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## Study Of Vitamin D Receptor Gene Polymorphism And Its Association With Vitamin D Levels In Healthy Individuals.

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### ABSTRACT

The study is aimed to evaluate the normal distribution of Taq1 gene polymorphism and its influence on vitamin D status in healthy individuals of Chennai population. The study population comprised of 100 normal individuals of age 25-60yrs. Person with diabetic, hypertensives, CAD are excluded. Fasting venous blood collected from each and base line investigations, 25 hydroxy vitamin D levels, and polymorphic studies were done. The genotype distribution among the study population of which the common genotype is homozygous tt genotype (94%) and heterozygous Tt genotype accounts for (6%), there is no homozygous TT genotype. Among the study population group-1 individuals have increased frequency of tt genotype (22.3%) and group-2 have Tt genotype of (66.7%). And there is no correlation between the genotype and vitamin D levels in this study population

**Keywords:** 25- hydroxy vitamin D, Taq1 genotype, vdr gene ,

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## INTRODUCTION

To the medical researchers all over the globe Vitamin D had become topic of interest. As evidenced by recent research the world is facing the problem of vitamin D deficiency pandemic. Deficiency of vitamin D is a risk factor in humans, right from conception and throughout lifespan. It is one of the common medical condition undiagnosed and under treated nutritional deficiency in the world. Globally about one billion people are known to have vitamin D insufficiency or deficiency [1]. In general population worldwide the prevalence of vitamin D deficiency is about 70-100%. In India the increased prevalence of about 50-100% is due to low intake of dietary calcium [2].

The medical fraternities across the world are curious in realizing that vitamin D plays a major role in health and disease. It causes skeletal as well as extra skeletal manifestations. Vitamin D not only regulates calcium and phosphorus homeostasis but also protects the individuals from many diseases like malignancies, chronic infections, and cardiac problems and reduce the risk autoimmune diseases. The study is to impress upon the physicians about the gravity of the vitamin D deficiency problem throughout India and to make appropriate diagnosis and treatment with care and caution[3]. As the vitamin D status is improved worldwide it would have remarkable effects on public health and decrease the healthcare expenses for several chronic diseases.

Vitamin D exerts its action in the body by binding to vitamin D receptor (VDR) which is a member of nuclear hormone receptor super family. It modulates the transcription of target genes by complexing with vitamin D responsive elements (VDRES) in the promoter region of target genes [4]. Excellent opportunities are provided by genetic studies to relate molecular insights with epidemiological data and it gained much interest. Polymorphism referred as variations in the DNA sequence or gene which occur in atleast 1% of population and can have only modest and subtle effects [5].

Many polymorphisms are known to exist in VDR gene according to recent studies, but the influence of this polymorphism on VDR protein function is not known. At the 3'end of the VDR gene three restriction fragments length polymorphisms (RFLP) for BsmI, ApaI and TaqI have been studied so far.

VDR gene polymorphisms could potentially influence the binding of 1,25(OH)<sub>2</sub> D and the anti-proliferative effects of vitamin. Many studies have been reported on VDR polymorphism and its influence on different disease throughout the world on the contrary in our country the reports are minimal.

The Taq1 polymorphism is a T/C nucleotide substitution (ATT to ATC) leading to a silent change at codon 352 (isoleucine) in exon IX [6].

Therefore, this study is to find the frequency of VDR (Taq1) gene polymorphism by PCR based restriction analysis of normal individuals and to determine the association of this polymorphism with 25-hydroxy vitamin D levels.

## MATERIALS AND METHODS

This study was conducted in the Department of Biochemistry, Kilpauk Medical College Hospital, Chennai. All procedures concerning human subjects or patients were permitted by the Institutional Ethical Committee. Explicit written consent was obtained from the study population. The study population comprised of normal individuals coming for master health check up, Kilpauk Medical College Hospital, Chennai. Known smokers, alcoholics, diabetes, hypertensives, coronary artery disease were excluded from the study.

For the study, 2 ml of 12 hours Fasting Venous Blood was collected under sterile conditions from the ante cubital vein. Serum was separated after centrifugation at 3000 rpm for 10 minutes, aliquoted, and stored at -20°C and were not thawed until the batch was analyzed for 25-hydroxy vitamin-D. 25-OH vitamin D in serum is determined using ADVIA CENTAUR vitamin D assay on ADVIA CENTAUR XP systems. The reference range of VIT D:10-30 ng/ml.

For gene polymorphic studies fasting blood was collected in 2 ml EDTA coated and DNA extraction done using mini preparation kit from Helini biomolecules, Chennai. Extracted DNA was identified by 1% agarose gel electrophoresis and comparison with a known molecular weight 1kb DNA (Lambda DNA)

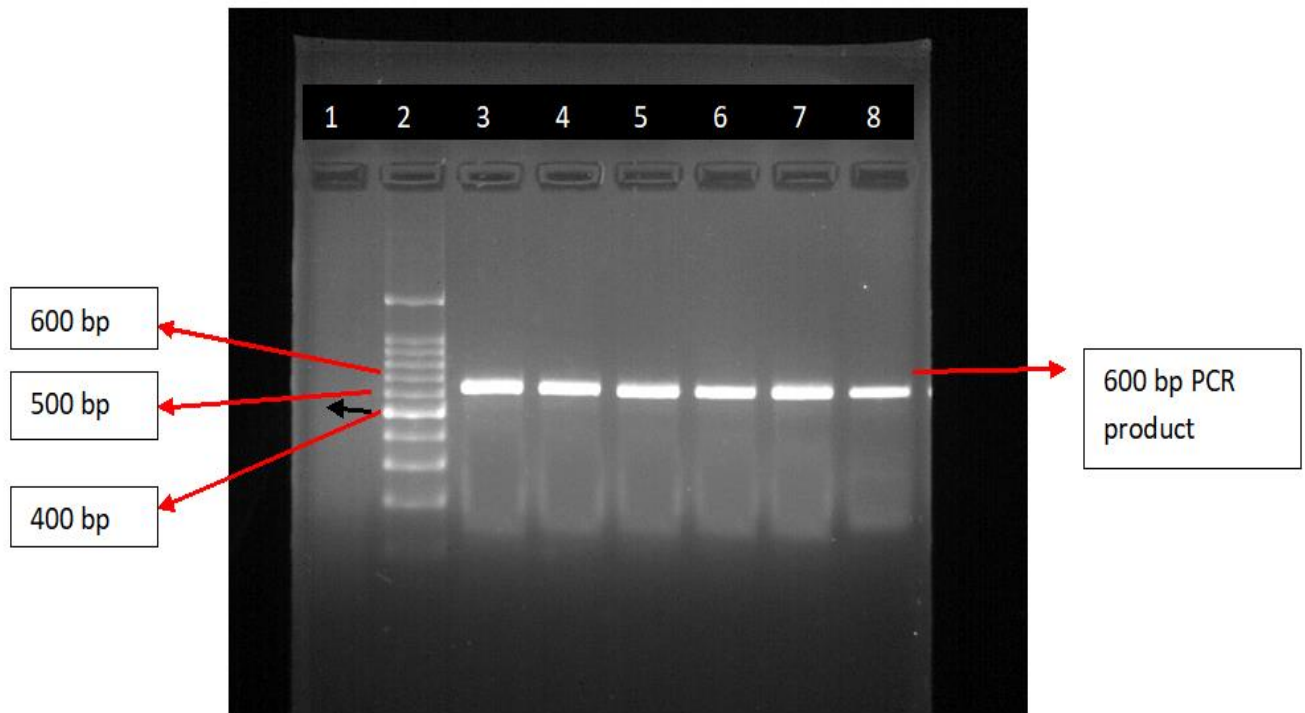
ladder .2xPCR Master mix was used in the following composition of Tris Hcl - pH 8.5 , (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>,MgCl<sub>2</sub> – 3 mM acts as catalyst and 0.2%Tween 20. dNTP's were used in a concentration of 0.4 mM each. Taq polymerase in a concentration of 0.2 U/ μl. Primers were used in a concentration of 10 pmol . PCR was carried out in a reaction in volume of 50μL with the following components in the following manner, 25 μL reconstituted PCR master mix (contains gel loading dye), 10 μL of reconstituted primers, 5.0 μl of DNA and 10 μL of distilled water.

Amplification of the extracted DNA was carried out in CYBERLAB SMART PCR-PRO thermal cyclor with the following cycling conditions of Initial denaturation for 95°C /5min, 34cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec , extension at 72°C for 45 sec and final extension at 72° C for 5 min.Amplicons of 600 bp was identified by 2.5% agarose gel electrophoresis by comparison with a known 100bp DNA ladder.

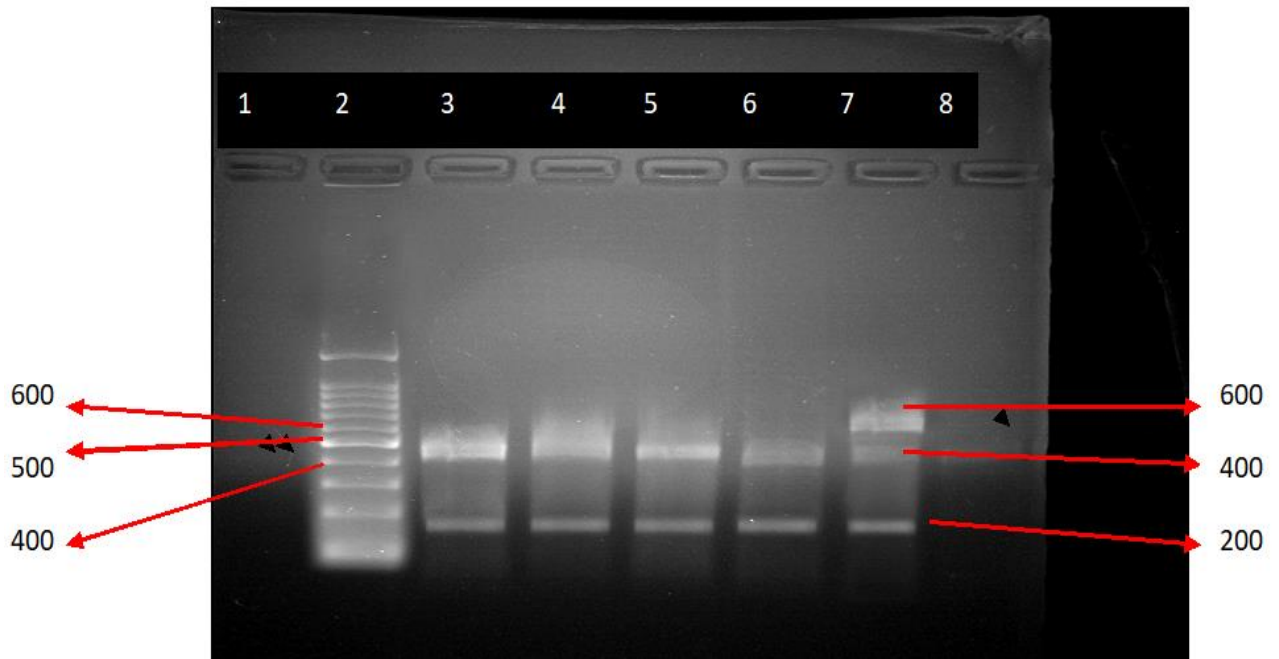
Taq1 gene polymorphism at the site was spotted by digestion of amplified PCR product with taq1 restriction enzyme using 5 μL of Taq1 buffer (1x), 2.5μl Taq1 enzyme,30.0 μL of PCR Product and 12.5μl of distilled water. The entire procedure was carried out in ice. The contents were mixed thoroughly. The eppendorf was then placed in a 65°C waterbath for 30minute.Restriction digested product was subjected to 2.5% agarose gel electrophoresis for genotyping. Two restriction site for Taq1 with in 600bp .

- t ALLELE- have restriction site,
- T ALLELE- absence of restriction site
- tt (homozygous) - yield 400bp,200 bp ,
- Tt(heterozygous) – yield 660bp ,400bp,200bp
- TT(homozygous) – yield 600bp

**Figure 1: PCR Product- rs731236**



**Figure 2: Restriction Digestion Product- rs731236**

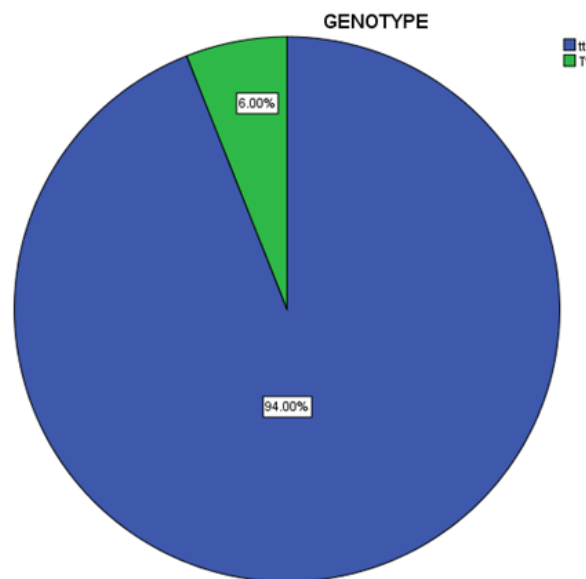


**Statistical Analysis**

**Table 1: Genotype frequency distribution of taq1 gene in study population**

Genotype	Frequency	Percent
tt	94	94.0
Tt	6	6.0
Total	100	100.0

This table shows the genotype distribution among the study population of which the common genotype is homozygous tt genotype which accounts for (94%) and the heterozygous Tt genotype accounts for (6%). There is no homozygous TT genotype in our study population.



**Table 2: Correlation between the genotype and vitamin D level in study population**

Genotype	Group1 N (%)	Group2 N (%)	Group3 N (%)	Total N (%)	p value
tt	21 (22.3%)	39 (41.5%)	34 (36.2%)	94 (100%)	0.49
Tt	0 (0%)	4 (66.7%)	2 (33.3%)	6 (100%)	

This shows among the study population, the group-1 individuals have increased frequency of tt genotype of (22.3%) whereas the group-2 individuals have increased frequency of Tt genotype of (66.7%)

### DISCUSSION

The present study is an attempt to evaluate the normal distribution of Taq1 gene polymorphism in unrelated normal healthy individuals. Of several allelic variants in the VDR gene, one which is present in 3'UTR is Taq1 gene polymorphism. It is a T/C nucleotide substitution (ATT to ATC) leading to a silent change at codon 352 both coding for isoleucine and is in linkage disequilibrium with other variants al[7].

The distribution of tt genotype is significantly higher among the study population. In this study population, we found 94% of tt genotype, 6% of Tt genotype and the homozygous TT genotype was not found. The presence ofTaq1 ' T ' allele in Asians is (8%) compared to Caucasians (43%) and Africans (31%) as stated in Kim Kostner et al [8] .

In this study the frequency of T allele is 6%. The finding of this study is similar to a study done in Austria (Ewald et al) where the frequency of tt genotype was found higher than TT genotype[9]. The genotype distribution in this study is not similar compared to the studies done so far in Japan (Tokita et al,1996),in china(kung et al 1996) American black Pennsylvania(zumudaetal,1997), NorthIndia (Hemant K Bid et al 2005). The genotype distribution was not found to be in agreement with Hardy Weinberg equilibrium in our study. Zumuda et al in his study describes that distribution of VDR genotypes was not in Hardy Weinberg equilibrium, the reason behind this may be, apart from genotyping errors, they may be due to chance fluctuations due to small samples, nonrandom mating, and migration into or out of the population, selective survivorship among genotypes, population stratification, and admixture of different ethnic group[10].

Hemant k bid et al in their studies says that there was a lack of data regarding the allelic variations in taq1 genotype from Indian subcontinent where population ethnicities are quite common.

The other hypothesis which focuses influence of polymorphism on the vitamin D status showed that Taq1 polymorphism does not influence vitamin D status. Further attempt should be mandatory in order to understand the molecular and cellular variations affected by the polymorphism and to execute observational studies in larger populations. Study of different haplotypes, instead of SNPs could reduce the inconsistencies found so far, until then the role of VDR polymorphism will still be a topic for debate.

### CONCLUSION

From this study we conclude that the predominant genotype in Chennai population is tt genotype. Also the homozygous genotype (TT) was not obtained in our study population. About 21% of individuals are vitamin D deficient. When genotypes are analysed with respect to 25(OH)Vitamin D no correlation was observed with Taq1 SNP

#### Compliance with ethical standards

**Conflict of interest:** Authors declare that they have no conflict of interest.

#### Ethical approval

All procedures performed in this studies involving human participants were in accordance with the ethical standards of Institution.



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