Null Results in Brief

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Common Genetic Variants in the Vitamin D Pathway Including Genome-Wide Associated Variants Are Not Associated with Breast Cancer Risk among Chinese Women

Tsogzolmaa Dorjgochoo[1], Ryan Delahanty[1], Wei Lu[2], Jirong Long[1], Qiuyin Cai[1], Ying Zheng[2], Kai Gu[2], Yu-Tang Gao[3], Wei Zheng[1], and Xiao Ou Shu[1]

Abstract

Background: Previous studies evaluating the association of vitamin D–related genetic variants with breast cancer risk have produced inconsistent results.

Methods: We evaluated the association between breast cancer risk and 559 single-nucleotide polymorphisms (SNP) in 12 vitamin D–related genes, including 6 genes associated with circulating 25-hydroxyvitamin D [25(OH)D] level identified by recent genome-wide association studies (GWAS), using directly observed and imputed GWAS genotyping data from 2,919 breast cancer cases and 2,323 controls recruited in the Shanghai Breast Cancer Study.

Results: Of the SNPs studied, only rs12570116 in the *ACADSB* gene, rs4760658 in the *VDR* gene and rs6091822, rs8124792, and rs6097809 in the *CYP24A1* gene, and rs10902845 in C10orf88 had a nominal association with breast cancer risk ($P < 0.05$ for all). None of these associations persisted after adjustment for multiple comparisons. The most extensively studied SNPs including rs10735810, also known as rs2228570 (Fok1, *VDR*), rs1544410 (Bsm1, *VDR*), and rs2296241 (*CYP24A1*), were not associated with breast cancer risk. GWAS-identified genetic variants that were associated with 25(OH)D were also not related to breast cancer risk.

Conclusions: Our data suggest that genetic polymorphisms in vitamin D–related genes do not play a major role in breast cancer risk in Chinese women.

Impact: Although our study confirms previously documented breast cancer risk factor associations, our null results suggest that common genetic variants in vitamin D genes and loci associated with control of vitamin D levels are not risk factors for breast cancer in Chinese women. Our data contribute to filling the gap in this field of research. *Cancer Epidemiol Biomarkers Prev; 20(10); 2313–6. ©2011 AACR.*

Introduction

In recent years, the role of vitamin D in the etiology of breast cancer has been increasingly recognized because of its importance in cell proliferation, apoptosis, and differentiation in normal and malignant tumor cells (1, 2). Numerous epidemiologic studies have suggested that vitamin D status or circulating 25-hydroxyvitamin D [25(OH)D] level (1) and common variants that affect vitamin D production and signaling may play a role in the development of breast cancer (2, 3); however, the results have been inconclusive. No epidemiologic study has yet simultaneously evaluated the association between polymorphisms in vitamin D pathway genes [25(OH)D-1-alpha hydroxylase (*CYP27B1*), vitamin D (3) 24-hydroxylase (*CYP27A1*), vitamin D 24-hydroxylase (*CYP24A1*), vitamin D binding protein (*GC*), cytochrome P450, family 3, subfamily A, polypeptide 4 (*CYP3A4*), cytochrome P450 arachidonic acid epoxygenase (*CYP2J2*), cytochrome P450, family 2, subfamily R, polypeptide 1 (*CYP2R1*), and vitamin D receptor gene (*VDR*); ref. 2], as well as in novel genes associated with 25(OH)D level that have been identified by recent genome-wide association studies [GWAS; NAD synthetase (*NADSYN1*), 7-dehydrocholestrol reductase (*DHCR7*), acyl-coenzyme A dehydrogenase (*ACADSB*), and the region of chromosome 10 harboring open-reading frame 88 (*C10orf88*); refs. 4, 5] and breast cancer risk. We comprehensively examined this association using data on more than 5,000 women from the Shanghai Breast Cancer GWAS (SBC-GWAS).

Authors' Affiliations: [1]Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, and Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee; [2]Shanghai Center for Disease Control and Prevention, Shanghai, China; and [3]Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China

Corresponding Author: Xiao Ou Shu, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, 2525 West End Avenue, 6th Floor, Nashville, TN 37203. Phone: 615-936-0713; Fax: 615-936-8291; E-mail: xiao-ou.shu@vanderbilt.edu

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Table 1. SNPs with a value of $P < 0.1$ in the genes of interest and breast cancer risk, the SBC-GWAS (2,919 cases and 2,323 controls)

Gene code SNPs	Chr	Base position	Effective allele	and frequency	Per allele OR (95% CIs)[a]	*P*	Data source
CYP2J2							
rs11572321	1	60134980	A	0.95	0.85 (0.70–1.01)	0.077	Imputed
rs11572307	1	60138925	C	0.05	1.18 (0.98–1.42)	0.078	Genotyped
rs11572305	1	60138974	C	0.95	0.85 (0.71–1.01)	0.079	Imputed
rs3738474	1	60,154,172	T	0.05	1.18 (0.98–1.41)	0.072	Genotyped
rs11572235	1	60156005	A	0.95	0.84 (0.70–1.01)	0.065	Imputed
rs11572227	1	60158049	C	0.05	1.18 (0.98–1.42)	0.066	Imputed
GC							
rs12640179	4	72831551	G	0.16	1.10 (0.99–1.22)	0.072	Genotyped
rs1491709	4	72832430	A	0.17	1.09 (0.98–1.21)	0.081	Imputed
ACADSB							
rs12570116	10	124766465	C	0.97	1.76 (1.18–2.62)	**0.004**	Imputed
C10orf88							
rs10902845	10	124673297	C	0.21	1.14 (1.01–1.30)	**0.039**	Imputed
VDR							
rs4760658	12	46582753	G	0.02	0.71 (0.52–0.97)	**0.033**	Genotyped
rs2254210	12	46559981	A	0.29	1.08 (0.99–1.17)	0.065	Genotyped
rs11168292	12	46579872	G	0.01	0.74 (0.54–1.01)	0.062	Genotyped
rs4077869	12	46591911	A	0.95	0.84 (0.69–1.02)	0.085	Imputed
rs7302038	12	46593908	A	0.04	1.20 (0.99–1.46)	0.058	Genotyped
CYP24A1							
rs6091822	20	52195842	G	0.71	1.11 (1.01–1.21)	**0.022**	Imputed
rs8124792	20	52200214	A	0.30	0.89 (0.82–0.98)	**0.016**	Genotyped
rs6097805	20	52201903	A	0.38	1.07 (0.98–1.16)	0.094	Genotyped
rs6097807	20	52202862	A	0.37	1.07 (0.99–1.16)	0.084	Imputed
rs6097809	20	52206917	C	0.31	0.90 (0.83–0.98)	**0.021**	Genotyped
rs4809959	20	52219266	A	0.47	0.92 (0.83–1.01)	0.094	Imputed
rs2585424	20	52231986	G	0.79	1.09 (0.98–1.20)	0.082	Imputed

NOTE: P_{trend} is from additive models of effect. SNPs associated with breast cancer risk ($P < 0.05$) are shown in bold; however, these *P* values were not significant after accounting for multiple comparisons (data not shown).
[a]Adjusted for age at interview and education.

Materials and Methods

Study population

This study includes data from 5,242 Chinese women, aged 25 to 70 years, in the SBC-GWAS, which drew its data from women who participated in the Shanghai Breast Cancer Study (SBCS, phases I and II), a population-based case–control study. Detailed methods for the SBCS and the SBC-GWAS have been published elsewhere (6). This study was approved by all participating institutions, and participants provided written informed consent.

Single-nucleotide polymorphism genotyping, selection, and imputation

Genotyping information was generated using the Affymetrix 6.0 array as described in detail previously (6). The 8 genes (*CYP27B1*, *CYP27A1*, *CYP24A1*, *GC*, *CYP3A4*, *CYP2J2*, *CYP2R1*, and *VDR*) evaluated in this study were selected on the basis of their potential biologic role in vitamin D metabolism and signaling as determined by literature review (2, 4), as well as an informatics tool, the STRING database (version 8.3). In addition, we included genes (*NADSYN1*, *ACADSB*, *DHCR7*, and *C10orf88*) that have been associated with 25(OH)D concentration, as identified by recent GWASs (4, 5). Single-nucleotide polymorphisms (SNP) were selected within the region 10 kb upstream of the transcription start site and 10 kb downstream from the end of each gene. A total of 559 SNPs (175 directly observed and 384 imputed) in these 12 vitamin D-related genes with a minor allele frequency of 5% or more were included in the analyses.

Statistical analysis

Descriptive statistics and genome-wide analyses were conducted within each sample set and in aggregate using SAS (version 9.2) and PLINK, respectively, as described

Table 2. GWAS-identified SNPs associated with circulating vitamin D [25(OH)D] levels and proxy-SNPs in relation to breast cancer risk in the SBC-GWAS (2,919 cases and 2,323 controls)

GWAS SNP	Chr	Genomic position	Gene location or nearest gene	Effective allele and frequency		Per allele OR (95% CIs)[a]	*P*	Data source
rs17467825[b]	4	72824381	*GC*	A	0.69	0.98 (0.90–1.08)	0.78	Imputed
rs2282679[b,c]	4	72827247	*GC*	G	0.31	1.01 (0.92–1.10)	0.82	Imputed
rs3755967[b]	4	72828262	*GC*	T	0.31	1.01 (0.92–1.09)	0.89	Genotyped
rs2298850[b]	4	72833131	*GC*	C	0.31	1.01 (0.92–1.09)	0.88	Genotyped
rs7041[b,c]	4	72837198	*GC*	A	0.72	1.03 (0.94–1.13)	0.44	Imputed
rs1155563[b,c]	4	72862352	*GC*	C	0.40	1.02 (0.94–1.12)	0.55	Imputed
rs1993116[b,c]	11	14866810	*CYP2R1*	A	0.35	0.98 (0.90–1.06)	0.58	Imputed
rs10500804[b]	11	14866849	*CYP2R1*	G	0.37	1.00 (0.92–1.08)	0.98	Genotyped
rs12794714	11	14870151	*CYP2R1*	A	0.37	0.99 (0.91–1.07)	0.87	Imputed
rs10741657[b,c]	11	14871454	*CYP2R1*	A	0.35	0.97 (0.90–1.05)	0.56	Imputed
rs2060793[b,c]	11	14871886	*CYP2R1*	A	0.35	0.98 (0.90–1.06)	0.56	Imputed
rs7944926[b]	11	70843273	*DHCR7/NADSYN1*	A	0.54	0.94 (0.87–1.02)	0.19	Imputed
rs12785878[b,c]	11	70845097	*DHCR7/NADSYN1*	T	0.46	1.02 (0.94–1.10)	0.58	Genotyped
rs4944957[b]	11	70845683	*DHCR7/NADSYN1*	G	0.45	1.01 (0.93–1.09)	0.79	Genotyped
rs12800438[b]	11	70848651	*DHCR7/NADSYN1*	A	0.45	1.04 (0.96–1.12)	0.30	Imputed
rs3794060[b]	11	70865327	*DHCR7/NADSYN1*	C	0.54	0.95 (0.88–1.03)	0.25	Imputed
rs4945008[b]	11	70898896	*DHCR7/NADSYN1*	A	0.55	0.95 (0.88–1.02)	0.21	Imputed
rs1790349[c]	11	70819998	*DHCR7/NADSYN1*	C	0.29	0.97 (0.89–1.06)	0.56	Genotyped
rs3829251[c]	11	70872207	*NADSYN1*	A	0.30	0.94 (0.86–1.02)	0.16	Imputed
rs10898193 *proxy for* rs11234027[c]	11	70874731	*NADSYN1*	T	0.30	0.94 (0.86–1.02)	0.16	Genotyped
rs17104498 *proxy for* rs6599638[c]	10	124786160	*C10orf88*	G	0.12	0.96 (0.85–1.08)	0.49	Genotyped
rs2762932 proxy for rs6013897[b]	20	52201798	*CYP24A1*	C	0.10	1.04 (0.91–1.18)	0.53	Genotyped

[a]Adjusted for age at interview and education.
[b]The SUNLIGHT GWAS of 15 cohorts (United Kingdom, United States, Canada, the Netherlands, Sweden, and Finland).
[c]Meta-analysis of 5 GWAS within 5 cohorts (European ancestry).

previously (6). Multivariate logistic regression was used to estimate ORs and 95% CIs for associations between breast cancer risk and polymorphisms under an additive genetic model, controlling for age and education. *P* values were not corrected for multiple testing (Tables 1 and 2). Linkage disequilibrium was assessed by Haploview.

Results

The mean age was 50.7 years for cases and 49.6 years for controls. As compared with controls, cases were more likely to have higher educational attainment, earlier age at menarche, later age at first birth and menopause, longer reproductive span, family history of breast cancer among first-degree relatives, and a higher waist-to-hip ratio (data not shown). Of the 559 SNPs in the 12 genes that we analyzed, only 6 SNPs (rs12570116 in *ACADSB*, rs10902845 in *C10orf88*, rs4760658 in *VDR*, and rs6091822, rs8124792, and rs6097809 in *CYP24A1*) were associated with breast cancer risk ($P < 0.05$ for all, Table 1). However, these nominally significant associations were not significant after accounting for multiple testing. We also found no association between breast cancer risk and the most extensively studied genetic polymorphisms (2), including Fok1 (rs10735810, also known as rs2228570), Bsm1 (rs1544410), Taq1 (rs731236), and Apa1 (rs7975232) in *VDR* and rs2296241 in *CYP24A1* (data not shown). Of the 22 SNPs associated with 25(OH)D level identified by prior GWAS, none were associated with breast cancer risk in our population (Table 2). Furthermore, in analyses stratified by menopausal status, physical activity, or dietary energy intake, no polymorphisms were associated with breast cancer risk (data not shown).

Discussion

To our knowledge, this is the first comprehensive examination of common genetic variations (559 SNPs) across 12 genes related to vitamin D metabolism and signaling and breast cancer risk. Overall, vitamin D

pathway gene polymorphisms were not associated with breast cancer risk in our population. The suggestive association for 6 SNPs in the *ACADSB*, *VDR*, and *CYP24A1* genes and *C10orf88* could be due to chance and need to be further investigated in other populations.

Among the vitamin D pathway genes, *VDR* SNPs (Apa1: rs7975232, Bsm1: rs1544410, Taq1: rs731236, and Fok1: rs10735810) have been investigated extensively in relation to cancer risk, particularly among European-ancestry populations (2, 3). However, none of these SNPs were associated with breast cancer risk in our study. In a meta-analysis of 21 case–control studies (2) and in a pooling analysis of 6 prospective studies (3), breast cancer risk was associated with the *VDR* Fok1 polymorphism, however, the Cancer Prevention Study (CPS) II Nutrition Cohort in the United States did not find a relationship between any of these *VDR* SNPs and breast cancer risk among postmenopausal women (7). In addition, prior studies showed that *CYP24A1* was overexpressed in breast carcinoma (8). In our study, we also found no associations of *CYP24A1* polymorphisms with breast cancer risk, consistent with the CPS II Nutrition Cohort (7).

In a recent GWAS conducted among 5 European-ancestry cohorts in the United States, several SNPs in the *GC*, *DHCR7/NADSYN*, and *CYP2R1* genes and *C10orf88* (in the vicinity of *ACADSB*) were associated with 25(OH)D level (4). Another GWAS [the Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits (SUNLIGHT)] of 15 cohorts among 33,996 individuals of European ancestry found an association for 3 other SNPs in *DHCR7/NADSYN1*, *GC*, and *CYP2R1* (5). None of these 25(OH)D-associated gene variants were related to breast cancer risk in our population.

Strengths of this study include its large sample size, population-based design, comprehensive analysis involving multiple genes, and control of potential confounding variables. A limitation of this study is that we have no direct measurements of circulating 25(OH)D level or vitamin D status. In addition, our analysis was conducted among Chinese women and the results may not be generalizable to other ethnic groups/populations.

In conclusion, this comprehensive survey provides no strong evidence for the hypothesis that genetic variants in a set of genes related to vitamin D level and metabolism play an independent role in breast cancer development among Chinese women.

Disclosure of Potential Conflicts of Interest

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the NIH. The authors have no conflicts of interest to disclose.

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