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HLA C Allele Associations in Mumbai Psoriasis Patients

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ABSTRACT Psoriasis a common autoimmune disorder characterized by T cell mediated keratinocyte hyperproliferation known to be associated with the presence of certain specific HLA alleles. In this study our aim was to evaluate distribution of HLACw susceptible allele of psoriasis from patients in Western India.The study included 84 Psoriasis patients and 91 normal individuals from same geographical region. HLA-C was typed using high resolution PCR-SSOP (Sequence Specific Oligonucleotide Probes) technique.The study revealed significant increase in frequencies of Cw*0202 (OR-6.508, P<0.0001) and Cw*0604 (OR-17.710, P=0.0153) when compared to controls while Cw*1502 (OR-0.130, P=0.0490) were significantly decreased in psoriasis patients.Our study shows a strong association of HLA Cw*0202 and Cw*0604 alleles with psoriasis conferring to its susceptibility to psoriasis patients from Western India.

INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder characterized by red, scaly plaques that features mirror the characteristic pathological changes, namely, T cell mediated keratinocyte hyperproliferation, loss of differentiation and impressive hyperplasia of the epidermis with poorly adherent stratum corneum and accumulation in the skin of neutrophils and T lymphocytes**.** Distribution of psoriasis in the world population varies according to ethnic groups and geographical locations, with a peak prevalence of approximately 2% of the population. The disease has a strong but complex genetic background with a concordance of approximately 60% in monozygotic twins (Krueger and Bowcock 2005). However the manner in which psoriasis susceptibility genes contribute to pathogenesis is still not clear. One of the most compelling susceptibility factors for psoriasis lies in the MHC region of chromosome 6 which code for (HLA) Human Leukocyte Antigens of a person. The association of psoriasis and HLA has long been reported on population and family studies. Psoriasis has been reported to be significantly associated with HLA - HLA-A*01, A*02, B13, B17, B39, B57, Cw*06, Cw*07, and DR7, DQA1*0201 (Rani et al. 1998; Pitchappan et al. 1989; Ikaheimo et al. 1996; Nakagawa et al. 1989; Gonzaga et al. 1996; Gonzaga et al. 1996). This relationship, however, tends to vary between patients of different racial and ethnic backgrounds. Studies from India have revealed the associations of HLA - B*57, DR*07 and HLA - Cw*0602 in psoriasis patients (Pitchappan et al. 1989; Ikaheimo et al. 1996).

The present study of this population was under-taken to evaluate distribution of HLA class I alleles and hence identify the susceptible allele of psoriasis from patients in this geographical region that is, Western India.

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MATERIALS AND METHODS

Patients and Controls*:* The study design included 84 psoriasis patients (59 males and 25 females) and 91 normal individuals as controls from Mumbai. The samples of psoriasis patients were collected from the Skin OPD of K.E.M. Hospital, Parel, Mumbai. The patients were being treated with chemotherapy and did not show signs of psoriatic arthritis. The ages of the patients ranged from 18-82 years with mean age of 44 years. Unrelated individuals of matched age groups and from same geographical area with no previous history of psoriasis were taken as controls.

HLA Typing*:* HLA-Cw alleles for the 84 patients and 91 controls were typed using PCR-SSOP (Sequence Specific Oligonucleotide Probes) technique. The genomic DNA was extracted using by phenol extraction method from 5 ml

of EDTA blood. Molecular subtyping was carried out using polymerase chain reaction-sequence-specific oligonucleotide probes (PCR-SSOP) reverse line strips hybridization method (Dynal kits Roche Molecular Systems). The RLS is an immobilized probe array/reverse lineblot system. It utilizes an immobilized probe array in which oligonucleotide probes (for high resolution HLA-C typing) are deposited in a linear array on a nylon membrane of the strip. The PCR product is generated using separate biotinylated primers for the second and third exons of the HLA C loci. The presence of biotinylated PCR product bound to a specific probe is detected using Streptavidin-horseradish (HRP) and a chromogenic, soluble substrate to produce a blue "line" at the position of the positive probe. Genotyping software can interpret the probe reactivity pattern as a genotype.

Statistical Analysis: The allele frequencies were estimated from the number of positive typing reactions divided by the total number of haplotypes tested (number of individuals studied X 2). Phenotype frequencies, odds ratio, probability value, chi square with Yates correction, and etiological and preventive fraction were estimated using our database and computer programs as described in Shankarkumar et al. (2003).

RESULTS

Some significant findings can be concluded from this study. Table 1 depicts the frequencies of HLA-Cw*0202, Cw*0104 and Cw*0604 were significantly increased as compared to the controls. Among these Cw*0202 (OR-6.508, P<0.0001) and Cw*0604 (OR-17.710, P= 0.0153) were strongly associated with psoriasis. HLA Cw*1502 showed significant decrease in frequency (OR-0.174, P=0.0063) among psoriasis patients. Among patients with Psoriasis vulgaris type the frequencies of Cw*0104 (OR-14.060, P=0.0107), Cw*0202 (OR-6.212, P= 0.0002) and Cw*0604 (OR-24.580, P=0.0086) were significantly increased as compared to controls while a significant decrease was seen in frequencies Cw*1502 (OR-0.130, P=0.0490).

DISCUSSION

This study shows the strong association of HLA-A2, B8, B17, Cw*0202 and Cw*0604 alleles with the disease conferring to its susceptibility. The alleles HLA-A28, B5, B12 and Cw* 1502 show strong negative association with the disease thus protective against it. We found the association of HLA-A2 (OR-3.976, P<0.0001), B8 (OR-5.647, P<0.0001) and B17 (OR-5.452, P<0.0001) alleles with psoriasis. These results are in accordance with a few studies in psoriasis patients amongst different ethnic populations of the world as seen from the Table 2.

Interestingly, psoriasis is the only chronic inflammatory disease that has a strong association with HLA-C, and about two-thirds of the patients carry the HLA-Cw*0602 allele compared to 10–15% in the population at large. In the present study, HLA-Cw*0202 (OR-6.508, P<0.0001) and Cw*0604 (OR-17.710, P= 0.0153) were strongly associated with psoriasis. Recent linkage and high resolution association studies strongly indicate that the specific allele HLA-Cw*0602 is a major susceptibility

Table 1: HLA-Cw distribution in Total Psoriasis patients

HLA Cw	*Patients (N=84) N+*	*Control (N=91)*			*OR*	*EF*	*PF*	*χ^2 Value*	*P Value*
		%AF	*N+*	*%AF*					
Cw *0104	**10**	**5.95**	**1**	**0.60**	**12.16**	**0.10**		**6.92**	**0.0085****
Cw *0202	**35**	**20.83**	**9**	**4.90**	**6.50**	**0.16**		**21.78**	**<0.0001****
Cw *0604	**7**	**4.17**	**0**	**0.00**	**17.71**	**0.03**		**5.878**	**0.0153**
Cw *1502	**3**	**1.79**	**16**	**8.60**	**0.17**		**0.14**	**7.47**	**0.0063****
	P. vulgaris (N=37)		*(N=91)*						
Cw *0104	5	6.76	1	0.60	14.06	0.12	6.50	0.0107	
Cw *0202	**15**	**20.27**	**9**	**4.90**	**6.21**	**0.34**		**14.27**	**0.0002****
Cw *0604	**4**	**5.41**	**0**	**0.00**	**24.58**	**0.10**		**6.89**	**0.0086****
Cw *0701	8	10.81	7	3.70	3.31	0.15		3.67	0.0551
Cw *1502	1	1.35	16	8.60	0.13		0.15	3.84	0.0490

N+ : Numbers Positive N : Total Number of samples AF : Allele Frequency
OR : Odds Ratio EF : Etiological Fraction PF : Preventive Fraction
χ^2 Value : Chi square with Yates correction ** Significant P value

Table 2: Distribution of HLA in different ethnic populations

S. No.	*Population*	*Sample size*	*Alleles associated*	*Alleles protective*	*Reference*
1.	Korean	84	HLA Cw*0602,	HLA Cw*1401,	Kim TG et al.
2.	Turkish	100	HLA Cw7, Cw6,	HLA Cw2, Cw4,	Kundakçi N et al.
3.	Chinese Hans	166	HLA Cw*0602,	HLA Cw*0304,	Zhang XJ et al.
4.	Northeastern Thais	140	HLA Cw*01, Cw*0602,	HLA Cw*04	Choonhakarn C et al.
5.	Croatian	_	HLA-Cw*0602	-	Kastelan M et al.
6.	Brazilian	92	HLA Cw*06, Cw*12,	-	Biral AC et al.
7.	Saudi	25	HLA - Cw*06, Cw*07	-	Abanmi A et al.
8.	Swedish	_	HLA - Cw*0602	-	Holm SJ et al.
9.	Canadian	_	HLA - Cw*0602	-	Rahman P et al.
10.	Finnish	124	HLA Cw06,	-	Ikaheimo I et al.
11.	Japanese	_	HLA Cw06, Cw11,	-	Nakagawa H et al.
12.	Northern Polish	78	HLA - Cw*06	-	Szczerkowska-DA et al.
13.	Indian (North)	38	HLA - Cw*0602	-	Rani R et al.
14.	Caucasian	29	HLA Cw*0602, Cw07,	-	Mallon E et al.
15.	Kuwait	305	HLA Cw01	-	Nanda A et al.
16.	Israeli Jewish	28	HLA - Cw06, Cw07	-	Roitberg-TA et al.
17	Western India	84	HLA Cw*0202, Cw*0604	HLA Cw*1502	Present study

risk allele for psoriasis (Shankarkumar et al. 2011**)**. Patients carrying this allele have been shown to have distinct clinical features including an earlier age of disease onset (Enerback et al. 1997; Gudjonsson et al. 2003). Furthermore, individuals who are homozygous for the HLA-Cw*0602 allele have about 2·5 times higher disease risk than heterozygotes (Valdimarsson et al. 1986). Although chronic plaque psoriasis is likely to be somewhat heterogeneous with respect to genetic and pathogenic components, available evidence indicates a final common pathogenic pathway involving specific antigen recognition by T cells that results in stimulation of keratinocyte proliferation (Gudjonsson et al. 2004). And that the cross primed CD8+ T cells respond to antigens in the HLA-Cw*0602 binding pocket of keratinocytes and thereby act as main effector cells in maintaining the pathogenic process (Mallon et al. 2000). HLA-Cw* 0602 is found to be associated amongst Caucasian patients with guttate psoriasis (Chablani et al. 1992).

HLA serological studies on Indian patients with psoriasis showed association with A1, B17 and Cw06 (Roitberg-Tambur et al. 1994), showing accordance with our findings in reference to the HLA-B and HLA-C loci. From North India, the results showed that Cw*0602 was the main allele that was increased in this group of patients. Cw*0602 was found in 71% of the patients. B*5701 and B*3701 were also increased but appeared to be secondary to linkage disequilibrium with Cw*0602 (Pitchappan et al. 1989). Eighty-three south Indian patients with psoriasis vulgaris were studied for HLA antigen frequencies and compared with 77 controls and found HLA Bw57 and DR7 association with the disease but no association with Cw*06 (Ikaheimo et al. 1996). HLA-Cw*06 and Cw*07 have a unique antigen-binding pocket containing both alanine at position 73 (Ala-73) and a negatively charged aspartic acid at position 9 (Asp-9). These residues are most probably important in determining the conformation of the C pocket and in turn the nature of the peptide bound to it. A study on Israeli Jewish psoriatic patients clearly showed that alanine in position 73 is significantly associated with psoriasis vulgaris in Jewish patients (Abanmi et al. 2005**)**. A similar study from Saudi Arabia was done in which Sequence-specific primers were used to specifically detect nucleotide coding for Ala-73 and Asp-9 in all the subjects. The results showed significantly higher frequency of Asp-9 (84.0 % versus 61.3 %) in patients as compared to controls ($P < 0.05$) (Kastelan et al. 2003). The alleles HLA-Cw*0202 (OR-6.508, $P<0.0001$) and Cw*0604 (OR-17.710, $P=0.0153$) are reported from India for the first time. HLA-Cw* 1502 (OR-0.130, $P=0.0490$) allele which is negatively associated and hence confers protection against psoriasis in this region, has also not been reported

CONCLUSION

Hence we conclude that novel alleles associated with psoriasis patients from Western India have been identified.

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