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HLA Class II Haplotypic Association and DQCAR Microsatellite Polymorphisms in Croatian Patients with Psoriasis

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A B S T R A C T

*The purpose of the present study was to investigate polymorphism of HLA class II haplotypic associations (HLA-DRB1, -DQA1, -DQB1) and DQCAR alleles in 78 Croatian patients with psoriasis. Patients were divided into two groups according to a family history of disease and age of onset: type I (positive family history and early onset) and type II (negative family history and late onset). The difference in frequency of HLA class II haplotypic associations between type I patients and controls was observed for the following combinations: HLA-DRB1*0701, -DQA1*0201, -DQB1*02 (23.6% vs. 7.2%; p < 0.001), HLA-DRB1*0701, -DQA1*0201, -DQB1*0303 (8.5% vs. 1.3%; p = 0.0018) and HLA-DRB1*1601, -DQA1*0102, -DQB1*0502 (2.8% vs. 9.3%; p = 0.06). The difference between type II psoriasis and controls for association: HLA-DRB1*1501, -DQA1*0102, -DQB1*0602 is not significant (20.0% vs. 8.9%; p = 0.06). The significantly higher frequency of DQCAR 113bp and 119bp alleles in patients with type I psoriasis is a result of linkage disequlibrium of these alleles with both HLA-DRB1*0701 haplotypic associations. Analysis of DQCAR alleles in the HLA-DRB1*0701 haplotypic associations in patients with psoriasis vulgaris and matched controls did not reveal any difference in polymorphism of DQCAR alleles. These data suggest that HLA-DRB*0701 haplotypic combinations are associated with type I but not for type II psoriasis in the Croatian population. DQCAR polymorphism is not useful genetic marker to distinguish susceptible HLA class II haplotypic association.*

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Introduction

Psoriasis vulgaris (PV) is a chronic hyperproliferative inflammatory skin disease affecting about 2% Caucasians[1]. The etiology of psoriasis has remained unclear, but genetic[2], immunological[3] and environmental factors are suggested to play important roles in the pathogenesis of PV. Numerous serologically defined studies showed that HLA-B13, B17, B27, B39, Cw2, Cw6, Cw7, and DR7 are risk HLA antigens for psoriasis[4]. The association between psoriasis and HLA antigens supports the concept of psoriasis being a T cell-mediated autoimmune disease. On the basis of these associations two types of psoriasis vulgaris can be differentiated: i) type I, manifesting early in life and frequently affecting other family members, is associated with HLA-B13, B17, B39 and DR7; ii) type II, having a late onset and not affecting other family members, is associated weakly with HLA-B27, and Cw2[5]. The largest and most consistently reported relative risk of developing psoriasis has been its association with Cw6 and DR7[6,7].

Molecular studies have reported about association between HLA-DRB1*07-DQA *0201-DQB1*0303 and psoriasis vulgaris[8,9]. The use of HLA genes as only genetic markers in studies of disease association with HLA is not ideal because of well known linkage disequlibrium between alleles of different loci[10]. So, additional genetic markers within HLA region are needed for the better characterization of association between HLA genes and disease. Such additional markers are microsatellite loci within the HLA region[11,12]. Studies using microsatellites located in the HLA region show how to better locate a disease susceptibility gene, or how to determine more precisely numerous markers involved in the genetic susceptibility[13–15]. For example, it is well known that many autoimmune diseases are HLA -DR and -DQ associated. In some cases is not possible to establish whether the primary association is to DR or DQ alleles, or influenced by some other polymorphisms in the DR-DQ region. To help resolve such kind of problems microsatellites located in that small region between DR-DQ loci can better explain association between disease and DR or DQ alleles. The role of microsatellite markers within HLA region for the analysis of linkage disequlibrium with HLA alleles has recently become of great importance in studies of HLA-associated diseases, including insulin-dependent diabetes mellitus (IDDM)[16], celiac disease (CD)[16], idiopathic nephrotic syndrome (INS)[16], rheumatoid arthritis (RA)[17], hemochromatosis[17]. In the present study, we analyzed HLA-DRB1, -DQA1, -DQB1 haplotypic associations and the frequency of DQCAR microsatellite polymorphism in a population of Croatian patients with two types of psoriasis and controls.

Subjects and Methods

Subjects

Seventy-eight patients with chronic stable psoriasis from the Department of Dermatology, University of Rijeka, Croatia, were assigned to either type I or type II psoriasis. Fifty-three patients with positive family history and age of onset less than 30 years were grouped to type I psoriasis[9], while 25 patients with a negative family history and age of onset later than 40 years were grouped to type II psoriasis[9]. Two control groups were used: i) C1 group for HLA class II haplotypic association study was composed of 135 healthy unrelated donors from the wider area of the Croatian capital city, Zagreb; ii) C2 group for studying DRB1*07 positive haplotypic associations was formed of 44 individuals undergoing paternity testing who were positive for DRB1*07 allele.

Methods

DNA was prepared from peripheral blood lymphocytes using standard salting out protocol[18]. Controls and patients were typed for HLA class II alleles by a high--resolution polymerase chain reaction (PCR) with sequence-specific oligo probes (PCR-SSOP) method, as described by Kimura et al.[19]. PCR amplification was performed with a set of specific primers for the second exons of DRB1, DQA1 and DQB1 genes as described elsewhere. PCR products were blotted on nylon membranes, and hybridized with biotinylated probes, as specified at the 12th International Histocompatibility Workshop (IHW)[20]. Forty-two DRB1 alleles, 8 DQA1 alleles, and 13 DQB1 alleles were tested, representing the most common Caucasoid HLA class II alleles.

Amplification of DQCAR microsatellite locus was performed as previously described[21]. The forward primer was labeled at the 5' end with Cyp5, and reverse primer was not labeled[22]. After the amplification, samples were run on a standard denaturing sequencing gel (6.0% polyacrilamide) in an Automated Laser Fluorescence DNA sequencer (ALF express, Pharmacia Biotech, Uppsala, Sweden). The data were analyzed by the Fragment Manager software (Pharmacia).

The frequencies of HLA-DRB1, -DQA1, -DQB1, and DQCAR alleles and haplotypic associations were estimated by the direct counting. The HLA class II haplotypes were assigned on the basis of known linkage disequilibrium as described in previous studies[23,24]. It was presumed that the DRB1, DQA1, DQB1, and DQCAR loci have no blanks and based on this presumption, when a single HLA class II allele or DQCAR allele was observed, the individual was considered as homozygous for that allele. The significance of differences in haplotypic association frequencies was evaluated using chi-square while Fisher's exact test was used if any value in a 2x2 table was lower than five[25]. Relative risk (RR) was calculated according to Woolf[26].

Results

The distribution of HLA class II haplotypic associations

Seventy-eight psoriatic patients were tested for HLA-DRB1, -DQA1, -DQB1 haplotypic associations (Table 1). Seventeen different HLA class II haplotypic associations could be identified in a sample of 58 patients with type I psoriasis, while 12 distinct combinations of HLA class II alleles were found in a sample of 25 patients with type II psoriasis. In the group of type I psoriatic patients, the association most frequently detected was HLA -DRB1*0701, -DQA1*0201, -DQB1*02. This association was present in 22 (41.5%) patients with type I psoriasis; three of them were homozygous for this haplotypic combination. In contrast, only four (16.0%) type II psoriatic patients were positive for this association. Among control individuals only 19 out of 118 individuals were positive for this association. At the chromosomal level, the numbers were 25 (23.6%) in type I and 4 (8.0%) in type II psoriasis, compared to 7.2% in the control population. This difference in frequency is statistically significant ($p < 0.001$). The HLA-DRB1*0701, -DQA1*0201, -DQB1 *0303 haplotypic association was found in 9 patients with type I disease, while among 25 type II psoriatic patients this association was not observed. Comparison of frequency of this association between type I psoriatic patients and controls revealed statistically significant difference (8.5% vs. 1.3; $p = 0.0018$).

Some haplotypic associations, e.g., HLA-DRB1*1501, -DQA1*0102, -DQB1 *0602, HLA-DRB1*1101, -DQA1*0501, -DQB1*0301, and HLA-DRB1*1302, -DQA1 *0102, -DQB1*0604, were found to be con-

TABLE 1
THE DISTRIBUTION (NO, %) OF HLA-DRB1, -DQA1, -DQB1 HAPLOTYPIC ASSOCIATIONS IN CROATIAN PATIENTS WITH TYPE I OR TYPE II PSORIASIS AND CONTROL INDIVIDUALS

Haplotypic association (allele)			No. (%) of patients with psoriasis		No. (%) of controls (N=118)
DRB1	DQA1	DQB1	Type I (N=53)	Type II (N=25)	
0101	0101	0501	12 (11.3)	6 (12.0)	21 (8.9)
1501	0102	0602	7 (6.6)	10 (20.0)	21 (8.9)
1501	0102	0502	1 (0.9)	1 (2.0)	1 (0.4)
1601	0102	0502	3 (2.8)	3 (6.0)	22 (9.3)
1602	0102	0502	1 (0.9)	0	3 (1.3)
0301	0501	0201	6 (5.7)	4 (8.0)	18 (7.6)
04	0301	0302	8 (7.5)	4 (8.0)	16 (6.8)
1101	0501	0301	6 (5.7)	6 (12.0)	16 (6.8)
1104	0501	0301	7 (6.6)	3 (6.0)	18 (7.6)
1301	0102	0604	6 (5.7)	3 (6.0)	13 (5.5)
1302	0102	0604	1 (0.9)	4 (8.0)*	7 (3.0)
1303	0501	0301	3 (2.7)	0	3 (1.3)
1401	0101	0503	1 (0.9)	2 (4.0)	8 (3.4)
0701	0201	02	25 (23.6)***	4 (8.0)	17 (7.2)
0701	0201	0303	9 (8.5)**	0	3 (1.3)
08	0401	0401	5 (4.7)	0	2 (0.9)
1001	0101	0501	5 (4.7)	0	5 (2.1)
x	x	x			42 (17.8)

***p<0.001; **p<0.01; *p<0.05;
x = different DRB1-DQA1-DQB1 haplotypic associations not observed in patients with psoriasis

siderably more frequent in type II compared to type I psoriasis. The difference in distribution of HLA-DRB1*1101, -DQA1 *0501, -DQB1*0301 haplotypic association is not significant ($p > 0.05$), while HLA-DRB1*1501, -DQA1*0102, -DQB1 *0602 association showed marginally significant p value ($p = 0.06$). In the case of HLA-DRB1*1302, -DQA1*0102, -DQB1 *0604 the p value was significant ($p = 0.034$).

The distribution of DQCAR alleles

Nine different DQCAR alleles were detected in our patients (Table 2). The statistically significant differences were detected for DQCAR 113bp allele in type I psoriasis in comparison to controls (15.1% vs. 3.3%; $p < 0.001$), as well as for DQCAR 119bp allele (12.3 vs. 5.6%; $p = 0.036$). DQCAR 107bp allele was less present in type I psoriasis than in controls (2.8% vs. 11.9%; $p = 0.006$). Comparison of DQCAR allele frequencies between two patient groups showed difference only for DQCAR 113bp allele, but without statistically significant p value (15.1% vs. 6.0%; $p = 0.08$).

Discussion

Our data on distribution of HLA class II haplotypic associations in a sample of 87 Croatian psoriatic patients confirmed statistically significant higher frequency of HLA-DRB1*0701, -DQA1*0201, -DQB1 *02 haplotypic association in type I psoriatic patients, as well as haplotypic association HLA-DRB1*0701, -DQA1*0201, -DQB1*0303. This is in concordance with the results obtained for patients from

TABLE 2
COMPARISON OF DQCAR ALLELE FREQUENCIES (NO, %) IN A SAMPLE OF CROATIAN PATIENTS WITH PSORIASIS (N=78) AND A SAMPLE OF UNRELATED CROATIANS (N=118)

DQCAR allele	No. (%) of patients with psoriasis		No. (%) of controls
	Type I (N=53)	Type II (N=25)	(N=118)
99bp	8 (7.5)	6 (12.0)	25 (10.7)
101bp	0	0	0
103bp	32 (30.2)	22 (44.0)	86 (36.4)
105bp	5 (4.7)	2 (4.0)	1 (0.3)
107bp	3 (2.8)**	4 (8.0)	28 (11.9)
109bp	0	0	1 (0.3)
111bp	7 (6.6)	5 (10.0)	28 (11.9)
113bp	16 (15.1)***	3 (6.0)	8 (3.3)
115bp	0	0	0
117bp	6 (5.7)	0	14 (5.9)
119bp	13 (12.3)*	3 (6.0)	13 (5.6)
121bp	15 (14.1)	5 (10.0)	32 (13.7)

***p<0.001; **p<0.01; *p<0.05

Germany[9] where the association between psoriasis and both DRB1*0701 haplotypic associations were found. We calculated relative risks for those two haplotypic associations as follows: RR = 4.65 for association HLA-DRB1*0701, -DQA1 *0201, -DQB1*02 and RR = 7.84 for combination HLA-DRB1*0701, -DQA1*0201, -DQB1*0303.

To define the existence of an additional genetic marker in the region between DR and DQ genes, which would be helpful to distinguish susceptible from not-susceptible haplotypic association, we have characterized DQCAR polymorphism in DRB1*0701 haplotypic associations in two groups of psoriatic patients, as well as in healthy controls. Namely, results of our previous population study about DQCAR polymorphism showed that HLA-DRB1*0701, -DQA1*0201, -DQB1 *0201 haplotypic association is not exclusively associated with one DQCAR allele as was observed for some of the other associations[22]. has the greatest DQCAR polymorphism[22]. We found 6 different DQCAR alleles (DQCAR 111bp, 113bp, 117bp, 119bp, or 121bp) in combination with HLA-DRB1*0701, -DQA1*0201, -DQB1*0201 haplotypic association. These alleles were present with a similar frequency in a sample of 30 unrelated HLA matched controls, 25 patients with type I psoriasis and 4 patients with type II (Table 3). On the basis of results listed in Table 3 we can only speculate about protective role of DQCAR 117bp allele in type II psoriasis, because this allele was not present among patients with this type of disease, but it is necessary to confirm on a bigger sample of patients. However, our findings of significant differences in distribution of DQCAR 113bp and DQCAR 119bp alleles between patients and controls are results of linkage disequilibrium between DQCAR alleles and HLA-DRB1 *0701 haplotypic associations[27,28]. It suggests that DR genes themselves are primary involved in the pathophysiology of psoriasis what is in concordance with data from Kwok et al.[29]. Similar findings are reported for some other autoimmune diseases (celiac disease, insulin dependent diabetes mellitus)[16].

TABLE 3
THE DISTRIBUTION (NO, %) OF DQCAR ALLELES ON HLA-DRB1*0701, -DQA1*0201, -DQB1*02 HAPLOTYPIC ASSOCIATIONS IN A SAMPLE OF CROATIAN PATIENTS WITH TYPE I (N=25) AND TYPE II PSORIASIS (N=4) AND HLA MATCHED CONTROLS (N=30)[a]

DQCAR allele	No. (%) of patients with psoriasis		No. (%) of controls
	Type I (N=25)	Type II (N=4)	(N=30)
111bp	3 (12.0)	-	-
113bp	15 (60.0)	3 (75.0)	18 (60.0)
117bp	–	–	3 (10.0)
119bp	3 (12.0)	1 (25.0)	5 (16.7)
121bp	4 (16.0)	–	4 (13.3)

[a] controls are positive for HLA-DRB1*0701-DQA1*0201-DQB1*02 haplotypic associations

In conclusion, the strongest correlation in the Croatian population exists between HLA-DRB1*0701, -DQA1*0201, -DQB1*02 haplotypic association and type I but not with type II disease. DQCAR polymorphism is not useful additional genetic marker for distinguishing disease associated DRB1*0701 haplotypic association. Our findings of association between psoriasis and HLA class II molecules further support the concept of type I psoriasis being a T-cell-mediated autoimmune disease[28].

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RAZNOVRSNOST HAPLOTIPSKIH VEZA HLA RAZREDA II (HLA-DRB1, -DQA1, -DQB1) I ALELA LOKUSA DQCAR U BOLESNIKA S PSORIJAZOM U HRVATSKOJ

S A Ž E T A K

Cilj rada bio je ispitati raznovrsnost haplotipskih veza HLA razreda II (HLA -DRB1, -DQA1, -DQB1) i alela lokusa DQCAR u skupini od 78 bolesnika s psorijazom. Bolesnici su bili podijeljeni u dvije skupine obzirom na obiteljsku anamnezu, kao i na vrijeme pojave bolesti: tip I (pozitivna obiteljska anamneza i rana pojava psorijaze) i tip II (negativna obiteljska anamneza i kasnija pojava psorijaze). Razlike u raspodjeli haplotipskih veza između bolesnika s tipom I psorijaze uočene su za slijedeće veze: HLA-DRB1*0701, -DQA1*0201, -DQB1*02 (23.6% vs. 7.2%; $p < 0.001$), HLA-DRB1*0701, -DQA1*0201, .DQB1*0303 (8.5% vs. 1.3%; $p = 0.0018$) i HLA-DRB1*1601, -DQA1*0102, -DQB1*0502 (2.8% vs. 9.3%; $p = 0.06$). Uočena razlika u raspodjeli haplotipske veze HLA-DRB1*1501, -DQA1*0102, -DQB1*0602 između skupine bolesnika s tipom II psorijaze i kontrole nije statistički značajna (20.0% vs. 8.9%; $p = 0.06$). Statistički značajno povišena učestalost alela DQCAR 113bp i 119bp posljedica je neravnoteže udruživanja tih alela s obje HLA-DRB1*0701 haplotipske veze. Analiza raznovrsnosti DQCAR alela unutar HLA-DRB1*0701 haplotipskih veza nije pokazala nikakvu razliku između bolesničke i kontrolne skupine. Naši rezultati ukazuju da su obje HLA-DRB1*0701 haplotipske veze u Hrvatskoj povezane s tipom I psorijaze, ali ne i s tipom II.