



Research Article

rs 10735810 of Vitamin D Receptor (VDR) Gene : Association with Pulmonary Tuberculosis in Children

Bindi Das Mohandas Sulaja,* Ugam Kumari Chauhan

Centre for Biotechnology Studies, A.P.S University, Rewa, MP, India.
Corresponding author: bindidas_ms@yahoo.com

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ABSTRACT

Tuberculosis (TB) in children is an important cause of morbidity and mortality worldwide. There is much risk of severe disease and death among young than adults, therefore, the pediatric tuberculosis remains a public health emergency. Vitamin D receptor (VDR) mediates the immunological function of vitamin D₃ which activates macrophages and its deficiency concerned to the risk of TB. So polymorphisms in VDR gene may influence the function of vitamin D and susceptibility to TB. This study is to investigate the existence of possible association between VDR *Fok I* polymorphism (rs 10735810) and pediatric pulmonary tuberculosis in Madhya Pradesh of central India, using polymerase chain reaction-restriction fragment length polymorphism (PCR - RFLP). The VDR *Fok I* polymorphism showed statistically significant association with pediatric pulmonary TB in central Indian population. The FF genotype and F allele showed lower risk to TB in children while the heterozygous Ff genotype could be associated with pathophysiology of pediatric TB (OR = 2.413). The ff genotype was statistically non-significant as the frequency of this genotype was very less in both cases (5.97%) and control (2.5%).

Key Words:

Fok-I, Genotype, Polymorphism, Polymerase Chain Reaction (PCR), Tuberculosis, Vitamine D Receptor(VDR)

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INTRODUCTION

Children are more likely to develop disease from a recent infection thus pediatric tuberculosis often reflects recent TB transmission within a community and consequently provides useful markers for monitoring and directing TB control programs (Reinhard et al., 1997). Many single-nucleotide polymorphisms (SNPs) have no effect on cell function, but some SNPs, especially if they affect the function of the gene, could predispose people to disease or influence their response to a drug (Sachidanandam et al., 2001). Significant associations between tuberculosis and gene polymorphisms have been studied in different populations, of which VDR polymorphism thought to be important due to its role in immune system and different studies reported that VDR *Fok I* polymorphism is associated with TB in North Central Indians (Sharma et al., 2011), Chinese (Wei et al., 2009) and South Indians (Selvaraj et al., 2003). So this work was conducted to study the association of *Fok I* VDR gene polymorphisms with genotype frequencies, allele frequencies and carriage rate distribution in patients with pulmonary tuberculosis among central Indian population to determine the association with pediatric tuberculosis.

VDR, a nuclear hormone receptor, is a member of the steroid receptor family present on monocytes and activated T and B lymphocytes (Provvedini et al., 1983) involved in a wide range of biological functions, including mediation of vitamin D₃ interactions with the immune system (Rostand

et al., 2008). Vitamin D is a critical immunoregulatory factor in inhibiting engulfed Mycobacterium from surviving in macrophage as its metabolism is closely related to macrophage activation (Manolagas et al., 1994). Vitamin D₃-VDR complex regulates the production of cathelicidin and defensin, two antimicrobial peptides, by the interaction with vitamin D response elements (VDRE) present on the promoter of cathelicidin and defensin genes (Nizet et al., 2001). Cathelicidin and defensin disrupt the integrity of the bacterial cell membrane resulting the death of the microbe (Ganz, 2004). So polymorphisms in the VDR gene may be a contributing factor to increase tuberculosis susceptibility. *Fok I* creates an alternative initiation codon (ATG), three codon from length by three amino acids, and the transcription of this allele is 1 - 7 times less efficient than the F allele, and can alter the amount of VDR produced (Uitterlinden et al., 2004).

MATERIALS AND METHODS

Study population

The study population consisted of 134 pulmonary TB patients (age > 14. Male - 60, Female - 73) and 160 controls from central India. Control subjects matched with patients by age (> 14), sex (Male - 74, Female - 87) and ethnicity (Central India).

Isolation of genomic DNA from blood samples

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller

and coworkers (Miller et al., 1988).

VDR Genotyping by PCR- RFLP

The *Fok I* region was amplified using forward primer 5'-AGCTGGCCCTGGCACTGACTCTGCTCT -3' and reverse primer 5'-AGGAAACACCTTGCTTCTTCTCCCTC -3' under the thermal conditions as follows: initial denaturation at 94°C for 3min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. The final extension was carried out for 3 min. Lambda DNA *EcoRI* double digest (Bangalore Genei, Bangalore, India) was used as molecular weight marker. Ladder with amplified product has been run on 1% agarose gel.

The PCR products were digested with *Fok I* (New England Biolabs, USA). The resultant products were analyzed on 2% agarose gel stained with ethidium bromide. A 1000 bp gene DNA ladder (Roche, Germany) was run concurrently as molecular weight marker. The products were visualized using an ultraviolet trans-illuminator. *Fok I* was amplified following the protocol described by Babb et al (2007).

Based on the *Fok I* cleavage pattern VDR genotype was determined as FF (presence of an uncleaved 265 bp product which represents the gene is homozygous for absence of restriction site), ff (shows two fragments of 196 bp and 69 bp which represents the gene is homozygous for presence of restriction site) and Ff (shows three fragments of 196 bp, 69 bp and 265 bp which represents the gene is

heterozygous for restriction site) (Figure 1).

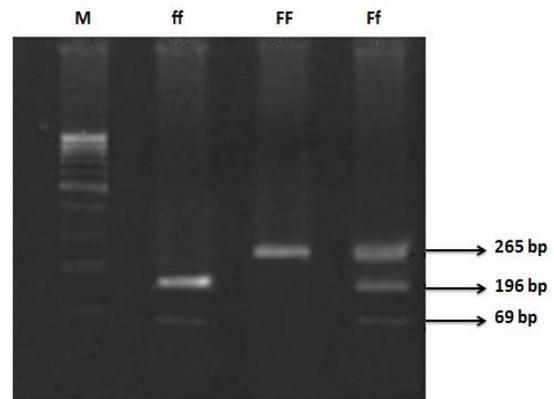


Figure 1: Representative gel picture of VDR *Fok I* gene polymorphism
 M = Molecular marker, ff = Homozygous mutant genotype
 FF = Wild genotype, Ff = Heterozygous genotype

Statistical analysis

Prism 5.0, San Diego, USA (www.graphpad.com) was used for statistical analysis.

VDR case	(n=134) control	(n= 160)	p value	odds ratio (95% CI)
Genotype				
FF	81 (60.45)	115 (71.87)	0.0173	0.5449 (0.3314-0.8958)
Ff	45 (33.58)	37 (23.12)	0.0682	1.626 (0.9721-2.720)
ff	8 (5.97)	4 (2.5)	0.2360	2.413 (0.7098-8.201)
Allele				
F	207 (77.24)	267 (83.44)	0.0129	0.5719 (0.3736-0.8756)
f	61 (22.76)	45 (14.06)		1.748 (1.142-2.677)
Carriage Rate				
F	126 (94.03)	152 (95.00)	0.0734	0.6413 (0.4003-1.027)
f	53 (39.55)	41 (25.62)		1.559 (0.9735-2.498)

* denotes the significant association between case and control, N - Number of individuals in study group, %- Genotype allele frequency and carriage rate expressed in percentage.

Table I: Fisher exact values of VDR gene polymorphism

RESULTS

The distribution of VDR-*Fok I* polymorphism (rs 10735810) was consistent with Hardy Weinberg equilibrium (HWE) in TB patients as well as in control subjects.

VDR- *Fok I* genotype distribution was significantly different in healthy control group with respect to TB disease group (table I). The common genotype (FF) was high in healthy control subjects than patients (71.87% vs. 60.45%). The odds ratio of FF genotype is 0.5449 shows its protective effect. The mutant genotype ff was less in TB patients (5.97%) as well as in control group (2.5%). The heterozygous Ff genotype was higher in patients (33.58%) with respect to control group (23.12%) and had an odds ratio of 1.626 and P value of 0.0682, the odds ratio advocates that it could be associated with pathophysiology of pediatric TB in the study population.

The frequency of allele F was lower in TB patients (77.24%) with respect to the case group (83.44%) whereas the f allele is higher in TB patients (22.76%) than control group (14.06%) ($\chi^2 = 6.711$; P = 0.0096). The odds ratio of F

allele was 0.5719 which indicates its protective role. The carriage rate of F allele was not different in patients and healthy subjects while the f allele was higher in TB patients with respect to control ($\chi^2 = 3.442$; P = 0.0636) but the values are non-significant even though the f allele shows an odds ratio of 0.6413. The frequency of FF genotype and F allele were higher in healthy controls which designates it has low risk (P = 0.0173; OR = 0.5449, 95% CI 0.314 - 0.8958 and P = 0.0129; OR = 0.5719, 95% CI 0.3736 - 0.8756 respectively).

DISCUSSION

Vitamin D status appears to be involved in the activation of macrophages and restricts the intracellular growth of mycobacteria (Vidyarani et al., 2007). This effect of vitamin D may be influenced by polymorphisms in the VDR gene (Haussler et al., 1998). Many studies suggest that single nucleotide polymorphisms (SNP) in the VDR gene are associated with TB in different populations (Roth et al., 2004). Therefore, we studied the association between

VDR *Fok I* polymorphism and pediatric pulmonary tuberculosis in Central Indian population. In our study the subjects with FF genotype and F allele show less risk to TB while the odds ratio of Ff (OR = 1.626) genotype suggests that it could be associated with the pathophysiology of pediatric TB and ff genotype was not associated with TB as it was not frequently observed among both TB patients (5.97 %) and control subjects (2.5%).

Our study results are supported by the report of (Sharma et al., 2011), in general population from North-Central India, the subjects with FF genotype of *Fok I* polymorphism appeared less likely ($p = 0.004$) to develop MDR TB whereas, those with Ff and ff genotypes were at high risk of MDR and smear positive disease, respectively. In both studies FF genotype didn't show susceptibility to TB. Ff genotype did show susceptibility to TB in the study of (Sharma et al.) but in our study it could be associated with pathophysiology of pediatric TB. While in the above mentioned study ff genotype showed susceptibility but in our study the ff genotype was not observed frequently either in patients or in control subjects

In the present study the F allele have shown protective role (OR = 0.5719) this was supported by the reports of (Alicia et al., 2007), reported their results had indicated that among the Ache (indigenous population from stern Paraguay) *Fok I* F allele of VDR gene protects individuals from mycobacterial infection, and (Wibur et al., 2007), reported the *Fok I* F allele associated with protection against infection in a large-scale genetic analysis of native South Americans. The VDR gene with FF genotype demonstrated an increased transcription rate and this has provided the main explanation for association between *Fok I* genotype and the development of diseases. Moreover, the production of vitamin D level may change depending on the environment as well as exposure to sunlight (ultraviolet rays).

Studies in South Indian pulmonary TB patients on *Fok I* polymorphisms of VDR gene showed an increased frequency of the genotype FF (homozygote) in male patients suggested the association with the susceptibility to TB (Selvaraj et al., 2003). The different results shown by Central Indian and South Indian populations may be due to the diversity of these two Indian populations and their difference in origins (Papiha, 1996). Wei et al. (2009) reported no association was found between VDR polymorphisms (either allele or genotype) and susceptibility to paediatric TB in the Chinese Han population. These reports suggest that the different effects of VDR genotypes in different ethnic populations may be due to gene-environment and gene-gene interactions. However, the internal relation between the environment and the host gene is unknown but it will help to explain the heterogeneity observed in studies and it should be addressed as an influencing factor for future studies. The polymorphic variants of VDR gene along with other gene and environmental factors may be responsible for an altered cell-mediated immunity against *M. tuberculosis* in a susceptible or resistant host.

Although several investigators reported and suggested that the VDR polymorphism may be of immune-regulatory significance for TB but it is not clear that the polymorphism determine susceptibility to the

development of clinical disease or susceptibility to infection. Further studies will be required to explore how VDR polymorphism may influence susceptibility to infectious disease or development of clinical disease.

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