

Vitamin D Receptor Genetic Variants among CKD Patients of South Indian Population

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Abstract

Background and Objectives: Vitamin D Receptor (VDR) gene polymorphism has long been known for its association with Chronic Kidney Disease (CKD). We aimed to investigate the potential role of VDR gene polymorphisms in CKD patients.

Design and Methods: The association of VDR gene polymorphisms in CKD patients (N=147; males =100 (68.03%) and females=47 (31.97%)) is investigated in this study. The patient samples were compared with healthy control subjects (N=210; males: 130 (61.90%) and females: 80 (38.10%)). Genotyping was carried out by polymerase chain reaction–restriction fragment length polymorphism method (PCR_RFLP). All the statistical analysis was carried out using the SPSS and PLINK-software.

Results: A significant difference in the genotype frequency of *Apal*-“CC” (p=0.015, OR=0.51, 95% CI=0.29–0.91) was observed in the patients vs. control subjects. Further, we observed that individuals with a/T haplotype were at a risk (0.25-fold higher; 95% CI=0.09–0.67). The serum calcium levels were increase in the patients with *Apal* (AC+CC) variants, but were significantly decreased in the AA variant (9.6 ± 1.16 vs. 9.07 ± 0.85 mg/dL, p=0.0005). The serum Hb levels were also increase in the patients with *TaqI* (TT) variants, but were significantly decreased in the (TC+CC) variants (8.36 ± 2.41 vs. 7.86 ± 2.20 mg/dL, p=0.03). We also observed that the “AC” genotype of the *Apal* polymorphism when present in a combination with the “TC” genotype of *TaqI* polymorphism conferred a 1.4 times higher risk (38/54) for developing CKD using MDR analysis.

Interpretations and Conclusions: *Apal* gene polymorphism of the VDR gene is significantly associated with CKD patients and the *Apal* “CC” variant could be a risk allele for CKD patients in South Indian population.

Keywords: Chronic kidney disease; Vitamin D receptor; SNP; Haplotypes

Introduction

Chronic Kidney Disease (CKD) is a progressive condition characterized by a decrease in the Glomerular Filtration Rate (GFR) over time which is less than 60 mL/min/1.73 m² for duration of 3 months or longer and can even progress to End Stage Renal Disease (ESRD) [1]. The pathogenesis of CKD is due to a combination of multiple genetic and environmental factors leading to ESRD [2-4]. It results from a profound hydroelectrolytical, metabolic and immunobiological imbalance and is associated with a number of systemic complications [5]. Gene polymorphism has reported as an important factor to increase the susceptibility of CKD. In the past decades, many epidemiologic studies have been conducted to understand the relationship between VDR polymorphism and CKD risk [6-8]. In several studies, an inverse relationship has been observed between the bone mineral density and renal function [9-13].

The active vitamin D (1,25(OH)₂D₃) is a steroid hormone which is metabolized in the liver and in the kidney. The active vitamin D interacted with its target nuclear receptor and exerts a wide variety of biological processes such as bone metabolism, immune response modulation, and regulation of cell proliferation and differentiation [14]. Further, VDR is also responsible for both positive and negative control of a few genes at the transcription level via VDR interaction [15].

The human VDR is located on chromosome 12 at *12q13-14* [16]. The VDR gene contains 11 exons along with introns, spanning

approximately 75 kb [17]. Approximately 100 polymorphisms are expected in the VDR region based on the Genome wide association study [18]. Polymorphisms are referred to as the presence of two or more alleles at a given locus, and if such alleles occur at a frequency of more than 1% in a population, the locus is known to be polymorphic. These polymorphisms are associated with several pathological conditions such as breast cancer [19], bone mass, bone turnover, bone mineral loss [20], osteoarthritis [21], and osteoporosis [22].

CKD has a genetic basis which combines interaction effects basically sequence variation of multiple genes with environment [23]. Vitamin D has played a major role among CKD patients as the bone metabolism modulator and it should be well balanced to avoid damage. The main aim of the present study is to evaluate the association of VDR polymorphisms and CKD. In this case-control study, we examined the possible associations between VDR gene polymorphisms in the South

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Indian CKD population between the age group of 30 and 70 years. We determined the genotypes and haplotype association of *Apal* and *TaqI* alleles of VDR in the study population.

Subjects and Methods

Study population

The work was carried out with the approval of the Institutional Ethical Committee. The patients were on regular follow-up in Lee kidney care hospital. Individuals with matched sex, and location were selected as the controls. All the participants belonged to the Dravidian ancestry of Tamilnadu population in South India. The inclusion criteria for patients selection were constantly elevated serum creatinine level above normal range (0.6 mg/dL-1.2 mg/dL) and creatinine clearance <60 mL/min/1.73 m². The CKD type was confirmed by ultrasound and/or Computed Tomography (CT) scan of the kidneys. The newly enrolled patients were excluded from the study. Controls with the risk factors such as family history of hypertension, diabetes mellitus, and hyperlipidemia were excluded from the study. Both the cases and controls were from the state of Tamil Nadu.

Blood collection, DNA extraction and Genotyping

A total of 5 mL of blood samples were collected from CKD patients and healthy volunteers between the age group of 30 and 70 years from clinics after obtaining informed consent from the participants. All the cases included in this study are under CKD stages (II-V) as diagnosed and identified by a nephrologist. The clinical data and family history were recorded in the questionnaire for all the participants. All the patient samples were collected from the Lee Kidney Care hospital. Control samples were also collected from the volunteers living in the same place and origin. Genomic DNA was extracted using modified Miller et al., protocol [24] and it was quantified spectrochemically by OD₂₆₀/OD₂₈₀ ratio. Genotyping was performed with PCR-RFLP according to Vupputuri et al. [25].

Statistical Analysis

Power and sample size calculations were conducted using genetic power test to estimate the Power of the study. Chi-square statistics was computed to compare the VDR genotype frequencies between the case and the control subjects. The strength of the association between the genotype frequencies was calculated by the Odds Ratio (OR) and the 95% Confidence Interval (CI). *P* value<0.05 was considered as a level of statistical significance for this study. Departure of Hardy-Weinberg Equilibrium (HWE) was tested for the frequencies of the marker alleles by the gene counting method. All the statistical calculations were carried out using PLINK 1.07 [26] and SPSS [27].

Genetic power test

We estimated the genetic power using the *Apal* polymorphism as an example; an 80% power is required to detect the linkage between CKD and the “a” allele at a type “I” error of 0.05 when the sample includes 147 cases and 210 control subjects. Further, we performed post hoc exploratory analyses to examine the relationships of the polymorphisms with the case and control subjects.

Results

Among the patients (n=147), 100 (68.03%) are male and 47 (31.97%) are female, whereas in the control subjects 130 (61.90%) are male and 81 (38.10%) are female. The mean age of the patients is 53.9 ± 12.3 years for male patients and 54.40 ± 10.42 years for female patients.

The mean age of the control subjects (n=210) is 43.71 ± 14.17 years and 43.90 ± 13.57, respectively, for males and females. Genetic power estimation showed that 147 cases and 210 controls had >80% power to detect the linkage between the *Apal* variant and CKD in South Indian population.

Distribution of VDR genotypes

The genotype distributions of *Apal* and *TaqI* polymorphisms were compatible with the HWE expectation in control subjects. The genotype, allele frequencies, and odds ratio were calculated for the variants to test the association of *Apal* and *TaqI* polymorphisms with CKD patients. The “CC” and “AA” genotype of *Apal* in the patients group was 13.61% and 32.65%. Among controls it was 23.33% and 31.42%, respectively, and both the groups (cases and controls) differed significantly (*p*=0.015; OR=0.51, 95% CI=0.29–0.91). In the case of *TaqI*, the “TT” genotype was present in 32.65%, while “CC” was observed only in 16.65%; compared to the control subjects (TT=41.90%; CC=16.19%), it was not significantly associated with CKD (*p*=1.00) after applying bonferroni corrections (Table 1).

Combined analysis and haplotype distribution

The genotypes of VDR genes were combined and compared in both the groups in order to evaluate the synergistic effect. The heterozygous and mutant types were combined together and compared with the wild type. The combined genotypes showed a significant difference in the frequency distribution of patients and control subjects (Table 2). In the double gene combination, we could not find significant changes.

Genotype	Patients, n=147 (%)	Control, n=210 (%)	<i>P</i> -value ^a	OR (95% CI)	
<i>Apal</i> (db SNP ID rs 7975232) polymorphism					
AA	48 (32.65)	66 (31.42)	0.44	1.06 (0.67 – 1.66)	
AC	79 (53.74)	95 (45.24)	0.07	1.41 (0.92 – 2.15)	
CC	20 (13.61)	49 (23.33)	0.015*	0.51 (0.29 – 0.91)	
Allele frequency					
Allele A	175 (59.52)	227 (54.05)	0.085	1.25 (0.92 – 1.69)	
Allele C	119 (40.47)	193 (45.95)			
Allele carriage frequency					
A allele carriage	127 (86.39)	161(76.67)	0.015*	1.93 (1.09 – 3.42)	
C allele carriage	99 (67.35)	144 (68.57)	0.45	0.95 (0.60 – 1.48)	
<i>TaqI</i> (db SNP ID rs 731236) polymorphism					
TT	48 (32.65)	88 (41.90)	0.05	0.67 (0.43 – 1.04)	
TC	76 (51.70)	88 (41.90)	0.045	1.48 (0.97 – 2.27)	
CC	23 (16.65)	34 (16.19)	0.50	0.96 (0.54 – 1.71)	
Allele frequency					
Allele T	176 (59.86)	264 (62.86)	0.17	0.85 (0.63 – 1.16)	
Allele C	122 (41.49)	156 (37.14)			
Allele carriage frequency					
T allele carriage	124 (84.35)	176 (83.81)	0.50	1.04 (0.73 – 0.58)	
C allele carriage	99 (67.35)	122 (58.10)	0.045	1.49 (0.96 – 2.31)	
SNP, Model & Test					
<i>Apal</i> CC + AC Vs AA	DOM	99/48	144/66	0.445	0.95 (0.60– 1.48)
CC Vs AC + AA	REC	20/127	49/161	0.015	0.52 (0.29 – 0.91)
<i>TaqI</i> CC + TC Vs TT	DOM	99/48	122/88	0.05	1.49 (0.96 – 2.31)
CC Vs TC + TT	REC	23/124	34/176	0.50	1.04 (0.58 – 1.85)

Note: a-Chi-squared test. DOM: Dominant Model; REC: Recessive Model; n: Sample Size; OR: Odds Ratio; CI: Confidence Interval; **p* < 0.025 statistically significant; After bonferroni correction the significance threshold is (0.05/2=0.025).

Table 1: Distribution of VDR polymorphism among CKD patients and healthy control group.

Genotype ^a	Patients, n=147 (%)	Controls, n=210 (%)	Corrected p – value ^a	OR (95% CI)
<i>TaqI and ApaI</i>				
T1 and A1	10 (6.80)	21 (10)	0.19	0.66 (0.30 – 1.44)
T1 and A0	38 (25.85)	67 (31.90)	0.13	0.74 (0.47 – 1.19)
T0 and A1	38 (25.85)	45 (30.61)	0.20	1.28 (0.78 – 2.10)
T0 and A0	61(41.50)	77 (36.67)	0.21	1.23 (0.80 – 1.89)

Note: a-Chi-squared test; [†]Significance value (p<0.05); 0=mutant+heterozygous; 1=wild type genotype; ^aAfter bonferroni correction the significance threshold is (0.05/2=0.025).

Table 2: Combined analysis of VDR genotypes among CKD patients and controls.

Haplotype ^a	Patients, n=147 (%)	Controls, n=210 (%)	p – value ^a	OR (95% CI)
A/t	13 (8.84)	16 (7.62)	0.41	1.18 (0.55 – 2.53)
a/T	5 (3.40)	26 (12.38)	0.0025*	0.25 (0.09 – 0.67)
A/T	10 (6.80)	21 (10)	0.18	0.66 (0.30 – 1.44)
a/t	9 (6.12)	5 (2.38)	0.065	2.67 (0.88 – 8.15)

Notes: a-Chi-squared test; For *ApaI*, the haplotypes are “A” (wild type) and “a” (mutant type); for *TaqI* the haplotypes are “T” (wild type) and “t” (mutant types); ^aAfter bonferroni correction the significance threshold is (0.05/2=0.025).

Table 3: Haplotype distribution of *ApaI* and *TaqI* gene polymorphisms among CKD patients and controls.

Haplotype analysis revealed that “a/T” haplotype is comparatively lower in the patients (3.40%) compared with controls (12.38%) and the difference was statistically significant (p=0.0025; OR=0.25; 95% CI=0.09–0.67) (Table 3). The plasma levels (mean ± SD) of creatinine, Glucose, calcium, Hb in CKD patients were compared with VDR genotypes. The serum calcium levels were increase in the patients with *ApaI* (AC+CC) variants, but were significantly decreased in the AA variant (9.6 ± 1.16 vs. 9.07 ± 0.85 mg/dL, p=0.0005). In case of Hb levels we could observe the changes in *TaqI* polymorphism. The serum Hb levels were increase in the patients with *TaqI* (TT) variants, but were significantly decreased in the (TC+CC) variants (8.36 ± 2.41 vs. 7.86 ± 2.20 mg/dL, p=0.03) (Table 4).

Characteristic	Genotype (W)	Genotype (H+M)	p-value ^a
<i>ApaI</i>	AA (n=48)	AC + CC (n=79 + 20)	
Hb	8.44 ± 2.39	7.80 ± 2.20	0.091
S. Creatinine mg/dL)	5.2 ± 3.12	4.93 ± 3.13	0.47
S. Glucose (mg/dL)	115.97 ± 50.14	130.20 ±75.88	0.051
S. Uric (mcmol/L)	105.94 ± 59.18	116.12 ± 83.06	0.343
S. Na (mmol/L)	119.26 ± 23.46	123.89 ± 12.77	0.064
S. K (mmol/L)	4.86 ± 1.82	5.37 ± 9.39	0.3795
S. Cl (mmol/L)	92.03 ± 23.62	86.68 ± 12.78	0.0905
S. Ca (mg/dL)	9.07 ± 0.85	9.6 ± 1.16	0.0005*
<i>TaqI</i>	TT (n=48)	TC+CC (n=76+23)	
Hb	8.36 ± 2.41	7.86 ± 2.20	0.035
S. Creatinine (mg/dL)	5.02 ±3.48	5.02 ± 2.97	0.4865
S. Glucose (mg/dL)	121.74 ± 69.79	125.65 ± 68.44	0.2755
S. Uric (mcmol/L)	110.57 ± 81.47	113.35 ± 73.67	0.327
S. Na (mmol/L)	122.04 ± 16.11	122.74 ± 17.59	0.3735
S. K (mmol/L)	4.35 ± 1.50	4.86 ± 1.45	0.1935
S. Cl (mmol/L)	90.82 ± 26.51	86.11 ± 20.85	0.193
S. Ca (mg/dL)	9.2 ±1.14	9.2 ± 1.18	0.256

The values are represented as mean ± standard deviation. ^aAfter bonferroni correction the significance threshold is (0.05/2=0.025).

Table 4: Biochemical parameter of patients (n=147) with VDR gene polymorphisms.

Analysis of Epistatic Interaction

MDR (Multi Drug Resistance) analysis was applied to detect the gene-gene interactions in the cases and controls in order to elaborate the findings of the analysis. Table 5 summarizes the results of the MDR analysis evaluated for the two possible combinations of polymorphisms studied on the risk of developing CKD. It exhibits the best model with a combination of polymorphisms in order along with its prediction error and Coefficient of Variation (CV) consistency (Table 5). The results reveal that the interaction of the *ApaI-TaqI* polymorphisms is the best model with the least prediction error of 0.39 and a CV consistency 10/10. For the present data, we set the threshold value of 1.0 (210/210), which determines the high-risk (dark gray) and low-risk (light gray) genotypic combinations. It was also observed that the “AC” genotype of the *ApaI* polymorphism when present in a combination with the “TC” genotype of *TaqI* polymorphism conferred a 1.4 times higher risk (38/54) for developing CKD (Figure 1).

Discussion

CKD is associated with major osteoporosis complications [28]. It affects the older population (>50 years) [29], therefore the mean age for male CKD patients is 53.90 ± 12.33 and for females it is 54.40 ± 10.42 in our study population. VDR acts as the bridging factor between CKD and osteoporosis [29,30]. The VDR gene comprises three components viz. 5’ promoter (from exon 1a to f), coding exons (exon 2–9), and a 3’ untranslated region in chromosome 12q13.1 [31]. Morrison et al. (1994) were the first to report that the VDR gene polymorphisms (*BsmI*, *ApaI*, and *TaqI*) were related to the Bone Mineral Density (BMD) [32]. Thus,

Model	Training bal. acc. (%)	Testing bal. acc. (%)	Cross-validation consistency	Prediction error
<i>TaqI</i>	0.570	0.530	8/10	0.430
<i>ApaI</i> and <i>TaqI</i>	0.606	0.547	10/10	0.394

Table 5: Summary of MDR analysis. MDR analysis revealed that combination of *ApaI* and *TaqI* could be a high risk for the prevalence of CKD.

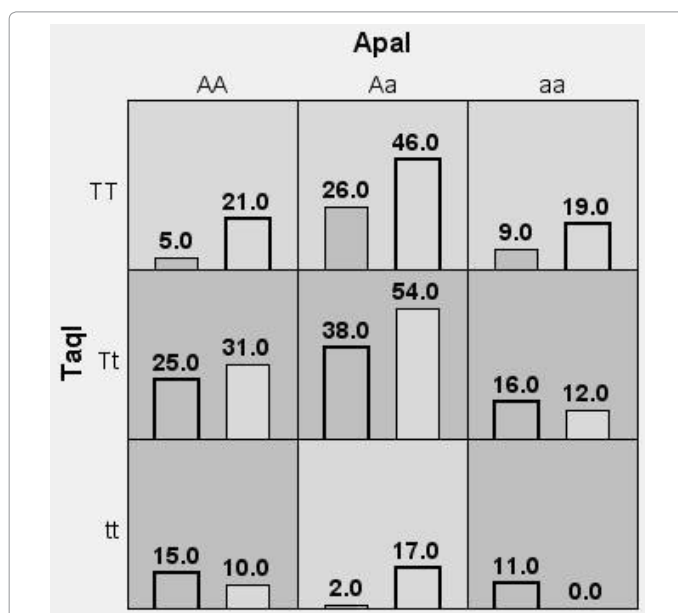


Figure 1: Distribution of high-risk (dark shaded) and low risk (light shaded) genotype among the markers studied.

VDR gene polymorphisms are considered as important markers for osteoporosis [33]. The main objective of the present study is to evaluate the association of VDR gene polymorphism in CKD. The VDR gene polymorphism has been investigated in different malignancies among the Indian population [34-36].

The frequency of the minor allele of *Apal* variant was 0.40 for this study among cases and this frequency were similar for different populations in 1000 Genome project, 0.36 in African, 0.55 in American, 0.46 in European and 0.41 in South Asian. Similarly, for the *TaqI* variant, the minor allele frequency was 0.41 and it is correlated with European (0.60) and South Asian (0.63) populations.

The VDR genotype differentiation may trigger a breakdown of the cytokine relationship directly or indirectly, and thus it may be associated with CKD pathogenesis, or it may cause the VDR structure alteration and ultimately lead to an altered receptor function that may increase or decrease the VDR expression and thereby cause disease [37]. Although the *Apal* polymorphisms lie in the intronic region, they may influence the VDR expression, which includes the disruption of a splice site for the VDR mRNA transcription. This may result in the truncated or alternatively spliced protein product [38] or it may alter the mRNA product [14]. However, we could not observe any significant changes in the *TaqI* polymorphism. The *TaqI* polymorphism may cause a silent mutation in exon 9 with both ATT and ATC coding for isoleucine [39]. Thus, it may not affect the mRNA transcripts. Such types of associations have been reported in ESRD [40] and type 2 diabetes patients [41] from north India.

Further, we performed haplotype analysis. A single gene contained multiple polymorphisms. Moreover, for multiple polymorphisms within the same gene, information contained at each polymorphic site is "linked" to its neighbors. Therefore, inheriting one polymorphism means a high likelihood of inheriting the neighboring polymorphism [42]. Rather than a single SNP analysis, a haplotype analysis provides more information and it has an advantage in disease association studies, since it gives the cumulative effect of SNPs in that gene [43]. The haplotype analysis revealed that individuals with "a/T" haplotype had a higher risk.

VDR polymorphisms have been reported to modify the parathyroid cell differentiation and function. Wide variations have been observed in the degree of secondary hyperparathyroidism. Some patients develop severe and uncontrolled hyperparathyroidism, while others develop moderate elevated levels of iPTH levels which may fail to promote sufficient bone turnover and finally result in a dynamic bone disease [44]. However, it is not well defined the reason behind the heterogeneity in the clinical behavior. The serum calcium levels were significantly higher ($p=0.0005$) in the "CC" genotype of *Apal*, showing correlation, which may act as a secondary check to the severity of secondary hyperparathyroidism.

Tripathi et al. studied and reported that *Apal*, *FokI*, and *BsmI* VDR polymorphism may be one of the genetic risk factors for End Stage Renal Disease (ESRD) in north Indian population. They also observed that the genotype of frequency of *Apal* (a) (C in our case), *FokI* (f), and *BsmI* (B) was significantly different among patients and controls. In our case, we could also observe *Apal* (C) genotype frequency was significantly differ among patients and controls in south Indian population. In both the studies, there is no significant difference in case of C at *TaqI* restriction site. Moreover, they observed that the serum calcium levels were significantly higher ($p=0.001$) in the BB genotype and in our study, we observed that serum calcium levels were

significantly higher ($p=0.0005$) in the AA genotype. Both the studies clearly indicated that serum calcium level play an important role among kidney disease populations in India.

Our study has a few limitations.

- The study was conducted with a low sample size
- We included only one kidney center in our study
- Controls were not age matched with the patients

More CKD patients from different kidney centers can be included for better interpretation of the role of VDR polymorphism or the progression of CKD. Moreover, there are no measurements are available to calculate the VDR levels in order to correlate with the VDR polymorphisms in this study. However, the samples are from a homogeneous genetic background, therefore, they may not be affected by the unmeasured confounding factors of population stratification.

In conclusion, we observed that VDR gene polymorphisms appear to be important genetic determinants associated with CKD patients. We observed that the "CC" of *Apal* were strongly associated with CKD patients among the Tamil Nadu population. We could not find a significant association with the *TaqI* polymorphism among CKD patients.

Declaration of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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