Vitamin D receptor gene polymorphisms are associated with adiposity phenotypes[1–3]

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ABSTRACT

Background: Emerging data suggest a role for the vitamin D receptor (VDR) in lipogenesis and adipocyte differentiation.

Objective: Our objective was to evaluate the association of VDR gene variants and adiposity phenotypes in an epidemiologic study.

Design: In a sample of 1773 healthy female adults recruited from western New York, we tested for the association of 14 *VDR* single nucleotide polymorphisms (SNPs) with the following 3 adiposity phenotypes: body mass index (in kg/m^2), waist circumference (in cm), and abdominal height (in cm). We examined age, education, total energy intake, smoking status, alcohol intake, and menopausal status as potential covariates.

Results: One SNP, rs3782905, remained associated with all 3 adiposity phenotypes after multiple-testing correction (Bonferroni-adjusted $P = 0.004$). The mean waist circumference for women with the rs3782905 homozygous rare genotype was 4.4 cm larger than for women with the common homozygous genotype. Two other *VDR* SNPs were associated with waist circumference and abdominal height, but the associations did not survive multiple-testing correction. Adjustment for covariates did not influence the results.

Conclusion: The study results and the biological activity of VDR in adipocyte differentiation suggest that 3′ *VDR* variants may play a role in adiposity phenotypes. *Am J Clin Nutr* 2011;93:5–10.

INTRODUCTION

Evidence from twin, family, and adoption studies suggests that total body adiposity is a highly heritable phenotype (1). Given the polygenic nature of adiposity, numerous quantitative trait loci and candidate genes have been identified (2, 3). Heritability estimates range from 16% to 85% for body mass index (BMI) (3–6) and 37–81% for waist circumference (3, 7–9), which suggest that, aside from environmental factors, genes also substantially contribute to obesity.

The active form of vitamin D, 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], binds the vitamin D receptor (VDR), which is a product of the VDR gene locus (*VDR*) on chr12q13.1 and a member of the steroid hormone receptor superfamily (10, 11). This receptor-ligand complex functions as a transcription factor binding to vitamin D–response elements and, thus, influences the transcription of vitamin D–responsive genes (10).

Obesity is now recognized as a risk factor for vitamin D deficiency (12–14). However, whether vitamin D status is at all causally associated with increased adiposity or vitamin D status results from increased adipose storage of vitamin D (14) remains to be determined. The role of 1,25$(OH)_2D_3$ in adipocyte metabolism is quite complex. One mechanism involves interaction with VDR. Some literature suggests that both 1,25$(OH)_2D_3$ and VDR are important players in adipocyte differentiation (15, 16). One study showed that 1,25$(OH)_2D_3$, via proposed interactions with VDR, inhibited the early phase of differentiation of preadipocytes to mature adipocytes in vitro (16). In *VDR* knockout mice, unlike in wild-type mice, 1,25$(OH)_2D_3$ was not able to block peroxisome proliferator–activated receptor γ expression and the corresponding adipocyte differentiation, which signified that VDR is a key mediator of the action of 1,25$(OH)_2D_3$ in adipocyte differentiation (16, 17). Other studies point to a role for VDR in adipogenesis on the basis of varying VDR messenger RNA concentrations during adipocyte differentiation (18–20).

The collective evidence to date along with the ability of bound VDR to influence expression of so many genes (21) and the accumulating evidence for involvement of vitamin D in a variety of chronic diseases and conditions (22) were the impetus for this

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study. To our knowledge, no large epidemiologic studies have examined the association of *VDR* polymorphisms and adiposity phenotypes.

We hypothesized a positive association of the *Cdx-2* variant and adiposity on the basis of a reduction of VDR transcription (23), which would thus result in an inability to suppress adipocyte differentiation. We hypothesized a positive association of the *FokI* variant and adiposity because of the decreased biological activity of the resulting VDR protein (24) that may suppress the inhibitory effect of $1,25(OH)_2D_3$ on adipogenesis. We were unable to hypothesize a direction for the remaining single nucleotide polymorphisms (SNPs) because they were tag SNPs and not known to be functional. We examined these associations in a sample of healthy women from western New York State.

SUBJECTS AND METHODS

Study population

We studied a random sample of healthy women (control subjects) aged 35–80 y and living in Erie and Niagara counties in western New York State who were recruited between 1996 and 2001 for a breast cancer case-control study. The details of the study were previously described (25). Women <65 y of age were selected from the New York State Department of Motor Vehicles roster, whereas women >65 y of age were selected from the Health Care Finance Administration list. Of 3396 eligible women identified, 2115 women participated. We excluded African American, Hispanic, Native American, or Asian women ($n = 205$) from this analysis because of small numbers as well as women missing genotype data for all 14 *VDR* SNPs ($n = 137$) and women missing all phenotype data ($n = 24$), which resulted in a final sample of 1749 women. The Institutional Review Board at the University of Buffalo approved the study protocol, and all women provided written informed consent to participate in the study.

Personal interview

Information was collected on sociodemographic factors, smoking history, menopausal status, alcohol intake, dietary intake, and physical activity of each participant by using interviewer-assisted interviews and self-administered questionnaires during a visit to the University at Buffalo Center for Preventive Medicine. A modified health habits and diet food-frequency questionnaire developed at the National Cancer Institute was used to obtain food-consumption information, as previously described (25).

Adiposity phenotype measurement

Interviewers measured height with a stadiometer and weight with a balance beam scale. We calculated BMI (in kg/m^2) and categorized BMI into the following categories for a portion of analyses: underweight (<18.5), normal weight (18.5–24.9), overweight (25–30), and obese (>30) (26). Because the number of women in the underweight category was low; we combined the underweight and normal weight categories.

To measure waist circumference, trained interviewers measured the narrowest distance between the inferior end of the rib cage and the superior end of the iliac crest to the nearest 0.5 cm after normal expiration. We categorized women according to waist circumference on the basis of the clinical criterion proposed for women of ≤88and >88 cm (27).

With the use of a Holtain-Kahn caliper, interviewers measured abdominal height with participants in a recumbent position to the nearest 0.5 cm (28). To minimize variability, we used the mean of the second and third (of 3 total) abdominal height measurements in our analyses. We categorized abdominal height into tertiles for some of the analyses.

Twenty-six women were missing BMI, 27 women were missing waist circumference, and 161 women were missing abdominal height measurements.

Selection of VDR SNPs and genotyping

We selected haplotype tagging SNPs by using the cell HapMap sample (data release #20/phase II Jan06, on NCBI build 35 assembly, dbSNP b125; www.hapmap.org) and Tagger (29). Two additional functional SNPs (*Cdx-2* and *FokI*) were selected for genotyping on the basis of the literature (23, 24) for a total of 14 SNPs. Genotyping was performed by using TaqMan methodology (ABI 7900HT; Applied Biosystems, Foster City, CA), direct sequencing (MegaBace capillary sequencer; Amersham Biosciences, Piscataway, NJ), or denaturing high-pressure liquid chromatography (Transgenomic Wave; Transgenomic Inc, Omaha, NE). The missing rate for the SNPs ranged from 0.79% to 2.88%.

Statistical analyses

We examined differences in demographic factors, lifestyle factors, and laboratory measurements across categories of BMI, waist circumference, and abdominal height. We used analysis of variance or the *t* test to test for differences in means for continuous variables and chi-square tests for categorical variables. We calculated Pearson's *r* and partial *r* of adiposity phenotypes with and without adjustment for age, education, total energy intake, smoking status, and menopausal status.

We estimated allele and genotype frequencies for all *VDR* SNPs and verified that all SNPs confirmed to Hardy-Weinberg proportions. After testing the normality assumption for continuous measures of adiposity phenotypes, we used linear regression to test the association of *VDR* SNPs with each adiposity phenotype in 3 completely separate models. We selected the aforementioned covariates, a priori, on the basis of previous literature. We set the significance level at $\alpha = 0.05$ and adjusted our results for multiple testing by using the Bonferroni method. We used Plink (version 1.05) (30), Haploview (version 4.1) (31), and SAS (version 9.2) (32) software for all analyses. We used Quanto (version 1.2) software for power calculations (33).

RESULTS

The sample had a mean age of 57.2 y with a mean BMI, waist circumference, and abdominal height of 27.9 and 86.6 and 20.4 cm, respectively. Descriptive characteristics stratified by waist circumference (≤88 and >88 cm) are shown in **Table 1**. Women with waist circumferences >88 cm had a larger BMI and abdominal height, were older, and had fewer years of education compared with women with waist circumferences ≤88 cm. Women with waist circumference >88 cm had high total energy intake, fasting glucose concentrations, HDL and LDL cholesterol,

TABLE 1
Variables stratified by categories of waist circumference (n = 1722)[1]

	Waist circumference		
Variable	≤88 cm (n = 1044)	>88 cm (n = 678)	P
Age (y)	56.3 ± 11.8[2]	58.4 ± 11.2	0.001
Years of education	13.7 ± 2.4	13.1 ± 2.3	0.001
BMI (kg/m^2)	24.3 ± 3.1	33.2 ± 5.4	0.001
Abdominal height (cm)	18.3 ± 2.2	23.8 ± 3.1	0.001
Energy intake (kcal/d)	1428.6 ± 552.3	1523.8 ± 552.8	0.001
Alcohol intake (L)[3]	84.72 ± 268.13	73.63 ± 172.55	0.30
Fasting glucose (mg/100 mL)	94.5 ± 14.4	113.2 ± 14.2	0.001
Systolic blood pressure (mm Hg)	116.8 ± 16.7	122.7 ± 16.4	0.001
Diastolic blood pressure (mm Hg)	69.9 ± 9.1	72.9 ± 9.4	0.001
Total cholesterol (mg/100 mL)	238.1 ± 54.0	243.7 ± 58.5	0.05
HDL cholesterol	66.0 ± 17.0	55.7 ± 14.1	0.001
LDL cholesterol	148.1 ± 47.1	153.2 ± 44.4	0.03
Triglycerides (mg/100 mL)	120.8 ± 74.3	172.7 ± 101.4	0.001
Menopausal status [n (%)]			
Premenopausal	355 (34.0)	172 (25.4)	0.001
Postmenopausal	689 (66.0)	506 (74.6)	0.001
Smoking status [n (%)]			
Never smoker	531 (51.0)	319 (47.2)	0.01
Former smoker	349 (33.5)	274 (40.5)	
Current smoker	162 (15.6)	83 (12.3)	

[1] A t test was used for continuous variables, and a chi-square test was used for categorical values.
[2] Mean ± SD (all such values).
[3] Lifetime alcohol consumption until 2 y before interview.

blood pressure, and triglycerides. Women with a larger waist circumference were also more likely to be postmenopausal and former smokers. Descriptive statistics were similar when stratified by BMI and abdominal height (data not shown).

Pearson's r for BMI, waist circumference, and abdominal height ranged from 0.87 to 0.88, with no effect after adjustment for the covariates examined (data not shown).

The minor allele frequencies and physical positions of the 14 *VDR* SNPs as well as the crude associations of *VDR* and adiposity phenotypes are presented in **Table 2**. We identified a statistically significant (at $\alpha = 0.05$) positive association for rs3782905 and an inverse association for rs3819545 with all 3 adiposity phenotypes. We also identified a positive association for *Cdx-2* (rs11568820) and rs2239179 with waist circumference and abdominal height only and a borderline significant association between these SNPs and BMI (P = 0.09 and 0.10, respectively).

For SNP rs3782905, we observed that each additional rare allele resulted in an increase of 0.87 in BMI, 2.21 cm in waist circumference, and 0.50 cm in abdominal height. After adjustment for multiple testing by using a Bonferroni correction, the rs3782905 was the only SNP that remained significant, with an adjusted P = 0.004. Results adjusted for covariates were similar to unadjusted results; thus, we present only crude models (data not shown).

Mean adiposity values for the 4 SNPs associated with adiposity phenotypes are presented in **Table 3.** Women with rare genotype for rs3782905 (GG) had a mean BMI that was 1.7 times larger and a 4.4-cm larger waist circumference compared with those of women with the common genotype. With the exception of rs3782905, differences in mean adiposity values were not significant for the remaining SNPs.

DISCUSSION

We identified a positive association of one *VDR* SNP (rs3782905) with BMI, waist circumference, and abdominal height, which survived adjustment for multiple testing. With regard to the 2 functional SNPs studied, we identified a positive association of *Cdx-2* (rs11568820) with waist circumference and abdominal height and a borderline positive association with BMI. We observed no significant associations for *FokI*.

To our knowledge, the observed associations between adiposity and the rs3782905 SNP were novel. The significant SNP (rs3782905) and the 2 borderline significant SNPs (rs3819545 and rs2239179) were located at the 3′ end of *VDR* gene. SNPs rs3782905, rs3819545, and rs2239179 were in high linkage disequilibrium (LD) ($r^2 \geq 0.80$), and a portion of the LD extended to the 3′ untranslated region (UTR) (34). Polymorphisms in the 3′ UTR region regulate *VDR* gene expression by modulating messenger RNA stability (35). Because the mentioned SNPs were tag SNPs in high LD, this suggested that functional variants in the 3′ UTR region or 3′ region of the gene may explain our associations.

Power calculations indicated that we had a 95% power to detect an association of rs3782905 with adiposity phenotypes and a 50–80% power for rs3819545 and rs2239179. The low power may explain the borderline significance for rs3819545 and rs2239179.

Cdx-2 (rs11568820) is an A-G transition in the intestine-specific binding site of transcription factor *Cdx-2* in the 5′ promoter region of *VDR* (23). This polymorphism results in a 30% reduction in transcriptional activity of the promoter, decreases intestinal VDR expression, and affects calcium absorption in the intestine (23). *Cdx-2* (rs11568820) may also have a biological role in adiposity because calcium amounts in cells

TABLE 2
VDR single nucleotide polymorphisms (SNPs), minor allele frequencies (MAFs), location, and association (crude) of adiposity phenotypes by using a log-additive model[1]

			BMI (kg/m^2) (n = 1723)		Waist circumference (cm) (n = 1722)		Abdominal height (cm) (n = 1588)	
SNP	MAF	Position[2]	$\beta \pm$ SE	Unadjusted P (adjusted P)	$\beta \pm$ SE	Unadjusted P (adjusted P)	$\beta \pm$ SE	Unadjusted P (adjusted P)
rs739837	0.47 (G)	46524488	−0.28 ± 0.21	0.20	−0.71 ± 0.50	0.16	−0.28 ± 0.13	0.03
rs1540339	0.37 (T)	46543593	−0.13 ± 0.22	0.56	−0.60 ± 0.51	0.23	−0.16 ± 0.13	0.24
rs2239179	0.43 (C)	46544033	0.36 ± 0.22	0.10	1.00 ± 0.51	0.04	0.30 ± 0.13	0.02
rs3819545	0.39 (G)	46551273	−0.43 ± 0.22	0.04	−1.14 ± 0.50	0.02	−0.26 ± 0.13	0.05
rs3782905	0.33 (G)	46552434	0.87 ± 0.23	0.001 (0.01)	2.21 ± 0.53	0.001 (0.0005)	0.50 ± 0.14	0.001 (0.01)
rs2239186	0.21 (G)	46555677	−0.31 ± 0.26	0.23	−1.09 ± 0.61	0.07	−0.09 ± 0.16	0.58
rs2228570 (*FokI*)	0.38 (A)	46559162	0.17 ± 0.22	0.43	0.22 ± 0.51	0.67	0.15 ± 0.13	0.27
rs2853564	0.39 (G)	46564754	−0.05 ± 0.22	0.84	−0.20 ± 0.51	0.70	−0.17 ± 0.13	0.19
rs4760648	0.42 (T)	46566932	0.23 ± 0.22	0.30	0.71 ± 0.51	0.16	0.27 ± 0.13	0.04
rs3890734	0.33 (A)	46575622	−0.14 ± 0.23	0.53	−0.49 ± 0.53	0.36	−0.25 ± 0.14	0.08
rs7136534	0.24 (T)	46580893	0.46 ± 0.25	0.07	1.06 ± 0.58	0.07	0.25 ± 0.15	0.11
rs10783219	0.36 (T)	46581755	−0.30 ± 0.23	0.19	−0.60 ± 0.53	0.26	−0.06 ± 0.14	0.66
rs7299460	0.29 (T)	46582535	0.38 ± 0.23	0.10	0.82 ± 0.53	0.12	0.24 ± 0.14	0.09
rs11568820 (*Cdx-2*)	0.20 (T)	46588812	0.46 ± 0.27	0.09	1.39 ± 0.62	0.03	0.32 ± 0.16	0.05

[1] Unadjusted P values were not adjusted for multiple testing; adjusted P values were adjusted for multiple testing by using a Bonferroni correction only where significant at $P = 0.05$.
[2] NCBI dbSNP Build 131; www.ncbi.nlm.nih.gov/snp.

influence lipogenesis, and lipolysis and are also involved in early and late phases of adipocyte differentiation (36, 37). We had only a 50–60% power to detect an association of *Cdx-2* with the 3 adiposity phenotypes in our study, which may explain the borderline significance.

The *FokI* polymorphism is a T-C transition at the translation initiation codon of *VDR* that results in a shorter protein with increased biological activity (24). We observed no association for *Fok1* with any of the adiposity phenotypes, which suggested that *Fok1* may not be associated with adiposity.

The genetic susceptibility for abdominal fat differs from total body fat (38, 39). However, our data do not support different roles for *VDR* variants in whole-body and abdominal adiposity, given the significant association of rs3782905 and all adiposity

TABLE 3
Adiposity phenotypes for top 4 *VDR* single nucleotide polymorphisms (SNPs)

	Common genotype	Heterozygous genotype	Rare genotype	Difference between common and rare	P[1]
Two SNPs associated with all 3 phenotypes					
rs3782905	*CC*	*CG*	*GG*		
n	758	786	182		
BMI (kg/m^2)	27.4 ± 6.0[2]	28.3 ± 6.3	29.1 ± 6.9	−1.7	0.001
Waist circumference (cm)	85.1 ± 13.8	87.4 ± 14.7	89.5 ± 15.9	−4.4	0.0002
Abdominal height (cm)	20.0 ± 3.5	20.6 ± 3.8	20.9 ± 3.8	−0.9	0.001
rs3819545	*AA*	*AG*	*GG*		
n	654	783	277		
BMI (kg/m^2)	28.2 ± 6.4	27.9 ± 6.4	27.3 ± 5.6	0.9	0.07
Waist circumference (cm)	87.4 ± 15.4	86.4 ± 14.1	85.0 ± 13.3	2.4	0.11
Abdominal height (cm)	20.5 ± 3.8	20.3 ± 3.6	20.0 ± 3.5	0.5	0.16
Two SNPs associated with waist circumference and abdominal height only					
rs2239179	*TT*	*CT*	*CC*		
n	540	849	315		
BMI (kg/m^2)	27.6 ± 6.2	28.1 ± 6.3	28.3 ± 6.4	−0.7	0.22
Waist circumference (cm)	85.4 ± 13.5	87.0 ± 14.8	87.3 ± 15.0	−1.9	0.08
Abdominal height (cm)	20.0 ± 3.4	20.4 ± 3.7	20.6 ± 3.9	−0.6	0.06
rs11568820 (*Cdx-2*)	*CC*	*CT*	*TT*		
n	1089	539	73		
BMI (kg/m^2)	27.8 ± 6.2	28.4 ± 6.5	28.2 ± 6.2	−0.4	0.15
Waist circumference (cm)	86.0 ± 14.4	87.7 ± 14.9	88.0 ± 14.3	−2.0	0.07
Abdominal height (cm)	20.2 ± 3.7	20.6 ± 3.6	20.8 ± 3.7	−0.6	0.14

[1] ANOVA.
[2] Mean ± SD (all such values).

phenotypes and borderline associations for rs3819545, rs2239179, and *Cdx-2* (rs11568820).

Few epidemiologic studies have investigated the association between *VDR* variants and adiposity, and existing studies are small (<400 individuals). Swedish women with LL *Poly*(*A*) (rs17878969) and BB *BsmI* (rs1544410) rare genotypes had higher fat mass measured by using dual-energy X-ray absorptiometry but no differences in BMI (40). Polish men with the BB *BsmI* rare genotype had higher BMI and waist circumferences; however there were no differences for *FokI* (rs10735810 or rs228570) (41). The linkage disequilibrium measured via r^2 between *BsmI* and rs3782905 is 0.42 in the Caucasian HapMap data, which may in part explain the similarity of our findings. In Polish women, *BsmI* and *FokI* were not associated with BMI or waist circumference (42).

The *BsmI* SNP was located at the 3′ end of *VDR* (26 kb) downstream from the most significant SNP (rs3782905) in our study. *BsmI* is not known to alter the structure or function of VDR (43), which suggested that LD with undiscovered functional SNPs in the 3′ region may explain our findings and those of the previously mentioned studies. Our results for *FokI* are consistent with the previous studies.

For 3 of the 4 most significant SNPs identified in our study, where individuals with the rare genotype were more obese, we speculated that the mechanism was mediated via binding of 1,25 $(OH)_2D_3$ to VDR. *VDR* variants may directly influence binding and mediate a host of downstream effects on VDR-responsive genes. Further studies are needed to study the functional significance of these variants, especially with regard to the downstream influence on adiposity-related endpoints.

Without having *VDR*-genotype data and data on circulating 25-hydroxyvitamin D concentrations together for the same individuals, we were unable to discern whether *VDR* polymorphisms could explain the reported correlation of 25-hydroxyvitamin D and BMI; however, future studies should investigate how *VDR* polymorphisms influence vitamin D status.

The large sample size and standardized phenotype measurements by trained interviewers were major strengths of our study. Because our study sample was limited to white women, the results may not be generalizable to other women.

Unbound VDR may exert independent effects from those of 1,25 $(OH)_2D_3$ (16); therefore VDR polymorphisms are important factors in their own right, however future studies that include circulating $1,25(OH)_2D_3$ concentrations and *VDR* genotypes would be interesting because there may be an interaction between genotypes and $1,25(OH)_2D_3$ concentrations. Also of interest would be other genes that are members of the hormone-receptor superfamily that can form heterodimers with the retinoid X receptor because these ligands and receptors may be interchangeable (20).

In conclusion, results from our study provide the first evidence, to our knowledge, of an association between *VDR* and adiposity. Replication studies are needed to further test this association. Future research should also investigate the biological significance of 3′ UTR polymorphisms in relation to adiposity.

The authors' responsibilities were as follows—HMO-B and RC: performed analyses and wrote the manuscript; and AEM, PGS, CM, MT, and JLF: contributed to the design and conduct of the study, contributed critically to the manuscript, and approved the final draft. None of the authors had a conflict of interest.

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