to vitamin D_3. As cholesterol is a precursor of sex steroids including estrogens and androgens, a breast cancer association with this gene, if substantiated, may have its basis in steroid hormone, not vitamin D, metabolism (7). The finding may, however, be due to chance.

Few studies have examined these SNPs in relation to breast cancer risk. One found no association for a polygenic score of the same four genes, but increased risk among women with two low vitamin D alleles for rs6013897 in *CYP24A1* (8). Our findings do not support an association between SNPs associated with vitamin D status and breast cancer risk, but the possible association of *DHCR7* with ER- disease should be examined further.

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Table 1. Individual SNP associations with risk of breast cancer

SNP	Gene	Frequency of low vitamin D allele	# Low vitamin D alleles	# Cases	# Controls	OR (95% CI)*	*p-value*†	*p for heterogeneity across cohorts*§
rs2282679	*GC*	0.28						
TT			0	4,531	5,100	1.0 (ref)		
GT			1	3,412	4,104	0.94 (0.88 – 1.00)		
GG			2	713	781	1.03 (0.92 – 1.15)	*0.08*	
Additive (per allele)						0.98 (0.94 – 1.03)	*0.39*	*0.10*
rs6013897	*CYP24A1*	0.21						
TT			0	5,759	6,551	1.0 (ref)		
AT			1	3,064	3,576	0.97 (0.91 – 1.03)		
AA			2	447	491	1.03 (0.90 – 1.18)	*0.48*	
Additive (per allele)						0.99 (0.94 – 1.04)	*0.65*	*0.84*
rs10741657	*CYP2R1*	0.61						
AA			0	1,301	1,486	1.0 (ref)		
AG			1	4,041	4,766	0.96 (0.88 – 1.05)		
GG			2	3,276	3,708	1.00 (0.91 – 1.09)	*0.41*	
Additive (per allele)						1.01 (0.97 – 1.05)	*0.72*	*0.62*
rs12785878	*DHCR7*	0.27						
TT			0	4,935	5,674	1.0 (ref)		
GT			1	3,620	4,052	1.03 (0.97 – 1.09)		
GG			2	669	834	0.92 (0.83 – 1.03)	0.16	
Additive (per allele)						0.99 (0.95 – 1.03)	0.60	0.13

*- Adjusted for age at baseline (continuous) and study cohort.

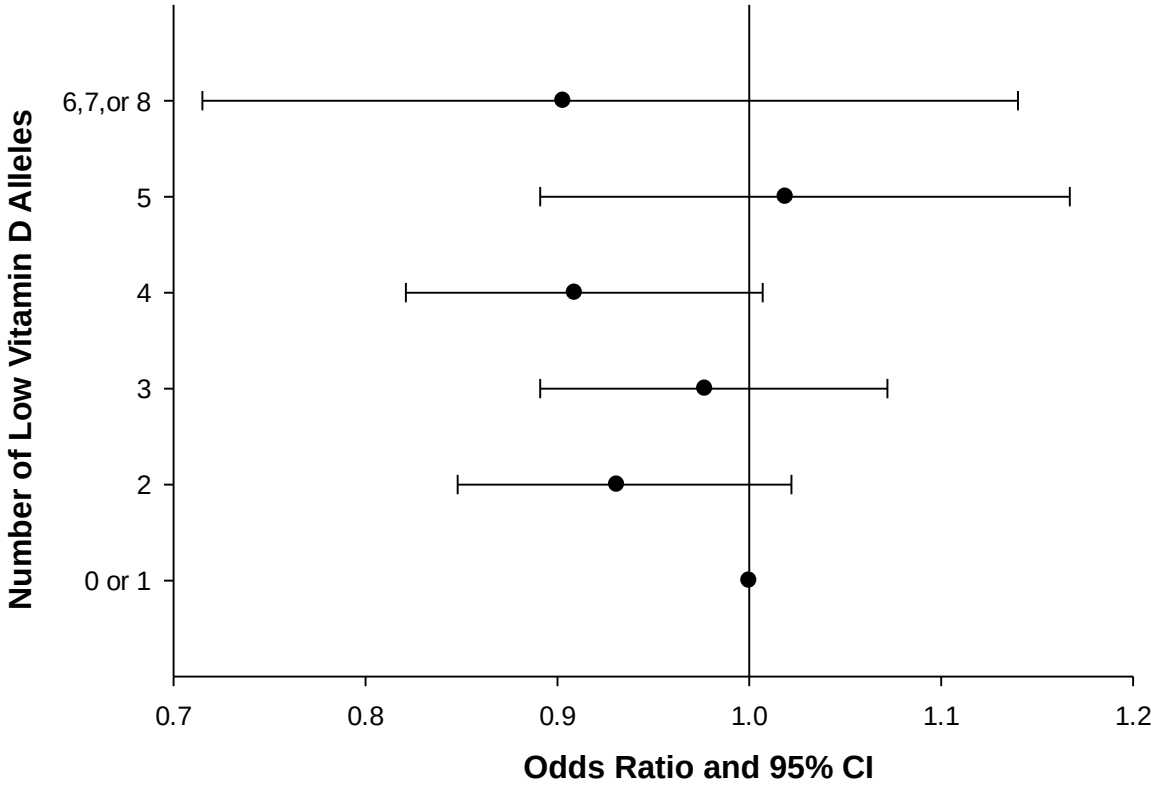
† - For categorical analyses of genotype, the p-value is a 2 degree of freedom test comparing a model containing indicator variables for genotype and covariates with a model containing only the covariates. For the per allele analyses, the p-value is a 1 degree of freedom test comparing a model containing the number of alleles and covariates to a model containing only the covariates.

§ - The p-value for heterogeneity across cohorts is based on the test for interaction between cohort and the additive.

Figure Legends:

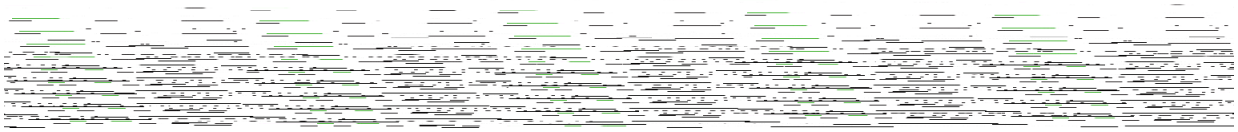
Figure 1: Association Between the 4-SNP Polygenic Score and Risk of Breast Cancer

Figure 1



	# Cases	OR (95% CI)	*p-value*
6,7,or 8	144	0.90 (0.72 – 1.14)	*0.39*
5	592	1.02 (0.89 – 1.17)	*0.78*
4	1,470	0.91 (0.82 – 1.01)	*0.07*
3	2,478	0.98 (0.89 – 1.07)	*0.62*
2	2,254	0.93 (0.85 – 1.02)	*0.13*
0 or 1	1,327	1.0 (ref)	

OR (95% CI) per low-vitamin D allele = 1.00 (0.97 – 1.02)



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