Vitamin D Pathway Polymorphisms in Relation to Mammographic Breast Density

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Introduction

Vitamin D may play a role in mammary gland development and carcinogenesis (1). Results from our team and others (2-4) suggest that high vitamin D intakes/levels may reduce breast cancer risk and mammographic breast density (MBD), a strong and highly heritable breast cancer risk factor (5). The protective action of vitamin D is mainly mediated through the vitamin D receptor (VDR) present in breast cells (1). Human mammary cells also express cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1), the enzyme that converts the major circulating form of vitamin D {25-hydroxyvitamin D [25(OH)D]} into biologically active 1,25-dihydroxyvitamin D [1,25(OH)D], the ligand for VDR (6). Recent data suggest that 25(OH)D may be delivered to and internalized by mammary cells in complex with vitamin D–binding protein (DBP; ref. 7). To further clarify the role of vitamin D in the etiology of breast cancer, we evaluated the association of 13 targeted single-nucleotide polymorphisms (SNP) in *VDR*, *CYP27B1*, and *DBP* genes with MBD in Caucasian women, including the well-studied *VDR Fok*I, *Bsm*I, *Apa*I, and *Taq*I previously found associated with breast cancer risk in some Caucasian studies (2).

Materials and Methods

Study subjects were premenopausal women who underwent screening mammography between February and December 2001 at the Clinique radiologique Audet (Québec, Canada; see ref. 8 for details). Among the 783 eligible women, 746 provided written informed consent to use their blood samples for assays other than those planned at recruitment (8). This study was approved by a research ethics committee.

Anthropometric measurements and blood samples were taken at recruitment by a trained nurse. Breast cancer risk factors were documented by telephone interview, and diet was evaluated with a food frequency questionnaire.

MBD was blindly assessed by one reviewer (C.D.) using a computer-assisted method (8). MBD is the proportion of the breast projection showing tissue density on one randomly selected craniocaudal view for each woman. The within-batch intraclass correlation coefficient and the between-batch coefficient of variation were 0.98% and 4%, respectively.

DNA was extracted from buffy coat ($n = 741$) using the PUREGene DNA extraction kit, and then DNA samples were blindly genotyped for 13 selected SNPs located in *VDR*, *CYP27B1*, and *DBP* genes using various platforms (see Table 1). Protocols can be provided upon request. Common SNPs were included in the present study if located in promoter regions or exons or found to be associated with breast cancer risk or its associated risk factors in previous studies (2, 9). Concordance within or between genotyping platforms was higher than 99%. No obvious deviation from Hardy-Weinberg equilibrium was observed for any of the SNPs ($P > 0.03$). The linkage disequilibrium strength was evaluated with Lewontin's D' statistic for pair-wise SNPs within each gene, and was 1.00 for those located in promoter region or intron 8 and exon 9 of *VDR*, *CYP27B1*, or *DBP*, and 0.02 to 0.78 otherwise.

Crude and adjusted means of MBD by category of genotypes were estimated using generalized linear models, and statistical differences were evaluated with P for heterogeneity. The associations between the number of copies of the rare allele entered as a continuous variable (0, 1, or 2) and MBD were evaluated by linear regression models (P_{trend}). Univariate and multivariate haplotype analyses were done based on all SNPs or those in haplotype blocks (region of strong

Table 1. SNPs evaluated in the present study

Gene name	Encoded product	*n*	Alleles (major > minor)	SNP reference ID*	Position in gene (other designation)	Genotyping platform	Call rate (%)
VDR	Vitamin D receptor	726	G > A	rs11568820	Promoter (Cdx2)	Sequenom	98.0
		732	C > G	rs7139166	Promoter (−1521)	Sequenom	98.8
		734	T > C	rs4516035	Promoter (−1012)	SNPstream	99.1
		736	G > A	rs2238136	Intron 1 (−4817)	SNPstream	99.3
		731	G > A	rs10735810	Exon 2 (*Fok*I)	Sequenom	98.7
		736	C > T	rs1544410	Intron 8 (*Bsm*I)	SNPstream	99.3
		707	A > C	rs7975232	Intron 8 (*Apa*I)	Sequenom	95.4
		737	T > C	rs731236	Exon 9 (*Taq*I)	Sequenom	99.5
CYP27B1	Cytochrome P450, family 27 subfamily B, polypeptide 1	739	A > G	rs703842	Promoter (−1918)	FP-SBE	99.7
		737	C > A	rs10877012	Promoter (−1260)	FP-SBE	99.5
		736	C > G	rs3782130	Promoter (−1077)	FP-SBE	99.3
DBP	Vitamin D–binding protein	733	G > T	rs7041	Exon 11 ($Asp^{416}Glu$)	Sequenom	98.9
		733	C > A	rs4588	Exon 11 ($Thr^{420}Lys$)	Sequenom	98.9

Abbreviation: FP-SBE, fluorescent polarization-single base extension.
*Polymorphisms are identified by their dbSNP accession number at http://www.ncbi.nlm.nih.gov/SNP/.

linkage disequilibrium) within gene using the method described by Stram et al. (10). For these analyses, the most common haplotype was used as the reference category. Assumption of normality of residuals from all analyses was met with untransformed variables. All statistical analyses were carried out using SAS version 9.1 (SAS Institute, Inc.).

Results

We observed no statistically significant association of any of the eight *VDR* SNPs with MBD (Table 2). No significant association was found between MBD and polymorphisms located in *CYP27B1* and *DBP* genes (Table 2). Moreover, we observed no significant association of any haplotypes within *VDR*, *CYP27B1*, or *DBP* genes with MBD (data not shown).

Discussion

Well-studied *VDR* *Fok*I:rs10735810, *Bsm*I:rs1544410, *Apa*I:rs7975232, and *Taq*I:rs731236 polymorphisms have been associated with breast cancer risk in some Caucasian populations (2, 11-14).[6] Knowing the strong link of MBD with breast cancer risk and because effects of vitamin D are mainly mediated via the *VDR* gene, we hypothesized that those *VDR* polymorphisms could be associated with MBD. However, our data suggest no evidence of associations between the above *VDR* polymorphisms and MBD.

The average frequencies of rare and common homozygote of those *VDR* polymorphisms are ~14% to 27% and 26% to 40%, respectively, in Caucasian populations (2, 11-13).[6] At such allele frequencies, the present study had 80% power to detect absolute differences of 6% to 8% in MBD comparing rare to common homozygote carriers (with two-sided $\alpha = 0.05$). It is estimated that a difference of 1% in MBD is associated with 0.4% to 2.7% difference in breast cancer risk (15). Assuming that breast cancer risk is totally due to MBD and using odds ratios of 1.33 to 1.34 for rare *Fok*I homozygote women from the same city[6] or from another North American Caucasian population (16), we expected at least 12% difference in MBD, therefore suggesting that our sample size was large enough.

The major strength of this study is the reliability of breast density measurements. Moreover, population stratification is not a major source of concern because our population was composed of 99.7% Caucasian women and more than 87.7% women of French-Canadian descent (17).

Vitamin D signaling pathway in mammary cells involves regulation of *VDR*, *CYP27B1*, and *DBP* genes (18). In addition to the above well-studied *VDR* polymorphisms, other SNPs in this gene (rs11568820, rs7139166, rs4516035, rs2238136) as well as in *CYP27B1* (rs703842, rs10877012, rs3782130) and *DBP* (rs7041, rs4588) genes were investigated, but no statistically significant association was observed with MBD. These polymorphisms were associated with breast cancer risk or its related factors, autoimmune Addison's disease, and diabetes or its related factors, respectively (9, 12, 19-21). Recently, lack of significant association was observed between both *DBP* polymorphisms and breast cancer risk (13).

In conclusion, we found no evidence that *VDR*, *CYP27B1*, and *DBP* polymorphisms studied here were associated with MBD among Caucasian premenopausal women of French descent.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

[6] M. Sinotte, et al. Vitamin D receptor polymorphisms (FokI and BsmI) and breast cancer risk: association replication in two case-control studies within French Canadian population. Submitted for publication.

Table 2. Associations of SNPs in vitamin D pathway genes with MBD

Gene name	rs number	Genotype	*n* (%)*	Breast density (%)					
				Crude models		Adjusted models 1 †		Adjusted models 2 ‡	
				Mean	*P*§	Mean	*P*§	Mean	*P*§
VDR	rs11568820	GG	418 (57.6)	41.9		41.7		41.9	
		GA	268 (36.9)	43.3	0.39 (2 *df*)	43.5	0.26 (2 *df*)	43.5	0.34 (2 *df*)
		AA	40 (5.5)	37.8	0.87 (1 *df*)	38.7	0.81 (1 *df*)	39.2	0.82 (1 *df*)
	rs7139166	CC	302 (41.3)	43.0		43.3		43.5	
		CG	315 (43.0)	42.2	0.80 (2 *df*)	42.6	0.21 (2 *df*)	42.5	0.32 (2 *df*)
		GG	115 (15.7)	41.3	0.51 (1 *df*)	39.5	0.12 (1 *df*)	40.1	0.15 (1 *df*)
	rs4516035	TT	302 (41.1)	43.0		43.2		43.3	
		TC	318 (43.3)	42.1	0.83 (2 *df*)	42.6	0.31 (2 *df*)	42.6	0.45 (2 *df*)
		CC	114 (15.5)	41.5	0.54 (1 *df*)	39.8	0.18 (1 *df*)	40.5	0.24 (1 *df*)
	rs2238136	GG	361 (49.0)	42.3		42.2		42.3	
		GA	308 (41.9)	42.8	0.73 (2 *df*)	43.1	0.45 (2 *df*)	43.4	0.35 (2 *df*)
		AA	67 (9.1)	40.2	0.75 (1 *df*)	39.8	0.76 (1 *df*)	39.5	0.70 (1 *df*)
	rs10735810	GG	271 (37.1)	42.1		42.6		42.2	
		GA	346 (47.3)	41.4	0.17 (2 *df*)	41.8	0.33 (2 *df*)	41.7	0.39 (2 *df*)
		AA	114 (15.6)	46.3	0.24 (1 *df*)	44.7	0.38 (1 *df*)	44.9	0.51 (1 *df*)
	rs1544410	CC	261 (35.5)	42.4		42.6		42.5	
		CT	347 (47.2)	41.7	0.64 (2 *df*)	41.5	0.40 (2 *df*)	41.9	0.49 (2 *df*)
		TT	128 (17.4)	44.1	0.63 (1 *df*)	44.3	0.62 (1 *df*)	44.4	0.56 (1 *df*)
	rs7975232	AA	224 (31.7)	43.0		43.5		43.3	
		AC	352 (49.8)	42.2	0.88 (2 *df*)	41.8	0.51 (2 *df*)	42.2	0.71 (2 *df*)
		CC	131 (18.5)	43.3	0.98 (1 *df*)	43.6	0.86 (1 *df*)	43.7	0.99 (1 *df*)
	rs731236	TT	265 (36.0)	42.1		42.4		42.4	
		TC	349 (47.3)	42.0	0.84 (2 *df*)	41.6	0.60 (2 *df*)	41.9	0.63 (2 *df*)
		CC	123 (16.7)	43.5	0.69 (1 *df*)	43.8	0.72 (1 *df*)	43.9	0.62 (1 *df*)
CYP27B1	rs703842	AA	392 (53.0)	42.8		43.1		43.3	
		AG	291 (39.4)	42.2	0.72 (2 *df*)	41.8	0.56 (2 *df*)	41.9	0.54 (2 *df*)
		GG	56 (7.6)	40.0	0.46 (1 *df*)	40.5	0.28 (1 *df*)	40.7	0.27 (1 *df*)
	rs10877012	CC	394 (53.4)	42.7		42.9		43.1	
		CA	288 (39.1)	42.4	0.69 (2 *df*)	42.0	0.59 (2 *df*)	42.1	0.59 (2 *df*)
		AA	55 (7.5)	39.7	0.50 (1 *df*)	40.2	0.32 (1 *df*)	40.5	0.31 (1 *df*)
	rs3782130	CC	394 (53.5)	42.4		42.8		43.0	
		CG	286 (38.9)	42.7	0.75 (2 *df*)	42.2	0.71 (2 *df*)	42.2	0.69 (2 *df*)
		GG	56 (7.6)	40.0	0.70 (1 *df*)	40.4	0.44 (1 *df*)	40.6	0.40 (1 *df*)
DBP	rs7041	GG	228 (31.1)	42.8		42.3		42.7	
		GT	377 (51.4)	42.9	0.50 (2 *df*)	42.8	0.69 (2 *df*)	43.0	0.51 (2 *df*)
		TT	128 (17.5)	40.0	0.38 (1 *df*)	41.1	0.70 (1 *df*)	40.6	0.45 (1 *df*)
	rs4588	CC	370 (50.5)	42.0		42.2		42.3	
		CA	296 (40.4)	42.8	0.92 (2 *df*)	42.5	0.97 (2 *df*)	42.7	0.97 (2 *df*)
		AA	67 (9.1)	42.2	0.80 (1 *df*)	42.8	0.80 (1 *df*)	42.4	0.89 (1 *df*)

Abbreviation: *df*, degrees of freedom.

*n values are slightly lower in models 2 because of nine missing values in additional adjustment variables.

†Adjusted models 1 are controlled for age and body mass index.

‡Adjusted models 2 are as models 1 with further adjustments for age at menarche, number of breast biopsies, family history of breast cancer in first-degree relatives, number of full-term pregnancies, age at first birth, past contraceptive and hormone replacement therapy uses, energy, vitamin D, calcium and alcohol intakes, smoking status, leisure-time physical activity, and education.

§2 *df P* value test for heterogeneity between the means of breast density. 1 *df P* value is the P_{trend}, testing genotype dosage.

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