VITAMIN D RECEPTOR (FOKI, BSMI AND TAQI) GENE POLYMORPHISMS AND TYPE 2 DIABETES MELLITUS: A NORTH INDIAN STUDY

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ABSTRACT

BACKGROUND: *The vitamin D receptor (VDR) gene is a candidate gene for susceptibility to several diseases. Studies on association between VDR polymorphisms and risk of type 2 diabetes (T2DM) in different ethnic populations are yet inconclusive.* ***AIMS:*** *This study was conducted to evaluate association between VDR polymorphisms and genetic susceptibility to T2DM in the north Indian population.* ***SETTINGS AND DESIGN:*** *One hundred clinically diagnosed T2DM patients and 160 healthy controls from the north Indian population were recruited for genetic association study.* ***MATERIALS AND METHODS:*** *Genomic DNA was extracted from blood and genotyped for the single nucleotide polymorphism SNPs of FokI (T/C) [rs2228570], BsmI (A/G) [rs1544410] and TaqI (C/T) [rs731236] by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis.* ***STATISTICAL ANALYSIS USED:*** *Genotype distribution and allelic frequencies were compared between patients and controls. Mean values and odds ratios (ORs) with 95% confidence interval (CI) were calculated using SPSS software (version 15.0).* ***RESULTS:*** *The genotype distribution, allele and haplotype frequencies of VDR polymorphism did not differ significantly between patients and controls. Mean age and waist-hip ratio of patients were found to be associated with VDR polymorphism. Combination studies showed FFBbtt increased the risk of T2DM in north Indians.* ***CONCLUSIONS:*** *Our data suggest that VDR gene polymorphism in combination of genotypes is associated with the risk of T2DM and thus requires further studies as a probable genetic risk marker for T2DM.*

Key words: *Diabetes, genetic polymorphisms, restriction fragment length polymorphisms, vitamin D receptor*

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INTRODUCTION

Environmental and genetic factors play important roles in the mechanisms involved in the development of T2DM. Studies on genes inducing susceptibility to T2DM have been carried out by various groups in different

populations.[1-9] An association study of candidate genes, *viz.,* fatty acid binding protein 2 (*FABP2*), uncoupling protein type 1 gene (*UCP1*), protein phosphatase type 1 (*PP1G*), β3 adrenergic receptor (*β3AR*), *VDR,* was carried out on T2DM patients in a migrant Indian population as well by Boullus-Sanchis *et al.*[10]

Genome-wide association studies have led to the identification of several single nucleotide polymorphisms in genes such as *CDKAL1, CDKN2B, HHEX/IDE, IGF2BP2, KCNJ11, SLC30A8, TCF2, TCF7L2* and *WFS1*.[11] Several genes were studied but only a handful showed confirmed association, such as resistin, alpha-endosulfine, calpain10, peroxisome proliferators–activated receptor-gamma 2 (PPARγ-2), abundant transcript 1 gene (apM1), TCF7L2, insulin-like growth factor–binding protein 5 (IGFBP5), to name a few.[9,12-19]

Earlier studies on genetic polymorphisms in T2DM candidate genes were carried out among south Indians, in addition to those in the *VDR* gene.[20-22] VDR is a member of the steroid/thyroid hormone receptor family.[23] It is known that vitamin D and especially its activated metabolite 1,25-dihydroxyvitamin D3 (1,25D3) are involved in controlling the normal functions of the endocrine pancreas, particularly insulin secretion; and the action of vitamin D is mediated through binding to its nuclear receptor (VDR).[24] Vitamin D and its receptor complex play the role of a transcription factor in regulating the β-cell insulin secretion. Vitamin-D deficiency has been shown to impair insulin synthesis and secretion in humans and in animal models of diabetes, which suggests it has a role in the development of type 2 diabetes.[25] There is evidence to suggest that altered vitamin D and calcium homeostasis also play a role in the development of T2DM; and recently, Knekt *et al.* reported that high vitamin D status provides protection against type 2 diabetes.[26-27]

In recent years, several polymorphisms, such as BsmI and FokI, have been described in the *VDR* genes that are able to alter the activity of VDR protein.[28] Some of the other polymorphisms in the *VDR* gene identified by allelic variation in restriction enzyme sites are Tru9I, TaqI, EcoRV and ApaI.[29] All these are located between exons 8 and 9 except that FokI is in exon 2. FokI polymorphism has been shown to have functional role in trascriptional activation of *VDR* gene.[30] However, the genetic background of the disease still remains unclear. Although these findings suggest that the *VDR* gene is a novel candidate gene contributing to susceptibility to T2DM [Table 1], there are no data on association of *VDR* gene polymorphism with T2DM in the north Indian population. In our present work, we studied the FokI, BsmI and TaqI restriction enzyme polymorphisms of the *VDR* gene in patients with T2DM in a north Indian population.

MATERIALS AND METHODS

Subject selection

The current study was carried out after obtaining prior approval from the institutional ethical committee of Chhatrapati Sahuji Maharaj Medical University (CSMMU), Lucknow, India. Patients (*n*= 100) with type 2 diabetes attending the Diabetes Clinic in the outpatient department of CSMMU, Lucknow, were enrolled for the present study.

Table 1: Data of genetic polymorphisms of VDR gene and T2DM risk from different countries

VDR site	*T2D patients*	*Mean age*	*Association*	*Country*	*Reference*
Intron 8 (BsmI, Tru9I, ApaI) and exon 9 (TaqI)	309	62 + 12	NS	France	Ye *et al* [36]
BsmI	293	NA	S	Germany	Ortlepp *et al* [37]
ApaI, BsmI, TaqI	242	71.7 + 8.6	NS	San Diego,	Oh *et al* [38]
BsmI	49	57 (29-77)	NS	Hungry	Speer *et al* [23]
FokI, BsmI, ApaI and TaqI	267	50.0 ± 9.2	NS	Poland	Cyganek *et al* [39]
ApaI, BsmI and TaqI	164	45.9 ± 10.3	S (ApaI) NS (BsmI and TaqI)	Bangladeshi Asians	Hitman *et al* [22]
FokI, BsmI and TaqI	100	49.32 ± 10.97	NS	North India	Our study

S= significant; NS= nonsignificant, VDR: vitamin D receptor

Screening and management of patients were done as per American Diabetes Association guidelines.[31] A questionnaire was used to record clinical history of patients, family history of diabetes and associated complications such as hypertension, etc.

A total of 160 age- and sex-matched normal healthy controls were enrolled for the study from among the healthy staff members of the institute and the university. They were screened with standard oral glucose tolerance test. Subjects having a history of coronary artery disease and other complications were not included in the control group.

DNA extraction and genotyping

After obtaining an informed consent, 5 mL of blood sample was collected in anticoagulant and plain vials from patients of both the groups. DNA was extracted from the blood samples by using QIAamp DNA mini kit (QIAGEN, Germany). Amplification was performed in a 20-µL reaction mixture containing genomic DNA (100-150 ng), 200 nM dNTPs (MBI-Fermentas, USA), 2-8 pmol of each primer and 0·5U of Taq DNA polymerase (MBI-Fermentas, USA) per tube in a gradient thermal Master cycler (Eppendorf, USA). Amplification reactions were set up separately for FokI, BsmI and TaqI polymorphic sites of *VDR* gene using primers given by Harris *et al.* and Morrison *et al.* [Table 2].[32-33] PCR-RFLP was used to identify the genotypic pattern of VDR family.

In order to improve the genotyping quality and validation, 20% of samples were re-genotyped by other laboratory personnel, and results were found to be reproducible with no discrepancy in genotyping. Genotyping of 10% randomly selected samples was confirmed by DNA sequencing.

Table 2: PCR-RFLP pattern of FokI, BsmI and TaqI polymorphisms of vitamin D receptor gene

Polymorphisms	*Primers*	*Annealing Temp (°C)*	*PCR-RFLP products (bp)*
Exon 2 (T/C)	F 5'– AGC TGG CCC TGG CAC TGA CTC TGC TCT – 3'	58	(TT) FF/ 196, 69
FokI	R 5'– ATG GAA ACA CCT TGC TTC TTC TCC CTC – 3'		(TC) Ff/265, 196, 69
rs2228570			(CC) ff/ 265
Exon 7/Intron 8 (A/G)	F 5'– CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA – 3'	63	(GG) BB/650,175
BsmI	R 5'– AAC CAG CGG GAA GAG GTC AAG GG – 3'		(GA) Bb/825,650,175
rs1544410			(AA) bb/825
Exon 9, codon 352 (C/T) TaqI	F 5'–CAG AGC ATG GAC AGG GAG CAA–3'	63	(TT) TT/495,245
rs731236	R 5'–GCA ACT CCT CAT GGC TGA GGT CTC–3'		(TC)Tt/495,290,245,205
			(CC) tt/290,245,205

PCR-RFLP = polymerase chain reaction and restriction fragment length polymorphism

Statistical analysis

Allele frequencies, genotype frequencies and carriage rates of the alleles in all the groups were compared by using Fisher's exact test. The Hardy-Weinberg equilibrium at individual loci was assessed by χ^2 statistics using SPSS software (version 15.0), and clinical association was calculated by paired *t* test. All *P* values were two sided, and differences were considered statistically significant for $P< 0.05$. Odds ratio (OR) at 95% confidence interval (CI) was determined to describe the strength of association by logistic regression model. Allele frequency was calculated as the number of occurrences of the test allele in the population divided by the total number of alleles. Carriage rate was calculated as the number of individuals carrying at least one copy of test allele divided by the total number of individuals.

RESULTS

Clinical characteristics of the patients are summarized in Table 3. The average age of the patient population was 49.32± 10.97 years, with mean BMI of 24.26± 4.30 kg/m^2. The allele and genotype frequency distribution and carriage rate of *VDR* (FokI, BsmI and TaqI) genes among patients and controls were as shown in Table 4. We did not observe any significant difference in *VDR* (FokI, BsmI and TaqI) genotypes between patient and control groups (P= 0.119, 0.428 and 0.416, respectively). Genotypes of each *VDR* marker were in Hardy–Weinberg equilibrium in each group separately and in the total of 100 T2DM patients.

Table 3: Clinical characteristics of patients with T2DM

No. of patients	*100*
Age (years)	49.32 ± 10.97
Duration of diabetes (years)	5.00 ± 5.70
BMI (kg/m^2)	24.26 ± 4.30
WHR (waist-hip ratio)	1.03 ± 0.90
Fasting plasma glucose (mg/dL)	174.30 ± 79.44
Postprandial plasma glucose (mg/dL)	264.50 ± 102.90
Total cholesterol (mg/dL)	225.13 ± 33.10
LDL cholesterol (mg/dL)	160.80 ± 30.41
HDL cholesterol (mg/dL)	42.60 ± 3.50
VLDL cholesterol (mg/dL)	23.31 ± 3.80
Triglyceride (mg/dL)	115.00 ± 14.10
Blood pressure (systolic) (mm Hg)	131.40 ± 15.25
Blood pressure (diastolic) (mm Hg)	81.44 ± 10.70
Serum creatinine (mg/dL)	1.04 ± 0.97

Data are mean ± standard deviation (SD) or frequencies (%).

Table 4: Distribution of genotype, allele frequencies and carriage rate of VDR (FokI, BsmI and TaqI) among patients and controls

Genotype	*FokI*			*P value*	*TaqI*			*P value*	*BsmI*			*P value*
	FF (%)	*Ff (%)*	*ff (%)*		*TT (%)*	*Tt (%)*	*tt (%)*		*BB (%)*	*Bb (%)*	*bb (%)*	
Patients (100)	38 (38.0)	60 (60.0)	2 (2.0)	0.119	36 (36.0)	49 (49.0)	15 (15.0)	0.416	30 (30.0)	52 (52.0)	18 (18.0)	0.428
Controls (160)	80 (50.0)	79 (49.4)	1 (0.63)		67 (41.87)	65 (40.63)	28 (17.5)		60 (37.5)	77 (47.13)	23 (14.38)	
Allele frequency												
Patients (100)	136 (68.0)	64 (32.0)	1.0 (Ref) 0.720 (0.488-1.063)	0.098	121 (60.5)	79 (39.5)	1.0 (Ref) 0.931 (0.648-1.338)	0.700	112 (56.0)	88 (44.0)	1.0 (Ref) 0.795 (0.555-1.138)	0.209
Controls (160)	239 (74.7)	81 (25.3)			199 (62.2)	121 (37.8)			197 (61.57)	123 (38.43)		
Carriage rate												
Patients (100)	98 (98.0)	62 (62.0)	1.0 (Ref) 0.795 (0.524-1.206)	0.281	85 (85.0)	64 (64.0)	1.0 (Ref) 0.798 (0.531-1.201)	0.280	82 (82.0)	70 (70.0)	1.0 (Ref) 0.981 (6.652-1.475)	0.926
Controls (160)	159 (99.37)	80 (50.0)			132 (82.5)	93 (58.13)			137 (86.0)	100 (62.5)		

In addition, we also compared the *VDR* (FokI, BsmI and TaqI) genotypes with different clinical parameters of T2DM but could not obtain association of any of the parameters except age (P= 0.002) and waist-hip ratio (P= 0.013) with *VDR* TaqI genotypes [Table 5].

Combining FokI, BsmI and TaqI genotypes resulted in 23 possible combinations. When each combination genotype was tested using $\chi 2$ against all other combination genotypes, there was a trend of increased risk of diabetes in the genotypes FfBbTt {P= 0.136, OR= 4.00 (0.64-24.69)} and FFBbtt {P= 0.334, OR= 2.50 (0.38-16.04)}.

Table 5: Association of various clinical parameters with different VDR (FokI, BsmI and TaqI) genotypes in T2DM patients

Clinical Parameters	*FokI*		*P value*	*BsmI*		*P value*	*TaqI*		*P value*
	FF	*Ff + ff*		*BB*	*Bb+bb*		*TT*	*Tt+tt*	
Age (years)	47.91 ± 10.16	49.53 ± 10.82	0.463	48.3 ± 9.99	49.56 ± 10.68	0.583	55.7 ± 12.08	48.63 ± 9.62	0.002
Duration of diabetes (years)	4.67 ± 4.60	5.09 ± 5.59	0.348	4.0 ± 5.6	3.21 ± 3.80	0.634	4.75 ± 5.57	5.54 ± 4.73	0.495
BMI (kg/m^2)	24.06 ± 3.36	24.17 ± 4.45	0.985	22.97 ± 5.69	23.95 ± 4.30	0.304	24.40 ± 4.15	23.27 ± 3.61	0.178
WHR (waist-hip ratio)	1.19 ± 1.58	0.92 ± 0.07	0.222	0.9 ± .08	1.01± 0.35	0.104	0.97 ± 0.05	0.93 ± 0.07	0.013
Fasting Plasma Glucose (mg/dL)	161.83 ± 88.53	172.10 ± 72.45	0.582	165.00 ± 83.1	175.86 ± 88.97	0.605	181.09 ±87.04	176.59 ± 90.50	0.820
Postprandial Plasma Glucose (mg/dL)	234.19 ± 96.97	274.68 ± 91.44	0.077	248.10 ± 116.80	263.64 ±109.3	0.576	267.03 ±117.9	267.59 ± 102.33	0.983
Total cholesterol (mg/dL)	221.51 ± 28.37	223.09 ± 28.44	0.811	220.52 ± 26.84	228.22 ± 30.32	0.289	249.90 ±16.25	216.92 ± 29.33	5.399
LDL cholesterol (mg/dL)	155.56 ± 27.40	157.49 ± 27.99	0.764	155.4 ± 26.17	162.52 ± 29.50	0.313	185.64 ±15.50	151.8 ± 28.19	8.954
HDL cholesterol (mg/dL)	42.16 ± 3.67	42.45 ± 3.36	0.721	42.90 ± 2.90	42.12 ± 4.41	0.471	41.71 ± 3.97	42.58 ± 3.52	0.284
VLDL cholesterol (mg/dL)	24.17 ± 3.40	22.97 ± 3.38	0.129	22.26 ± 2.90	23.84 ± 4.07	0.093	22.91 ± 3.69	22.58 ± 3.39	0.668
Triglyceride (mg/dL)	117.83 ± 12.63	115.02 ± 14.14	0.381	110 ± 14.66	115.88 ± 13.03	0.093	112.70 ±13.84	111.69 ± 13.72	0.739
Blood pressure (systolic)(mm Hg)	132.48 ± 12.58	132.35 ± 17.34	0.978	134.4 ± 17.29	137.75 ± 15.87	0.526	131.25 ±20.30	136 ± 16.36	0.424
Blood pressure (diastolic)(mm Hg)	81.52 ± 6.48	81.30 ± 13.08	0.945	80.93 ± 11.38	88.79 ± 24.39	0.247	80.50 ±11.40	81.83 ± 11.41	0.723
Serum creatinine (mg/dL)	1.01 ± .08	1.05 ± 0.096	0.133	1.01 ± 0.083	1.09 ± 0.24	0.169	1.04 ± 0.07	1.04 ± 0.09	0.951

DISCUSSION

Allelic differences in the *VDR* gene may contribute to the genetic predisposition to certain diseases. As vitamin D modulates insulin secretion, it is feasible that genetic variants of the *VDR* gene may contribute to the development of T2DM. Since patients with T2DM exhibit subtle alterations in glucose metabolism long before onset of the disease, genetic factors contributing to its pathogenesis or development could be detected early in the disease process.[34]

Our study revealed significant association of age (P= 0.002) and waist-hip ratio (P= 0.013) with TaqI genotypes of *VDR* gene polymorphism. This may indicate the association of *VDR* polymorphism with both modifiable and nonmodifiable risk factors of diabetes.

Our investigation showed the nonsignificant association between *VDR* (FokI, BsmI and TaqI) genotypes and diabetes risk in the north

Indian population. Previous investigations of *VDR* polymorphisms and diabetes risk by other groups have produced inconsistent results [Table 1].

Two studies have reported link between *VDR* polymorphism and diabetes risk — one in Caucasian subjects, for BsmI site; and the other in Bangladeshi Asians, for ApaI site.[23] However, our study, like the one in Polish population, showed no association between *VDR* FokI, ApaI, BsmI and TaqI polymorphism and diabetes risk.[35]

Further studies involving genetic linkage assessment are needed to evaluate the direct effect of these polymorphisms on T2DM; also, the studies should be carried out on a large cohort of population. The major limitation of the current study is its moderately low sample size. While polymorphisms of the *VDR* gene might play a role in the pathogenesis of T2DM, the non-association of FokI, BsmI and TaqI need to be interpreted considering this limitation. Finally, our data affirms the association of *VDR* polymorphism in combination of genotypes with risk of T2DM in the north Indian population.

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