

The FokI Polymorphism of the VDR Gene is a Protective Factor for Psoriasis Vulgaris

Ricardo E Vega-Hernandez¹, Marina MJ Romero-Prado¹, Lucila Sandoval-Ramirez², Maria G. Moreno-Trevino³, Oscar RFajardo-Ramirez³ and Julio C Salas-Alanis^{3*}

¹Department of Physiology, University Center of Health Sciences (CUCS), Guadalajara, Mexico

²Mexican Social Security Institute, Genetics Division, Biomedical Research Centre of the West (Cibo), Guadalajara, Mexico

³Department of Basic Sciences and Dermatology, Monterrey University, School of Medicine, Nuevo Leon, Mexico

Abstract

Introduction: Vitamin D receptor (VDR) plays a key role in the metabolism and differentiation of keratinocytes; thus, associations between VDR polymorphisms with Psoriasis vulgaris (PsV) onset have been sought for many years. The results of these studies have not been conclusive. To date there are no studies in Mexico regarding the association between these polymorphisms and the clinical manifestations of PsV.

Objective: The aim of this study was to determine the association between polymorphisms in the VDR gene (FokI, BsmI, ApaI and TaqI) and the clinical manifestations of PsV in a group of Mexican patients.

Methods: The study group consisted of 52 patients from north (NPsV n=24) and western (OPsV n=28) regions of the country diagnosed with PsV. This group of patients were compared with healthy donors from the western (POc n=50) and northern (PN n=50) regions. RFLPs were identified to determine allelic and genotypic frequencies for all the groups. Hardy-Weinberg equilibrium (HWE) as well as haplotype distributions were estimated. Statistical tests were χ^2 and Fisher's exact test. Haplotype distribution was carried out with SNPstats software.

Results: There was no significant difference when the genotypic frequencies between patients and controls are compared; however, there is an association between the TT(ff) genotype of FokI polymorphism and clinical manifestations. The most frequently observed haplotypes of polymorphisms (FokI, BsmI, ApaI and TaqI) have significantly different distributions ($p > 0.0001$) between patients with PsV and controls.

Conclusions: Our results show that the polymorphisms FokI, ApaI, BsmI and TaqI in the VDR gene are not associated with the risk of presenting PsV in Mexican population, but the TT(ff) genotype of the FokI polymorphism is significantly more common in patients with late onset of PsV (after age 40) and those without nail affection. More studies including a greater number of samples and other polymorphisms must be analyzed.

Keywords: Psoriasis vulgaris; Vitamin D receptor; Polymorphisms; Mexican patients

Introduction

Psoriasis vulgaris (PsV) is a multifactorial disease characterized by well-circumscribed erythematous plaques, constant skin flaking and intense pruritus, that affects specific skin areas. PsV worldwide prevalence ranges from 0.4 to 4.8% and in Mexico 2% of the population presents this disease. It is well known that Vitamin D Receptor (VDR) plays a key role in both the metabolism and differentiation of keratinocytes [1]. The gene encoding the VDR is located on chromosome 12cen-q12, contains 11 exons and spans approximately 75 kb of genomic DNA. Several polymorphisms have been identified in the gene including the Fok I polymorphism located in exon 2 at the 5' coding region of the gene [2]. For this reason, researchers have attempted to establish an association between VDR gene polymorphisms and the onset, presence and response of treatment of psoriasis vulgaris. The aim of this study was to determine the association between four polymorphisms on the VDR gene (FokI, BsmI, ApaI and TaqI) and the clinical manifestations of PsV in a sample of Mexican patients.

Materials and Methods

Samples

Peripheral blood from a total of 52 patients (aged 5-68 years) and 100 healthy donors was taken after obtaining informed consent. The diagnosis was based on the clinical history, classic features, and a positive Auspitz's sign. The Psoriasis Area Severity Index (PASI) was also assessed. At least one first-degree relative diagnosed with PsV was reported by 48% of the patients.

Analysis of polymorphisms

Total DNA was obtained from blood by the Gustincich method [3]. Three sets of primers previously described were used to amplify exons 4 and 11, and intron 10 of the VDR gene [4]. Amplified fragments were analyzed by RFLP using the enzymes FokI (rs2228570), TaqI (rs731236), ApaI (rs7975232) and BsmI (rs1544410); the latter SNP was confirmed with the enzyme HhaI and by direct sequencing (sequencer model ABI PRISM 310, Applied Biosystems, Foster City, CA). All PCRs were performed according to the manufacturer's protocol (New England Biolab, Ipswich, MA) and the products were visualized on 1% agarose gels stained with bromide after electrophoresis.

Statistical analysis

The genotype and allele frequencies were analyzed using the Chi square test (χ^2); in addition, the relative risk for disease and clinical variables was established. Models of dominant, co-dominant or

***Corresponding author:** Julio Cesar Salas Alanis, Calle Otomie 206, Colonia Azteca, Guadalupe, Nuevo Leon, CP 67150, Mexico, Tel: +52 (81) 83676060; E-mail: drjuliosalas@gmail.com

Received May 16, 2015; Accepted May 25, 2015; Published May 27, 2015

Citation: Vega-Hernandez RE, Romero-Prado MMJ, Sandoval-Ramirez L, Moreno-Trevino MG, RFajardo-Ramirez O, et al. (2015) The FokI Polymorphism of the VDR Gene is a Protective Factor for Psoriasis Vulgaris. J Clin Case Rep 5: 528. doi:10.4172/2165-7920.1000528

Copyright: © 2015 Vega-Hernandez RE, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

recessive binary association were used to estimate the OR with a 95% CI analyzed by binary logistic regression [5]. The estimation of haplotype frequencies was performed using the expectation-maximization algorithm. A p value <0.05 was considered significant. The platform used for statistical analysis was SNPSstats [6].

Results

Of the total of patients (n=52) with PsV, 78% (n=38) corresponded to early onset PsV; 65% (n=39) did not have nail involvement and 48% of patients (n=25) reported at least one first-degree relative diagnosed with psoriasis.

The PASI score obtained by the study universe was mild in 81%, moderate in 13%, and severe in 6% with a mean of 5.04, range 0.3 to 32. The polymorphisms BsmI (p=0.042) and ApaI (p=0.011) did not show HWE in the reference population. By analyzing the distribution of allelic and genotypic frequencies regarding the clinical variables mentioned above, it is observed that under the dominant model, the TT(ff) genotype was significantly more frequent in patients with late onset PsV (40 or older) (p=0.033) and in patients without nail involvement (p=0.015). The TT(aa) genotype was significantly more frequent, under the recessive model, in patients with a negative family history (p=0.049) (Table 1). According to the haplotype analysis in the SNPstats platform, we observed significant differences in the most common haplotype in the reference population HpVDR1 (FbaT, 21.1%) compared to patients with PsV HpVDR2 (FbAT, 24.1%) (p<0.001). Considering only 3 of the polymorphisms located just before the 3' region of the VDR gene (BsmI, ApaI and TaqI), we found that haplotypes significantly different between healthy donors and PsV patients were HpBAT1 (baT 32.4%) vs. HpBAT2 (bAT, 49.7%), respectively (p<0.001). Although we found no association of any haplotype in particular with the presence of PsV, we did find a significant difference (p<0.001) between the distribution of the haplotypes composed of the recessive alleles FokI and ApaI (f and a) in patients with PsV vs. the reference population (Table 2). This is the first report of a possible association of the ff genotype in VDR gen (rs2228570) with the clinical variables, late age of onset, and no nail involvement, and also the first time a protective effect of the "f" allele is suggested, which contradicts with that demonstrated in Egyptian population, since this same allele, both homozygous (ff) and heterozygous (Ff), proved to be associated with Type 1 Diabetes Mellitus in Egyptian children [7]. In addition, contrary to the protective

effect found in this study, Swapna et al. reported that the VDR gene FokI polymorphism is associated with the risk of developing essential hypertension [8].

Similarly, there is no previous history that addresses a possible association between genotype "aa" (rs7975232) and a family history for the disease according to the recessive model. On the other hand, there are reports that the "aa" allele lessens the risk of tuberculosis (p=0.006), according to a meta-analysis published in 2014, which also suggests that it may be a protector allele [5]. In this study, the genotype frequencies obtained in Mexican population (AA 22%, Aa 35%, aa 43%) show that this polymorphism is in HW disequilibrium with BsmI (P=0.011), which is consistent with previous reports in African population (D'0.974, P<0.00001) [9]. Furthermore, we found that the bAT haplotype was present in 49.7% of our patients vs. 17.2% as reported by Rucevic in Croatia [10]. In contrast with Acikbas et al. [11] we found no haplotype associated with susceptibility to PsV in patients vs. healthy donors [11].

Finally, vitamin-D is a member of the steroid receptor family and mediates the effects of the active metabolite 1, 25(OH) 2 vit D3 by regulating transcription of a number of different cellular genes. The action of vitamin D is mediated through its binding to nuclear receptor (VDR) [12]. The FokI Vitamin D Receptor (VDR) polymorphism results in different translation initiation sites on VDR. In the VDRff variant, initiation of translation occurs at the first ATG site, giving rise to a full length VDR protein of 427 amino acids. Conversely, in the VDRFF variant, translation begins at the second ATG site, resulting in a truncated protein with three less amino acids [13]. This study found an association in FokI polymorphism of VDR gene and two major clinical variables, suggesting that the genotype [T/T]/ff may be a protective factor for nail involvement and age of onset in Mexican patients with PsV.

References

1. Yuan FN, Valiyaparambil J, Woods MC, Tran H, Pant R, et al. (2014) Vitamin D signaling regulates oral keratinocyte proliferation in vitro and in vivo. *Int J Oncol* 44: 1625-1633.
2. Whitfield GK, Remus LS, Jurutka PW, Zitzer H, Oza AK, et al. (2001) Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. *Mol Cell Endocrinol* 177: 145-159.
3. Gustincich S, Manfioletti G, Del Sal G, Schneider C, Carninci P (1991) A fast method for high-quality genomic DNA extraction from whole human blood. *Biotechniques* 11: 298-300, 302.

Model	[TT]/ff genotype of the FokI polymorphism				[TT]/aa genotype of the ApaI polymorphism				
	Clinical variable	Positive	Negative	OR (95% CI)	p	Positive	Negative	OR (95% CI)	p
Dominant	Nails	46.10%	82%	5.33 (1.36-20.84)	*0.015	69.20%	64.40%	0.79 80.21-3.05)	0.098
	Age of onset	65.80%	92.90%	6.76 (0.79-57.55)	*0.033	65.80%	64.30%	0.56 (0.06-5.42)	0.74
Recessive	Family history	80%	81.50%	0.91 (0.23-3.61)	0.89	48%	22.20%	0.31 (0.09-1.03)	*0.049

Table 1: Differences between FokI and ApaI in a sample of patients with PsV and Mexican Mestizos.

Estimate of haplotype frequencies in Mexican Mestizos (N=100)								PsV(N=52)	
Haplotype		FokI	BsmI	ApaI	TaqI	Total	AF	Total	AF
Frequent haplotypes	VDRHp1	F	b	a	T	21	21	7	7
	VDRHp2	F	b	A	T	16	37	13	20
	VDRHp3	f	b	A	T	15	52	12	32
Haplotypes with the recessive alleles FokI (f) and ApaI (a)*	VDRHp4	f	b	a	T	11	11	3	3
	VDRHp9	f	B	a	T	3	14	1	4
	VDRHp11	f	b	a	t	2	16	1	5
	VDRHp12	f	B	a	t	2	18	5	10

*X² test: p<0.001; PsV=Psoriasis Vulgaris; AF: Accumulated Frequency.

Table 2: Haplotype estimates with the T(f) and T(a) alleles of the FokI, BsmI, ApaI y TaqI polymorphisms in a sample of Mexican patients with PsV.

4. Miyamoto K, Kesterson RA, Yamamoto H, Taketani Y, Nishiwaki E, et al. (1997) Structural organization of the human vitamin D receptor chromosomal gene and its promoter. *Mol Endocrinol* 11: 1165-1179.
5. Areeshi MY, Mandal RK, Panda AK, Haque S (2014) Vitamin D receptor Apal gene polymorphism and tuberculosis susceptibility: a meta-analysis. *Genet Test Mol Biomarkers* 18: 323-329.
6. Iniesta R, Guinó E, Moreno V (2005) [Statistical analysis of genetic polymorphisms in epidemiological studies]. *Gac Sanit* 19: 333-341.
7. Abd-Allah SH, Pasha HF, Hagrass HA, Alghobashy AA (2014) Vitamin D status and vitamin D receptor gene polymorphisms and susceptibility to type 1 diabetes in Egyptian children. *Gene* 536: 430-434.
8. Swapna N, Vamsi UM, Usha G, Padma T (2011) Risk conferred by FokI polymorphism of vitamin D receptor (VDR) gene for essential hypertension. *Indian J Hum Genet* 17: 201-206.
9. Bornman L, Campbell SJ, Fielding K, Bah B, Sillah J, et al. (2004) Vitamin D receptor polymorphisms and susceptibility to tuberculosis in West Africa: a case-control and family study. *J Infect Dis* 190: 1631-1641.
10. Rucevic I, Stefanic M, Tokic S, Vuksic M, Glavas-Obrovac L, et al. (2012) Lack of association of vitamin D receptor gene 3'-haplotypes with psoriasis in Croatian patients. *J Dermatol* 39: 58-62.
11. Acikbas I, Sanla B, Tepeli E, Ergin S, Aktan S, et al. (2012) Vitamin D receptor gene polymorphisms and haplotypes (Apa I, Bsm I, Fok I, Taq I) in Turkish psoriasis patients. *Med Sci Monit* 18: CR661-666.
12. Merke J, Milde P, Lewicka S, Hügel U, Klaus G, et al. (1989) Identification and regulation of ,25-dihydroxyvitamin D3 receptor activity and biosynthesis of ,25-dihydroxyvitamin D3. Studies in cultured bovine aortic endothelial cells and human dermal capillaries. *J Clin Invest* 83: 1903-1915.
13. Alimirah F, Peng X, Murillo G, Mehta RG (2011) Functional significance of vitamin D receptor FokI polymorphism in human breast cancer cells. *PLoS One* 6: e16024.