ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 10, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

VITAMIN D RECEPTOR GENE POLYMORPHISMS AND HAPLOTYPE ANALYSIS IN TYPE 2 DIABETES MELLITUS PATIENTS FROM NORTH INDIA

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Received: 05 June 2017, Revised and Accepted: 29 June 2017

ABSTRACT

Objective: Vitamin D receptor (VDR) mediated Vitamin D signaling is important for expression of insulin gene and glucose transporters, which help in glucose uptake by cells. Current evidence suggests that four common polymorphisms (*FokI, BsmI, ApaI, TaqI)* of VDR gene are associated with Type 2 diabetes mellitus (T2DM) in different populations. However, there is a scarcity of data on VDR polymorphisms from Indian population.

Methods: In the current study, total genomic DNA was isolated from 100 well-characterized T2DM patients and 100 healthy controls. We investigated the prevalence of *FokI* and *ApaI* polymorphisms in VDR gene of these patients by polymerase chain reaction-restriction fragment length polymorphism-based method. Taking help of our previous published data on *TaqI* and *BsmI* polymorphisms in same patients, the haplotype study was also conducted. Statistical analysis of data was performed using SPSS 21.0 software. Haplotype and linkage disequilibrium analysis was performed by Haploview software.

Results: Both the wild (TT) and mutant (CC) genotype of *FokI* polymorphism showed a significant difference between patients and controls ($p<0.001$ and $p<0.001$, respectively). The frequency of mutant allele (C) was also significantly higher in T2DM patients than the controls ($p<0.001$). In case of *ApaI*, frequency of wild (GG) and mutant (CC) genotype was significantly different in patients and controls ($p=0.017$ and $p=0.034$). As per haplotype analysis, the CACT haplotype was predicted to be of significance in patients and consists of mutant alleles of three polymorphisms (*FokI, BsmI, ApaI*).

Conclusion: Our study supports the association of *FokI* and *ApaI* polymorphism in T2DM. The haplotype analysis also indicates that the combinations of mutant allele of different VDR polymorphisms are probably responsible for increased susceptibility of these individuals toward T2DM.

Keywords: Type 2 diabetes, Vitamin D, Vitamin D receptor, Polymorphisms, Haplotype.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i10.20453

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex, polygenic disease where individuals experience high blood glucose levels. It is a progressive disease, in which either the cells become resistant to insulin or steadily cells lose its ability of insulin production in the pancreatic β cells. Pathogenesis of T2DM involves various genetic and environmental factors. Out of various environmental factors, Vitamin D has allured extensive importance as a potent factor in prognosis and evasion of T2DM [1,2]. The major role of Vitamin D is calcium homeostasis which is an important for both insulin secretion from the pancreatic β cell and its action on target cell [3-5]. The intracellular signaling of Vitamin D requires its binding to Vitamin D receptor (VDR). VDR is present on the nuclear membrane to perform the majority of the biological actions [6]. VDR after binding with the Vitamin D acts as a transcription factor and controls the expression of many genes involved in glucose metabolism such as insulin and glucose transporters [7-9]. VDR is encoded by the VDR gene located on the chromosome 12 (12q12-q14). Several VDR polymorphisms have been reported in case of different populations suffering from different metabolic diseases [10-13]. In the recent years, these polymorphisms are also known to be associated with both the risk of occurrence of diabetes in the various diabetic populations [14]. Among all the known VDR polymorphisms, the most commonly found single nucleotide polymorphisms (SNPs) are rs7975232 (*ApaI*) G64978C, rs2228570 (*FokI*) T30920C, rs731236 (*TaqI*) T65058C, and rs1544410 (*BsmI*) G63980A are identified using *ApaI*, *FokI*, *TaqI*, and *BsmI* restriction enzymes, respectively. Among the four polymorphisms *TaqI, FokI* are exonic mutations reported in (exon 9) and (exon 2), respectively. On the other side, *ApaI* (intron 8) and *BsmI* (intron 8) are intronic polymorphisms. *FokI* is also known as start codon polymorphisms that alter size of VDR protein [15]. *ApaI*, *BsmI* are non coding changes but are known to affect the stability of VDR mRNA [16]. Latest findings suggest that these allelic variations of VDR gene are associated with incidence, clinical symptoms, and severity or susceptibility of diabetic phenotype [17-20]. Furthermore, these polymorphisms are reported to be in strong linkage disequilibrium (LD) in different phenotypes including T2DM. Most of these studies have published the data from different parts of the world population, and very few data are available in context to the Indian population. To the best of our knowledge, till date, there is only one literature published, which suggests the association of only three VDR gene polymorphisms (*FokI, BsmI, TaqI*) with T2DM in Indian patients [21]. To study the association of all these four polymorphisms with T2DM in India, recently we cared for total 100 well characterized T2DM patients, and the same number of age matched healthy controls from New Delhi, India. As a preliminary analysis, firstly we studied *TaqI* and *BsmI* polymorphisms. As a result, we found that the frequency of TT and TC genotype of *TaqI* as well as GG and GA genotype of *BsmI* polymorphism was significantly different in patients and controls [22]. In the current study, the association of remaining two important polymorphisms such as *FokI* and *ApaI* is investigated. As per literature, *BsmI*, *ApaI* and *TaqI* polymorphisms are shown to be in strong LD [23]. Hence, by taking help from our published data [22], the relationship between these four polymorphisms (LD and haplotype analysis) was also studied further in our patients.

METHODS

Study population

A total of 100 well-characterized Type 2 diabetes (T2D) patients (as per ADA Guidelines (2012) were selected for the study. All the patients had higher fasting plasma glucose levels (≥126 mg/dl), postprandial glucose >200 mg/dl and hemoglobin A1c >6.5% and were between age of 35 and 55 years. Along with patients, a total of 100 respective age

matched controls were recruited from All Indian Institute of Medical Sciences (AIIMS), New Delhi, India. The study was approved by the ethical committee AIIMS, New Delhi and JIIT, Noida. All the subjects were nonsmokers and non alcoholic. After taking the informed consent, the clinical details and blood samples were collected from all the subjects selected for the study.

Genomic DNA extraction

Blood sample (2 ml) was collected in ethylenediaminetetraceatic acid coated BD vacutainer. From all the blood samples, total genomic DNA was isolated using the standard protocol of salting, out method [24] and stored at −20°C for further experiments.

Genotyping/polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

PCR-RFLP was used to analyze the *ApaI* and *FokI* polymorphism in T2DM patients and controls. PCR amplification was carried out using the Bio-Rad thermal cycler, the reaction mix was prepared using 50 ng of DNA, 10 pmol of each forward (F) and reverse (R) primers (Sigma-Aldrich), 2 mM of deoxynucleotide triphosphates's (Sigma-Aldrich), 0.5 U *Taq* polymerase (Sigma-Aldrich) and nuclease free water was used to make up the volume to 30 µl. PCR conditions were initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 1 minute, annealing at 69°C (*FokI*) and 60°C (*ApaI*) for 1 minute, extension at 72°C for 5 minutes, and final extension at 72°C for 5 minutes. The PCR product obtained was 267 bp for *FokI* and 200 bp for *ApaI*; these PCR products were digested with 10 U of *FokI* (65°C/4 hrs, Thermo Scientific) and *ApaI* (37°C/4 hrs, Thermo Scientific) restriction enzyme. These digested products were then resolved in 3.5% agarose gel stained with ethidium bromide and analyzed (Table 1).

Statistical analysis

All the statistical analysis was performed using a commercially available software program (SPSS 21.0, SPSS Inc., Chicago, Illinois, USA). Clinical data were expressed as mean and standard deviation. To determine whether any significant differences in polymorphism frequencies occurred between the case and the control populations, the allele and genotype frequencies were compared, using the Chi-square (χ^2) method. Fischer's exact test was used to compare the genotype and allelic frequencies in all the groups. Associations between the T2DM phenotype and genotypes were assessed by calculating the p value and 95 confidence intervals. Haplotype and LD analysis was performed using Haploview 4.2. The p≤0.05 was considered to be statistically significant.

RESULTS

General characteristics of study population

In case of T2D patients, 45% of the population was women. The mean age for T2D patients and the controls were (46.8±11.33 years vs. 39.34±11.12 years), respectively. Fasting glucose level of T2D patients was 158.25±48.85 mg/dl, whereas postprandial plasma glucose level was 235.57±86.0 mg/dl.

Distribution of *FokI* and *ApaI* polymorphisms in VDR gene

After PCR amplification of different fragments of VDR gene, the RFLP study was performed using *FokI* and *ApaI* restriction enzymes. Based on the PCR/RFLP results (Figs. 1 and 2), the analysis was conducted further. For *FokI* polymorphism, genotype frequencies were TT (3%), TC (36%), CC (61%) in patients and TT (29%), TC (39%), and CC (32%) in controls (Table 2). Thus, mutant genotype (CC) was most prevalent in patients whereas the heterozygous genotype (TC) was common in control. As a result of statistical analysis, the frequency of wild (TT) and mutant (CC) genotype of *FokI* polymorphism showed a significant difference between patients and controls (χ^2=16.903, 25.149, p<0.001and p<0.001, respectively). The frequency of mutant allele (C) was also significantly higher in patients than the controls (χ^2=19.531, p<0.001*) (Table 3). Whereas the frequency of heterozygous profile (TC) was almost similar among these two groups (χ^2=0.343, p=0.558) (Table 2).

RFLP analysis of *ApaI* polymorphisms revealed that in case of patients, frequency of wild (GG), heterozygous (GC), mutant (CC) genotypes were 2%, 40%, 58%, respectively. In case of controls, distribution of genotypes was GG (10%), GC (46%), and CC (44%) (Table 2). Although the mutant genotype was widespread in both the groups, its frequency was relatively higher in patients. The frequency of wild type genotype was low in both groups. In case of *ApaI* polymorphisms, statistical results showed that frequency of wild (GG) and mutant (CC) genotype was significantly different in patients and controls (p=0.017 and

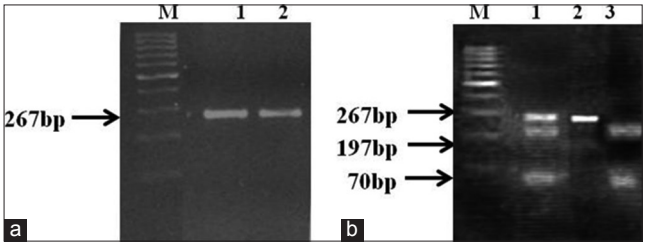

Fig. 1: (a) *FokI* polymerase chain reaction product at 267 bp. (b) Ethidium bromide *FokI* digested products electrophoresed on 3.5% agarose gel. Lane M: Molecular weight marker (100 bp), Lane 1: Heterozygous genotype (267 bp, 197 bp, 70 bp), Lane 2: Mutant genotype (267 bp), Lane 3: Wild genotype (197 bp, 70 bp)

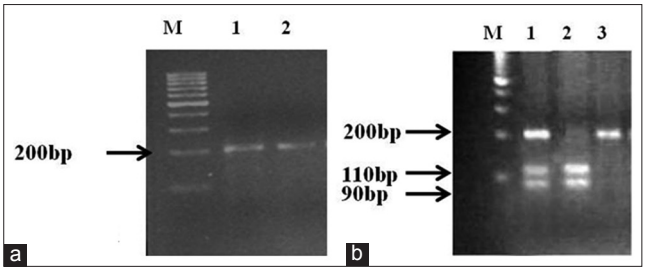

Fig. 2: (a) *ApaI* polymerase chain reaction product at 200 bp. (b) Ethidium bromide *ApaI* digested products electrophoresed on 3.5% agarose gel. Lane M: Molecular weight marker (100 bp), Lane 1: Heterozygous genotype (200 bp, 110 bp, 90 bp), Lane 2: Wild genotype (110 bp, 90 bp), Lane 3: Mutant genotype (200 bp)

Table 1: Primer sequences and fragment lengths details obtained after digestion of PCR products with *ApaI* and *FokI* restriction enzymes

SNP	Position/db SNP	Base change	Primer sequence	Annealing	Amplicon length (bp)	Genotype/RFLP products (bp)
ApaI	Intron 8/rs7975232	G/C	F-5'-CGGTCAGCAGTCATAGAGG -3' R-5'-CAGTGTGTTGGACAGGCG -3' [25]	60°C	200	GG/110, 90 GC/200, 110, 90 CC/200
FokI	Exon 2/rs2228570	T/C	F-5'-AGCTGGCCCTGGCACTGACTCTGCTCT -3' R-5'-ATGGAAACACCTTGCTTCTTCTCCCTC -3' [26]	68°C	267	TT/197, 70 TC/267, 70, 197 CC/267

SNP: Single nucleotide polymorphisms, PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphism

p=0.034). However, the heterozygous genotype was almost equally distributed in both the groups. The number of the mutant allele (C) was higher in patients group as compared to the control group, but the difference was not statistically significant (χ^2=3.04, p=0.082) (Table 3).

Haplotype analysis

After completing the genetic study of *ApaI* and *FokI* polymorphisms in the VDR gene of these subjects, the earlier published data of *TaqI* and *BsmI* polymorphisms of these subjects (Supplementary Table 1) were pooled together, and haplotype analysis was performed for all four polymorphisms. As a result, total 11 common haplotypes were present in patients and controls, though with different frequencies (Table 4). The most prevalent haplotype in both the groups was CGCT which was present in 19% of the T2DM patients and 12% control individuals (Fig. 3). There was no significant difference in the frequency of all 11 haplotypes except CACT. The frequency of this haplotype was significantly higher in patients (p=0.0267) as compared to the control group. Apart from those 11 common haplotypes, some haplotypes though with the low frequency were exclusively present in either of the group. For example, TGGT and TAGT were present only in the controls. However, TACC and CAGC were present in few patients.

LD plot in T2DM patients revealed a moderate LD between *ApaI* and *TaqI* (r^2=0.42) as well as between *FokI* and *BsmI* (r^2<0.60) polymorphisms. While very weak LD (r^2<0.3) was observed between these four polymorphisms in the control group (Fig. 4).

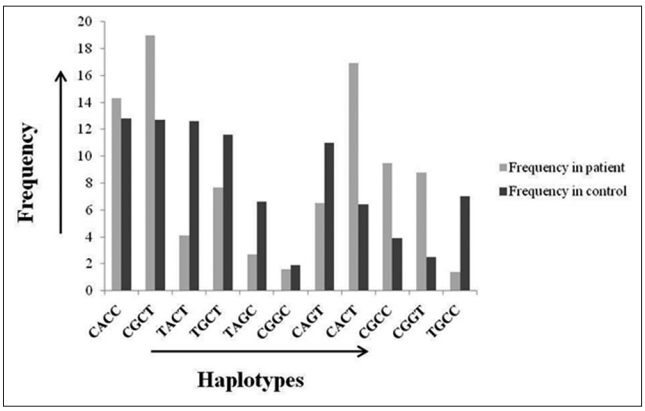

Fig. 3: Vitamin D receptor polymorphisms (*FokI, BsmI, ApaI, TaqI*) haplotypes and estimated prevalence (%) in Type 2 diabetes mellitus patients and healthy subjects

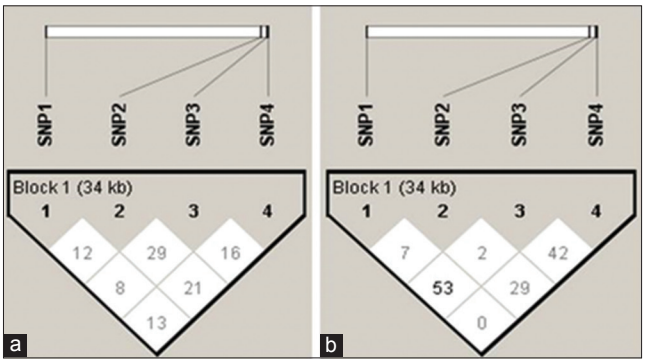

Fig. 4: Linkage disequilibrium plot showing the relationship between four Vitamin D receptor polymorphisms (*FokI, BsmI, ApaI, TaqI* as single nucleotide polymorphisms 1, 2, 3 and 4 respectively) in case of (a) controls and (b) Type 2 diabetes mellitus patients. Numbers in the boxes represents correlation coefficients value of linkage disequilibrium (r^2) multiplied by 100

DISCUSSION

VDR gene is known to express in different types of cells like pancreatic β cells, adipocytes and the SNP's in the VDR gene is known to affect the function of VDR protein in these cells. The SNP's in the VDR gene is also known to be associated with several diseases such as neurological diseases, different types of cancer, and diabetes in different populations [10-14]. Role of VDR polymorphisms in diabetes may be justified by making the argument that due to allelic differences in the VDR gene; the VDR mediated signaling of Vitamin D may get affected. It may further lead to low expression of insulin gene and compromised action of insulin on target cells, leading to diabetes [23,27,28]. Although, different VDR gene polymorphisms have been screened for their association with diverse T2DM patients hailing from different ethnic background. However, their association status is still conflicting worldwide [14,19,21,29-34]. These differences could be due to the diverse ethnicity, genetic and environmental factors leading to the T2DM.

Table 2: Distribution of *ApaI* and *FokI* polymorphism in T2DM patients and respective control

Genotype	N=100 (%)		χ^2	p value
	Patients	Controls		
ApaI				
GG (wild)	2 (2)	10 (10)	5.674	0.017*
GC (heterozygous mutant)	40 (40)	46 (46)	0.734	0.391
CC (mutant)	58 (58)	44 (44)	4.504	0.034*
FokI				
TT (wild)	3 (3)	29 (29)	25.149	<0.001*
TC (heterozygous mutant)	36 (36)	39 (39)	0.343	0.558
CC (mutant)	61 (61)	32 (32)	16.903	<0.001*

Statistical evaluation was made using the Chi-square test, *p<0.05.
T2DM: Type 2 diabetes mellitus

Table 3: Comparison of allelic frequencies of *ApaI and FokI* in T2DM patients and control

Allele	Patients (%)	Controls (%)	χ^2	p value
ApaI				
G	44 (22)	66 (33)	3.034	0.082
C	156 (78)	134 (67)		
FokI				
T	42 (21)	103 (51.5)	19.531	<0.001*
C	158 (79)	97 (48.5)		

Statistical evaluation was made using the Chi-square test, *p<0.05.
T2DM: Type 2 diabetes mellitus

Table 4: Frequencies of haplotypes representing the four SNP loci (*FokI, BsmI, ApaI, TaqI*), in the respective order, among the T2DM patients and controls

S.No.	Haplotype	Frequency in patient	Frequency in control	χ^2	p value
1	CACC	0.143	0.128	0.044	0.8339
2	CGCT	0.19	0.127	1.34	0.2408
3	TACT	0.041	0.126	3.32	0.0681
4	TGCT	0.077	0.116	0.549	0.4585
5	TAGC	0.027	0.066	1.172	0.279
6	CGGC	0.016	0.019	0	1
7	CAGT	0.065	0.11	1.029	0.31
8	CACT	0.169	0.064	4.91	0.0267
9	CGCC	0.095	0.039	1.316	0.2513
10	CGGT	0.088	0.025	2.623	0.1047
11	TGCC	0.014	0.07	3.255	0.0712

T2DM: Type 2 diabetes mellitus, SNP: Single nucleotide polymorphisms

Supplementary Table 1: Distribution of TaqI and BsmI polymorphism in T2DM patients and respective control [22]

Genotype	(N=100) %		χ^2	p value
	Patients	Controls		
(TaqI)				
TT(Wild)	36 (36)	50 (50)	5.195	0.023
TC(Heterozygous mutant)	54 (54)	37 (37)	37.72	<0.001
CC(mutant)	10 (10)	13 (13)	0.442	0.506
(BsmI)				
GG(Wild)	20 (20)	9 (9)	4.880	0.027
GA(Heterozygous mutant)	56 (56)	77 (77)	10.945	0.001
AA(mutant)	24 (24)	14 (14)	3.249	0.071

Statistical evaluation was made using the Chi square test, *p<0.05, **p<0.01
T2DM: Type 2 diabetes mellitus

Our current study is also an effort to further understand the association of VDR polymorphisms with T2DM. Out of all the four common VDR polymorphisms, *FokI* polymorphism located in the 5'end near the promoter region of the VDR gene, it is proposed to attenuate the Vitamin D function as well as its binding efficiency to the VDR [35] and further affects the insulin action leading to the diabetic phenotype [30]. In our study, T2DM patients are found to be significantly associated with the *FokI* polymorphisms (p<0.001**).** The mutant allele frequency (C) of *FokI* polymorphisms was also high in the T2DM patients as compared to the control (p<0.001). Similar to our study, *FokI* polymorphism was also found to be associated with T2DM risk in the studies conducted by Wang *et al.* and Li *et al.* [14,36]. According to the meta-analysis done by Li *et al.* in 2012 observed that the subjects carrying mutant allele or heterozygous genotype of *FokI* polymorphism had 1.51-fold increased risk of developing T2DM [37]. On the other hand in Caucasians and some Indian T2DM patients, *FokI* polymorphism was found to be associated with the T2DM in the study conducted by Malecki *et al.* and Bid *et al.*, respectively [18,21].

Like *FokI,* in case of *ApaI* polymorphism also, our results demonstrated that the genotype differences were significant in the diabetic and control groups (p=0.022). Our observations were in support with the study conducted in Caribbean T2DM patients who had hypovitaminosis D and showed their association *ApaI* polymorphism [38]. Howard *et al.* in 1995 also showed that *ApaI* may affect the circulatory Vitamin D levels [39]. The mutant genotype of *ApaI* polymorphism was associated with the lower insulin secretion in the Bangladeshi population in a research study conducted by Hitman *et al.* [30]. A correlation was also observed between *Apa I* polymorphism and fasting plasma glucose of the healthy adults. As *ApaI* site is located in an intronic region of the VDR gene and studies have also proposed that alterations in these sequences may influence expression of the VDR protein [40].

As per our earlier study, the same T2DM patients had significantly a lower number of wild type genotype (TT) 36% of *TaqI* polymorphism as compared to (TT) 50% in the controls [22]. As the TT genotype is known to be associated with high copy number of VDR mRNA [27,30] and hence low occurrence of wild type genotype in these patients indicate that they might have low VDR protein levels and may have compromised glucose metabolism too. In case of *BsmI* polymorphisms, the frequency of heterozygous GA genotype was also significantly different among patients and controls (p=0.001).

As per haplotype analysis of all these four polymorphisms (*FokI, BsmI, ApaI and TaqI)* in our study, the frequency of CACT haplotype was significantly elevated in T2DM patients (p=0.0267). This haplotype consisted of three mutant alleles of (*FokI, BsmI, ApaI)* polymorphisms and one wild type allele of *TaqI* polymorphism, which suggested the contribution of mutant alleles toward increasing the susceptibility to the T2DM phenotype. *FokI* polymorphism is known to affect the function and binding of Vitamin D to VDR protein and decreases the action and functioning of the insulin leading to the diabetic phenotype [41]. The meta-analysis studies also indicated the mutant allele of *FokI* polymorphism as a risk factor for T2DM [37,42]. The mutant allele of *FokI* polymorphism was further found to be associated with the diabetes retinopathy in Han Chinese population [43]. According to the meta-analysis by Yu *et al.*, the mutant allele and mutant homozygous (AA) of the *BsmI* polymorphism was also significantly associated with the T2DM [41]. The TACC, CAGC haplotypes were found to be present in patients only. In these two haplotypes, 3 out of 4 alleles were mutant (*BsmI, ApaI, TaqI* and *FokI, BsmI, TaqI,* respectively). These mutant alleles may be thought to be a factor, increasing the susceptibility of these individuals toward T2DM. In the similar lines of our observation, the VDR haplotype consisted of mutant allele *BsmI, ApaI, TaqI* has been shown to be associated with decreased VDR protein levels [44,45]. Studies in the humans have also suggested that VDR allelic variations alter the pancreatic β cells function [3]. In Bangladeshi populations *BsmI, ApaI, TaqI* SNP's were significantly associated with the insulin secretion and considered at risk to develop T2DM [30]. The mutated genotypes of *BsmI, ApaI, TaqI* were associated with decreased insulin levels as compared to the subjects carrying wild genotypes, and intermediate insulin levels were observed in the heterozygous profile [27]. These polymorphisms (*BsmI, ApaI, TaqI*) in 3' untranslated region of VDR gene is associated with the variable gene transcriptions and mRNA stability, leading to altered VDR activity. In Bangladeshi Asians, the frequent haplotypes of *BsmI, ApaI, TaqI* polymorphisms were associated with variation in the VDR gene transcription, stability of the mRNA and protein levels in the subjects [27]. The study further observed that in the peripheral blood cells, these genotypes significantly determines the VDR mRNA and protein levels [27,28,46]. Two rare haplotypes TGGT and TAGT were limited to the control group only. Both these haplotypes consist of at least three wild type alleles of *FokI, BsmI, ApaI, TaqI* and thus may be proposed to be associated with the lower the risk of T2DM in these individuals.

CONCLUSION

As per our current study, *FokI* and *ApaI* VDR polymorphisms are associated with T2DM patients, though with different frequency. The frequency of mutant allele of *FokI* is was significantly very high in these patients, indicating it possible role in causing diabetes. Further, the frequency of wild type genotype of both the polymorphisms was significantly low in patients. It may suggest that due to the absence of wild type allele these individuals are susceptible toward T2DM. Haplotype analysis also indicates the pathogenic significance of the four common VDR polymorphisms and their associating with diabetes. The combination of mutation alleles of *FokI, BsmI, ApaI* polymorphisms seems to be crucial in case of our patients. However, a large number of patients should be studied to strengthen the study and confirm the association of these polymorphisms in diabetic patients.

ACKNOWLEDGMENT

Authors acknowledge the subjects who participated in the study and AIIMS, New Delhi and JIIT, Noida for providing the infrastructure and financial support to conduct the study.

REFERENCES

1. Chantal M, Conny G, Annapaula G, Roger B. Vitamin D and diabetes. Diabetologia 2005;48:1247-57.
2. Takiishi T, Gysemans C, Bouillon R, Mathieu C. Vitamin D and diabetes. Endocrinol Metab Clin North Am 2010;39(2):419-46.
3. Palomer X, González-Clemente JM, Blanco-Vaca F, Mauricio D. Role of vitamin D in the pathogenesis of Type 2 diabetes mellitus. Diabetes Obes Metab 2008;10(3):185-97.
4. Mitri J, Pittas AG. Vitamin D and diabetes. Endocrinol Metab Clin North Am 2014;43(1):205-32.
5. Kalsi A, Singh S, Taneja N, Kukal S, Mani S. Current treatment for Type 2 diabetes, their side effects and possible complementary treatments. Int J Pharm Pharm Sci 2015;3:13-8.
6. Carlberg C. The concept of multiple vitamin D signaling pathways. J Investig Dermatol Symp Proc 1996;1(1):10-4.
7. Hossein-Nezhad A, Spira A, Holick MF. Influence of vitamin D status

and vitamin D3 supplementation on genome wide expression of white blood cells: A randomized double-blind clinical trial. PLoS One 2013;8(1):e58725.
8. Montecino M, Stein GS, Stein JL, Lian JB, van Wijnen AJ, Carvallo L, *et al.* Vitamin D control of gene expression: Temporal and spatial parameters for organization of the regulatory machinery. Crit Rev Eukaryot Gene Expr 2008;18(2):163-72.
9. Zittermann A. Vitamin D in preventive medicine: Are we ignoring the evidence? Br J Nutr 2003;89(5):552-72.
10. McCullough ML, Stevens VL, Diver WR, Feigelson HS, Rodriguez C, Bostick RM, *et al.* Vitamin D pathway gene polymorphisms, diet, and risk of postmenopausal breast cancer: A nested case-control study. Breast Cancer Res 2007;9(1):R9.
11. Cicek MS, Liu X, Schumacher FR, Casey G, Witte JS. Vitamin D receptor genotypes/haplotypes and prostate cancer risk. Cancer Epidemiol Biomarkers Prev 2006;15:2549-52.
12. Mittal RD, Mishra DK, Srivastava P, Manchanda P, Bid HK, Kapoor R. Polymorphisms in the vitamin D receptor and the androgen receptor gene associated with the risk of urolithiasis. Indian J Clin Biochem 2010;25(2):119-26.
13. Györffy B, Vásárhelyi B, Krikovszky D, Madácsy L, Tordai A, Tulassay T, *et al.* Gender-specific association of vitamin D receptor polymorphism combinations with Type 1 diabetes mellitus. Eur J Endocrinol 2002;147(6):803-8.
14. Li HM, Miao H, Lu YB, Gen HF, Jiang XQ. Association between DNA polymorphism of human vitamin D receptor gene and Type 2 diabetes mellitus. China J Mod Med 2005;15:989-92.
15. Miyamoto K, Kesterson RA, Yamamoto H, Taketani Y, Nishiwaki E, Tatsumi S, *et al.* Structural organization of the human vitamin D receptor chromosomal gene and its promoter. Mol Endocrinol 1997;11(8):1165-79.
16. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, *et al.* Prediction of bone density from vitamin D receptor alleles. Nature 1994;367(6460):284-7.
17. Cyganek K, Mirkiewicz-Sieradzka B, Malecki MT, Wolkow P, Skupien J, Bobrek J, *et al.* Clinical risk factors and the role of VDR gene polymorphisms in diabetic retinopathy in Polish Type 2 diabetes patients. Acta Diabetol 2006;43(4):114-9.
18. Malecki MT, Frey J, Moczulski D, Klupa T, Kozek E, Sieradzki J. Vitamin D receptor gene polymorphisms and association with Type 2 diabetes mellitus in a Polish population. Exp Clin Endocrinol Diabetes 2003;111(8):505-9.
19. Ye WZ, Reis AF, Dubois-Laforgue D, Bellanné-Chantelot C, Timsit J, Velho G. Vitamin D receptor gene polymorphisms are associated with obesity in Type 2 diabetic subjects with early age of onset. Eur J Endocrinol 2001;145(2):181-6.
20. Al-Daghri NM, Al-Attas OS, Alkharfy KM, Khan N, Mohammed AK, Vinodson B, *et al.* Association of VDR-gene variants with factors related to the metabolic syndrome, Type 2 diabetes and vitamin D deficiency. Gene 2014;542(2):129-33.
21. Bid HK, Konwar R, Aggarwal CG, Gautam S, Saxena M, Nayak VL, *et al.* Vitamin D receptor (FokI, BsmI and TaqI) gene polymorphisms and Type 2 diabetes mellitus: A North Indian study. Indian J Med Sci 2009;63(5):187-94.
22. Taneja N, Khadgawat R, Mani S. BsmI and TaqI polymorphisms in Vitamin D receptor gene of Type 2 diabetes mellitus patients from north India. Asian J Pharm Clin Res 2016;9:186-9.
23. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of Vitamin D receptor polymorphisms. Gene 2004;338(2):143-56.
24. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16(3):1215.
25. Naito M, Miyaki K, Naito T, Zhang L, Hoshi K, Hara A, *et al.* Association between Vitamin D receptor gene haplotypes and chronic periodontitis among Japanese men. Int J Med Sci 2007;4(4):216-22.
26. Harris SS, Eccleshall TR, Gross C, Dawson-Hughes B, Feldman D. The Vitamin D receptor start codon polymorphism (FokI) and bone mineral density in premenopausal American black and white women. J Bone Miner Res 1997;12(7):1043-8.
27. Ogunkolade BW, Boucher BJ, Prahl JM, Bustin SA, Burrin JM, Noonan K, *et al.* Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to Vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. Diabetes 2002;51(7):2294-300.
28. Reis AF, Hauache OM, Velho G. Vitamin D endocrine system and the genetic susceptibility to diabetes, obesity and vascular disease. A review of evidence. Diabetes Metab 2005;31:318-25.
29. McDermott MF, Ramachandran A, Ogunkolade BW, Aganna E, Curtis D, Boucher BJ, *et al.* Allelic variation in the Vitamin D receptor influences susceptibility to IDDM in Indian Asians. Diabetologia 1997;40(8):971-5.
30. Hitman GA, Mannan N, McDermott MF, Aganna E, Ogunkolade BW, Hales CN, *et al.* Vitamin D receptor gene polymorphisms influence insulin secretion in Bangladeshi Asians. Diabetes 1998;47(4):688-90.
31. Speer G, Cseh K, Winkler G, Vargha P, Braun E, Takács I, *et al.* Vitamin D and estrogen receptor gene polymorphisms in Type 2 diabetes mellitus and in android type obesity. Eur J Endocrinol 2001;144(4):385-9.
32. Filus A, Trzmiel A, Kuliczkowska-Plaksej J, Tworowska U, Jedrzejuk D, Milewicz A, *et al.* Relationship between Vitamin D receptor BsmI and FokI polymorphisms and anthropometric and biochemical parameters describing metabolic syndrome. Aging Male 2008;11(3):134-9.
33. Ortlepp JR, Lauscher J, Hoffmann R, Hanrath P, Joost HG. The Vitamin D receptor gene variant is associated with the prevalence of Type 2 diabetes mellitus and coronary artery disease. Diabet Med 2001;18(10):842-5.
34. Oh J, Barrett-Connor E. Association between VDR polymorphism and Type 2 diabetes or metabolic syndrome in community-dwelling older adults: The Rancho Bernardo study. Metabolism 2002;5:356-9.
35. Leandro AC, Rocha MA, Cardoso CS, Bonecini-Almeida MG. Genetic polymorphisms in Vitamin D receptor, Vitamin D-binding protein, Toll-like receptor 2, nitric oxide synthase 2, and interferon-gamma genes and its association with susceptibility to tuberculosis. Braz J Med Biol Res 2009;42(4):312-22.
36. Wang H, Craig RL, Schay J, Chu W, Das SK, Zhang Z, *et al.* Alpha-endosulfine, a positional and functional candidate gene for Type 2 diabetes: Molecular screening, association studies, and role in reduced insulin secretion. Mol Genet Metab 2004;81:9-15.
37. Li L, Wu B, Liu JY, Yang LB. Vitamin D receptor gene polymorphisms and Type 2 diabetes: A meta-analysis. Arch Med Res 2013;44(3):235-41.
38. Vélayoudom-Céphise FL, Larifla L, Donnet JP, Maimaitiming S, Deloumeaux J, Blanchet A, *et al.* Vitamin D deficiency, vitamin D receptor gene polymorphisms and cardiovascular risk factors in Caribbean patients with Type 2 diabetes. Diabetes Metab 2011;37(6):540-5.
39. Howard G, Nguyen T, Morrison N, Watanabe T, Sambrook P, Eisman J, *et al.* Genetic influences on bone density: Physiological correlates of vitamin D receptor gene alleles in premenopausal women. J Clin Endocrinol Metab 1995;80(9):2800-5.
40. Mocharla H, Butch AW, Pappas AA, Flick JT, Weinstein RS, de Togni P, *et al.* Quantification of vitamin D receptor mRNA by competitive polymerase chain reaction in PBMC: Lack of correspondence with common allelic variants. J Bone Miner Res 1997;12:726-33.
41. Yu F, Cui LL, Li X, Wang CJ, Ba Y, Wang L, *et al.* The genetic polymorphisms in vitamin D receptor and the risk of Type 2 diabetes mellitus: An updated meta-analysis. Asia Pac J Clin Nutr 2016;25(3):614-24.
42. Wang Q, Xi B, Reilly KH, Liu M, Fu M. Quantitative assessment of the associations between four polymorphisms (FokI, ApaI, BsmI, TaqI) of vitamin D receptor gene and risk of diabetes mellitus. Mol Biol Rep 2012;39(10):9405-14.
43. Zhong X, Du Y, Lei Y, Liu N, Guo Y, Pan T. Effects of vitamin D receptor gene polymorphism and clinical characteristics on risk of diabetic retinopathy in Han Chinese Type 2 diabetes patients. Gene 2015;566(2):212-6.
44. Selvaraj P, Prabhu Anand S, Harishankar M, Alagarasu K. Plasma 1,25 dihydroxy vitamin D3 level and expression of vitamin D receptor and cathelicidin in pulmonary tuberculosis. J Clin Immunol 2009;29(4):470-8.
45. Mallayasamy M, David HA, Pandian PR. Comparison of angiotensin receptor blocker alone and in combination with vitamin D analogue in reducing proteinuria in diabetic nephropathy patients. Int J Pharm Pharm Sci 2014;6:201-4.
46. Morrison NA, Yeoman R, Kelly PJ, Eisman JA. Contribution of trans-acting factor alleles to normal physiological variability: Vitamin D receptor gene polymorphism and circulating osteocalcin. Proc Natl Acad Sci U S A 1992;89(15):6665-9.