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# Analysis of Human Leukocyte Antigen class II Gene Polymorphism in Iranian Patients with Papillon-Lefevre Syndrome: a Family Study

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## ABSTRACT

**Background:** Papillon-Lefevre syndrome (PLS) is a rare autosomal recessive disorder characterized by palmoplantar hyperkeratosis and early development of aggressive periodontitis. Although cathepsin C (CTSC) gene mutations have been established in about 70-80% of PLS patients, it is assumed that the patients may have dysfunctioning of immune defense mechanisms. **Objective:** To assess the association of HLA class II genes and PLS. **Methods:** HLA class II genes were typed in nine Iranian PLS patients and their family members and the results were compared to 816 Iranian healthy subjects. **Results:** The results of this study revealed that DRB1\*0101 and DRB1\*0301 alleles were more frequent in PLS patients than in normal controls. However, there was no significant difference between PLS patients and normal controls. Moreover, the same haplotypes and genotype combinations were also observed in some patients and their healthy siblings. **Conclusion:** The results of this study showed no strong association between HLA class II alleles and PLS.

**Keywords:** HLA class II, Iran, Papillon-Lefevre Syndrome

## INTRODUCTION

PLS is a rare autosomal recessive disorder manifesting palmoplantar keratoderma combined with early development of aggressive periodontal inflammation which extends to both deciduous and permanent dentition (1). An increased susceptibility to infections has been reported in approximately 20% of patients with PLS (2, 3). Although painful fissures and recurrent pyogenic infections of the skin seem to be the most common medical complications (3), a number of patients with abscesses or pseudo-tumors of the liver have also been reported (4, 5).

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However the exact etiology of PLS has not yet been determined. It has been recommended that the presence of pathogens is not sufficient for the expression of PLS and the periodontal problems are the result of an improperly regulated immune response to infections (6, 7).

HLA genes encode the highly polymorphic molecules critically involved in immune responses (8). These genes are also associated with more than 100 diseases, most of which are immune-mediated (9). As exposure to infectious agents and chemicals may trigger PLS in genetically susceptible individuals, identification of genetically susceptible individuals might be helpful in attenuating the disease development by preventing exposure to environmental factors (10). Due to the important role of HLA gene products in many immune-related diseases, this study was aimed at finding a probable association between HLA class II alleles and PLS.

## MATERIALS AND METHODS

Nine patients with a confirmed diagnosis of PLS were included in this study. The patients (3 boys and 6 girls with mean age of 13 years) were from eight unrelated families and parental consanguinity was observed in six of the studied families. Blood samples were collected with informed consent from the patients and their family members (25 individuals). HLA-DQA1, DQB1, and DRB1 typing were performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method (11). Allele and haplotype frequencies were analyzed using PyPop. Significant variation of allele frequencies between PLS patients, their family members and normal controls (816 individuals) (12-16), were calculated by Chi-square and Fisher exact test using EPI6. P-values less than 0.05 were considered as significant. Corrected p-values ( $P_c$ ) were obtained by multiplying p-values by the number of alleles in each locus according to Bonferroni's correction (17).

## RESULTS

HLA class II allele frequencies in PLS patients and their family members as well as healthy controls are shown in table 1. As shown, DRB1\*0101 and DRB1\*0301 alleles were more frequent in PLS patients than in normal controls (0.167 vs. 0.038,  $p=0.031$  & 0.333 vs. 0.126,  $p=0.020$ ; respectively). However, these differences were not statistically significant when p-values were corrected by Bonferroni's correction ( $P_c=1.271$  &  $P_c=0.82$ , respectively). DRB1\*1103/04-DQA1\*0501-DQB1\*0301 and DRB1\*0301-DQA1\*0501-DQB1\*0201 were the most common haplotypes in PLS family members and normal controls. These haplotypes were also observed with high frequencies in PLS patients followed by DRB1\*0101, DQA1\*0101/02, and DQB1\*0501 haplotypes.

Distribution of HLA class II genotypes in PLS patients and their family members is shown in Table 2. In the first family the patient carried a quite similar genotype as one of her healthy brothers and the same haplotype as her another healthy brother. There were two PLS patients in the fourth family who carried the same genotype as their healthy brother. Identical genotypes were also observed in the fifth family between the patient and his healthy sister, in the sixth family between the patient and one of her healthy brothers and in the seventh family between the patient and her both healthy

sisters. In the eighth family, there was just a common haplotype between the patient and her three healthy brothers (Table 2).

**Table 1. HLA-DRB1 (a), DQA1 (b), and DQB1 (c) allele frequencies in PLS patients and their family members in comparison to normal controls**

Table 1, a							
DRB1	Normal population (F)	PLS Family members (F)	PLS Patients (F)	DRB1	Normal population (F)	PLS Family members (F)	PLS Patients (F)
0101	62 (0.038)	3 (0.060)	3 (0.167) <sup>1</sup>	1102	19 (0.012)	—	—
0102	37 (0.023)	—	—	1103/04	285 (0.175)	8 (0.160)	4 (0.222)
0103	22 (0.013)	—	—	1112	1 (0.001)	—	—
0301	206 (0.126)	14 (0.280)	6 (0.333) <sup>2</sup>	1201	12 (0.007)	—	—
0302	35 (0.021)	—	—	1202	2 (0.001)	—	—
0401	2 (0.001)	—	—	1301	101 (0.062)	—	—
0402	55 (0.034)	8 (0.160)	2 (0.111)	1302	38 (0.023)	—	—
0403	50 (0.031)	—	—	1303	15 (0.009)	—	—
0404	9 (0.006)	—	—	1304	2 (0.001)	—	—
0405	6 (0.004)	—	—	1305	13 (0.008)	—	—
0407	11 (0.007)	—	—	1401	49 (0.030)	1 (0.020)	—
0408	1 (0.001)	—	—	1403	1 (0.001)	—	—
0409	10 (0.006)	—	—	1404	3 (0.002)	—	—
0411	2 (0.001)	—	—	1405	6 (0.004)	—	—
0701	148 (0.091)	—	—	1501	84 (0.051)	5 (0.100)	1 (0.056)
0801	11 (0.007)	—	—	1502	81 (0.050)	4 (0.080)	—
0803	3 (0.002)	—	—	1503	1 (0.001)	—	—
0804	5 (0.003)	—	—	1601	43 (0.026)	1 (0.020)	—
0901	8 (0.005)	—	—	1602	65 (0.040)	6 (0.120)	1 (0.056)
1001	22 (0.013)	—	—	1605	6 (0.004)	—	—
1101	100 (0.061)	—	—	Total	1632 (1.000)	50 (1.000)	18 (1.000)

<sup>1</sup>p=0.031, Pc= 1.271; <sup>2</sup>p=0.020, Pc=0.82

Table 1, b				Table 1, c			
DQA1	Normal population (F)	PLS Family members (F)	PLS Patients (F)	DQB1	Normal population (F)	PLS Family members (F)	PLS Patients (F)
0101/02	430 (0.263)	12 (0.240)	5 (0.278)	0201	344 (0.211)	14 (0.280)	6 (0.333)
0103	217 (0.133)	8 (0.160)	2 (0.111)	0301	444 (0.272)	9 (0.180)	4 (0.222)
0201	140 (0.086)	—	—	0302	9 (0.006)	—	—
0301	172 (0.105)	7 (0.140)	1 (0.056)	0303	156 (0.096)	7 (0.140)	1 (0.056)
0401	33 (0.020)	—	—	0402	39 (0.024)	—	—
0501	638 (0.391)	23 (0.460)	10 (0.556)	0501	86 (0.053)	3 (0.060)	3 (0.167)
0601	2 (0.001)	—	—	0502	143 (0.088)	7 (0.140)	1 (0.056)
Total	1632 (1.000)	50 (1.000)	18 (1.000)	0503	90 (0.055)	1 (0.020)	—
				0601	115 (0.070)	8 (0.160)	3 (0.167)
				0602/03	158 (0.097)	1 (0.020)	—
				0604	48 (0.029)	—	—
				Total	1632 (1.000)	50 (1.000)	18 (1.000)

## DISCUSSION

Although knowledge about PLS has increased during the last decades, the cause of the clinical expression in this disease is still not fully understood. Immunological abnormalities and susceptibility to infectious agents are considered to be involved in the pathoetiology of PLS; however the link between the cutaneous and gingival manifestations is still in doubt. CTSC gene mutations have recently been suggested as responsible factors for PLS (18). Most PLS patients are homozygote for CTSC mutations (19) and clinical manifestations are present when CTSC activity is completely absent. Also asymptomatic subjects with homozygous CTSC gene mutations have been reported (20). Heterozygote carriers of

**Table 2. Distribution of HLA-DRB1, DQA1, and DQB1 alleles in PLS patients and their family members**

<b>Family 1</b>	<b>DRB1</b>	<b>DRB1</b>	<b>DQA1</b>	<b>DQA1</b>	<b>DQB1</b>	<b>DQB1</b>
P1	0402	0402	0101/02	0301	0601	0303
B	0402	0402	0101/02	0301	0601	0303
B	0402	0402	0301	0301	0303	0303
M	0402	1401	0101/02	0301	0503	0303
<b>Family 2</b>						
P2	1103/04	0301	0501	0501	0201	0301
F	1103/04	0402	0301	0501	0303	0301
M	0301	1601	0101/02	0501	0502	0201
<b>Family 3</b>						
P3	0301	0301	0501	0501	0201	0201
F	0301	1602	0101/02	0501	0502	0201
M	0301	0301	0501	0501	0201	0201
<b>Family 4</b>						
P4	0301	0101	0101/02	0501	0501	0201
P5	0301	0101	0101/02	0501	0501	0201
B	0301	0101	0101/02	0501	0501	0201
F	0301	0301	0501	0501	0201	0201
M	0101	0101	0101/02	0101/02	0501	0501
<b>Family 5</b>						
P6	1103/04	1103/04	0501	0501	0301	0301
S	1103/04	1103/04	0501	0501	0301	0301
F	1103/04	1602	0101/02	0501	0502	0301
M	1103/04	0301	0501	0501	0301	0201
<b>Family 6</b>						
P7	1501	1602	0101/02	0103	0502	0601
B	1501	1602	0101/02	0103	0502	0601
B	1501	1501	0103	0103	0601	0601
B	0402	1602	0101/02	0301	0502	0303
F	1501	1602	0101/02	0103	0502	0601
M	0402	1501	0103	0301	0601	0303
<b>Family 7</b>						
P8	1103/04	1502	0103	0501	0601	0301
S	1103/04	1502	0103	0501	0601	0301
S	1103/04	1502	0103	0501	0601	0301
F	1502	1502	0101/02	0103	0502	0601
M	1103/04	1103/04	0501	0501	0301	0301
<b>Family 8</b>						
P9	0301	0101	0101/02	0501	0501	0201
B	0301	0301	0501	0501	0201	0201
B	0301	0301	0501	0501	0201	0201
B	0301	0301	0501	0501	0201	0201

P: patient, M: mother, F: father, S: sister, B: brother

the CTSC gene mutation are clinically unaffected while symptomatic heterozygote patients have also been reported (21). Although lack of CTSC activity could possibly explain the severe periodontitis in PLS, the mechanisms leading to hyperkeratotic skin lesions are still unclear. According to different reports, a causative role for CTSC mutations in PLS has just been established in about 70-80% of the patients (19, 22). In this regard, Van Steensel and his coworkers observed no mutations in either coding or non-coding

sequences of the CTSC gene in some cases of PLS (23). Therefore, it seems that other genes may be involved in this disease in addition to CTSC. Since there are complex interactions among different cells and molecules of the immune system, the possible effect of other genetic predisposing factors in this system should also be considered in the pathoetiology of PLS. While HLA gene products play a key role in immune responses, it is important to determine if there is a correlation between HLA genes and periodontal and skin involvement in PLS. Furthermore, the roles of HLA molecules in some disorders characterized by periodontal or skin abnormalities e.g. early onset periodontitis and psoriasis have been established (24, 25). In this regard, Nitta and his coworkers suggested HLA class II antigens as possible host factors in the pathogenesis of PLS (26). In this study, HLA class II genes were considered as candidate genes. The results of this study showed that DRB1\*0101 and DRB1\*0301 were the most frequent alleles in PLS patients than in normal controls but the differences were not statistically significant (Table 1). Moreover, the same HLA class II genotypes were also observed in patients and their healthy siblings (Table 2). Therefore, other genes might be implicated in genetic susceptibility of the PLS patients. Recently, HLA genes were extended to a region with an approximate length of 8 Mbp and about 28% of their corresponding transcripts were immune related (27). However, due to strong linkage disequilibrium between the genes in HLA region, the identification of the responsible disease gene is complicated (28). Since the chance of PLS involvement in the HLA of identical siblings is not the same, other genes may also contribute to genetic susceptibility to PLS. Also, incomplete penetrance of HLA genes might be explained by epigenetic mechanisms or by environmental triggering of the disease following exposure to certain infectious agents (29, 30). Inconsistent findings between the results of this study and previous reports might be explained by HLA gene variations in different populations or the low sample size in this study. While PLS is a rare disease, the results of different studies can be helpful in meta-analyses. Although the results of this study showed no strong association between HLA class II genes and PLS, future studies in PLS as well as other periodontal and skin disorders with the same manifestations can shed more light on the genetic factors linking the periodontal and skin involvement in PLS.

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