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Association of Vitamin D Deficiency and Vitamin D Receptor Gene Polymorphisms with Type 1 Diabetes Risk: A South Indian Familial Study

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What is already known on this topic?

Type 1 diabetes mellitus (T1D) is an autoimmune disease characterized by the depletion of pancreatic β-cells, hypoglycemia, and elevated inflammatory cytokines in the case of serum vitamin D deficiency (VDD). Vitamin D receptor (VDR) haplotype polymorphisms are associated with T1D in several population.

What this study adds?

The genotype frequency of *VDR* gene polymorphisms FokI (rs2228570 C/T)-FF is significantly higher in T1D patients than controls and this relationship is reversed in the FokI-Ff genotype. VDD appears to be contributing risk factor to T1D development in South Indian children.

Abstract

Objective: Vitamin D is a potent immune modulator and is associated with autoimmune diseases, including type 1 diabetes (T1D). The vitamin D levels and its receptor gene polymorphisms together in T1D are not yet investigated in the South Indian population. The present study focused on exploring the significance of vitamin D levels and vitamin D receptor (VDR) gene polymorphisms with the risk of developing T1D in the South Indian population.

Methods: Patients with T1D and unaffected first-degree relatives (FDRs) were included in this study. Genotyping of VDR polymorphisms at four different loci (FokI- F/f, BsmI- B/b, TaqI- T/t, and ApaI- A/a) was assessed through the amplification refractive mutation system-polymerase chain reaction method. Serum vitamin D levels were measured in 98 T1D patients and 75 age- and sex-matched siblings.

Results: A total of 120 patients with T1D and 214 FDRs were included. Vitamin D deficiency (VDD) was observed in a higher proportion of T1D patients than in controls (52% vs. 32%; $p < 0.03$). The frequency of the FokI-FF genotype was significantly higher [odds ratio (OR) = 1.66; $p < 0.03$] in T1D patients conferring a susceptible association with the disease. Nevertheless, the increased frequency of heterozygous Ff genotype (OR = 0.57; $p < 0.02$) among controls may confer a protective association with T1D. Furthermore, the transmission disequilibrium test revealed over-transmission of ApaI-A (T: U = 15/5; $p < 0.006$) and BsmI-B alleles (T: U = 17/5; $p < 0.01$) and under-transmission of BsmI-b/ApaI-a/TaqI-T haplotype (T: U = 5.4/14.4; $p = 0.04$) from parents to T1D patients.

Conclusion: The present study concludes that VDD is the major contributing risk factor to T1D development in the South Indian population. Furthermore, the FokI-FF genotype, BsmI-B, and ApaI-A alleles were positively associated with T1D. In contrast, the FokI-Ff genotype and BsmI-b/ApaI-a/TaqI-T haplotype were negatively associated with T1D.

Keywords: Vitamin D, type 1 diabetes, autoimmunity, polymorphism, vitamin D receptor, β-cells

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Introduction

Type 1 diabetes mellitus (T1D) is characterized by the destruction of insulin-producing β-cells of pancreatic islets due to aberrations in both humoral and cell-mediated immunity (1,2). As a result of this autoimmune process, the pancreas produces very little or no insulin, which leads to development of T1D. In 2021 approximately 8.4 million individuals were reported to have T1D worldwide. Of these ∼1.5 million were younger than 20 years, 5.4 million were in the age group of 20-59 years, and 1.6 million were aged 60 years or older. Furthermore, there were 0.5 million new cases diagnosed, and about 35,000 undiagnosed individuals died within 12 months of symptomatic onset in 2021. One-fifth of individuals with T1D are living in low-income and lower-middle-income countries. Alarmingly, this group has predicted an increase in prevalent cases to 13.5-17.4 million in low-income and lower-middle-income countries by 2040 (3). The exact pathophysiology of T1D is still not yet understood but is associated with a wide range of genetic and environmental factors, especially viral infection and nutrition, or a combination of both genetics and environment (4,5,6). Among the various risk factors, having a family history of T1D is associated with an increased risk of developing the disease.

Vitamin D and its receptor are well known to play a prominent role in T1D development (6). Vitamin D deficiency (VDD) is a major health issue in various populations, including India, even with abundant sunshine (7,8). The rate of T1D incidence is reported to be steadily increasing and is associated with VDD across global populations (9,10). The ability of vitamin D to prevent T1D could be attributed to its immunoregulatory effect. Indeed, vitamin D plays a dynamic role in the inhibition of macrophage stimulation, antigen-presenting cell maturation, and dendritic cell differentiation, affecting the cytokine paradigm, and reducing the expression of human leukocyte antigen (HLA) class I molecules and Fas, thereby leading to reduced pancreatic β-cell damage (4,11). Although HLA class I and II loci are known to play a major role in T1D development, non-HLA genes such as the genes for proinflammatory (tumor necrosis factor-α, interferon-γ, IL-1, and IL-6) and anti-inflammatory cytokines (IL-10, IL-12, IL-13 and transforming growth factor-β) and vitamin D associated factors (VDR, CYP2R1, CYP27B1, CYP24A1, and DBP) have also been reported as high-risk factors (12,13,14,15).

VDR is a member of the nuclear hormone receptor family that exhibits a functional effect upon binding to vitamin D. Furthermore, the VDR-vitamin D heterodimerizes with retinoid X receptor. This binds to the vitamin D response element located in the promoter region of vitamin D responsive genes leading to the recruitment of co-activators or co-repressors to regulate the transcription of the genes (16). The *VDR* gene is located on chromosome 12q13.11. This gene contains nine exons and spans ∼ 75 kb of genomic DNA. Principally, exon 2-9 encode 427 amino acids containing VDR protein isoforms that consequence to include the DNA-binding (2-3 exon) and the vitamin D-binding (5-9 exon) regions (17). More than 8700 *VDR* gene polymorphisms have been described in healthy and various disease conditions among various populations (https://www.ncbi.nlm.nih.gov/pmc/). Particularly, *VDR* gene single nucleotide polymorphisms (SNPs) at four loci, namely FokI- F/f (rs2228570 C/T), BsmI- B/b (rs1544410 T/C), ApaI- A/a (rs7975232 T/C) and TaqI- T/t (rs731236 T/G) are known to be closely involved in vitamin D metabolism and vitamin D levels, and may thereby act as risk factors for developing T1D (18). Previous studies have reported one or more VDR polymorphisms associated with T1D (19,20,21,22,23,24,25,26). However, other studies have failed to lend support to this association (19,27,28,29,30). Vitamin D status and *VDR* gene polymorphisms are yet to be investigated in T1D in various ethnicities, including South Indians. Hence, the present study set out to explore the significance of vitamin D levels and *VDR* gene polymorphisms and the risk of T1D in the South Indian population.

Methods

Study Subjects

Participants including patients diagnosed with T1D and unaffected relatives [comprising parents, first-degree relatives (FDR), and 36 trios] belonging to 120 families were recruited from Government Rajaji Hospital, Madurai, Tamil Nadu, India. The T1D patients were diagnosed and stratified for inclusion in this study based on the American Diabetes Association guidelines (31). Approximately 5 mL of venous blood was obtained from all participating individuals throughout the year because of the climate in the country.

DNA Extraction and Serum Separation

The serum was separated from ∼1.5 mL of clotted blood. In addition, genomic DNA was extracted from ∼ 3.5 mL of ethylenediaminetetraacetic acid blood by the salting-out method (32). Serum and genomic DNA were stored at - 80 °C and -20 °C, respectively, for further analysis.

Estimation of Serum 25 (OH)-vitamin D Levels

Serum 25-hydroxyvitamin D_2 and D_3 [25 (OH)-D_2 and 25 (OH)-D_3] levels were measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions (DIA source Immunoassays S.A., Belgium). The serum 25 (OH) vitamin D status was classified as VDD ≤10 ng/dL, vitamin D insufficiency (VDI) 11-30 ng/dL, vitamin D sufficiency (VDS) 31-50 ng/dL and vitamin D toxicity (VDT) > 100 ng/dL, based on the Clinical Practice Guidelines of the Endocrine Society (33).

VDR Genotyping

The VDR FokI-F/f (rs2228570 T > C), BsmI-B/b (rs1544410 A > G), ApaI-A/a (rs7975232 C > A), and TaqI-T/t (rs731236 T > C) polymorphisms were analyzed using amplification refractive mutation system-polymerase chain reaction method, as previously described (34).

This study was approved by the Institutional Ethics Committees of Madurai Kamaraj University (MKU/IRB/11/11) and Government Rajaji Hospital (ref no: 23339/E4/3/10, date: 12.04.2011). The participant's written informed consent was obtained along with a detailed questionnaire.

Statistical Analysis

Vitamin D levels were compared between T1D patients and siblings by Student's t-test. Prior to the familial association analysis, genotype data of 36 trio families were assessed for quality control. Out of 36 families, 33 trios qualified for further analyses. Single-point and multi-point association analyses were carried out for the transmission disequilibrium test (TDT). To obtain empirical p values, 10,000 permutations were run for each analysis, and a p value of < 0.05 was considered statistically significant. TDT and Linkage disequilibrium (LD) analyses were performed using Haploview 4.2v (35).

The association of alleles, genotypes, and haplotypes with T1D was tested through odds ratio (OR) with a 95% confidence interval. Logistic regression analysis was conducted to correlate VDR SNPs with serum vitamin D status. Continuous and categorical variables are presented as mean ± standard deviation and percentage, respectively. All exploratory data analyses were performed using Epi-info 7v (https://www.cdc.gov/epiinfo/index.html), R programme (www.R-project.org/), and STATA 14v (College Station, TX: StataCorp LLC). The level of significance was set at a $p < 0.05$.

Bioinformatics Analysis

Pathogenicity prediction scores of nonsynonymous (ns) VDR-FokI (rs2228570) SNP was performed using SIFT, SIFT4G, Polyphen2_HDIV, Polyphen2_HVAR, LRT, MutationTaster, MetaSVM, MetaLR, PrimateAI, DEOGEN2, BayesDel_addAF, BayesDel_noAF, ClinPred, and LIST-S2 tools, which are genome-scale based. These query variants can be classified as likely pathogenic/deleterious or neutral/benign properties by the above 14 tools through dbNSFP V4.1a on VannoPortal (36,37). This portal also archives five major classes of annotations including basic SNP information, evolutionary annotation, disease association, SNP regulatory potential, and SNP pathogenicity and variant pathogenicity.

Table 1. Demographical and clinical categorization of T1D patients and their family members in the study

	T1D patients and age at onset				First degree relatives	
Characteristics	**All patients**	**< 10 yrs**	**11-20 yrs**	**> 20 yrs**	**Parents**	**Siblings**
No. of patients	120	34	56	30	120	94
Age (year)						
Mean ± SD	24.10 ± 10.07	16.11 ± 6.42	30.31 ± 14.08	30.31 ± 14.21	42.92 ± 9.92	20.55 ± 12.33
Range	4-50	4-32	13-40	23-50	25-72	1.5-65
Gender						
Male	70 (57.78%)	20 (58.82%)	32 (56.90%)	17 (56.67%)	44 (36.67%)	56 (59.57%)
Female	51 (42.14%)	14 (41.18%)	24 (43.10%)	13 (43.33%)	76 (63.33%)	38 (40.42%)
Disease duration						
< 2 yrs	4 (3.28%)	2 (5.88%)	10 (17.24%)	2 (6.67%)	-	-
2-5 yrs	41 (34.43%)	6 (17.65%)	17 (32.76%)	7 (23.33%)	-	-
6-10 yrs	37 (30.33)	13 (38.23%)	16 (27.59%)	8 (26.67%)	-	-
11-15 yrs	19 (15.57%)	5 (14.71%)	7 (12.07%)	7 (23.33%)	-	-
> 15 yrs	20 (16.39%)	8 (23.53%)	6 (10.34%)	6 (20.00%)	-	-

First-degree relatives are comprised of parents and siblings; continuous variables are expressed as mean ± SD; categorical variables are expressed as n (%).
T1D: type 1 diabetes, yrs: years, SD: standard deviation

Results

A total of 334 participants (120 T1D patients and 214 controls) belonging to 120 families (comprising parents, FDR, and 36 trios) were recruited. The detailed demographic and clinical variables of the T1D patients and their FDRs are presented in Table 1. All 334 individuals were characterized by VDR genotyping. Furthermore, serum vitamin D levels were measured in 173 (51.7%), including 98 (81.7%) T1D patients and 75 age- and sex-matched siblings.

Vitamin D Status

Serum vitamin D levels and their status distribution in T1D patients and siblings are presented in Table 2 and Figure 1. Vitamin D [25 (OH)] level was significantly lower in T1D patients (9.73 ± 7.82 ng/dL) than in healthy siblings (16.17 ± 11.48 ng/dL). The distribution of vitamin D status among T1D patients and siblings was: VDD 52% vs 36% respectively, VDI 43.9% vs 53.1%, respectively, and VDS 4.1% vs 13%, respectively. However, no participant was classified as VDT in the study population.

VDR Polymorphisms in Trios

TDT analysis revealed that VDR-ApaI-A (T: U = 15/5; p = 0.006) and VDR-BsmI-B (T: U = 17/5; p = 0.01) alleles were significantly over-transmitted from parents to T1D patients. However, the VDR-FokI-F and VDR-TaqI-T alleles did not show any deviation in allele transmission. Furthermore, haplotype BsmI-b/ApaI-a/TaqI-T (baT) was significantly under-transmitted (T: U = 5.4/14.4; p = 0.04) from parents to T1D patients (Table 3).

LD analysis identified a single haplotype block, comprising VDR TaqI-ApaI-BsmI (Figure 2). High LD was observed between TaqI-ApaI (D' = 1.0; LOD = 14.02; r2 = 0.45), TaqI-BsmI (D' = 0.92; LOD = 19.9; r2 = 0.68) and BsmI-ApaI (D' = 1.0; LOD = 18.73; r2 = 0.62).

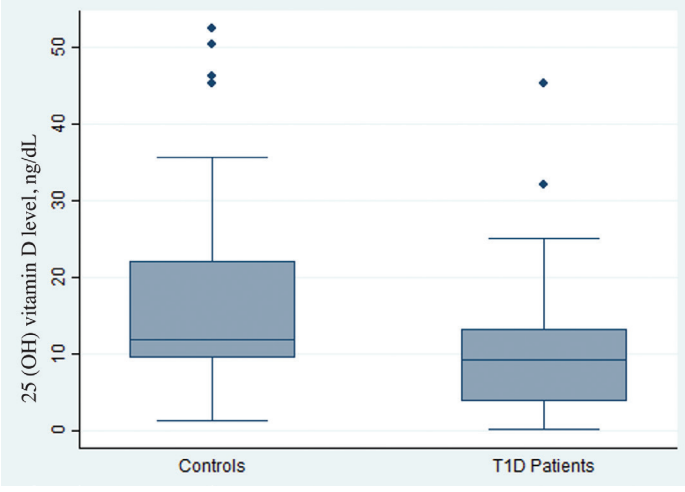

Figure 1. Serum 25 (OH) vitamin D levels (ng/dL) in T1D patients and controls

T1D: type 1 diabetes

VDR Polymorphisms in Patients and FDRs

Allele and genotype frequencies of VDR polymorphisms (FokI, BsmI, ApaI, and TaqI) are presented in Table 4. The genotype frequency of FokI-FF (OR = 1.66; p = 0.03) was significantly more common in T1D patients than FDRs, while the FokI-Ff genotype frequency (OR = 0.5; p = 0.02) was significantly lower in T1D patients than FDRs. However, genotype and allele frequencies of BsmI, ApaI,

Table 2. Vitamin D status and its distribution among T1D patients and siblings

Parameter	T1D patients (%)	Siblings (%)	p
Subjects	98	75	-
Vitamin D, ng/mL	9.73 ± 7.82	16.17 ± 11.48	**0.00001**
Deficiency (%)	51 (52.0)	27 (36.0)	**0.03**
Insufficiency (%)	43 (43.9)	39 (52.0)	0.29
Sufficiency (%)	4 (4.1)	9 (12.0)	0.05

T1D: type 1 diabetes

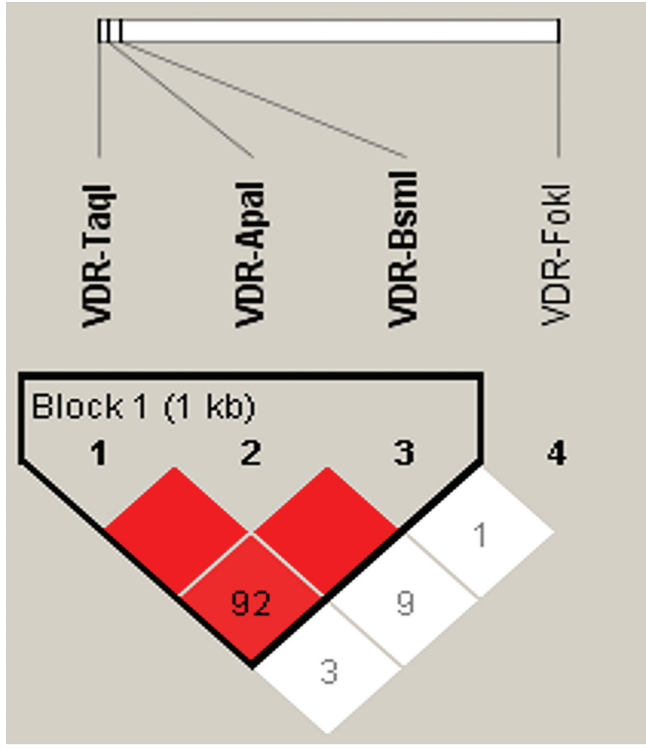

Figure 2. LD plots of the *VDR* gene polymorphism associated with T1D: The physical position of each SNP is shown above the plot as a white bar. The black outline denotes the haplotype block. Color intensity indicates the value of D' from white (D' = 0) to red (D' = 1)

VDR: vitamin D receptor, LD: Linkage disequilibrium, SNP: single nucleotide polymorphism

Table 3. TDT analysis of VDR alleles and haplotypes transmission from parents to siblings in trio families

Genotype/haplotype	T:U	MAF	χ^2 value	p
VDR-Allele				
TaqI-T	18:14	0.23	0.5	0.48
ApaI-A	18:05	0.47	7.3	**0.006**
BsmI-B	17:05	0.38	6.5	**0.01**
FokI-F	12:10	0.41	0.18	0.67
VDR-Haplotype				
BsmI-b/ApaI-A/TaqI-T	14.9:8.8	0.39	1.6	0.2
BsmI-b/ApaI-a/TaqI-T	5.4:14.4	0.38	4.1	**0.04**
BsmI-B/ApaI-A/TaqI-t	7.4:8.6	0.11	0.09	0.76
BsmI-B/ApaI-A/TaqI-T	5.4:1.2	0.07	2.64	0.1
BsmI-b/ApaI-A/TaqI-t	1.1:1.2	0.02	0.91	0.91

T: U copies of the minor allele transmitted (T) and non-transmitted (U) from heterozygous parents to affected offspring; MAF minor allele frequencies in type 1 diabetes (T1D) patients.
VDR: vitamin D receptor, TDT: transmission disequilibrium test

and TaqI did not show significant differences between the study groups. Likewise, there were no significant differences in the distribution of *VDR* genotypes and haplotypes based on the age at onset of the disease (data not shown). Logistic regression analysis of *VDR* genotypes and vitamin D status (VDD, VDI, and VDS) did not show a significant association with T1D (Supplementary Table 1).

Discussion

Vitamin D status is reported to be associated with a predisposition to immunological disorders such as T1D, rheumatoid arthritis, dermatomyositis, multiple sclerosis, systemic lupus erythematosus, hepatitis, asthma, inflammatory bowel disease, and microbial infections (38). Indeed, Vitamin D exerts immune-modulatory

Table 4. Distribution of genotype and allele frequencies of *VDR* gene polymorphisms in T1D patients and controls

VDR-SNPs	Patients, n = 120 (%)	Controls, n = 214 (%)	OR	95% CI	p
FokI-genotype					
FF	76 (63.33)	109 (50.94)	1.6639	1.0526-2.6302	**0.03**
Ff	39 (32.5)	98 (45.79)	0.5699	0.3572-0.9093	**0.02**
ff	5 (4.17)	7 (3.27)	1.2857	0.3990-4.1429	0.67
Allele					
F	191 (79.58)	316 (73.83)	1.3816	0.9441-2.0217	0.09
f	49 (20.42)	112 (26.17)			
BsmI-genotype					
BB	34 (28.33)	76 (35.51)	0.7179	0.4416-1.1669	0.18
Bb	50 (41.67)	81 (37.85)	1.1728	0.7433-1.8506	0.49
bb	36 (30.0)	57 (26.64)	1.1805	0.7201-1.9351	0.51
Allele					
B	118 (49.17)	233 (54.44)	0.8095	0.5898-1.1110	0.19
b	122 (50.83)	195 (45.56)			
ApaI-genotype					
AA	20 (16.67)	34 (15.89)	1.0588	0.5788-1.9371	0.85
Aa	53 (44.16)	102 (47.66)	0.8686	0.5543-1.3611	0.53
aa	47 (39.17)	78 (36.45)	1.1226	0.7085-1.7788	0.62
Allele					
A	93 (38.75)	170 (39.72)	0.9601	0.6945-1.3274	0.8
a	147 (61.25)	158 (60.28)			
TaqI-genotype					
TT	25 (20.83)	35 (16.36)	1.3459	0.7608-2.3807	0.3
Tt	54 (45.00)	102 (47.66)	0.8984	0.5736-1.4071	0.63
tt	41 (34.17)	77 (35.98)	0.9234	0.5775-1.4764	0.73
Allele					
T	104 (43.33)	172 (40.19)	1.1382	0.8263-1.5677	0.42
t	136 (56.67)	256 (59.81)			

OR: odds ratio; 95% CI: 95% confidence interval, T1D: type 1 diabetes, VDR: vitamin D receptor, SNPs: single nucleotide polymorphisms

effects, such as successive reduction in T-cell infiltration, decrease in proinflammatory cytokines, and suppression of the autoimmune process, which could be expected to delay the development of T1D (38). Moreover, vitamin D supplementation for women during pregnancy and infant life has been recognized as a protective factor against T1D (39,40,41). Similarly, vitamin D treatment potentially improves glycemic control in T1D children and adolescents (42). Although the significance of vitamin D status in T1D patients is widely recognized, the influence of VDD on T1D incidence remains unclear (43). The present study findings show that VDD is more common in diagnozed patients with T1D than in their unaffected relatives, which is in agreement with the earlier reports from Swedish, Qatari, North Indian, Iranian, American, Egyptian, Australian, Italian, Saudi Arabian, and Bangladesh populations (44,45,46,47,48,49,50,51,52 ,53). Further, a meta-analysis study also reported that VDD was significantly more common in the T1D group (10). Nevertheless, a lower VDD frequency is reported in Quatrain and Egyptian populations (24,44).

Vitamin D pathway genes, such as *VDR, CYP27A1, CYP27B1, CYP2R1, CYP24A1*, and vitamin D binding protein (*VDBP*) not only control vitamin D biosynthesis and its transport but also influence the cytokine levels in various autoimmune diseases, including T1D (54,55,56). Vitamin D plays a major role in the regulation of insulin secretion from pancreatic β-cells (57,58). VDR polymorphisms in the 3´UTR region are well known to affect its translation and mRNA stability. Predominantly, the BsmI, ApaI, TaqI, and FokI SNPs are the most commonly studied *VDR* gene polymorphisms in association with non-skeletal outcomes, including T1D (59). However, limited familial studies are reporting the transmission of VDR alleles, genotypes, and haplotypes from parents to T1D-affected children (27,30,60,61,62,63,64,65,66).

In the present study, the BsmI-B and ApaI-A alleles were over-transmitted, apparently conferring susceptibility and BsmI-b/ApaI-a/TaqI-T (baT) haplotype was under-transmitted, suggesting protection to T1D. Further, the study also revealed a strong LD between TaqI-ApaI, TaqI-BsmI, and BsmI-ApaI. This is in accord with previous reports on VDR TaqI-ApaI in German, British and Egyptian populations (60,61,67). The VDR FokI polymorphism affects immune cell behavior and possibly plays a role in immune-mediated diseases, including T1D (68). In the present study, FokI-FF and FokI-Ff genotypes were significantly associated with susceptibility and protection to T1D, respectively. The T1D susceptibility association with the FokI-FF genotype has been previously reported in various populations (19,20,21,22,23,24,25,26). However, this is not consistently found. Several studies have shown a lack of association of the VDR-Folk-FF genotype with T1D susceptibility (28,68,69,70,71,72,73,74). The alleles and haplotypes of VDR FokI, BsmI, ApaI, and TaqI and genotypes of BsmI, ApaI, and TaqI were not associated with T1D in the current study. Similarly, the BsmI/ApaI/TaqI alleles and genotypes have not shown an association with T1D in several other populations (21,22,75,76,77). Furthermore, VDR haplotypes did not show an association with T1D in Spanish, Portuguese, North Indian, and Turkish populations (22,28,69,71). However, several studies point out that VDR BsmI/ApaI/TaqI polymorphisms are associated with T1D (21,24,25,29,70,72,75,78). In addition, meta-analysis studies in Asian populations revealed an association between VDR-BsmI polymorphism and T1D (79,80,81).

The VDR-FokI (rs2228570) SNP leads to alteration in the amino acid sequence that may further affect protein function. This T → C substitution changes the first start codon of ATG by an alternative start site codon of ACG leading to a different-sized protein, such as short-form (424 aa; C allele or F allele, methionine at the 4th position) and long-form (427 aa; T allele or f allele, methionine at 1st position). The 424 aa (mutant) containing VDR is somewhat more active than the 427 aa (wild type) VDR (82,83). Moreover, this transition is predicted to enhance VDR protein stability (84). In this study, VDR-FokI (rs2228570) was slightly associated with T1D in terms of higher f → F allele transition in patients than controls. Although the VDR protein was more stable in T1D patients, they might be susceptible to disease because of the low Vitamin D levels in the patients.

Study Limitations

As this study was structured for a family-based approach, the small sample size is the foremost limitation. A comprehensive familial study with large sample size, DNA sequencing, and gene expression evaluations are necessary to clarify the role of the *VDR* gene variants on T1D in the future. Furthermore, factors possibly influencing serum vitamin D synthesis, such as intake of supplements, obesity, liver and kidney diseases, and cutaneous factors, were not investigated.

Conclusion

The present study found that VDD was more common in patients with T1D than their unaffected relatives in the South Indian population. Furthermore, the VDR polymorphisms FokI-FF genotype, BsmI-B, and ApaI-A alleles were positively associated with T1D. However, the

FokI-Ff genotype and BsmI-b/ApaI-a/TaqI-T haplotype were negatively associated with the disease. Although VDR protein stability is enhanced in subjects harboring the F allele, and was more common in the patients with T1D, this suggests some other Vitamin D associated mechanism or function may be associated with the development of T1D in this population, rather than the simple presence of the FokI-Ff genotype, which was also present in more than 50 % of unaffected related controls.

Ethics

Ethical Committee Approval: This study was approved by the Institutional Ethics Committees of Madurai Kamaraj University (MKU/IRB/11/11) and Government Rajaji Hospital (ref no: 23339/E4/3/10, date: 12.04.2011).

Informed Consent: The participant's written informed consent was obtained along with a detailed questionnaire.

Authorship Contributions

Surgical and Medical Practices: Ayyappan Chitra, Arthur Asirvatham, Concept: Ramasamy Thirunavukkarasu, Ayyappan Chitra, Arthur Asirvatham, Mariakuttikan Jayalakshmi, Design: Ramasamy Thirunavukkarasu, Ayyappan Chitra, Arthur Asirvatham, Mariakuttikan Jayalakshmi, Data Collection or Processing: Ramasamy Thirunavukkarasu, Mariakuttikan Jayalakshmi, Analysis or Interpretation: Ramasamy Thirunavukkarasu, Literature Search: Ramasamy Thirunavukkarasu, Mariakuttikan Jayalakshmi, Writing: Ramasamy Thirunavukkarasu, Mariakuttikan Jayalakshmi.

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