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Vitamin D Receptor fok I Gene Polymorphism in Angiographically Proven Coronary Artery Disease Subjects: Case - Control Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author BS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors BPK and JNN managed the literature searches, analyses of the study performed by PCR, RFLP method. Authors VS, BS, DB and DRNK analyzed the samples by PCR- RFLP. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Coronary artery disease (CAD) is multifactorial disease resulting from modifiable and non modifiable risk factors. Gene polymorphism is one of the non modifiable risk factors, which may contribute to disease susceptibility. Identifying genetic polymorphisms is essential for better understanding of pathophysiology and treatment strategies for a particular disease. The objective of our study was to evaluate the association of vitamin D receptor (VDR) fok I polymorphism with CAD.

Place and Duration of the Study: The study samples were collected at Narayana Medical College Hospital, Nellore and genetic analysis done at Sri Ramachandra University, Chennai, India, from Nov 2013 to June 2014.

Materials and Methodology: The study included 40 angiographically proven CAD subjects as

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cases and 40 normal healthy controls .VDR fok I polymorphism was analysed by PCR-RFLP method. Chi Square and odds ratio was used to find the association.
Results: F allele frequency is 66.25% in CAD vs 52.5% in controls. There is no significant association of FF (p= 0.099), Ff (p= 0.851), ff (p= 0.138) with CAD.
Conclusion: There is no significant association of VDR fok I polymorphism with CAD in south Indian population. According to our study F allele frequency is more in CAD than in controls.

Keywords: Coronary artery disease; vitamin D receptor; fok I; South Indian.

1. INTRODUCTION

Cardiovascular disease is the number one cause of mortality globally. According to World Health Organization (WHO) ischemic heart disease is the leading cause, accounting for 1.4 million deaths in the developed and 5.7 million deaths in developing regions [1]. Coronary artery disease is a multifactorial disorder due to the interaction of genetic and environmental risk factors [2]. In some individuals genetic factors may contribute to coronary atherosclerosis and thrombotic complications [3]. Vitamin D deficiency is not uncommon in India [4]. Due to ethnic differences, it is important to examine polymorphisms related to CAD. VDR gene is a candidate gene for susceptibility to several diseases [5]. Identifying gene variants provide better understanding of the pathophysiology, also elucidate the biochemical and physiological pathways that link various risk factors to the disease condition [6]. VDR is located on Chr $12q^{12,}$ contains 11 exons and spans 75 Kb of genomic DNA. Gene for fok I am located in exon 2 at the 5^{1}coding region [7]. Fok I polymorphism results in long and short variants. VDR ff comprises 427 amino acids; FF is a truncated protein with 424 aminoacids. Fok I is the only polymorphism that results in functional and structural variation in the VDR protein. Studies have demonstrated that VDR are present in the aortic endothelial, vascular smooth muscle cells etc. VDR polymorphisms may influence the susceptibility to CAD.

There are very few studies in India focusing VDR polymorphism in CAD [8-10]. Fok I being functionally significant [11,12], altered VDR may be the reason for the vitamin D insufficiency and related disorders [13]. The present study is a pilot study, focusing on fok I polymorphism association with CAD in angiographically proven cases.

2. MATERIALS

The study included 40 randomly selected normal healthy individuals (18-65 yrs), without any known disease as controls. CAD subjects admitted in the Cardiology department of Narayana Medical College Hospial were selected as the cases.40 angiographically proven CAD subjects (18-65 yrs) with > 50% stenosis in one of the coronary arteries or its major branches were randomly selected for the study. Both males and females were included. Subjects with pregnancy, PCOD (Poly Cystic Ovarian Disease), any known endocrine disorders and those on vitamin D supplementation, malignancies were excluded. The study was approved by the institutional ethics committee. After taking an informed written consent, whole blood was collected in EDTA tubes for genotyping. Plasma and serum samples were stored at -20°c until analysis. The EDTA samples were analyzed by PCR -RFLP method in the department of Human Genetics, Sri Ramachandra University, Chennai.

3. METHODS

3.1 Genotyping

Genomic DNA was extracted from peripheral blood using the Bioserve kit as per the manufacturer's protocol. Amplification of the gene of VDR was performed using primers described previously by Harris et al. [14], forward primer AGCTGGCCCTGGCACT GACTCTGCTCT and reverse primer ATGGAAACACCTTGCTTCTTCTCCCTC.

Reaction was setup with standard PCR reagents in a 20 µl reaction volume containing 50ng of genomic DNA, 30-50 pM of each oligonucleotide primer, 2 µl of 1X PCR buffer, 200 µM dNTP and 3units/ µl of Taq DNA polymerase. The PCR reactions were carried out as follows: 95°C for 2min, 56°C for 45 sec, 72°C for 35 sec; 30cycles and final extension 72°C for 5 min. PCR amplification was confirmed by 2% agarose gel electrophoresis. 100bp molecular weight marker was used to confirm the amplicon size and the gel was visualized in the gel documentation system. Fok I genotypes were analyzed by using

a PCR –RFLP. The PCR product was digested with 1.0 unit of Fok-I restriction enzyme and the reaction buffer and incubated at 37°C for 4 hours; 10ml of the digested reaction mixture was then loaded into 4% agarose gel containing ethidium bromide. The FF genotype lacked a Fok-I site and showed only one band of 265 bp. The ff genotype generated two fragments of 196 and 69 bp. The heterozygote displayed three fragments of 265, 196 and 69 bp, designated as Ff. Genotype quality and validation was done by performing repeated assay on 20% of the samples selected at random. Samples with known genotypes were included in the reaction to confirm the genotypes.

3.2 Statistical Analysis

The expected genotype and allele frequencies for the observed variations were calculated for the cases and controls. These frequencies were used to test if the population followed Hardy-Weinberg equilibrium. The interaction between the genotypes was evaluated by calculating the odds ratio and confidence intervals (CI). A difference was considered to be statistically significant when P values were <0.05. All the analysis was done using SPSS 12.0 software (SPSS Inc., Chicago. Il USA).

4. RESULTS

Table 1 shows the distribution of VDR Fok I genotype. Among 40 cases FF homozygous genotype was observed in 19, Ff genotyping in 15 and ff in 6 subjects. In controls, among the 40 subjects 11 were FF, 20 were Ff and 9 were ff genotypes. 95% CI and odds ratio were calculated, which does not show significant association with CAD.

Table 2 shows the allele frequency in cases and controls. The odds ratio is not significant for the development of CAD with all the genotypes. Fig. 1 shows the Ff, FF, ff genotype bands on gel.

5. DISCUSSION

Vitamin D is known primarily as a hormone of bone metabolism. VDR has a role in various other biophysiological functions besides mineral homeostasis [15], can affect the transcription of a number of genes which play a pivotal role in the pathogenesis of acute coronary syndrome [16]. VDR is identified in different tissues such as vascular smooth muscle cells, cardiomyocytes, system of fibrinolysis and thrombosis. According to a study by J. Ruth Wu – Wong et al, the anti atherogenic effects of Vitamin D is due to suppressing the expression of thrombospondin1 and inducing the expression of thromdomodulin in Human Coronary Artery Vascular Smooth Muscle Cells [17]. Research on VDR knockout mice found that the function of VDR associates with cardiac hypertrophy [18], cardiac fibrotic lesions [19], TIF 1 α, MMP2 & MMP9 [20], and renin angiotensin system [21]. These findings are important in understanding vitamin D signal transduction in heart cells and an evidence that the VDR plays a role in cardiovascular structure and function. Several polymorphisms have been identified in the VDR gene, such as Apa I, Bsm I, Fok Iand Taq I etc, Singlenuleotide polymorphisms (SNP) are scattered throughout the genome. High degree of variability make their functional significance and potential effects on disease susceptibility [22,23]. Kim et al. [24] showed significant association of VDR polymorphism in cancer breast, prostate, Skin, bladder and renal cell carcinoma. A study by Zhao et al. [25] proved the association of Bsm I and Fok I with Metabolic syndrome in Chinese population. These studies have also pointed out substantial allelic variations of VDR gene in different populations.

Table 1. Distribution of VDR Fok- I genotype

Genotypes	Controls (N=40)	Cases (N=40)	Odds ratio & 95% CI		*P value*
FF	11	19	1	-	-
Ff	20	15	0.43	0.16 – 1.18	NS (0.099)
ff	9	6	0.39	0.11 – 1.38	NS (0.137)

NS – Non significant

Table 2. Fok I Allele frequency in cases and controls

		Genotypes			Allele frequency		*P value*
		FF(TT)	Ff(TC)	ff(CC)	F(T)	f(C)	
Controls	Observed	11	20	9	0.52	0.48	NS(0.089)
(N=40)	Expected	11	20	9			
Cases	Observed	19	15	6	0.66	0.34	NS(0.097)
(N=40)	Expected	17.6	17.9	4.6			

NS – Non-significant

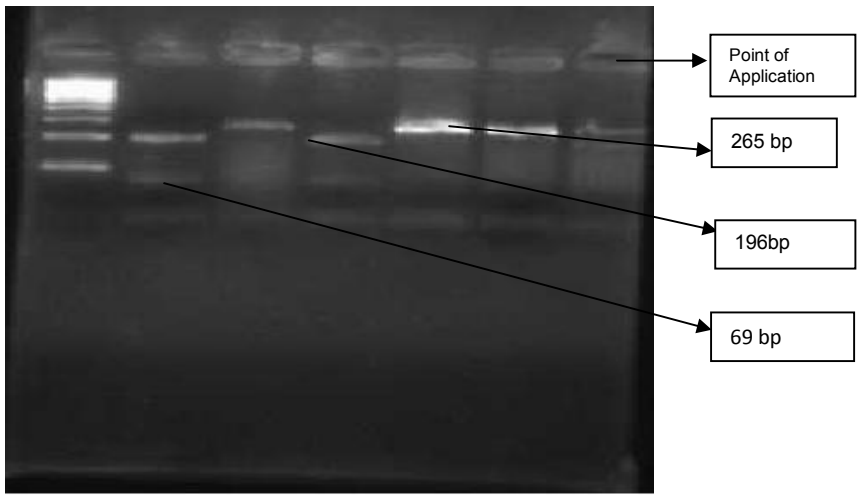

Fig. 1. Is the gel showing the genotype bands

FF genotype – single band with 265 bp , ff genotype - two bands with 196 bp and 96bp, Ff genotype – three bands with 265 bp, 196 bp, & 96 bp

Van Schooten et al. [26] reported an association between VDR BsmI polymorphism and CAD of European whites in Netherlands. White Ortlepp et al, reported no association of VDR polymorphism on CAD of Aachen in Germany [27]. A study from Chinese population by Pan et al, reported no significant association of VDR fok I polymorphism in CAD [28].

According to IARS (Indian Atherosclerosis Research study) by Shanker et al. [29] VDR genotypes did not show any association with either Vitamin D levels or CAD. A North Indian study by HK Bid et al, in type 2 diabetes subjects found no significant difference in fok I between cases and controls [30]. According to a study by HK Bid et al, allelic frequency of F vs f in healthy north Indian individuals were 58% vs 42% respectively [31]. Another study by Swapna et al. [6] proved FF genotype have elevated production of renin and angiotensin II leading to the development of hypertension. But there is no single study in south Indians. Our study being first of its order in South Indian & CAD subjects found that F allele frequency is 66.25% in CAD vs 52.5% in controls. Hence F allele frequency is more in CAD than in controls, but there is no significant association of genotype with CAD. The results associating VDR polymorphism with different diseases is conflicting. The influence of polymorphism may not be related to changes in the protein structure, but to difference in stability / translation efficiency of the RNA or even changes in a totally different gene [8]. Therefore further large population based studies are required on VDR and genes in close proximity to VDR location like insulin receptor gene. This may enlighten the significance of vitamin D & VDR on disease susceptibility and hence the treatment strategies.

There are certain limitations of the present case – control study 1) Small sample size for genetic polymorphism. 2) Other non functional polymorphisms like Apa I, BsmI, Taq I etc were not included 3) Being small sample and pilot study phenotype and genotype association not studied. 4) We have not included Vitamin D levels and focused only on ethnic and allele variations of fok I polymorphism in CAD. But our study gives an insight for further progress on genetic polymorphisms in CAD. Prospective and

population based studies can be planned for better understanding on Vitamin D and VDR.

6. CONCLUSION

According to our study we conclude that there is no significant association of fok I polymorphism with CAD. But we proved F allele frequency is more in CAD than in Controls. There are limited studies in India focusing VDR polymorphism with CAD. Globally large population based and multicentric studies are required to explain the pathophysiological mechanisms linking VDR and Vitamin D deficiency in CAD. Studies are needed on VDR protein and some other gene in close proximity to VDR like insulin receptor gene which may influence disease susceptibility.

COMPETING INTERESTS

The authors declared no conflict of interest.

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