Evaluation of *HCP5* and *Chemokine C Receptor type* 5 Gene Polymorphisms in Indian Psoriatic Patients

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Abstract

Background: Genetic variations associated with nonprogression of HIV infection to AIDS are enriched in psoriasis patients. *HCP5* gene 335 T > G and *chemokine C receptor type 5* (*CCR5*) gene Δ32 polymorphisms are associated with HIV nonprogression phenotype. **Aim:** The aim of this study was to determine the association of HCP5 gene 335 T > G (rs2395029) and *CCR5* gene Δ32 (rs333) polymorphisms with psoriasis vulgaris (PV). **Materials and Methods:** Genotype of *HCP5* gene 335 T > G and CCR5 gene Δ32 polymorphisms were determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism and allele-specific PCR methods, respectively. **Results:** The frequency of *HCP5* gene 335 T > G SNP was ~7 times higher in PV patients than in the control group ($P = 1.49 \times 10^{-8}$; odds ratio [OR] = 10.2; 0.95 confidence interval [CI]: 3.9–26.8). OR for the occurrence of *HCP5* 335 G allele in either homozygous or heterozygous genotype in PV patients was 13.1 (0.95 CI: 4.7–36.1). The strength of association was higher with moderate-to-severe subgroup ($P = 3.29 \times 10^{-9}$; OR = 18.4; 0.95 CI: 6.2–54.9) than with mild subgroup ($P = 2.1 \times 10^{-4}$; OR = 8.3; 0.95 CI: 2.6–23.3). In addition, the strength of association was higher with Type I ($P = 9.53 \times 10^{-8}$; OR = 15.3; 0.95 CI: 5.1–46.5) than with Type II subgroup ($P = 6.8 \times 10^{-6}$; OR = 11.0; 0.95 CI: 3.6–33.9). Type I gene Δ32 polymorphism was observed neither among psoriatic nor among healthy individuals. **Conclusions:** Our results indicate that *HCP5* gene 335 T > G polymorphism and not *CCR5* gene Δ32 polymorphism is associated with the increased risk of developing PV.

Key Words: *Chemokine C receptor type 5 gene Δ32, gene polymorphism, HCP5 gene, HIV infection, long-term nonprogression, psoriasis vulgaris*

Introduction

Psoriasis is an autoimmune inflammatory disorder characterized by hyperproliferation and abnormal differentiation of keratinocytes. Psoriasis is a multifactorial disorder with polygenic inheritance. Results from numerous studies indicate that immune dysregulation appears to play a major role in the disease development. Twin studies indicate that genetic factors influence the development of psoriasis by about 68%.[1] Linkage studies in affected families have indicated up to 10 genetic loci for the genetic predisposition.[2] These loci are designated as PSORS1, PSORS2, …, PSORS10. *HLACw06* allele is now established as the variant responsible for PSORS1 and SNPs in *CARD14* gene as the basis for PSORS2.[3,4] In addition to unique markers, several autoimmunity-associated polymorphisms mostly in the inflammatory pathways are also common.[5] An interesting aspect of the genetic landscape of psoriasis is the preponderance of variants that are associated with the resistance to HIV infection or delay in progression into AIDS.[6] Patients who do not develop symptoms of AIDS for over 10 years after initial HIV infection are described as long-term nonprogressors. Also of particular interest is the relationship between psoriasis and HIV infection. Psoriasis is the common secondary complication seen in HIV-infected patients.[7]

Recent studies have found that human leukocyte antigen (HLA) alleles associated with HIV long-term nonprogression phenotype such as HLA-B57 × 01 and -B13 × 02 are commonly enriched in psoriasis patients.[6] There are also non-HLA genetic markers such as polymorphisms in *HCP5* and chemokine C receptor

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type 5 (*CCR5)* genes that are also associated with HIV nonprogression phenotype.[8-10] *HCP5* gene codes for HLA complex P5 protein which is an endogenous retroviral element. It is located in the HLA Class I region. *HCP5* gene 335 T > G variant (rs2395029) is associated with HIV nonprogression phenotype and with hypersensitivity to antiretroviral agents such as abacavir.[11] *HCP5* gene 335 T > G variant was found to be strongly associated with psoriasis in a genome-wide study.[12] CCR5 is a cell surface receptor which is used by HIV to enter lymphocytes. The receptor is coded by *CCR5* gene. A 32 base-pair deletion (Δ32) within the coding region results in a frame shift and generates a nonfunctional receptor that does not support membrane fusion or infection by HIV-1 strains.[12] No functional CCR5 receptors are expressed in individuals homozygous for Δ32 mutations, and these individuals are resistant to HIV-1 infection, despite multiple high-risk exposures.[13,14] Expression of CCR5 receptor is reduced by half in individuals heterozygous for the mutant allele, and these individuals show reduced viral load and slower progression to AIDS.[15] To the best of our knowledge, there is a paucity of information on the status of these variants among Indian patients with psoriasis. This study was, therefore, undertaken to fill the gap in the literature.

Materials and Methods

Study design and participants

This was a group comparison study involving two groups, namely, patient group and control group. Patient group comprised of 74 psoriasis vulgaris (PV) patients, while the control group comprised of 74 healthy individuals with no history of psoriasis or other chronic diseases. Psoriasis other than vulgaris subtype was excluded from the study. The study was approved by the Institutional Ethics Committee.

Inclusion criteria were (i) clinically diagnosed psoriatic patients and (ii) both male and female patients of age ≥18 years. Exclusion criteria were (i) patients with any chronic disease other than PV, (ii) chronic infection, (iii) malignancy, (iv) pregnancy or lactation, and (v) use of anti-psoriatic/anti-inflammatory medications in previous 6 months. Details regarding exclusion criteria were obtained from patients' history. Healthy individuals without any history of chronic diseases were included as controls. Both patients and healthy volunteers were recruited from Kolar area of Karnataka state in India, from March 2014 to March 2016. Patients were diagnosed to have PV based on history and clinical examination and enrolled in the study after obtaining informed consent in writing. PV was classified as early-onset (Type I) if skin symptoms occurred before 40 years of age, and late-onset (Type II) if age of onset was more than 40 years. Disease severity was assessed at study entry by determination of the Psoriatic Area and Severity Index (PASI).[16] Patients with Psoriasis Area and Severity Index (PASI) score <10 were designated as mild and those with PASI score ≥10 were designated as moderate-to-severe.

Sample collection and DNA isolation

About 3 ml of peripheral venous blood was collected in ethylenediaminetetraacetic acid vacutainer and stored at 4°C until processing. DNA was isolated from the peripheral blood lymphocytes using salting-out method. Purity of the sample was determined by ultraviolet spectrophotometry (Perkin Elmer model Lambda 35, USA).

Genotyping HCP5 335 T > G polymorphism

Genomic DNA was amplified by polymerase chain reaction (PCR) on Bio-Rad C1000 Touch Thermal Cycler. The primer pairs used were 5′-TCA TTG TGT GAC AGC AGC C-3′ (forward) and 5′-TCC CAT TCC TTC AAC TCA CC-3′ (reverse). About 25 μl reaction mixture included 1× assay buffer, 150 ng genomic DNA, 0.2 mM dNTP, 1 picomole of each primer, 1.5 mM $MgCl_2$, and 1 unit *Taq* DNA polymerase (Bangalore Genei, India). The PCR program was as follows: initial denaturation, 95°C – 3 min; cycle denaturation, 95°C – 30 s; annealing, 60°C – 30 s; extension, 72°C – 1 min; final extension 72°C – 5 min; and no of cycles 35. The PCR product was analyzed on 2% agarose gel. The 268 bp amplicon was subjected to restriction digestion with 10 units of XcmI (New England Biolabs, USA) at 37°C for 2 h and analyzed on 1% agarose gel with ethidium bromide staining. Band patterns of the three *HCP5* 335 T > G genotypes were identified as follows: homozygote TT – 117 + 151 bp, homozygote GG – 268 bp, and heterozygote TG – 117 + 151 + 268 bp. About 10% of the samples were randomly selected for confirmation and the results were 100% concordant.

CCR5 gene Δ32 polymorphism

Genomic DNA was amplified by PCR using the primer pairs 5′-CTT CAT TAC ACC TGC AGC TCT-3′ (forward) and 5′-CAC AGC CCT GTG CCT CTT CTT C-3′ (reverse). Reaction composition was as in the previous section. The PCR program was as follows: initial denaturation, 95°C – 3 min; cycle denaturation 95°C – 30 s; annealing 62°C – 30 s; extension 72°C – 1 min; final extension 72°C – 5 min; and no of cycles 35. The 182 bp PCR product was analyzed on 2% agarose gel.

Statistical analysis

The *post hoc* power of the study was calculated using OpenEpi web tool (Available at www.openepi.com) using 95% confidence interval.[17] Normal approximation with continuity correction was considered for power calculation. Statistical analysis was done using the Statistical Packages for the Social Sciences software (version 22, SPSS Inc., Chicago, IL, USA). Allele and genotype frequencies of the two groups were compared

by calculating two-tailed *P* value using Fisher's exact test. The study population was tested for conformity to Hardy–Weinberg equilibrium using the web program by Rodriguez *et al*.[18] $P < 0.05$ was considered as statistically significant.

Results

Clinical parameters of the study population are listed in Table 1. All patients and control subjects were genotyped for the *HCP5* 335 T > G and *CCR5* Δ32 polymorphisms. The distribution of the genotypes in the control group was in agreement with Hardy–Weinberg equilibrium.

The frequency of *HCP5* 335G allele among PV patients was 26.3% which is 7.7 times higher than the frequency of 3.4% seen in the control group [Table 2]. Difference in the distribution of the two alleles in the patient and control groups was compared by means of contingency table and was found to be statistically significant ($P = 1.49 \times 10^{-8}$). The power of the study based on normal approximation with continuity correction was found to be 99.9%.

Table 1: Baseline demographic and clinical parameters of the study participants

Parameter	Observation
Gender	
Patients (*n*=74)	Male: 54 - female: 20
Controls (*n*=74)	Male: 56 - female: 18
Age (years)	
Patients	41.0±13.9
Controls	42.0±13.8
Clinical type	
Type I	*n*=38
Type II	*n*=36
Severity	
Mild	*n*=32
Moderate-to-severe	*n*=42

Table 2: Distribution of *HCP5* 335 T>G alleles in the study groups

Allele	Patients (*n*=74), *n* (%)	Controls (*n*=74), *n* (%)
T	109 (73.6)	143 (96.6)
G	39 (26.4)	5 (3.4)
*P**	1.49×10^{-8}	

*Chi-squared test - Fisher's exact test

We also compared the distribution of the genotype frequency among PV and non-PV groups [Table 3]. *HCP5* 335G allele was observed mostly in the heterozygous condition. The frequency was 44.6% in PV patients and 6.7% in non-PV individuals. *HCP5* 335G allele was seen in homozygous condition in 4.1% of psoriatic patients and none among nonpsoriatic individuals. The distribution of the genotypes in the two groups was compared by means of contingency table. The two groups showed statistically significant difference only in dominant genetic model ($P = 9.47 \times 10^{-9}$) and not in recessive genetic model ($P = 0.24$). The lack of association in the recessive genetic model is probably due to the low frequency of *HCP5* 335GG genotype.

The distribution of the genotypes was stratified on the basis of the type of PV, and the data were analyzed for disease association by means of contingency table [Table 3]. The two subgroups did not show any major difference when they were tested separately. However, the association with Type I PV was higher than that with Type II by an order of 2. In this context, it is interesting to note that the small fraction of PV patients who showed homozygous GG genotype was with Type I form of PV.

The data were also analyzed by stratifying the distribution of the genotypes on the basis of severity of PV. Significant association was found with both mild and moderate-to-severe subgroups. The association was found to be stronger with the moderate-to-severe subgroup than with the mild subgroup [Table 3]. The *P* value for association with the moderate-to-severe subgroup was higher than that for the mild subgroup by an order of 5.

Familial incidence of PV was seen in four patients. All the four patients were suffering from Type I PV. Among these patients, one showed mild PV and the remaining three showed moderate-to-severe PV. Genotype of *HCP5* T > G SNP was TG in all the four patients.

Discussion

HIV infection is known to increase the chance of developing psoriasis. However, the underlying mechanism that triggers psoriasis in such patients is not clearly understood. Both HIV infection and psoriasis involve an immunological component, particularly in the role of T-lymphocytes.[19] Genetic factors affecting T-lymphocyte

Table 3: Statistical evaluation of *HCP5* 335 T>G genotype in psoriasis vulgaris groups in dominant genetic model

Genotype	Control (*n*=74)	Patients (*n*=74)	Mild (*n*=32)	Moderate-to-severe (*n*=42)	Type I (*n*=38)	Type II (*n*=36)
TT	69	38	20	18	18	20
TG	5	33	11	22	17	16
GG	0	3	1	2	3	0
*P**	-	9.47×10^{-9}	2.1×10^{-4}	3.29×10^{-9}	9.53×10^{-8}	6.81×10^{-6}

*Chi-squared test - Fisher's exact test

function can, therefore, be expected to be the link between the two diseases.

An interesting aspect of the association between HIV infection and psoriasis is the enrichment of genetic variants associated with "long-term nonprogression" phenotype in psoriatic patients.[6] "Long-term nonprogression" comprises of <5% of the total HIV population.[20] Several genetic factors are associated with "long-term nonprogression" phenotype.[20] These factors can be broadly divided into three categories: (a) genes-encoding cell-surface receptors or their cognate ligands, (b) HLA genes that regulate immune response, and (c) cytokine and other immune response genes. Δ32 *CCR5* polymorphism belongs to the first category. The mechanism by which *HCP5* G allele influences the progression of HIV to AIDS or the development of psoriasis is not known. Increased antiviral inflammatory response in "long-term nonprogression" has been indicated as a putative basis for induction of psoriasis in predisposed patients.[12] This idea is compatible with the fact that psoriasis is an inflammatory disorder characterized by elevated plasma levels of pro-inflammatory cytokines.[21]

HCP5 or HLA Complex P5 is an endogenous retroviral element, that is, remnant of an ancient retroviral lysogenic DNA. *HCP5* gene is expressed primarily in the lymphocytes.[22] 335 T > G substitution in the cDNA results in 112 Val > Gly switch in the protein chain. It is not known how this substitution affects the functionality of HCP5 protein. Patients carrying *HCP5* G allele are known to show hypersensitivity and adverse drug reaction to antiretroviral therapeutics such as abacavir.[11] Genome-Wide Association Studies with the US and UK psoriasis cohorts found *HCP5* T > G as the second most strongly associated SNP among psoriasis patients ($P = 2.13 \times 10^{-26}$).[12] Positive association was also seen in the Chinese population ($P = 0.0005$).[23] The results obtained in the present study indicate that *HCP5* G allele is significantly enriched in psoriasis patients, and therefore, constitutes a risk factor in the Indian patients. The allele was associated with both Type I and Type II forms and also with mild and moderate-to-severe subgroups. However, the disease association was relatively higher with the Type I and moderate-to-severe groups. This indicates that the strength of association is influenced by both the type and severity of psoriasis. The variability between the subgroups compels us to assume that *HCP5* 335 G allele may not be the primary predisposing genetic factor but a modifier. That is, the polymorphism increases the probability of early onset and severity of PV when triggered in a predisposed individual.

There are indications that the chemokine receptor CCR5 and its ligands play an important role in the pathogenesis of psoriasis.[24-26] Expression of CCR5 on regulatory T-cells plays a crucial role in limiting Th1 inflammation associated with autoimmunity and bacterial infections. Recent studies have found that CCR5 expression in regulatory T-cells is decreased in psoriatic patients.[27] Impairment in CCR5 is assumed to affect the long-term failure of regulatory T-cells in the psoriatic patients to suppress the expansion of effector T-cell response. These findings encourage the hypothesis that *CCR5* Δ32 polymorphism may be associated with psoriasis. Survey of literature revealed only one study that has examined this issue. The said study, based out of Spain, found that the frequency of the deletion was similar among both psoriatic and nonpsoriatic individuals.[28] In the present study, we found *CCR5* Δ32 polymorphism neither in patient nor control groups. The possible reason for this observation could be the global variability in the distribution of this polymorphism. *CCR5* Δ32 polymorphism is common in the Caucasian population with an allelic frequency of about 10%.[29] The deletion allele occurs at a frequency of about 1% in heterozygous condition in some Indian communities.[30] The low frequency in our population combined with the essentiality of the receptor for the development of psoriasis may be the reasons for our negative observation.

Conclusions

The results obtained in this study indicate that *HCP5* 335 T > G and not *CCR5* Δ32 is a risk factor for the development of PV in the Indian population.

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Conflicts of interest

There are no conflicts of interest.

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