

FOK 1 (rs10735810 id) Variants in Pemphigus Vulgaris: A Pilot Investigation in North Indians

Sir,

Pemphigus vulgaris (PV) is a blistering autoimmune, potentially life-threatening disease. It is characterized by intraepithelial acantholysis due to pathogenic desmoglein-3 (DSG3) autoantibodies.^[1] Vitamin D, as an immunoregulator has a variety of anti-inflammatory or regulatory effects and several reports have linked vitamin D deficiency with PV predisposition.^[1,2] Furthermore, vitamin D has been shown to reduce DSG3 expression in keratinocytes.^[3] Vitamin D facilitates several immuno-modulatory functions through vitamin D receptor (VDR), a ligand-activated transcription factor that controls or triggers expression of a variety of genes which impart stimulatory and protective effects on keratinocytes thereby signifying implication of VDRs in skin diseases^[1-5] Single-nucleotide polymorphisms (SNPs) in *VDR* (12q12-14) have been extensively studied in various dermatological disorders^[4,5]; however, SNPs remain neglected in PV. Therefore, a pilot case-control study was conducted with 204 North Indian subjects (recruited over a period of 9 months) including PV patients ($n = 74$, after a detailed medical and family history) and age- and sex-matched healthy controls ($n = 130$), to investigate the genetic association of *VDR-FokI* variants in PV patients.

Sample size was calculated using EPI Info 7 online calculator at an alpha error of 0.05, power of 80% and ratio of controls and cases of 1.75:1, taking the expected odds ratio of 5.71 for *FOK1* gene polymorphisms and assumption that proportion of controls and cases with *FOK1* gene polymorphisms 85% and 97%, respectively. Total sample size calculated (Fleiss with continuity correction) was 178 with 65 cases and 113 controls. For possible dropouts it was decided to include extra subjects so the final sample size was 74 cases and 130 healthy controls.

Under all aseptic precautions, peripheral blood samples were obtained consecutively from patients presenting to the dermatology outpatient clinics, with histopathology and/or direct immunofluorescence proven PV and from healthy unrelated voluntary controls. An informed consent was obtained from all the enrolled cases and healthy controls.

Genomic DNA was isolated from collected venous blood (3 ml) and quantified. Using primers,^[2] polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays were performed for the identification of *FOK1* variants. *FokI* being functional and important polymorphism of *VDR* gene results in different translation initiation sites due to thymine (T) to cytosine (C) substitution in the first translation initiation codon ATG which generates long and short forms of VDR. The *FokI*

polymorphism affects function of VDR resulting in altered efficiency of binding to vitamin D. Individuals with the C allele initiate translation at the second ATG site and lack the three NH₂-terminal amino acids of the full-length VDR protein. In contrast, individuals with the T allele initiate translation at the first ATG site and synthesize a full-length VDR protein. The short and long protein forms are associated with a different ability to induce transcription of vitamin D-dependent genes.

The disease was commoner in females compared to males (male:female = 1:1.47). The mean age of patients was 46.68 ± 12.9 years. The most common clinical subtype of PV was mucocutaneous (70%). Forty-two patients presented with active disease at enrolment. Distribution followed Hardy-Weinberg equilibrium [Table 1] and "CC" genotype (mutant) was observed to be significantly associated with the risk of PV in patients (58.1% vs. 30.7% controls; OR = 3.12; C.I. = 1.7-5.60; $P = 0.000$). TT genotype (wild) did not show any significant difference ($P = 0.98$) between the groups. 61.2% controls were heterozygous (TC) carriers ($P = 0.0000^*$). The allelic distribution showed "C" allele was the most abundant in patients vs. healthy controls (77% vs. 63.84%; OR = 1.89; CI = 1.2- 3.0; $P = 0.008$) whereas T allele occurred more frequently in healthy controls (OR = 0.53; CI = 0.33-0.83; $P = 0.008$). The carriage rate of "C" allele was more than 90% in all the subjects but controls possessed a significantly higher proportion of heterozygous "TC" genotype with the protective allele "T" (69.23%, $P = 0.0002$) compared with patients. These findings indicate that individuals with mutant allele "C" were more susceptible to develop PV, indicating that "C" allele might be a risk factor. Meanwhile, presence of T allele might prevent synthesis of transcriptionally more active truncated VDR (protein) in their skin, thereby generating a protective response in the keratinocytes and modulating the disease. The absence of data on serum vitamin D precluded a confirmation of our findings. However, a previous study^[1] on low vitamin D levels among North Indian PV strengthens our observations. Lacking data on VDR mediation in PV pathogenesis, we compared our healthy population data with that of the other studies and found concordance with Hasan *et al.*^[4] and incongruity with Itu Singh *et al.*^[5] A meta-analysis of *FOK1* variations across different Indian populations indicates genetic evidence of disease susceptibility in different ethnicities.^[6] In conclusion, our analysis highlights that "C" allele carriers are more susceptible to develop PV. Further studies can support this correlation if a corresponding decrease in Vitamin D levels is observed.

Table 1: Representative frequency distribution of FOK1 genotypes and alleles in *Pemphigus vulgaris* Patients and healthy controls

FOK1 genotypes and alleles	Patients n=74 (%)	Controls n=130 (%)	Odds ratio (OR)	Confidence interval (CI)-95%	P
TT (Wild homozygous)	3 (4.05%)	4 (3.07%)	1.331	0.29 to 6.116	0.975
TC (Heterozygous carrier)	28 (37.83%)	86 (66.15%)	0.311	0.172 to 0.564	0.0000*
CC (Mutant homozygous)	43 (58.1%)	40 (30.7%)	3.121	1.724 to 5.649	0.0000*
T (Wild allele)	34 (22.9%)	94 (36.15%)	0.527	0.333 to 0.833	0.008*
C (Mutant allele)	114 (77.02%)	166 (63.84%)	1.899	1.2 to 3.005	0.008*
Carriage rate "T"	31 (41.89%)	90 (69.23%)	0.3204	0.1770 to 0.5799	0.0002*
Carriage rate "C"	71 (95.94%)	126 (96.3%)	0.7513	0.1635 to 3.4523	0.7133

* $P \leq 0.05$ was considered significant

Ethical clearance

The study has been approved by the Institute extramural ethics committee, PGIMER, Chandigarh, India (PGI/IEC/2013/P-400) dated 26/02/2013.

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Conflicts of interest

There are no conflicts of interest.

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