

Association of HLA-DRB1, DQB1 Alleles with Chronic Urticaria

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Summary: In order to investigate the association of genotypes of HLA-DRB1 and HLA-DQB1 alleles with the genetic susceptibility of chronic urticaria (CU), genotypes of HLA-DRB1 and HLA-DQB1 genes were detected by polymerase chain reactions with sequence-specific primers (PCR-SSP) in 42 patients with CU (19 men and 23 women, mean age 30.67 ± 12.45 y old as well as 193 racially matched healthy persons in ethnic Han from Hubei province. Gene frequencies of HLA-DRB1 * 12, * 0901 ($RR=3.11$, $\chi^2=7.579$, $P=0.006$; $RR=2.47$, $\chi^2=5.684$, $P=0.017$) were significantly increased in CU patients as compared with that in healthy people. Gene frequencies of HLA-DQB1 * 05 ($RR=0.26$, $\chi^2=6.683$, $P=0.01$) were significantly decreased in CU patients. It was suggested that CU was found strongly associated with HLA-DRB1 * 12, * 0901 and HLA-DQB1 * 05, the former might be the genetic markers for susceptibility to CU, but the latter might play a resistive role.

Key words: urticaria, chronic; genes, MHC class II; polymerase chain reaction-sequence specific primer

Chronic urticaria (CU) is a common disease in the Department of Dermatology, so far we have no idea of the etiology and the exact mechanism of CU. Recent researches has focused primarily on an autoimmune basis. Hide^[1] *et al* reported that, demonstration of the functionally important IgG antibodies directed to the a subunit of the high affinity IgE receptor (Fc ϵ RIa) on basophils is the most direct evidence of autoimmune process, like found in CU. Genetic background is thought to be a relevant factor in autoimmune diseases. A recent study in a large population of CU patients showed a marked increase in prevalence of the disease among first-degree relatives, which suggested that the episode of CU was associated with genetic factors^[2]. In this study, polymerase chain reactions with sequence-specific primers (PCR-SSP) was used to analyze gene polymorphism of HLA-DRB1 and HLA-DQB1 alleles in patients with CU and healthy controls of ethnic Han population in Hubei province. The aim of this research was to find the correlation between CU and HLA-II, and to further discuss the immunological disorder mechanism of CU.

1 MATERIALS AND METHODS

1.1 Patients

Forty-two patients (19 men, 23 women; age 8—67 y old, with a mean age of 30.67 ± 12.45) were people who had no relationship one another in ethnic Han (they were followed up between 2002 and 2004 in our hospital). In addition, they had urticarial lesions of unknown etiology for more than 6 weeks, and this symptom occurred almost

everyday. None of the patients had features for vasculitic urticaris like abdominal or joint pain, persistent lesions for more than 24 h, increase in erythrocyte sedimentation rate or decrease in complement levels. The control population, comprising 193 unrelated Chinese subjects, was drawn from health examination, with no personal or family history of CU.

1.2 Materials and Specimen Collection

1.2.1 Reagents PCR primers were provided by Boya Biological Techniques Co., Ltd. (China). Taq polymerase was purchased from Promega Corporation (USA), deoxyribonucleoside triphosphate from Pharmacia (Sweden), Protease K from amresco (USA), and Agarose from Oxoid., Ltd. (England).

1.2.2 Collection and Preservation of Specimens

400 μ l blood containing EDTA as anticoagulant was taken from the recipient. The genome DNA of peripheral blood lymphocytes was extracted with phenol/chloroform by the conventional method. The yield and purity of DNA were finally quantified by an ultraviolet spectrophotometer (Pharmacia Biotech RNA/DNA calculator,). The A_{260}/A_{280} of them was between 1.6—1.8.

1.3 Methods

The primers to aim directly at gene polymorphism in exon 2 of HLA-DRB1 and HLA-DQB1 were designed^[3, 4]. The amplified products had the specificity of corresponding alleles. The components of PCR system were as follows: the final volume of each reaction was 10 μ l, consisting of 0.5 μ mol/L each allele-specific primer and 0.2 μ mol/L internal control primers, 200 μ mol of each deoxyribonucleoside triphosphate, 2 $\times$ PCR buffer (10 mmol/L Tris base, pH 8.3, 50 mmol/L potassium chloride, 1.5 mmol/L magnesium chlo-

ride, 0.01 % gelatin), 0.5 units of Taq polymerase, 50–100 ng DNA. Cycling parameters employed in PTC100 DNA Engines (MJ Research, USA) were as follows: 94 °C for 5 min followed by 30 s at 94 °C, one min at 65 °C, then one min at 72 °C, to add up to 30 cycles. After PCR amplification, the contents of each reaction were electrophoresed on 2 % agarose gels containing ethidium bromide and visualized using ultraviolet irradiation.

1.4 Statistical Analysis

By counting directly, the frequency of allele was calculated. All statistical analyses were performed by using SPSS(11.0) for Windows. The χ^2 -test were employed for data evaluation. If the theoretical value (T) was less than 5, correction for χ^2 -test was used. If the T was less than 1, exact probabilities way (Fisher) was used. Relative

risk was calculated by Woolf formula. A $P < 0.05$ was considered to be statistically significant.

2 RESULTS

2.1 Association of HLA-DRB1 Alleles with CU

Gene frequencies of HLA-DRB1 * 12, * 0901 were significantly increased in CU patients as compared with those in healthy people and the difference was statistically significant ($RR = 3.11$ and 2.47 respectively, $P < 0.05$, table 1).

2.2 Association of HLA-DQB1 Alleles with CU

Gene frequencies of HLA-DQB1 * 05 were significantly decreased in CU patients as compared with those in healthy people and the difference was statistically significant ($RR = 0.26$, $P < 0.05$, table 2).

Table 1 Frequency (percentage) of HLA-DRB1 alleles in CU and control population

HLA-DRB1	CU group (n=42)		Control group (n=193)		χ^2	P	RR
	N	GF	N	GF			
DRB1 * 01	1	0.012	19	0.049	1.531	0.216	0.22
DRB1 * 0301	3	0.036	28	0.073	1.405	0.236	0.45
DRB1 * 04	12	0.143	42	0.109	0.786	0.375	1.44
DRB1 * 07	4	0.048	29	0.075	0.800	0.371	0.60
DRB1 * 08	7	0.083	31	0.080	0.008	0.927	1.05
DRB1 * 0901	20	0.238	52	0.135	5.684	0.017	2.47
DRB1 * 1001	0	0	14	0.036	2.011	0.156	0
DRB1 * 11	6	0.071	24	0.062	0.099	0.753	1.17
DRB1 * 12	12	0.143	22	0.057	7.579	0.006	3.11
DRB1 * 13	2	0.024	28	0.073	2.741	0.098	0.29
DRB1 * 14	2	0.024	19	0.049	0.533	0.465	0.46
DRB1 * 15	13	0.155	56	0.145	0.052	0.820	1.10
DRB1 * 16	2	0.024	22	0.057	0.958	0.328	0.39

GF: Gene frequency; RR: Relative risk

Table 2 Frequency (percentage) of HLA-DQB1 alleles in CU and control population

HLA-DRB1	CU group (n=42)		Control group (n=193)		χ^2	P	RR
	N	GF	N	GF			
DQB1 * 02	7	0.083	37	0.096	0.127	0.721	0.84
DQB1 * 04	10	0.119	24	0.062	3.325	0.068	2.20
DQB1 * 0	55	0.060	66	0.171	6.683	0.010	0.26
DQB1 * 06	23	0.274	87	0.225	0.902	0.342	1.47
DQB1 * 0301/4	20	0.238	74	0.092	0.928	0.335	1.46
DQB1 * 0302	5	0.060	31	0.080	0.421	0.516	0.71
DQB1 * 0303	14	0.167	67	0.174	0.023	0.879	0.94

3 DISCUSSION

A growing body of evidence shows that CU have autoimmune basis. In 1993, Hide^[1] *et al* reported first time that the serum of about 25 % of the patients with CU had autoantibodies against a subunit of the high affinity IgE receptor (Fc ϵ RIa)

and in addition a 5 % of the patients had autoantibodies against IgE. It is well known that patients with one autoimmune disease are at higher risk for having a second autoimmune disease. Anti-thyroglobulin or anti-thyroid peroxidase antibodies can be detected in 25 %–30 % of CU patients^[5] and no obvious explanation for this association has been proposed. The HLA typing of large group of

patients with various autoimmune diseases has demonstrated that some HLA alleles occur at higher frequencies in specific diseases than in the general population. In overseas literatures, several HLA studies were found on urticaria related diseases such as hereditary angioedema, mastocytosis, familial exercise-induced anaphylaxis and dermatographism^[6-10]. However, only two studies have already been performed on the association between CU and HLA^[11, 12].

Our investigation showed that CU in ethnic Han from Hubei province was strongly associated with HLA-DRB1 * 12, * 0901 and HLA-DQB1 * 05, which is different from the results of Europe. They believed CU was related to HLA-DRB1 * 04, * 15 and HLA-DQB1 * 08, * 06^[11, 12]. In our opinion, in terms of the allele of HLA, there is distribution frequency differences in races, regions and groups (gene frequency, linkage disequilibrium, different combinations of cis-acting and trans-acting between chain α and chain β , and interaction between DR-DQ). This is the main reason. Some data showed there was obvious correlation between Graves disease (GD) and HLA-DRB1 * 09, HLA-DQB1 * 0303 in Chinese from Hongkong^[13, 14], which was also different from the results of Europe. They believed GD was associated with HLA-DQA1 * 0501^[15]. Our study showed that the gene frequency of HLA-DRB1 * 0901 were significantly increased, whether there is genetic background in common between CU and GD or not is that we have to make a further research.

A considerable number of studies have shown that particular HLA class II alleles are the risk factors for or protective factors against the development of sensitization to specific allergens. This is suggested as being due to the following reasons^[16, 17]: (1) HLA molecules may act as receptors for viruses and toxins which may lead to disease onset. (2) Some HLA molecules may be selective for the disease agent's peptide and people carrying these HLA molecules are at risk for that disease. (3) HLA molecules and some etiological agents may have similar antigenic determinants.

The importance of HLA associations in mechanisms of autoimmunity is not completely clear. Many questions still remain to be answered in CU such as the autoimmune process, temporary spontaneous remissions, the genetic heterogeneity of the patients and other genetic or environmental factors and further studies with larger population from different parts of the world are still needed.

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