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Association between HLA-DR B1 and clinical features of Adult Onset Still's Disease in Korea

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ABSTRACT

Objective. *To determine whether HLA-DR alleles are associated with the development and clinical features of Adult Onset Still's Disease (AOSD) in Korea.*

Methods. *Forty-seven patients (41 women, 6 men, mean age at diagnosis 31.6 yr) meeting Yamaguchi's criteria for AOSD and 144 healthy controls were enrolled in this study. The patients with AOSD were subdivided into groups according to their chronicity: monocyclic systemic, polycyclic systemic, and chronic destructive type, and were furthermore classified as non-articular, oligoarticular or polyarticular types (having arthritis involving 5 or more joints) according to the extent of articular involvement. HLA-DRB1 genotypes were assessed by PCR-SSOP.*

Results. *Patients with AOSD had more frequent DRB1*12 (p = 0.028, relative risk (RR) = 2.27, 95% confidence interval (CI): 1.08-4.80) and DRB1*15 (p=0.013, RR=2.16, 95% CI: 1.17-4.00). They had less frequent DRB1*04 (p=0.006, RR=0.35, 95% CI: 0.16-0.75) compared to controls. DRB1*14 (p=0.011, RR=3.80, 95% CI: 1.27-11.31) were associated with the monocyclic systemic type.*

Conclusion. *Korean AOSD patients had more frequent DRB1*12 and DRB1*15, and less frequent HLA-DRB1*04. The patients with the monocyclic systemic type had more frequent DRB1*14 alleles. This study suggests that Korean AOSD patients have distinct immunogenetic profiles, and that it would be valuable to assess the relationships between HLA-DRB1 genes and polymorphisms of proinflammatory cytokines in the pathogenesis of AOSD.*

Introduction

Adult Onset Still's Disease (AOSD) is a systemic inflammatory disease characterized by high fever, sore throat, typical Still's rash, and arthralgia or arthritis. AOSD is highly heterogeneous in terms of its clinical expression and may include neutrophilic leukocytosis, non-specific hyperferritinemia, negative results for rheumatoid factor and antinuclear antibody, serositis, splenomegaly and hepatic dysfunction. The etiology of AOSD is not precisely known. There have been some reports on its association with infections, environmental factors, immune reactions and genetic factors. There are sporadic case reports suggesting an association with infective organisms including viruses, Toxoplasma gondii and Yersinia enterocolitica (1-3). Active stage AOSD patients have high levels of interleukin-6 (IL-6), interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), TNFR2 and IL-18 (4, 5). There is some evidence for the role of Fc gamma receptors (FcγRs) in the pathogenesis of AOSD (6). These results support the hypothesis that immune systems are involved in pathogenesis of AOSD.

Studies of genetic predisposition in AOSD have limitations and have shown inconsistent results. This may be due to the small number of patients studied given the rarity of disease, heterogeneous clinical expression, possible unknown inter-racial genetic backgrounds, different diagnostic criteria applied and study biases. Study of the possible association of AOSD and HLA molecules has shown inconsistent data; HLA-B17, B18, B35, Bw35, Cw4, DR2, DR4, DR5, DR7, DQ1 were reported to be increased in AOSD (5, 7-10). FcγR IIa, IIIa polymorphisms were not associated with the development of AOSD in Korean (6). In this study we examined the association of HLA-DRB1 genotypes with AOSD in 47 Korean patients with AOSD, and compared our results with the data in the literature.

Patients and methods

Forty-seven Korean patients fulfilling Yamaguchi's criteria (11) were recruited from four university-hospital based rheumatology departments throughout the country who were being followed between August 1990 and November 2001. The controls were 144 disease-free Korean medical students and laboratory workers in Hanyang University Medical Center. We reviewed the medical records retrospectively to collect clinical data, and laboratory data at diagnosis and follow-up. HLA-DRB1 genotyping was performed using the

patient's genomic DNA after receiving informed consent.

We classified the disease course into three categories. The monocyclic-systemic type was defined as an initial, single bout of systemic disease of variable duration followed by complete remission, and the polycyclic-systemic type as 2 or more bouts of systemic disease. The chronic-destructive type was defined as recurrent systemic disease followed by bony erosion and deformities. The patients were also divided into 3 types according to the extent of arthritis: the non-articular type, the oligoarticular type involving 4 or less joints, and the polyarticular type with involvement of 5 or more joints.

HLA-DRB1 typing

HLA-DRB1 typing was performed using the polymerase chain reaction-sequence specific oligonucleotide probe (PCR-SSOP) method, which was essentially the same as that described at the 12th International Workshop with minor modifications (12). Specific HLA-DRB1 primers were used to amplify the DRB1 products, which were then denatured and immobilized on a nylon membrane and probed with a series of digoxigenin labeled oligonucleotides specific for the known hyper-variable sequences. Stringent washing was performed in the presence of tetramethyl ammonium chloride (TMAC, Sigma chemical Co., St. Louis, USA). The hybridized probe was detected according to the manufacturer's instructions with the anti-digoxigenin antibody conjugated with alkaline phosphatase and followed by the addition of chemiluminescent substrate CSPD (Boehringer Mannheim, GmbH, Germany). Chemiluminescence was detected by exposure to X-ray film.

Statistical analysis

The Chi-square test was used for comparison of the non-parametric data and Fisher's exact test was used when the numbers were small. For comparison of the parametric data, Student's t-test was used. We employed the SPSS for Windows version 11.0 software program.

Results

Clinical characteristics of AOSD

Of 47 patients, 41 (87.2%) were women. The mean age at diagnosis was 31.6 ± 12.6 yr. The mean duration from the initial manifestation to diagnosis was 12.0 ± 16.8 months and the duration of follow-up was 36.4 ± 33.1 months. Clinical manifestations were fever in 97.8%, myalgia in 97.7%, and arthritis in 84.4%.

In terms of the disease course, 24 patients (51.1%) had the polycyclic-systemic type, 19 (40.4%) had the monocyclic-systemic type, and 4 (8.5%) had the chronic-destructive type. With regard to the extent of articular involvement, 22 (46.8%) had the polyarticular, 19 (40.4%) the oligoarticular and 6 (12.8%) the non-articular type (Table I).

Association of the HLA-DRB1 genotype with AOSD

AOSD patients had higher frequencies of DRB1*12 (p=0.028, RR=2.27, 95% CI:1.08-4.80) and DRB1*15 (p=0.013, RR=2.16, 95% CI: 1.17-4.00), and a

Table I. Clinical characteristics of Adult Onset Still's Disease.

Characteristics		
Sex ratio (F:M)		6.83 (41:6)
Mean age (years)		31.6 ± 12.6
Time to diagnosis (months)		12.0 ± 16.8
Follow up duration (months)		36.4 ± 33.1
Disease type (n = 47)	Monocyclic systemic (%)	19 (40.4)
	Polycyclic systemic (%)	24 (51.1)
	Chronic destructive (%)	4 (8.5)
Articular type (n = 47)	Nonarticular (%)	6 (12.8)
	Oligoarticular (%)	19 (40.4)
	Polyarticular (%)	22 (46.8)

Table II. Distribution of HLA-DRB1 alleles in AOSD patients and controls.

HLA-DRB1 type	AOSD n=94 (%)	AOSD Chronic type n=56 (%)	AOSD Non-chronic type n=38 (%)	AOSD Polyarticular type n=44 (%)	AOSD Non-polyarticular type n=50 (%)	Controls n=288 (%)
01	6 (6.4)	4 (7.1)	2 (5.3)	2 (4.6)	4 (8.0)	31 (10.8)
03	1 (1.1)	1 (1.8)	0	0	1 (2.0)	6 (2.1)
04	8 (8.5)[a]	4 (7.1)[d]	4 (10.5)	6 (13.6)	2 (4.0)[i]	61 (21.2)
07	5 (5.3)	4 (7.1)	1 (2.6)	2 (4.6)	3 (6.0)	16 (5.6)
08	14 (14.9)	8 (14.3)	6 (15.8)	7 (15.9)	7 (14.0)	29 (10.1)
09	6 (6.4)	4 (7.1)	2 (5.3)	2 (4.6)	4 (8.0)	30 (10.4)
10	2 (2.1)	2 (3.6)	0	1 (2.3)	1 (2.0)	7 (2.4)
11	5 (5.3)	3 (5.4)	2 (5.3)	1 (2.3)	4 (8.0)	12 (4.2)
12	13 (13.8)[b]	6 (10.7)	7 (18.4)[f]	5 (11.4)	8 (16.0)[j]	19 (6.6)
13	5 (5.3)	4 (7.1)	1 (2.6)	2 (4.6)	3 (6.0)	28 (9.7)
14	9 (9.6)	3 (5.4)	6 (15.8)[g]	5 (11.4)	4 (8.0)	17 (5.9)
15	20 (21.3)[c]	13 (23.2)[e]	7 (18.4)	11 (25.0)[h]	9 (18.0)	32 (11.1)

[a-c] AOSD versus controls; a: RR=0.35 (0.16-0.75), p=0.006, b: RR=2.27 (1.08-4.80), p=0.028, c: RR=2.16 (1.17-4.00), p=0.013.

[d-e] AOSD chronic type versus controls; d: RR=0.29 (0.10-0.82), p=0.014, e: RR=2.42 (1.18-4.97), p=0.014.

[f-g] AOSD non-chronic type versus controls; f: RR=3.20 (1.25-8.21), p=0.01, g: RR=2.99 (1.10-8.13), p=0.025.

[h] AOSD polyarticular type versus controls; h: RR=2.67 (1.23-5.79), p=0.011.

[i-j] AOSD non-polyarticular type versus controls; i: RR=0.16 (0.04-0.66), p=0.003, j: RR=2.70 (1.11-6.55), p=0.024.

Table III. Studies of HLA in patients with AOSD.

Study	Subjects	Controls	Conclusion
1981, Terkeltaub (ref. 8)	Eastern Canadian n=25 (24 whites, 1 Asian Indian)	260	HLA-Bw35 and Cw4 were increased in AOSD
			HLA-Bw35 may be a favorable prongnostic factor in AOSD
1986, Wouters (ref. 9)	Netherlander n=42 (41 whites, 1 Asian Indian)	277	HLA-DR4 was increased in AOSD
			HLA-DRw6 may be a poor prognostic factor in AOSD
1991, Pouchot (ref. 7)	Eastern Canadian n=55	323	HLA-B17, B18, B35, and DR2 were associated with AOSD
2001, Fujii (ref. 5)	Japanese n=30	63	HLA-DRB1*1501(DR2), DRB1*1201(DR5), and DQB1*0602(DQ1) were associated with AOSD
			DRB1*1501 and DRB1*1201 were more frequent in chronic articular AOSD[$]
This study	Korean n=47	144	HLA-DRB1*12 and DRB1*15 were frequent in AOSD
			HLA-DRB1*04 was decreased in AOSD
			HLA-DRB1*12 were more frequent in non-chronic and non-polyarticular AOSD
			HLA-DRB1*14 were more frequent in non-chronic AOSD
			HLA-DRB1*15 were more frequent in chronic and polyarticular AOSD

[$] Chronic arthritis involving at least one joint area, lasting longer than 6 months.

lower frequency of DRB1*04 (p=0.006, RR=0.35, 95% CI: 0.16-0.75) compared to controls (Table II).

Association of HLA-DRB1 genotype with AOSD subtypes

Chronic and non-chronic types versus controls. We categorized the patients with the polycyclic-systemic or chronic-destructive types as having chronic AOSD (N=28) and those with the monocyclic type as having non-chronic AOSD (N=19). Compared with normal controls, chronic AOSD was associated with a higher DRB1*15 and a lower DRB1*04. Those with non-chronic AOSD had higher DRB1*12 and DRB1*14 (Table II).

Polyarticular and non-polyarticular types versus controls

We redefined the patients with the oligoarticular and non-articular types of disease as non-polyarticular patients (N=25). Compared with normal controls, the polyarticular patients (N=22) showed an association with DRB1*15 and the non-polyarticular type had higher DRB1*12 and lower DRB1*04 (Table II).

Discussion

There have been controversies about the association between HLA molecules and AOSD (Table III). Terkeltaub *et al.* (8) reported that HLA-Bw35 and Cw4 were significantly frequent and that Bw35 was favorable prognostic factor. On the contrary, Wouter *et al.* (9) found no association with Bw35, but that DR4 was significantly frequent and DRw6 was more frequent in axial joint involvement. Marc *et al.* (10) reported a frequent association with DR7 and Pouchot *et al.* (7) reported that there were increased frequencies of B17, B18, B35 and DR2.

In recent report by Fujii *et al.* (5), 35 Japanese patients (13 men and 22 women) fulfilling Yamaguchi's criteria (11) were studied and classified as having either chronic articular AOSD (with chronic arthritis lasting longer than 6 months) or systemic AOSD (without chronic arthritis). Seventeen (49%) had chronic articular AOSD and 18 (51%) had systemic AOSD. The MHC class II alleles were evaluated in 30 of them, and the authors wrote that DRB1*1501 (DR2) and DRB1*1201 (DR5) were more frequent in chronic articular AOSD and in AOSD overall than in controls. DQB1*0602 (DQ1) was more frequent in both types of AOSD than in controls. Also, the chronic articular type had more frequent DRB1*1201 than the systemic type. Our results showed increased frequencies of DRB1*12 and DRB1*15, findings similar to Fujii *et al.* (5) In addition, DRB1*04 had a lower frequency and the non-chronic type had more frequent DRB1*14. Although the number of patients studied should be higher to achieve more consistent results, it may be supposed that Korean and Japanese AOSD is associated with DRB1*12 and DRB1*15. Whether DRB1*04 represents a genuine protective factor in AOSD in Korea needs to be evaluated further.

Recently, increased IL-6 or γδ T-cell activation has been reported in the acute phase of AOSD (13). Fujii *et al.* (5) reported that TNF-α, TNF receptor 2 and IL-18 were increased in AOSD, irrespective of disease activity and soluble IL-2 receptors, IL-4 and IL-18 were correlated with disease activity or C-reactive protein (CRP) values only in

chronic articular AOSD. Fujii *et al.* also suggested that INF-γ and IL-8 remained increased only in chronic articular AOSD, even when disease activity, including IL-6 and CRP, was low. More recent promising reports about treatments with TNF-α blocking agents (14, 15) support the hypothesis that cytokines, especially TNF-α, are important pathogenetic factors for the development of AOSD. Further evaluation of whether proinflammatory cytokines including TNF-α related genetic factors are involved, and whether these genes are independent or not would be important to identify possible genetic risk factors for AOSD.

In conclusion, Korean patients with AOSD had a distinct immunogenetic pattern and it would be valuable to assess the relationships between HLA-DRB1 genes and polymorphisms of proinflammatory cytokines in the pathogenesis of AOSD.

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