HLA-DRB1 Alleles and Henoch-Schönlein Purpura: Susceptibility and Severity of Disease

OGUZ SOYLEMEZOGLU, HARUN PERU, SEVIM GONEN, AYSUN CETINYUREK, and NECLA BUYAN

ABSTRACT. ***Objective.*** The genetic basis of susceptibility to Henoch-Schönlein purpura (HSP) may be conferred by a number of gene loci, including the MHC. Associations between human leukocyte antigen (HLA) and disease can help to establish a basis for susceptibility and assist in the prediction of the outcome and clinical heterogeneity. We aimed to investigate the implications of the HLA-DRB1 locus and the susceptibility to HSP, and to determine if there are associations with joint, gastrointestinal, and renal manifestations of the disease.

Methods. We studied 110 Turkish patients (men/women: 66/44) with HSP. Patients and ethnically matched controls with respect to age and sex (n = 250) were HLA-DRB1 genotyped from DNA determined using molecular based methods.

Results. HLA-DRB1 genotype differences between patients with HSP and controls were observed. The frequency of HLA-DRB1*11/14 was higher [odds ratio (OR) 1.97, 95% confidence interval (95% CI) 1.25–3.12, p = 0.003; OR = 1.83, 95% CI = 1.02-3.28, p = 0.035, respectively] and the frequency of HLA-DRB1*10/17 was lower (OR = 1.04, 95% CI = 1.01–1.86, p = 0.035; OR = 3.96, 95% CI = 1.17–13.33, p = 0018, respectively) in patients with HSP compared to controls. No HLA-DRB1 associations with gastrointestinal and renal manifestations were found (p > 0.05). In contrast, HLA-DRB1*11 positivity was increased and HLA-DRB1*14 positivity reduced in HSP patients with joint manifestations (OR = 2.68, 95% CI = 1.09–6.66, p = 0.029; OR = 9.34, 95% CI = 3.38–25.64, p = 0.000, respectively). Also, HLA-DRB1*13 positivity was found to be increased in patients with nephrotic proteinuria (OR = 3.76, 95% CI = 1.25–11.23, p = 0.025).

Conclusion. These results suggest that genetic factors from HLA-DRB1 genotypes might be related to the susceptibility to HSP for Turkish children but not to the severity of this disease. Additional studies are required to confirm the association of alleles encoded in the HLA region with the disease progression and severity. (First Release April 15 2008; J Rheumatol 2008;35:1165–8)

Key Indexing Terms:
HENOCH-SCHÖNLEIN PURPURA HUMAN LEUKOCYTE ANTIGEN SUSCEPTIBILITY

Henoch-Schönlein purpura (HSP) is the most common vasculitic syndrome in childhood. This vasculitis may affect several organ systems, but mainly affects the skin, joints, gastrointestinal (GI) tract, and kidneys. Longterm morbidity is almost entirely due to renal disease[1-3]. The pathogenesis of HSP remains unknown; however, it is generally considered to be an immune complex-mediated disease[4-6]. Hence, MHC genes may contribute to the susceptibility to this disease. Although it has been considered that immunogenetic factors have a role in the etiopathogenesis of vasculitic syndromes, a specific genetic abnormality has not been reported yet.

Associations between human leukocyte antigens (HLA) and disease can help to establish a basis for susceptibility and assist in the prediction of the outcome and clinical heterogeneity. Jin, *et al*[7] suggested that presence of decreased complement C4 due to complement C4 gene deletion increased the susceptibility to HSP. Presence of HLA-DRB1*01 or HLA-DRB*11 was found to be a factor for susceptibility to disease, and HLA-DRB1*07 allele was reported as a risk-decreasing allele for susceptibility to HSP[8]. Amoli, *et al*[9] showed that HLA-B alleles were not associated with GI manifestations, whereas an increased frequency of HLA-B35 allele was observed in patients with renal manifestations compared to those without. In contrast, in a previous study[10], we reported that HLA-A2, A11, and B35 antigens are associated with the increased susceptibility to HSP. However, increased HLA-B35 positivity had no effect on the severity of renal involvement and proteinuria.

Clinical, laboratory, and genetic risk factors in high-risk groups were investigated to determine the longterm progno-

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sis in limited studies[7-11]. There have been quite a few studies on the role of HLA antigens in children with HSP in different populations. Our study was performed to investigate the implications of the HLA-DRB1 phenotypes in the susceptibility to HSP that is very common in the Turkish population, and to determine if there were associations with clinical findings and severity of disease.

MATERIALS AND METHODS

Patients. The study was performed between January 2003 and May 2005. One hundred ten patients with a clinical diagnosis of HSP based on American College of Rheumatology criteria[12] were enrolled. The control group consisted of 250 children from different families who were bone marrow transplant donors, and the group was age-matched with the patient group. Organ involvement of patients with the diagnosis of HSP was classified as skin, joint, GI, and renal involvement. The joint, GI, and kidney symptoms were scored using a grading scale ranging from 0 to 3 (Table 1)[13]. Patients were divided into 2 groups according to their clinical scores [high clinical score (HCS) group: patients with clinical scores ≥ 4; and low clinical score (LCS) group: patients with clinical scores < 4]. We also investigated the HLA-DRB1 alleles in HSP patients with and without renal involvement.

Erythrocyte sedimentation rate, blood urea nitrogen, creatinine, C-reactive protein, IgA, C3 and C4 levels, urinalysis, and stool blood analysis were determined in every subject. 24 h urine was collected from patients who had erythrocytes and/or protein in spot urine tests and creatinine clearance and daily protein excretion were calculated. In the first 2 weeks of disease, which is defined as the acute period, renal biopsy was performed on patients who had nephrotic proteinuria and/or nephrotic syndrome and acute renal failure. If there was diffuse and persistent abdominal pain with or without bloody diarrhea, bowel angina was considered. GI bleeding was defined as the presence of melena, hematochezia, or a positive test for occult blood in the stool.

HLA-DRB1 typing. HLA Class II tissue antigens were studied in the tissue typing laboratory of our hospital. Three-milliliter blood samples were taken from all patients and the control group. HLA-DRB1 typing was performed on DNA extracted from anticoagulated blood collected in EDTA using a commercial DNA extraction kit (Qiagen, Valencia, CA, USA). The samples were kept at –70°C. All patients with HSP and healthy individuals were typed for HLA-DRB1 at the DNA sequence level. Genetic typing of HLA-DRB1 polymorphism was performed using polymerase chain reaction (PCR) with sequence-specific primers. A total of 24 sequence-specific oligonucleotide primers were used to identify 23 A types (Olerup SSP AB, Saltsjobaden, Sweden). Using the Perkin Elmer 9600 thermal cycler, 1 cycle of incubation at 94°C (2 min), 10 cycles at 94°C (10 s), 10 cycles at 65°C (1 min), 20 cycles at 94°C (10 s), 20 cycles at 61°C (50 s), and 20 cycles at 72°C (30 s) were performed. The PCR products were resolved by electrophoresis in 2% agarose gel, stained with ethidium bromide, and visualized and photographed under ultraviolet light.

Statistics. Statistical analysis was performed by using SPSS for Windows Version 12.0. All results were presented as mean ± standard deviation (SD). Significance levels were determined using contingency tables by either chi-square test or Fisher's exact analysis. Statistical significance was defined as $p \leq 0.05$. Hardy-Weinberg equilibrium was tested by grouping the allele frequencies that were < 5. The strength of association between HSP and HLA-DRB1 allele frequency was estimated using odds ratios (OR) and corresponding 95% confidence intervals (95% CI).

Table 1. Clinical scores to assess the degree of organ involvement during the acute phase of Henoch-Schönlein purpura (HSP).

Organ Involvement	Degree of Severity
Joint	0 = no symptoms 1 = pain and/or swelling of slight grade 2 = pain and/or swelling of moderate grade 3 = pain and/or swelling of severe grade
Gastrointestinal	0 = no symptoms 1 = slight pain and/or occult blood in the stool 2 = moderate pain and/or occult blood in the stool (+2, +3) 3 = severe pain and/or melena
Kidney	0 = no proteinuria and/or hematuria 1 = proteinuria < 40 mg/m^2 per hour and/or hematuria 2 = proteinuria 40–80 mg/m^2 per hour and/or hematuria 3 = proteinuria > 80 mg/m^2 per hour and/or hematuria

RESULTS

The mean age of the children with HSP was 8.65 ± 3.59 years. Of 110 children with HSP, 66 were boys and 44 were girls. Male to female ratio was 1.5. The mean age of the control group was 10.25 ± 2.75 years. Of 250 children in the control group, 141 were boys and 109 were girls. Male to female ratio was 1.3. All of the patients presented with the purpuric rash and 32.7% showed renal involvement. Mean age of 36 patients with HSP nephritis was 9.00 ± 3.26 years (24 boys, 12 girls). Nineteen (52.8%) of these 36 patients had nephrotic and 17 (47.2%) had non-nephrotic proteinuria. Demographic and clinical features of patients with HSP, HCS, and LCS are presented in Table 2.

There were no differences in the mean age and sex of patients with HCS and LCS. Also no difference was observed regarding sex between nephrotic and non-nephrotic patients with HSP ($p = 0.320$). In patients with HCS and LCS, significant differences were seen regarding the nephrotic and non-nephrotic proteinuria ($p < 0.001$).

When HLA Class II antigens of patients with HSP and controls were compared, there were statistically significant differences in the frequencies of HLA-DRB1*10, DRB1*11, DRB1*14, and DRB1*17 antigens. HLA-DRB1*11 and DRB1*14 antigens were found to be more common in patients than in controls, while HLA-DRB1*10 and DRB1*17 antigens were less common in patients compared to controls (Table 3). HLA-DRB1*11 and DRB1*14

Table 2. Demographic and clinical features of patients with HSP, HCS, and LCS.

	HSP, n = 110	HCS, n = 28	LCS, n = 82
Sex (male/female)	66/44	15/13	51/31
Age, yrs	8.65 ± 3.59	9.75 ± 3.32	8.26 ± 3.47
Joint involvement, n (%)	82 (74.5)	26 (92.8)	56 (68.2)
GI involvement, n (%)	58 (52.7)	23 (82.1)	35 (42.6)
Renal involvement, n (%)	36 (32.7)	25 (89.2)	11 (13.4)

HCS: high clinical score; LCS: low clinical score; GI: gastrointestinal.

Table 3. Frequency of HLA-DRB1 antigens in patients with HSP and control group.

Antigens	Control (250), n (%)	Patient (110), n (%)	p (OR, 95% CI)
DRB1*01	31 (12.4)	11 (10)	0.595
DRB1*04	78 (31.2)	31 (28.2)	0.619
DRB1*07	39 (15.6)	21 (19.1)	0.444
DRB1*08	14 (5.6)	2 (1.8)	0.164
DRB1*09	8 (3.2)	—	0.113
DRB1*10	10 (4)	—	0.035 (1.04, 1.01–1.86)
DRB1*11	84 (33.6)	55 (50)	0.003 (1.97, 1.25–3.12)
DRB1*12	13 (5.2)	2 (1.8)	0.164
DRB1*13	42 (16.8)	21 (19.1)	0.652
DRB1*14	33 (13.2)	24 (21.8)	0.035 (1.83, 1.02–3.28)
DRB1*15	42 (16.8)	14 (12.7)	0.349
DRB1*16	23 (9.2)	6 (5.5)	0.295
DRB1*17	25 (10)	3 (2.7)	0.018 (3.96, 1.17–13.33)
DRB1*18	11 (4.4)	5 (4.5)	1.000

HLA: human leukocyte antigen.

antigens showed an increased risk for predisposition to HSP (OR = 1.97, 95%CI = 1.25–3.12, p = 0.003; OR = 1.83, 95% CI = 1.02–3.28, p = 0.035, respectively), while HLA-DRB1*10 and DRB1*17 antigens revealed decreased risk for predisposition to HSP (OR = 1.04, 95% CI = 1.01–1.86, p = 0.035; OR = 3.96, 95% CI = 1.17–13.33, p = 0.018, respectively).

No HLA-DRB1 associations with GI and renal manifestations were observed (p > 0.05). In contrast, HLA-DRB1*11 positivity showed a significant increase and HLA-DRB1*14 showed a significant decrease in HSP patients with joint manifestations (OR = 2.68 95% CI = 1.09–6.66, p = 0.029; OR = 9.34, 95% CI = 3.38–25.64, p = 0.000, respectively). Also, HLA-DRB1*13 frequency increased in patients with nephrotic proteinuria (OR = 3.76, 95% CI = 1.25–11.23, p = 0.025). When antigen frequencies were assessed in patients with HCS and LCS, no significant differences were observed.

DISCUSSION

The genetic basis of susceptibility to HSP may be conferred by a number of gene loci, including the MHC[11]. The implication of HLA Class II genes in the susceptibility to systemic vasculitis has been described. Recent reports have confirmed the previously reported association of giant cell arteritis with HLA-DRB1*04 alleles[14-18]. Moreover, the risk of visual complications was also associated with HLA-DRB1*04 alleles[19]. Our study was performed to investigate whether HLA Class II antigens had an effect on the susceptibility, severity, and clinical heterogeneity of the disease.

There have been studies from different parts of the world regarding the HLA Class II antigens association with HSP in children. It has been reported that HLA-DQA1*301, DRB1*01, and DRB1*11 are associated with susceptibility to HSP, while HLA-DRB1*07 may be a protective allele against the development of this small-vessel vasculitis[7,8]. Increased susceptibility to HSP was found in Italian and Spanish patients who were positive for HLA-DRB1*01. However, it had been postulated that HLA-DRB1*01 was not a genetic factor associated with the severity of disease[8,11]. In our study, when HLA Class II antigens of patients with HSP and the control group were compared, statistically significant differences were found in the frequencies of HLA-DRB1*10, DRB1*11, DRB1*14, and DRB1*17 antigens. Compared with the control group, lower frequency of HLA-DRB1*10 and DRB1*17 antigens and higher frequency of HLA-DRB1*11 and DRB1*14 antigens were detected in patients with HSP. These results were statistically significant and revealed that HLA-DRB1*10 and DRB1*17 antigens are associated with the decreased relative risk of susceptibility to this disease, while DRB1*11 and DRB1*14 antigens are associated with increased relative risk of disease occurrence. Although there is a discrepancy with the Italian and Spanish patients and our patients regarding the HLA-DRB1 antigens and susceptibility, there are also some reports describing the HLA-DRB1*11[8]. Ethnic differences in various populations may explain this discrepancy.

There are only a few studies reporting the effect of antigenic frequency on clinical symptoms and severity of HSP. Although conflicting results have been reported, the possible involvement of HLA Class I polymorphism in HSP has also been examined[8-10,20-22]. Amoli, *et al*[9] found a significant relation between the severity of renal involvement and HLA-B35 antigen, but HLA-B35 antigen positivity had no effect on susceptibility to this disease. Glass, *et al*[20] reported an association between HLA-B35 and cutaneous vasculitis in the setting of other autoimmune diseases. In 1990, Ostergaard, *et al*[21] observed no association between HLA Class I antigens and HSP. In our previous study[10], we found no significant difference between the antigen frequencies of patients with nephrotic and non-nephrotic proteinuria in our patient population. However, HLA-A3 and B44 antigen positivity were significantly higher in patients with joint involvement, but not in patients with GI and renal involvement.

As for other vasculitides, there is scant information on the implication of HLA-DRB1 in the susceptibility to HSP. Increased susceptibility to HSP was found in Italian and Spanish patients who were positive for HLA-DRB1*01[8,11]. However, HLA-DRB1*01 was not a genetic marker for disease severity. In our study, no HLA-DRB1 associations with GI and renal manifestations were observed (p > 0.05). In contrast, HLA-DRB1*11 was increased and HLA-DRB1*14 was reduced in HSP patients with joint manifes-

tations (OR = 2.689, 95% CI = 1.091–6.66, p = 0.029; OR = 9.34, 95% CI = 3.38–25.64, p = 0.000, respectively). When antigen frequencies were assessed, there were no significant differences in patients with HCS and LCS. However, HLA-DRB1*13 was found to be increased in patients with nephrotic proteinuria (OR = 3.76, 95% CI = 1.25–11.23, p = 0.025). Freycon, *et al*[23] evaluated HLA Class I and II antigens in 121 children with rheumatoid purpura who had nephropathy or no nephropathy. HLA-B35 antigen frequency was found to be 38.2% in patients with nephropathy and 20.4% in patients without nephropathy, revealing a significant difference. On the other hand, HLA-B35 and DR4 association was significantly higher in patients with rheumatoid purpura with or without nephropathy than in controls.

Our study shows that the increased frequency of HLA-DRB1*11/14 alleles in an unselected pediatric HSP patient population and miscarrying of HLA-DRB1*10/17 could be considered as a risk factor for susceptibility to HSP. However, there was no significant difference regarding HLA-DRB1 genotypes between the HCS and LCS groups. These results suggest that genetic factors from HLA-DRB1 genotypes might be related to the susceptibility of Turkish children to HSP, but not to the severity of this disease. While some of our results were similar to the results of previous reports, some results were different. It is possible that different results arise as a result of genetic, racial, regional, or environmental factors. We think that every ethnic population should perform studies to determine their own associations of HLA antigens with the disease. Additional studies are required to confirm the association of HSP alleles encoded in the HLA region and confirm which locus is primarily associated with disease susceptibility and severity.

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