Association Between Vitamin D Receptor Polymorphism and Type 2 Diabetes or Metabolic Syndrome in Community-Dwelling Older Adults: The Rancho Bernardo Study

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Vitamin D receptor (VDR) polymorphism influences susceptibility to type 1 diabetes, but the association with type 2 diabetes is not clear. We investigated the association between VDR polymorphism and type 2 diabetes and metabolic syndrome in a community-based study of unrelated older adults without known diabetes. Oral glucose tolerance test (75 g), plasma glucose and insulin measurement, homeostasis model assessment (HOMA), and VDR genotyping were performed. The distributions of genotype frequencies of *Apa*I, *Bsm*I, and *Taq*I polymorphism did not differ between persons with and without diabetes, but the frequency of aa genotype of *Apa*I polymorphism was marginally higher in persons with type 2 diabetes ($P = .058$). Fasting plasma glucose ($P < .05$) and prevalence of glucose intolerance ($P < .05$) were significantly higher in nondiabetic persons with aa genotype compared with those with AA genotype. The bb genotype of *Bsm*I polymorphism was associated with insulin resistance as assessed by HOMA after adjustment for age, sex, body mass index (BMI), and calcium and vitamin D use in persons without diabetes ($P < .05$). Our research suggests that *Apa*I polymorphism may be associated with glucose intolerance independent of defective insulin secretion and *Bsm*I polymorphism with insulin resistance in a nondiabetic Caucasian population.
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VITAMIN D HAS important immunomodulatory properties and may influence insulin secretion.[1,2] For example, Gedick and Akalin[3] reported that insulin secretion, but not glucagon secretion, was significantly lower before vitamin D_3 treatment in patients with vitamin D deficiency compared with healthy subjects, and vitamin D-treatment increased insulin secretion in these patients. Kumar et al[4] also reported that β-cell function, but not insulin sensitivity, improved after low-dose vitamin D treatment of a patient with vitamin D deficiency.

Vitamin D receptor (VDR) polymorphism has been reported to be related to type 1 diabetes.[5-7] In the nonobese diabetic mouse model for insulin-dependent diabetes mellitus, vitamin D is necessary for normal insulin release and maintenance of glucose tolerance.[8] Recently, associations have been described between allelic variants of the VDR gene and impaired insulin secretion in type 1 and type 2 diabetes.[5-7,9] *Bsm*I polymorphism was shown to be associated with type 1 diabetes in South Indians,[5] and combinations of *Bsm*I/*Apa*I/*Taq*I influenced susceptibility to type 1 diabetes in Germans.[6] In a Taiwanese population, the AA genotype of *Apa*I polymorphism was associated with type 1 diabetes.[7] The aa genotype was associated with defective insulin secretion in Bangladeshi Asians, a population at increased risk for type 2 diabetes.[9] These findings suggest that the VDR gene is a novel candidate gene contributing to susceptibility to both types of diabetes. However, there are no data reporting the direct effect on type 2 diabetes.

We evaluated the association between VDR polymorphism and metabolic syndrome and type 2 diabetes in relation to insulin secretion or insulin resistance in older Caucasian men and women without known diabetes.

SUBJECTS AND METHODS

Subjects

A total of 1,545 older adults participated in this cross-sectional study. All participants were members of the Rancho Bernardo cohort, a community-based study of homogenously ethnic Caucasian adults unrelated to each other. None had a history of diabetes. In the 1984 to 1987 visit, participants were given a 75-g oral glucose tolerance test in the morning after a 12-hour fast. Height and weight were measured with persons wearing light clothing and no shoes; body mass index (BMI) was calculated as [weight(kg)/height(m)^2]. Waist and hip girth were measured in centimeters, as previously described.[10] Waist-to-hip ratio (WHR) was used to estimate upper body obesity. Waist circumference was used as an integrated measure of obesity and fat distribution on the basis of studies suggesting that waist circumference is more highly correlated with both total and visceral body fat when measured by computed tomography or magnetic resonance imaging than by WHR.[11,12] Systolic and diastolic blood pressures were measured in sitting position. Personal history of diabetes and behavioral factors, including alcohol consumption, and current use of vitamin D or calcium were determined by standard interviews. Type 2 diabetes was defined by a fasting plasma glucose level $\geq$ 7.0 mmol/L or a 2-hour postchallenge glucose level $\geq$ 11.1 mmol/L.[13] Persons with a previous physician diagnosis of type 2 diabetes or those using antidiabetic medication were excluded. Persons with a fasting plasma glucose level between 6.1 and 7.0 mmol/L were considered to have impaired fasting glucose (IFG), and those with a 2-hour postchallenge glucose level between 7.8 and 11.1 mmol/L were considered to have impaired glucose tolerance (IGT). Persons with either IFG or IGT were considered to have glucose intolerance. Hypertension was defined as an average measured systolic blood pressure $\geq$ 160 mm Hg or a diastolic blood pressure $\geq$ 90 mm Hg, a previous diagnosis of hypertension by a physician, or the use of antihypertensive medications. Dyslipidemia was defined as fasting plasma triglycerides $\geq$ 2.3 mmol/L or high-density lipoprotein (HDL) cholesterol levels $\leq$ 1.4 mmol/L. The metabolic syndrome was defined as the simultaneous presence of abnormal glucose tolerance, hypertension, and dyslipidemia. Informed consent was obtained from all subjects for the study purpose, and potential risks

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Submitted May 14, 2001; accepted July 9, 2001.

Supported by Grant No. DK31801 from the National Institute of Diabetes and Digestive and Kidney Diseases.

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0026-0495/02/5103-0038$35.00/0

doi:10.1053/meta.2002.29969

Table 1. Clinical Characteristics by Diabetic Status

Variables	Diabetes (n = 242)	No Diabetes (n = 1,303)	*P* Value
Age (yr)	71.7 ± 8.6	68.8 ± 9.2	.000
% Male	47.9	40.4	.031
BMI (kg/m^2)	25.5 ± 3.9	24.8 ± 3.5	.007
Waist (cm)	91.4 ± 59.9	85.4 ± 37.7	.040
WHR	0.91 ± 0.05	0.86 ± 0.09	.048
Systolic blood pressure (mm Hg)	143.1 ± 20.6	135.7 ± 21.2	.000
Diastolic blood pressure (mm Hg)	76.3 ± 9.9	76.2 ± 9.0	.845

NOTE. Data are means ± SD.

of the study were explained. The study was approved by the institutional review board of University of California, San Diego.

DNA Studies

A 4-mL blood sample, drawn by venipuncture from each individual, was stored at -70°C until DNA extraction using the Qiagen (Valencia, CA) blood DNA extraction kit. Purified DNA was stored in 0.1 × TE solution at -20°C. Previously published primers were used to amplify a 900-bp fragment of DNA using the polymerase chain reaction (PCR) technique to determine the *Bsm*I genotype.[14] The fragment was subsequently digested using the restriction endonuclease *Bsm*I. The *Apa*I and *Taq*I genotypes were determined by digestion of a 280-bp fragment amplified using the primers 5′GGTGGGATTGAGCAGTG3′ and 5′ATGCTGCACTCAGGCTG3′. In each case, PCR was performed using 20 ng of genomic DNA in a final reaction volume of 20 μL, and amplification occurred during 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. Of the PCR product, 15 μL was incubated with 7.5 U of restriction endonuclease for 2 hours in a final volume of 20 mL. Digestion with *Taq*I (Stratagene, La Jolla, CA) and *Bsm*I (Stratagene) occurred at 65°C and with *Apa*I (Stratagene) at 37°C. All products were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining under ultraviolet light (UV) light. The genotype of each sample was determined by 2 technicians working independently, and 10% of samples were genotyped at each site more than once to ensure that 100% reproducibility was achieved.

For the statistical analysis, capital letters denote the absence and lowercase letters denote the presence of the site of the restriction enzymes *Bsm*I (B/b), *Apa*I (A/a), and *Taq*I (T/t) on each of the alleles.

Biochemical Measurements

Fasting and 2-hour postchallenge plasma glucose levels were measured using a glucose oxidase assay. Fasting insulin was determined by double-antibody, as previously described.[15] Lipids were measured in a lipid research laboratory certified by the Centers for Disease Control, Atlanta, GA. Total cholesterol and triglyceride were measured by enzymatic methods using an ABA-200 biochromatic analyzer (Abbott, Irving, TX). HDL cholesterol was measured by precipitation according to Lipid Research Clinic protocol,[16] and low-density lipoprotein (LDL) cholesterol was calculated using the formula of Friedewald et al.[17] HOMA-insulin resistance (HOMA-IR) was calculated as {fasting glucose (mmol/L) × fasting insulin (μU/mL)}/22.5, and HOMA–β-cell function (HOMA-β) as 20 × fasting insulin (μU/mL)/{fasting glucose (mmol/L)-3.5}, as described by Levy et al.[18]

Statistical Analyses

Statistical analyses were performed using SPSS (SPSS Inc, Chicago, IL; SPSS Base 10.0 for Windows). Student's *t* tests were used for continuous variables, and χ^2 tests for trend were used for categorical variables to test for statistical significance. Data for fasting insulin, HOMA-β, and HOMA-IR were log transformed to reduce skewness of the data. An analysis of covariance (ANCOVA) with age, sex, BMI, and calcium and vitamin D intake as covariates was used to examine the association between VDR polymorphism and glucose metabolism, insulin secretion, and insulin resistance.

RESULTS

Of the total 1,545 participants who had no personal history of diabetes, 242 (15.7%) were newly diagnosed with diabetes by oral glucose tolerance test. Persons with newly diagnosed type 2 diabetes were significantly older and more obese, had more unfavorable body fat distribution and higher systolic blood pressure, and also were more likely to be male than persons without diabetes (Table 1). Table 2 shows the genotype frequency in persons with and without type 2 diabetes. Genotype frequencies of *Bsm*I and *Taq*I polymorphisms did not differ by diabetes status, but persons with diabetes had a higher prevalence of aa genotype compared with those without diabetes with marginal significance (27.4% *v* 20.3%, $P = .058$).

After excluding all persons with newly diagnosed type 2 diabetes, we evaluated the association between VDR polymorphism and insulin secretion defect or insulin resistance. We found no association between *Apa*I polymorphism and the insulin secretion index, calculated by the HOMA-β, in persons with normal and impaired glucose tolerance (IFG and IGT). We also found no association between VDR polymorphism and metabolic syndrome, defined as the combination of glucose intolerance, hypertension, and dyslipidemia (Table 3). However, the prevalence of glucose intolerance increased significantly from AA to aa genotype (30.9% in AA, 33.8% in Aa, and 39.9% in aa, *P* for trend < .05). After adjustment for age, sex, BMI, and calcium and vitamin D use, fasting plasma glucose was significantly higher in persons with aa genotype compared with those with AA genotype (5.43 ± 0.56 mmol/L *v* 5.35 ± 0.57 mmol/L, $P < .05$, Table 4). Fasting insulin, HOMA-β, and HOMA-IR did not differ significantly among nondiabetic persons with AA, Aa, and aa genotypes.

Although the frequency of metabolic syndrome did not differ significantly between BB, Bb, and bb genotypes, persons with bb genotype showed more insulin resistance. HOMA-IR was significantly higher in persons with bb genotype compared with those with BB genotype after adjustment for age, sex, BMI, and calcium and vitamin D use (2.8 ± 1.5 in BB, 3.0 ± 2.0 in Bb,

Table 2. Genotype Distribution (%) of VDR Polymorphism Among Persons With and Without Diabetes

Genotype Distribution		Diabetes (n = 242)	No Diabetes (n = 1,303)
*Apa*I*	AA	34.8	37.4
	Aa	37.8	42.4
	aa	27.4	20.3
*Bsm*I	BB	20.2	19.4
	Bb	44.2	45.3
	bb	35.6	35.3
*Taq*I	TT	38.6	38.6
	Tt	44.5	44.6
	tt	16.9	16.8

*$P = .058$ between persons with diabetes and those without diabetes. The distribution of the VDR alleles was in the Hardy-Weinberg equilibrium.

Table 3. Prevalences of Metabolic Syndrome by VDR Polymorphism in Persons Without Diabetes

		Glucose Intolerance (%)	Dyslipidemia (%)	Hypertension (%)	Metabolic Syndrome (%)
*Apa*I	AA	30.9	37.5	36.2	5.5
	Aa	33.8	40.8	33.8	7.7
	aa	39.9*	39.9	32.7	8.5
*Bsm*I	BB	32.5	40.1	36.1	6.3
	Bb	32.0	38.9	34.6	7.0
	bb	36.4	40.1	33.3	7.2
*Taq*I	TT	35.2	40.0	32.4	6.4
	Tt	32.6	40.0	35.4	7.6
	tt	33.0	37.2	37.2	6.4

NOTE. Metabolic syndrome is defined as glucose intolerance, hypertension, and dyslipidemia; see text.

*$P < .05$ using χ^2 tests for trend.

and 3.2 ± 2.2 in bb, $P < .05$, Table 4). This association was not significant after adjusting for age, sex, and BMI.

DISCUSSION

The genotype distribution and allele frequency of persons without diabetes in this study of unrelated community-dwelling adults was similar to healthy Caucasian persons in other studies.[9,19] We observed no significantly different genotype distribution and allele frequency between persons with newly diagnosed type 2 diabetes and persons without diabetes.

We found a consistent association between aa genotype of *Apa*I polymorphism and glucose intolerance. Persons with newly diagnosed type 2 diabetes had higher frequency of aa genotype with marginal significance compared with persons without diabetes. After exclusion of persons with diabetes, we also found that prevalence of glucose intolerance (IFG and IGT) was significantly higher in nondiabetic persons with aa genotype compared with those with Aa and AA genotypes. Fasting plasma glucose was also significantly higher in nondiabetic persons with aa genotype after adjustment for age, sex, BMI, and calcium and vitamin D use. However, insulin secretion or insulin resistance as assessed by HOMA did not differ significantly between 3 genotypes of *Apa*I polymorphism. Therefore, these data do not suggest that abnormal glucose tolerance in aa genotype is associated with an insulin secretion defect or insulin resistance. We cannot, at present, suggest the possible mechanism of the association between *Apa*I polymorphism and glucose metabolism. Functional studies of molecular basis will further elucidate the potential causality of this polymorphism and glucose metabolism.

We found no association between VDR polymorphism and metabolic syndrome in persons without diabetes. The relation of VDR polymorphism and metabolic syndrome has not been reported. Other evidence for a vitamin D-metabolic syndrome association was found in a study of Pima Indians, in which linkage was reported between prediabetic metabolic traits and a few microsatellite markers on chromosome 4q in the region containing the vitamin D-binding protein gene.[20]

We observed that persons with bb genotype of *Bsm*I polymorphism showed significantly higher levels of HOMA-IR compared with those with BB and Bb genotypes only after additional adjustment for calcium and vitamin D use. This result supports a report by Kiel et al[21] who found an association between dietary calcium intake and bone mineral density only in the bb genotype. They suggested that *Bsm*I genotype might play a role in the absorption of dietary calcium. Although vitamin D plays a role in insulin secretion, the exact mechanism is unclear. The effects of 1,25-dihydroxyvitamin D_3 directly influence insulin secretion in the β cell through an increase in intracellular free calcium concentration via the nonselective calcium channel.[22] In addition, 1,25-dihydroxyvitamin D_3 has been shown to inhibit β-cell growth by upregulation of vitamin D receptors, suggesting VDR variants might alter insulin responses through early β-cell differentiation.[23] We did not investigate the direct effect of calcium and vitamin D intake on insulin secretion or insulin sensitivity according to VDR polymorphism. These studies are restricted to the chronic effects of vitamin D on insulin secretion through a signal transduction system involving the nuclear VDR. Recently, Kajikawa et al[24] reported a rapid nongenomic effect of vitamin D, via membrane VDR, on pancreatic β-cell function.

There are several limitations to this study. First, it is cross-sectional, which limits the investigation of causal relationships. Second, there is the possibility that the sequence differences in linkage dysequilibrium are responsible for our observations. Because linkage dysequilibrium only occurs over very short genetic distances in outbred populations, association study is

Table 4. Metabolic Parameters According to VDR Polymorphism in Persons Without Diabetes

	*Apa*I			*Bsm*I			*Taq*I		
	AA	Aa	aa	BB	Bb	bb	TT	Tt	tt
SBP (mm Hg)	136.1 ± 20.7	135.8 ± 21.6	135.1 ± 21.0	136.2 ± 21.2	136.2 ± 21.3	134.7 ± 20.8	134.4 ± 20.6	136.2 ± 21.5	137.4 ± 21.4
DBP (mm Hg)	76.7 ± 9.4	75.6 ± 8.9	76.1 ± 9.1	77.1 ± 9.5	76.1 ± 8.9	75.8 ± 9.1	75.7 ± 9.1	76.1 ± 8.8	77.5 ± 9.4
FPG (mmol/L)	5.35 ± 0.57	5.43 ± 0.57	5.43 ± 0.56*	5.35 ± 0.60	5.42 ± 0.56	5.40 ± 0.56	5.38 ± 0.57	5.43 ± 0.55	5.34 ± 0.61
PCPG (mmol/L)	6.76 ± 1.76	6.78 ± 1.88	7.02 ± 1.76	6.77 ± 1.88	6.77 ± 1.78	6.86 ± 1.81	6.84 ± 1.79	6.78 ± 1.79	6.75 ± 1.89
FPI (pmol/L)	73.1 ± 42.7	74.7 ± 50.0	75.3 ± 52.2	69.2 ± 34.8	73.3 ± 47.1	77.9 ± 52.6	76.0 ± 50.1	74.2 ± 48.2	69.2 ± 35.6
HOMA-β	144.2 ± 198.3	130.5 ± 131.6	135.0 ± 97.3	141.4 ± 245.6	137.1 ± 134.0	138.0 ± 139.6	144.6 ± 216.2	138.1 ± 134.7	125.3 ± 91.3
HOMA-IR	3.0 ± 1.8	3.1 ± 2.1	3.1 ± 2.0	2.8 ± 1.5	3.0 ± 2.0	3.2 ± 2.2†	3.1 ± 2.1	3.0 ± 2.1	2.8 ± 1.6

NOTE. Data are means ± SD. Fasting insulin, HOMA-β, and HOMA-IR were log-transformed for ANCOVA. Data are adjusted for age, sex, BMI, and calcium and vitamin D use.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; PCPG, postchallenge glucose; FPI, fasting plasma insulin; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance.

*$P < .05$ *v* AA.

†$P < .05$ *v* BB.

not suitable for studying random markers in a genome scan, but is the current method of choice for candidate gene study. Further studies are needed to evaluate the direct effect of this polymorphism on type 2 diabetes by genetic linkage study. Finally, *Apa*I, *Bsm*I, and *Taq*I restriction sites are localized to introns and are unlikely to cause disease. Therefore, DNA sequencing studies are indicated in subjects possessing these diabetes-related VDR genotypes and haplotypes.

In summary, aa genotype of *Apa*I polymorphism in nondiabetic persons was associated with a significantly higher prevalence of glucose intolerance independent of insulin secretion defect. Persons with bb genotype of *Bsm*I polymorphism showed significantly more insulin resistance. Further studies are needed to elucidate the association between vitamin D, vitamin D receptors, and vitamin D-binding protein with type 2 diabetes and the metabolic syndrome.

REFERENCES

1. Manolagas SC, Hustmyer FG, Yu XP: Immunomodulating properties of 1,25-dihydroxyvitamin D3. Kidney Int Suppl 29:S9-S16, 1990
2. Norman AW, Frankel JB, Heldt AM, et al: Vitamin D deficiency inhibits pancreatic secretion of insulin. Science 209:823-825, 1980
3. Gedick O, Akalin S: Effects of vitamin D deficiency and repletion on insulin and glucagon secretion in man. Diabetologia 29:142-145, 1986
4. Kumar S, Davies M, Zakaria Y, et al: Improvement in glucose tolerance and beta-cell function in a patient with vitamin D deficiency during treatment with vitamin D. Postgrad Med J 70:440-443, 1994
5. McDermott MF, Ramachandran A, Ogunkolade BW, et al: Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. Diabetologia 40:971-975, 1997
6. Pani MA, Knapp M, Donner H, et al: Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. Diabetes 49:504-507, 2000
7. Chang TJ, Lei HH, Yeh JI, et al: Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population. Clin Endocrinol 52:575-580, 2000
8. Mathieu C, Laureys J, Sobis H, et al: 1,25-dihydroxyvitamin D3 prevents insulitis in NOD mice. Diabetes 41:1491-1495, 1992
9. Hitman GA, Mannan N, McDermott MF, et al: Vitamin D receptor gene polymorphisms influence insulin secretion in Bangladeshi Asians. Diabetes 47:688-690, 1998
10. Goodman-Gruen D, Barrett-Connor E: Sex differences in measures of body fat and body fat distribution in the elderly. Am J Epidemiol 143:898-906, 1996
11. Borkan GA, Hults DE, Gerzof SG, et al: Relationships between computed tomography tissue areas, thicknesses and total body composition. Ann Hum Biol 10:537-545, 1983
12. Ross R, Shaw KD, Rissanen J, et al: 1994 Sex differences in lean and adipose tissue distribution by magnetic resonance imaging: Anthropometric relationships. Am J Clin Nutr 59:1277-1285, 1994
13. World Health Organization: Definition, diagnosis and classification of diabetes mellitus and its complications: Report of a WHO Consultation. Part 1. Diagnosis and classification of diabetes mellitus. Geneva, World Health Organization, 1999
14. Morrison NA, Qi JC, Tokita A, et al: Prediction of bone density from vitamin D receptor alleles. Nature 367:284-287, 1994
15. Desbuquios B, Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J Clin Endocrinol Metab 33:732-738, 1971
16. Lipid Research Clinics Program (US), Laboratory Methods Committee: Manual of Laboratory Operations (prepared by the Laboratory Methods Committee of the Lipid Research Clinics Program) Bethesda, MD, National Heart and Lung Institute, National Institues of Health, 1974 (DHEW publication no. 75-628)
17. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499-502, 1972
18. Levy JC, Matthews DR, Hermans MP: Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 21:2191-2192, 1998
19. Riggs BL, Nguyen TV, Melton LJ III, et al: The contribution of vitamin D receptor gene alleles to the determination of bone mineral density in normal and osteoporotic women. J Bone Miner Res 10:991-996, 1995
20. Prately RE, Thompson DB, Prochazka M, et al: An autosomal genomic scan for loci linked to prediabetic phenotypes in Pima Indians. J Clin Invest 101:1757-1764, 1998
21. Kiel DP, Myers RH, Cupples LA, et al: The BsmI vitamin D receptor restriction fragment length polymorphism (bb) influences the effect of calcium intake on bone mineral density. J Bone Miner Res 12:1049-1057, 1997
22. Sargeev IN, Rhoten WB: 1,25-dihydroxyvitamin D3 evokes oscillations of intracelluar calcium in a pancreatic β–cell line. Endocrinology 136:2852-2861, 1995
23. Lee S, Clark SA, Gill RK, et al: 1,25-dihydroxyvitamin D3 and pancreatic β–cell function: Vitamin D receptors, gene expression and insulin secretion. Endocrinology 134:1602-1610, 1994
24. Kajikawa M, Isida H, Fujimoto S, et al: An insulinotropic effect of vitamin D analog with increasing intracellular Ca^{2+} concentration in pancreatic β–cells through nongenomic signal transduction. Endocrinology 140:4706-4712, 1999