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Study of Alanine-73 and Aspartate-9 of HLA-C Locus in Saudi Psoriasis Patients, Using Sequence-specific Primers (PCR-SSP)

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Alanine at residue 73 (Ala-73) and aspartate at residue 9 (Asp-9) are characteristic to both Cw6 and Cw7 alleles of HLA-C gene and have been suggested as possible markers for psoriasis vulgaris (PsV). However, the results from various ethnic groups/populations are contradictory and inconclusive. In this study, an attempt has been made to examine the association between HLA-C (Ala-73 and Asp-9) and susceptibility to PsV among Saudi patients. Genomic DNA was extracted from 25 Saudi PsV patients and 75 control subjects. Polymerase chain reaction (PCR) was performed to amplify HLA-C sequences using earlier reported primers, C133P and C243PR. 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 PsV patients as compared to controls ($p < 0.05$, 2-tailed Fisher's exact test). The frequencies of Ala-73 among PsV patients (92%) and controls (88%) did not differ significantly.

Keywords: Alanine-73, Aspartate-9, HLA, PCR, Psoriasis

Introduction

Earlier investigators have suggested that alanine at position 73 (Ala-73) and aspartate at position 9 (Asp-9) of HLA-C molecules can be good markers for PsV (Asahina *et al*., 1991, 1996; Ikaheimo *et al*., 1994; Roitberg Tambur *et al*., 1994). Serologic typing of HLA-C locus is difficult because of its low surface expression, structural similarities of alleles and lack of proper typing reagents (Zemmour and Parham, 1992; Bunce and Welsh, 1994; Petersdorf *et al.*, 1994). Moreover, in about 20-50% of individuals, at least one allele of HLA-C locus is not serologically defined and called C blank (Bunce and Welsh 1994). On the other hand, HLA typing by polymerase chain reaction (PCR) has been successfully employed for accurate and efficient detection of HLA-C alleles (Bunce and Welsh 1994; Kennedy *et al*., 1995). The frequencies of Ala-73 and Asp-9 within the HLA-C gene have been determined in PsV patients from diverse populations by sequence-specific primers (Asahina *et al*., 1996; Mallon *et al*., 1997) or sequence-specific probes (Asahina *et al*., 1991; Ikaheimo *et al*., 1994; Roitberg Tambur *et al*., 1994). However, to our knowledge no such attempt has been made so far to investigate HLA associations with PsV in Saudi population despite a high prevalence of this disease among Saudis (Fatani *et al*., 2002).

It has been noticed that HLA frequencies differ in various populations and these racial differences may have significant impact on HLA associations with diseases (Eastmond, 1994). Studies from various countries have clearly shown that degree of association between Ala-73 and PsV varies from one population to another (Asahina *et al*., 1991; Roitberg Tambur 1994; Mallon *et al*., 1997; Ikaheimo *et al*., 1996; Rani *et al*., 1998). Although the groups of Asahina (1996) and Nakagawa (1992) have described a significant association between Asp-9 and PsV in Japanese patients, its potential as a PsV susceptibility marker has not been explored in other populations. In this study, an attempt was made to study a possible association of Ala-73 and Asp-9 with PsV in Saudi patients.

Materials and Methods

Twenty-five Saudi patients with PsV and 75 Saudi control subjects were selected for this study. Genomic DNA was extracted from the blood samples using GenomicPrep Blood DNA Isolation Kit (Amersham Biosciences, Piscataway, NJ, USA).

Specific amplification of HLA-C fragments (residues 45 to 88) was accomplished with primer pair C133P (coding for amino acid residues 45 to 52 of the a1 domain of HLA-C molecules) and

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C243PR (residues 82 to 88) as suggested by earlier investigators (Asahina *et al*., 1991; Ikaheimo *et al*., 1994; Roitberg Tambur *et al*., 1994). Ala-73 was specifically detected with a combination of sense primer 5'-ACAAGCGCCAGGCACAGG (HLA-C, exon 2, 199-216) and the antisense primer 5'-GCTCTGGTTGTAGTAGCC GCG (exon 2, 243-263), which produced a 65 base-pair (bp) amplicon when Ala-73 was present in HLA-C locus allele. Specific detection of Asp-9 was carried out using the primers, 5'-TCCCACT CCATGAGGTATTTCG (exons 2, 3-24) and 5'-TCCGGCCCCTC CTGCTCCAC (exon2, 153-172). The resulting amplified segment of 170 bp confirmed the presence of Asp-9 (Asahina *et al*., 1996). Control primers were used as described earlier (Bunce and Welsh 1994).

For PCR amplification, genomic DNA (0.2-0.5 μg), respective primers (200 pmoles) and 0.5 ml of deionized formamide were added to thin-walled PCR tubes containing Ready-to-Go PCR beads (Amersham Biosciences). Each PCR bead contained 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP and 1.5 units of Taq DNA polymerase in a final reaction volume of 25 μl. The reaction mixture was overlayed with 40 μl of Nujol mineral oil (Applied Biosystems, Foster City, USA) and denatured by heating at 95°C for 5 min. Thirty PCR cycles of denaturation at 94°C for 30 s, annealing at 65°C for 60 s and extension at 72°C for 45 s were carried out followed by a final extension at 72°C for 7 min using a programmable DNA thermal cycler (Model 480, Perkin Elmer, Norwalk, USA). Ten microliters of amplified PCR products or size marker (50 or 100 base-pair ladder) were electrophorsed in 1.5% agarose gel containing 0.6 μg/ml ethidium bromide. The gels were run for 60 min at 180 Volts in 1xTBE buffer using Hoefer HE 99X Max Submarine Unit connected to electrophoresis power supply EPS-600 (Pharmacia Biotech, USA). The DNA fragments were visualized on the Gel Documentation System (Ultraviolet Products Ltd, Cambridge, UK) and printed with a Digital Graphic Printer (Model UP-D890, Sony Corp., Japan). The data were analyzed by Fisher's exact test (2-tailed). *P* values less than 0.05 were considered significant.

Results

The DNA samples from all the 25 PsV patients and 75 controls were successfully amplified with the primers C133P and C243PR, which was confirmed by the detection of 131 bp fragments on the gel (data not shown). The results of PCR-SSP for the detection of Ala-73 showed positive amplifications in 23 out of 25 PsV patients (92%) as compared to 66 among 75 controls (88%) (Fig. 1). Asp-9 was detected in 21 (84%) PsV patients and 46 (61.3%) control subjects (Fig. 1). All the patients with Asp-9 were also positive for Ala-73 (Fig. 2). Similarly, all the controls with positive amplification of Asp-9 were also positive for Ala-73, except one control (No. 45, Fig. 2), who was positive for Asp-9 but negative for Ala-73. A comparative view of our findings and the frequencies of Ala-73 and Asp-9 in PsV patients versus controls, reported by other investigators, is given in Table 1.

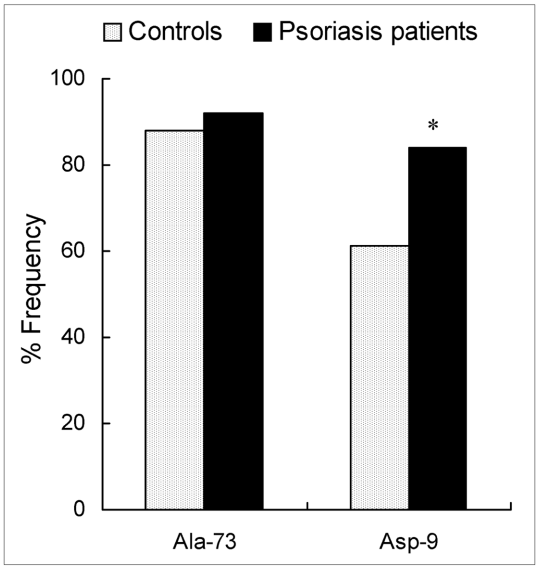

Fig. 1. Percent frequency of Ala-73 and Asp-9 in Saudi psoriasis patients and normal controls. *$p < 0.05$ versus controls using 2-tailed Fisher's exact test.

Discussion

Earlier studies on Japanese (Asahina *et al*., 1991), Finnish (Ikaheimo *et al*., 1994) and Jewish (Roitberg Tambur *et al*., 1994) patients have shown a strong association between specific nucleotide sequence of HLA-C (Ala-73) and the susceptibility to PsV, whereas studies on other ethnic groups including British Caucasians (Mallon *et al*., 1997) and Indians (Rani *et al*., 1998) failed to observe such an association. The results of this investigation demonstrated that frequencies of Ala-73 did not differ significantly among Saudi PsV patients and controls (Fig. 1). On the other hand, the frequency of Asp-9 was significantly higher in PsV patients as compared to controls (Fig. 1), which is in agreement with the findings of Asahina *et al*., (1996) who have reported an association between Asp-9 and PsV in Japanese patients.

The mechanism behind high disease vulnerability of certain HLA-C variants is not clearly understood. However, it has been suggested that these HLA-C variants may fail to bind and present psoriasis epitopes to $CD8^+$ T-cells with suppressor function, thereby causing persistent inflammation (Gottlieb and Krueger 1990; Valdimarsson *et al*., 1995). Crystallographic analysis of HLA class I molecules has revealed that these molecules have a receptor like shape with a peptide binding groove (Bjorkman *et al*., 1987; Garrett *et al*., 1989). The residues 9 and 73 are located close to each other, the former on the bottom and the later on one side of the putative antigens binding cleft. Any alteration in the amino acid composition of the groove may cause dimensional or polarity changes, leading to differential ability of the HLA molecule to bind and present antigens (Mallon *et al*., 1997). Thus, the PsV triggering effect of Ala-73 and Asp-9 might be synergistic in

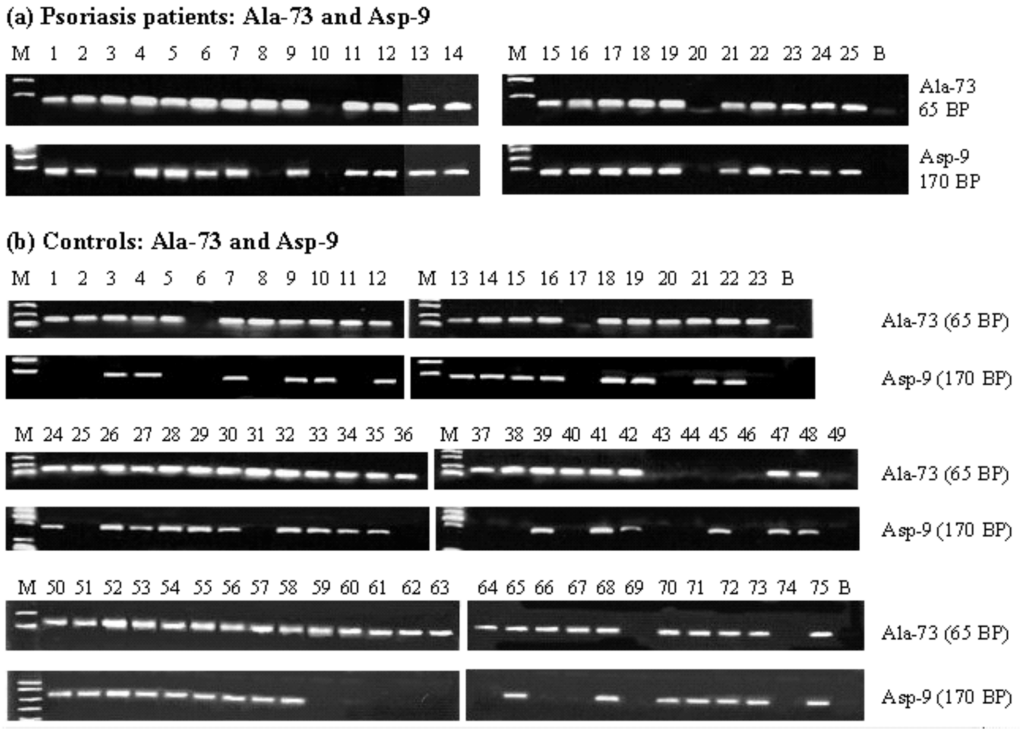

Fig. 2. Specific detection of Ala-73 and Asp-9 using PCR-SSP. Amplification of a 65 base-pair fragment confirmed the presence of Ala-73 in 23 of 25 patients and 66 of 75 controls. Amplification of a 170 base-pair fragment confirmed the presence of Asp-9 in 21 patients and 46 controls. Abbreviations are: M, 50 or 100 base-pair size marker; B, negative control (blank, without DNA); BP, base-pair.

Table 1. Frequencies of Ala-73 and Asp-9 among psoriasis patients and healthy controls from various ethnic groups: a comparison with present study.

Study	Ethnic group	% Frequency		P
		Patients	Controls	
Ala-73				
Ikaheino *et al*. (1994)	Finnish	92.6	62.7	*
Asahina *et al*. (1991)	Japanese	81.3	48.0	*
Roitberg *et al*. (1994)	Jewish	100.0	76.9	*
Mallon *et al*. (1997)	British	88.5	84.3	NS
Rani *et al*. (1998)	Indian	100.0	90.6	NS
Abanmi *et al*. (this study)	Saudi	92.0	88.0	NS
Asp-9				
Asahina *et al*. (1996)	Japanese	48.0	20.0	*
Nakagawa *et al*. (1992)	Japanese	63.0	20.0	*
Abanmi *et al*. (this study)	Saudi	84.0	61.3	*

*$p < 0.05$ psoriasis patients versus controls.
Abbreviations: *P*, probability (significance); NS, not significant.

favor of disease vulnerability.

Both Cw6 and Cw7 have strong association with the susceptibility to PsV in various ethnic groups (Tiilikainen *et al*., 1980; Ozawa *et al*., 1988; Rani *et al*., 1998; Enerback *et al*., 2000; Guedjonsson *et al*., 2002). Both the alleles have a unique antigen binding pocket containing alanine at position 73 and a negatively charged aspartic acid at position 9. Cw6 has also been shown to significantly influence the onset (Enerback *et al*., 1997) and clinical features (Guedjonson *et al*., 2002) of PsV. On the other hand, Cw1, which is strongly associated with PsV (Nakagawa *et al*., 1990) does not possess Ala-73 or Asp-9, indicating that another sequence might also be involved in determining susceptibility to PsV. Thus, to elucidate whether Ala-73 and/or Asp-9 are the important amino acids determining susceptibility to PsV, it is necessary to identify triggering agents and analyze T-cell clones recognizing the MHC class I-antigen complex.

We conclude that although 92% of PsV patients were

positive for Ala-73 it failed to reach the significance level due to high frequency of Ala-73 among Saudi controls as well. On the other hand, significantly high frequency of Asp-9 in PsV patients points towards its possible application as a marker for detecting susceptibility to PsV in Saudi population.

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