Vitamin D receptor gene polymorphisms are associated with adiposity phenotypes1–3

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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/m²), 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 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 D₃ [1,25(OH)₂D₃], 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)₂D₃ in adipocyte metabolism is quite complex. One mechanism involves interaction with VDR. Some literature suggests that both 1,25(OH)₂D₃ and VDR are important players in adipocyte differentiation (15, 16). One study showed that 1,25(OH)₂D₃, via proposed interactions with VDR, inhibited the early phase of differentiation of pre-adipocytes to mature adipocytes in vitro (16). In VDR knockout mice, unlike in wild-type mice, 1,25(OH)₂D₃ 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)₂D₃ 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)2D3 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/m²) 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 <88 and ≥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 α = 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,
significant for the remaining SNPs.

With the exception of rs3782905, differences in mean adiposity values were not significant for women with the common genotype. With the exception of 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 rare genotype. 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 and a 50–80% power for rs3782905 with BMI, waist circumference, and abdominal height and a borderline positive association with BMI. We observed no significant associations for Cdx-2 (rs11568820) with 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.

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.

<table>
<thead>
<tr>
<th>SNP</th>
<th>MAF</th>
<th>Position</th>
<th>β ± SE (adjusted P)</th>
<th>Unadjusted P</th>
<th>β ± SE (adjusted P)</th>
<th>Unadjusted P</th>
<th>β ± SE (adjusted P)</th>
<th>Unadjusted P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs79837</td>
<td>0.47</td>
<td>G</td>
<td>-0.28 ± 0.21</td>
<td>0.20</td>
<td>-0.71 ± 0.50</td>
<td>0.16</td>
<td>-0.28 ± 0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>rs2239179</td>
<td>0.43</td>
<td>C</td>
<td>0.36 ± 0.22</td>
<td>0.10</td>
<td>1.00 ± 0.51</td>
<td>0.04</td>
<td>0.30 ± 0.13</td>
<td>0.02</td>
</tr>
<tr>
<td>rs3819545</td>
<td>0.39</td>
<td>G</td>
<td>-0.43 ± 0.22</td>
<td>0.04</td>
<td>-1.14 ± 0.50</td>
<td>0.02</td>
<td>-0.26 ± 0.13</td>
<td>0.05</td>
</tr>
<tr>
<td>rs2239186</td>
<td>0.21</td>
<td>G</td>
<td>-0.31 ± 0.26</td>
<td>0.23</td>
<td>-1.09 ± 0.61</td>
<td>0.07</td>
<td>-0.09 ± 0.16</td>
<td>0.58</td>
</tr>
<tr>
<td>rs228570</td>
<td>0.38</td>
<td>A</td>
<td>0.17 ± 0.22</td>
<td>0.43</td>
<td>0.22 ± 0.51</td>
<td>0.67</td>
<td>0.15 ± 0.13</td>
<td>0.27</td>
</tr>
<tr>
<td>rs285364</td>
<td>0.39</td>
<td>G</td>
<td>-0.05 ± 0.22</td>
<td>0.84</td>
<td>-0.20 ± 0.51</td>
<td>0.70</td>
<td>-0.17 ± 0.13</td>
<td>0.19</td>
</tr>
<tr>
<td>rs4760648</td>
<td>0.42</td>
<td>T</td>
<td>0.23 ± 0.22</td>
<td>0.30</td>
<td>0.71 ± 0.51</td>
<td>0.16</td>
<td>0.27 ± 0.13</td>
<td>0.04</td>
</tr>
<tr>
<td>rs3890734</td>
<td>0.33</td>
<td>A</td>
<td>-0.14 ± 0.23</td>
<td>0.53</td>
<td>-0.49 ± 0.53</td>
<td>0.36</td>
<td>-0.25 ± 0.14</td>
<td>0.08</td>
</tr>
<tr>
<td>rs7136534</td>
<td>0.24</td>
<td>T</td>
<td>0.46 ± 0.25</td>
<td>0.07</td>
<td>1.06 ± 0.58</td>
<td>0.07</td>
<td>0.25 ± 0.15</td>
<td>0.11</td>
</tr>
<tr>
<td>rs10783219</td>
<td>0.36</td>
<td>T</td>
<td>-0.30 ± 0.23</td>
<td>0.19</td>
<td>-0.60 ± 0.53</td>
<td>0.26</td>
<td>-0.06 ± 0.14</td>
<td>0.66</td>
</tr>
<tr>
<td>rs799460</td>
<td>0.29</td>
<td>A</td>
<td>0.38 ± 0.23</td>
<td>0.10</td>
<td>0.82 ± 0.53</td>
<td>0.12</td>
<td>0.24 ± 0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>rs11568820</td>
<td>0.20</td>
<td>T</td>
<td>0.46 ± 0.27</td>
<td>0.09</td>
<td>1.39 ± 0.62</td>
<td>0.03</td>
<td>0.32 ± 0.16</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1 Unadjusted P values were not adjusted for multiple testing; adjusted P values were adjusted for multiple testing by using a Bonferroni correction where significant at P = 0.05.

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 FokI with any of the adiposity phenotypes, which suggested that FokI 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)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Common genotype</th>
<th>Heterozygous genotype</th>
<th>Rare genotype</th>
<th>Difference between common and rare</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3782905</td>
<td>CC</td>
<td>CG</td>
<td>GG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>758</td>
<td>786</td>
<td>182</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.4 ± 6.0</td>
<td>28.3 ± 6.3</td>
<td>29.1 ± 6.9</td>
<td>-1.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>85.1 ± 13.8</td>
<td>87.4 ± 14.7</td>
<td>89.5 ± 15.9</td>
<td>-4.4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Abdominal height (cm)</td>
<td>20.0 ± 3.5</td>
<td>20.6 ± 3.8</td>
<td>20.9 ± 3.8</td>
<td>-0.9</td>
<td>0.001</td>
</tr>
<tr>
<td>rs3819545</td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>654</td>
<td>783</td>
<td>277</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.2 ± 6.4</td>
<td>27.9 ± 6.4</td>
<td>27.3 ± 5.6</td>
<td>0.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>87.4 ± 15.4</td>
<td>86.4 ± 14.1</td>
<td>85.0 ± 13.3</td>
<td>2.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Abdominal height (cm)</td>
<td>20.5 ± 3.8</td>
<td>20.3 ± 3.6</td>
<td>20.0 ± 3.5</td>
<td>0.5</td>
<td>0.16</td>
</tr>
<tr>
<td>rs2239179</td>
<td>TT</td>
<td>CT</td>
<td>CC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>540</td>
<td>849</td>
<td>315</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6 ± 6.2</td>
<td>28.1 ± 6.3</td>
<td>28.3 ± 6.4</td>
<td>-0.7</td>
<td>0.22</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>85.4 ± 13.5</td>
<td>87.0 ± 14.8</td>
<td>87.3 ± 15.0</td>
<td>-1.9</td>
<td>0.08</td>
</tr>
<tr>
<td>Abdominal height (cm)</td>
<td>20.0 ± 3.4</td>
<td>20.4 ± 3.7</td>
<td>20.6 ± 3.9</td>
<td>-0.6</td>
<td>0.06</td>
</tr>
<tr>
<td>rs11568820</td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>1089</td>
<td>539</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8 ± 6.2</td>
<td>28.4 ± 6.5</td>
<td>28.2 ± 6.2</td>
<td>-0.4</td>
<td>0.15</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>86.0 ± 14.4</td>
<td>87.4 ± 14.9</td>
<td>88.0 ± 14.3</td>
<td>-2.0</td>
<td>0.07</td>
</tr>
<tr>
<td>Abdominal height (cm)</td>
<td>20.2 ± 3.7</td>
<td>20.6 ± 3.6</td>
<td>20.8 ± 3.7</td>
<td>-0.6</td>
<td>0.14</td>
</tr>
</tbody>
</table>

1 ANOVA.
2 Mean ± SD (all such values).
phenotypes and borderline associations for rs3819545, rs2239179, and Cdx-2 (rs11566820).

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 r2 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)2D3 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 BMI; however, future studies should investigate how 25-hydroxyvitamin D concentrations together for the same individual may explain our findings and those of the previously mentioned studies. Our results for FokI are consistent with the previous studies.

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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