

HLA CLASS II ALLELES IN JAPANESE PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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Abstract. The distribution of HLA-DRB1 alleles and DQB1 alleles in 30 Japanese patients with non-Hodgkin's lymphoma (NHL) was analyzed using polymerase chain reaction with the sequence-specific primer (PCR-SSP) method, and the association between the disease and the presence of certain HLA class II alleles was investigated. The frequencies of HLA-DRB1*0803, DRB1*0802 and DRB1*1502 were increased while those of DRB1*1501 and DRB1*0405 were decreased. On the other hand, the incidence of HLA-DQB1 alleles was similar to that in the normal population. However, none of these HLA class II alleles showed significant positive or negative associations with NHL. In addition, when allele frequencies of NHL Japanese patients were compared to Thai patients, only DRB1*0803 was significantly increased in Japanese patients. These results indicate that DRB1*0803 may not contribute to NHL susceptibility in the Japanese population. However, further studies with larger numbers of NHL Japanese patients are needed to confirm our preliminary findings.

INTRODUCTION

The prevalence of non-Hodgkin's lymphoma (NHL) shows wide geographic variation throughout the world. In the United States, NHL is among the five leading causes of cancer mortality in young men and women. Not only in the United States but also worldwide, the incidence rates for NHL have increased 3 to 4% each year, which is greater than other cancer types except melanoma and lung cancer in women (Devesa and Fears, 1992). Although no common etiologic agent can be associated with all cases of NHL, both host and environmental factors seem to be important to the etiology and underlying immunodeficiency syndrome, which may set the stage for the development of lymphoproliferative disorders. Environmental agents such as chemicals, immunosuppressive drugs, radiation or viruses may also be predisposed to lymphoma development. The difference in genetic and racial incidence may reflect occupational risks that are experienced more preferentially than the more frequently affected groups (Persson *et al*, 1993; Bierman *et al*, 1995). Interestingly, recent studies show that HLA class II alleles have been associated with NHL Thai patients, especially the HLA-DRB1*1502 allele has been reported to be significantly increased in patients

45 years and under as well as in males (Nathalang *et al*, 1997; 1999). The aim of the present study is to analyze HLA class II alleles in NHL Japanese patients and investigate the association between the disease and the presence of certain HLA alleles.

MATERIALS AND METHODS

Subjects

The sample studied consisted of 30 Japanese patients with NHL; 15 males and 15 females. Their ages ranged from 17 to 76 years; the mean age was 50.5 and the median was 49.5. They came from Department of Hematology, Osaka City University Medical School, Japan. All patients were diagnosed histopathologically. Informed consent was obtained from all subjects. The control group consisted of 916 healthy unrelated individuals, which had been reported (Hashimoto *et al*, 1994).

HLA DNA typing and allele frequency (AF) determination

Genomic DNA was extracted from peripheral blood cells using the QIAamp blood kit (Qiagen, Hilden, Germany). Whole blood was centrifuged at 3,000 rpm for 10 minutes and the buffy coat was used for DNA extraction. The second exon of the DRB1 and DQB1 genes was amplified by the PCR-SSP method. Approximately, 100 ng/ μ l of each DNA sample was tested using the micro SSP

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DRB/DQB generic kit and the micro SSP DRB1, 3, 4, 5 high resolution kit (One Lambda, Inc, Canoga Park, USA). Briefly, the DNA sample was amplified with 31 and 95 different primer sets, which had been optimized and dispensed into each well of a thin-walled 96 well PCR plate for HLA class II low resolution typing and HLA-DRB high resolution typing. The SSP-DNA reaction set was placed in the Perkin-Elmer 9600 thermal cycler (Perkin Elmer, Norwalk, USA). The cycle parameters of the PCR program began with a first step of 1 cycle of 130 seconds at 94°C and 60 seconds at 63°C followed by 9 cycles of 10 seconds at 94°C, 60 seconds at 63°C and finally, 20 cycles of 10 seconds at 94°C, 50 seconds at 59°C and 30 seconds at 72°C. The last step was to hold the sample at 4°C. After amplification, 10 µl of each PCR reaction was transferred in sequence to a 2.5% agarose gel with 0.5 µg/ml ethidium bromide and electrophoresed at 150 volts for 4 minutes. The reaction pattern was photographed and the assessment of HLA alleles was performed by analysis of the gel banding pattern using a reaction pattern typing grid. Allele frequencies (AF) were calculated using the following formula : $AF(\%) = \frac{\text{sum of the allele}}{2n} \times 100$; where n is the sum of the total number of individuals analyzed (Chandanayingyong *et al*, 1997).

Statistical analysis

Allele frequencies in patients and controls were compared by chi-square contingency table analysis with Yates' correction as well as by standard p-value and Fisher's test. A level of $p < 0.05$ was accepted as statistically significant. The significance of the association between HLA class II alleles and NHL was calculated using the odds ratio (OR) and 95% confidence intervals (CI) (Ingelfinger *et al*, 1994).

RESULTS

The distribution of HLA-DRB1 and DQB1 alleles as defined by PCR-SSP in NHL Japanese patients is summarized in Tables 1 and 2. Overall, 16 DRB1 alleles were detected in NHL patients. Among them, DRB1*0803, DRB1*1502 and DRB1*09012 were the most frequent. In addition, the most common DRB1 alleles in healthy Japanese individuals were DRB1*0405, DRB1*09012 and DRB1*1502. Also, seven DQB1 alleles were identified in this study. The most common DQB1 alleles in NHL patients; were DQB1*06 and

DQB1*04. When allele frequencies of the patients and the controls were compared, the frequencies of DRB1*0803, DRB1*0802 and DRB1*1502 were increased while those of DRB1*1501 and DRB1*0405 were decreased in the NHL patients; however, there was no statistical difference in each allele ($p > 0.05$). In contrast, the incidence of HLA-DQB1 alleles was similar to the control group. We have also included for comparison in Table 3, DRB1 AFs for the NHL Thai patients and controls studied in a previous report (Nathalang *et al*, 1999). It was found that the AFs of DRB1*0101, *0405, *0410, *0802, *0803 and *1403 were significantly higher in NHL Japanese patients while DRB1*0301, *0404, *0701, *1001, *1106, *1202, *1312, *1404 and *1602 were significantly higher in the Thai control group. Interestingly, the DRB1*0803 allele was significantly increased in NHL Japanese patients compared to NHL Thai patients (13% vs 1.5% ; $p = 0.00$; OR = 0.10, CI = 0.03-0.39) while its incidence was similar in both control groups.

Additionally, a comparison of DQB1 AFs determined in both Japanese and Thai groups, is given in Table 4. The AFs of DQB1*06 and*04 were significantly higher in the Japanese groups, whereas DQB1*05,*0301/04 and*02 were significantly frequent in the Thai groups.

DISCUSSION

The present study is the first to determine the frequencies of HLA class II alleles using the PCR-SSP method in Japanese patients with NHL. The frequencies of HLA-DRB1*0803, DRB1*0802 and DRB1*1502 were increased while those of DRB1*1501 and DRB1*0405 were decreased in NHL patients compared to healthy controls. However, none of these alleles showed significant positive or negative associations with NHL. Moreover, the most frequent alleles in NHL patients were DRB1*0803, DRB1*1502 and DRB1*09012, which is similar to other studies in the Japanese population (Hashimoto *et al*, 1994; Tanaka *et al*, 1997). Additionally, in this study the age distribution of the patients with NHL was comparable to those reported in Japan and Thailand (Kadin *et al*, 1983; Tajima *et al*, 1985; Intragumtornchai *et al*, 1996). Previous studies in Thai population have found significant association with DRB1*1502 and NHL, especially in male patients 45 years and under (Nathalang *et al*, 1999). Since the distribution and prevalence of HLA class II alleles are different in

Table 1
Distribution of HLA-DRB1 alleles in Japanese patients with NHL.

| DRB1 allele | NHL (N = 30) | | Controls (N=916) | | p-value | Odds ratio | 95% CI |
|-------------|--------------|------|------------------|------|---------|------------|-----------|
| | observed | % | observed | % | | | |
| DRB1*0101 | 4 | 6.7 | 85 | 4.8 | ns | | |
| DRB1*1501 | 2 | 3.3 | 108 | 6.1 | ns | 0.55 | 0.13-2.28 |
| DRB1*1502 | 7 | 11.7 | 153 | 8.7 | ns | 1.45 | 0.65-3.24 |
| DRB1*1602 | 0 | 0 | 10 | 0.5 | ns | | |
| DRB1*0301 | 0 | 0 | 4 | 0.2 | ns | | |
| DRB1*0401 | 0 | 0 | 21 | 1.2 | ns | | |
| DRB1*0403 | 2 | 3.3 | 54 | 3.0 | ns | | |
| DRB1*0404 | 0 | 0 | 4 | 0.2 | ns | | |
| DRB1*0405 | 6 | 10 | 262 | 15.5 | ns | 0.67 | 0.28-1.56 |
| DRB1*0406 | 0 | 0 | 58 | 3.2 | ns | | |
| DRB1*0407 | 0 | 0 | 8 | 0.4 | ns | | |
| DRB1*0410 | 2 | 3.3 | 33 | 1.8 | ns | | |
| DRB1*1101 | 3 | 5.0 | 53 | 2.9 | ns | | |
| DRB1*1201 | 0 | 0 | 70 | 3.9 | ns | | |
| DRB1*1202 | 0 | 0 | 48 | 2.6 | ns | | |
| DRB1*1301 | 0 | 0 | 16 | 0.9 | ns | | |
| DRB1*1302 | 3 | 5.0 | 95 | 5.3 | ns | | |
| DRB1*1307 | 0 | 0 | 2 | 0.1 | ns | | |
| DRB1*1401 | 3 | 5.0 | 81 | 4.4 | ns | | |
| DRB1*1402 | 0 | 0 | 1 | 0.1 | ns | | |
| DRB1*1403 | 2 | 3.3 | 29 | 1.6 | ns | | |
| DRB1*1405 | 3 | 5.0 | 47 | 2.6 | ns | | |
| DRB1*1406 | 1 | 1.7 | 24 | 1.3 | ns | | |
| DRB1*1407 | 0 | 0 | 5 | 0.3 | ns | | |
| DRB1*0701 | 1 | 1.7 | 24 | 1.3 | ns | | |
| DRB1*0801 | 0 | 0 | 3 | 0.2 | ns | | |
| DRB1*0802 | 6 | 10.0 | 89 | 5.0 | ns | 2.18 | 0.91-5.19 |
| DRB1*0803 | 8 | 13.3 | 133 | 7.6 | ns | 1.97 | 0.92-4.22 |
| DRB1*09012 | 7 | 11.7 | 216 | 12.4 | ns | | |
| DRB1*1001 | 0 | 0 | 11 | 0.5 | ns | | |
| BL | 0 | 0 | 94 | 1.9 | ns | | |

N = Number of individuals tested; ns = Not significant; BL = Undetermined allele

Table 2
Distribution of HLA-DQB1 alleles in Japanese patients with NHL.

| DQB1 allele | NHL (N = 30) | | Controls (N = 916) | | p-value |
|-----------------|--------------|------|--------------------|------|---------|
| | observed | % | observed | % | |
| DQB1*05 | 10 | 16.7 | 232 | 12.8 | ns |
| DQB1*06 | 20 | 33.3 | 499 | 28.5 | ns |
| DQB1*02 | 1 | 1.7 | 19 | 1.0 | ns |
| DQB1*0301/04 | 5 | 8.3 | 228 | 13.1 | ns |
| DQB1*0302/05/07 | 4 | 6.7 | 207 | 11.8 | ns |
| DQB1*03032/06 | 8 | 13.3 | 196 | 11.2 | ns |
| DQB1*04 | 12 | 20.0 | 338 | 19.4 | ns |
| BL | 0 | 0 | 113 | 2.2 | ns |

N = Number of individuals tested; ns = Not significant; BL = Undetermined allele

Table 3
Distribution of selected DRB1 allele frequencies in NHL Japanese and Thai patients, compared to the controls.

| DRB1 allele | NHL | | p-value | OR | 95% CI | Controls | | p-value | OR | 95% CI |
|-------------|----------|------|---------|------|------------|----------|------|---------|-------|--------------|
| | Japanese | Thai | | | | Japanese | Thai | | | |
| DRB1*0101 | 6.7 | 0.5 | 0.002 | 0.07 | 0.008-0.64 | 4.8 | 1.0 | ns | 0.21 | 0.03-1.51 |
| DRB1*1502 | 11.7 | 16.0 | ns | | | 8.7 | 7.0 | ns | | |
| DRB1*1602 | 0.0 | 4.5 | ns | | | 0.5 | 4.0 | 0.00 | 7.59 | 2.34-24.65 |
| DRB1*0301 | 0.0 | 5.5 | ns | | | 0.2 | 8.0 | 0.00 | 39.74 | 11.75-134.39 |
| DRB1*0404 | 0.0 | 0.0 | ns | | | 0.2 | 3.0 | 0.00 | 14.13 | 3.12-64.04 |
| DRB1*0405 | 10.0 | 3.0 | 0.02 | 0.28 | 0.09-0.90 | 15.5 | 5.0 | 0.01 | 0.32 | 0.13-0.78 |
| DRB1*0410 | 3.3 | 0.0 | 0.01 | | | 1.8 | 0.0 | ns | | |
| DRB1*0701 | 1.7 | 6.5 | ns | | | 0.8 | 11.0 | 0.00 | 14.97 | 6.68-33.54 |
| DRB1*0802 | 10.0 | 0.5 | 0.00 | 0.05 | 0.01-0.38 | 5.0 | 0.0 | 0.024 | | |
| DRB1*0803 | 13.0 | 1.5 | 0.00 | 0.1 | 0.03-0.39 | 7.6 | 7.0 | ns | 0.96 | 0.44-2.12 |
| DRB1*09012 | 11.7 | 14.5 | ns | | | 12.4 | 7.0 | ns | | |
| DRB1*1001 | 0.0 | 1.5 | ns | | | 0.5 | 3.0 | 0.006 | 5.12 | 1.41-18.65 |
| DRB1*1101 | 5.0 | 1.0 | 0.048 | 0.19 | 0.03-1.18 | 2.9 | 1.0 | ns | | |
| DRB1*1106 | 0.0 | 1.0 | ns | | | 0.0 | 4.0 | 0.00 | | |
| DRB1*1202 | 0.0 | 19.5 | 0.00 | | | 2.6 | 14.0 | 0.00 | 6.05 | 3.21-11.40 |
| DRB1*1312 | 0.0 | 2.5 | ns | | | 0.0 | 3.0 | 0.00 | | |
| DRB1*1403 | 3.3 | 0.0 | 0.01 | | | 1.6 | 0.0 | ns | | |
| DRB1*1404 | 0.0 | 2.0 | ns | | | 0.6 | 2.0 | 0.00 | | |
| DRB1*1405 | 5.0 | 1.0 | 0.048 | | | 2.6 | 3.0 | ns | | |

ns = Not significant

Table 4
Distribution of DQB1 allele frequencies in NHL Japanese and Thai patients, compared to the controls.

| DQB1 alleles | NHL | | p-value | Controls | | p-value |
|-----------------|----------|------|---------|----------|------|---------|
| | Japanese | Thai | | Japanese | Thai | |
| N= | 30 | 100 | | 916 | 50 | |
| DQB1*05 | 16.7 | 35.5 | 0.006 | 12.8 | 27.0 | 0.00 |
| DQB1*06 | 33.3 | 10.0 | 0.00 | 28.5 | 14.0 | 0.004 |
| DQB1*02 | 1.7 | 11.0 | 0.026 | 1.0 | 17.0 | 0.00 |
| DQB1*0301/04 | 8.3 | 22.0 | 0.018 | 13.1 | 20.0 | 0.03 |
| DQB1*0302/05/07 | 6.7 | 2.5 | ns | 11.8 | 7.0 | ns |
| DQB1*03032/06 | 13.3 | 16.0 | ns | 11.2 | 10.0 | ns |
| DQB1*04 | 20.0 | 3.0 | 0.00 | 19.4 | 5.0 | 0.001 |
| BL | 0.0 | 0.0 | ns | 2.2 | 0.0 | ns |

N = Number of individuals tested; ns = Not significant; BL = Undetermined allele

Thai and Japanese populations, we have also compared AFs of NHL patients and controls in both groups. DRB1*1502 and DRB1*09012 were common among Thai and Japanese populations. DRB1*0803 was the most frequent allele in NHL Japanese patients while DRB1*0405 was the most frequent allele in the control group. DRB1*1202 was the most frequent allele in NHL Thai patients

as well as controls. Although there were significant differences between HLA-DRB1 and DQB1 alleles in both Thai and Japanese populations; however, when the AFs of the NHL patients were compared, only DRB1*0803 was significantly increased in Japanese patients compared to Thai patients while its frequency was similar in both control groups.

In conclusion, our results indicate that DRB1*0803 may not contribute to a strong susceptibility to NHL in the Japanese, which may be due to the small number of patients investigated. However, we demonstrated an association with DRB1*0803 and NHL in Japanese patients, compared to Thai patients, which could be a result of the differences in the ethnic groups. Further studies with larger samples of NHL Japanese patients are needed to confirm our preliminary findings.

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