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• BRIEF REPORT •

# Relationship between autoimmune hepatitis and HLA-DR4 and DRβ allelic sequences in the third hypervariable region in Chinese

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## Abstract

AIM **To analyze the association of HLA-DRB1 with autoimmune hepatitis (AIH) in patients from China.**

METHODS **In 32 patients and 48 healthy controls, polymerase chain reaction amplification with sequence-specific primers (PCR-SSP) was performed to examine the association of certain alleles or polymorphic sequences of HLA-DRB1 with AIH.**

RESULTS **HLA-DRB1 typing by PCR-SSP showed that DR4 had a significantly increased frequency among patients with AIH versus healthy control (46.9% versus 20.8%; relative risk = 3.35, *P* = 0.014). In subtypes of DR4, there was a trend of increase in the gene frequency of DRB1 *0405 in patients with AIH versus healthy controls (21.9% *vs* 6.3%, *P* = 0.04, but $P_c$ = 0.08). In addition, a significant increase was found in the alleles frequency encoding QRRAA from the third hyperpolymorphic region of DR4 in the patients with AIH (86.7% of DR4 positive patients *vs* 40.0% in DR4 positive controls, *P* = 0.016, $P_c$ = 0.028, RR = 9.75).**

CONCLUSION **AIH in Chinese is associated with HLA-DR4. There is a relationship between QRRAA sequence within the third hyperpolymorphic region of the DRB allele and AIH in Chinese.**

**Subject headings** hepatitis, autoimmune/immunology; HLA-DR antigen/genetics; alleles; sequence analysis; polymerase chain reaction

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## INTRODUCTION

Autoimmune hepatitis (AIH) is an inflammation of the liver characterized by the presence of periportal hepatitis in microscopic examination (piecemeal necrosis or interface hepatitis), hypergammaglobulinemia, and serum autoantibodies[1-5]. Infiltration of the liver by T lymphocytes and a peripheral defect in T-suppressor cell function suggest an immune basis for the pathogenesis and liver injury in AIH[6-9]. Among northern European and North American white populations, various studies have shown that the serologically defined HLA-DR3 and DR4 antigen are the primary determinants of HLA-encoded susceptibility to AIH[10]. The principal susceptibility allele for type 1 autoimmune hepatitis among white northern Europeans and Americans is HLA-DRB1 *0301, and a second, independent risk factor is HLA-DRB1*0401, which encodes DR3 and DR4 respectively[11-13]. In Japan, where DR3 is very rare in the normal population, the primary HLA associated with DR4 and almost all patients are in the older age groups, with a peak onset at 50-60 years of age[14-16]. Patients from Argentina[17], Brazil[18], and Mexico[19] have susceptibility alleles for type 1 autoimmune hepatitis that are different from those in white northern European and American patients. These discrepant observations emphasize the importance of studying ethnically homogeneous populations[20]. In present study, we performed HLA genotyping with respect to DRB1 in Chinese patients with AIH using PCR amplification by the sequence-specific primers (PCR-SSP) method to investigate the relationship between the distribution of the HLA-DRB1 allele and the susceptibility to autoimmune hepatitis.

## MATERIALS AND METHODS

### *Subjects*

A total of 32 patients, all female, who satisfied the diagnostic criteria for AIH[1,2,21-23], were enrolled in this study. They were followed up at Shanghai Institute of Digestive Disease, Shanghai Renji Hospital from 1996 to December 2000. Each patient denied illicit drug use, contact with jaundiced individuals, family history of liver disease, alcohol abuse, and exposure to hepatotoxic medication or chemicals. Each lacked evidence of infection with hepatitis B and C virus. All patients were seropositive at a titer of at least 1:40 by indirect immunofluorescence for antinuclear antibodies or smooth muscle antibodies. The control population comprised 48 healthy female Chinese who were unrelated to the patients.

### *Methods*

**DNA extraction** High molecular weight DNA was isolated from peripheral blood leukocytes by phenol/chloroform extractions.

**Amplification primers** Primer pairs used for HLA-DRB1 and subtypes of HLA-DR4 were synthesized by the Shanghai Branch, Canadian Sangon Bioengineer Company, according to REFERENCES[24,25]. In each PCR reaction a primer pair was included that amplified the third intron of DRB1 genes. These two primers matched non-allelic sequences and thus functioned as an internal positive amplification control. 5'-prime C5 $^{5'}$-TGCCAAGTGGAGCACCCAA$^{3'}$ (Tm 60℃, complementary to codons 173-179 in the 3'end of exon 3) and 3'-primer

C3 5' GCATCTTGCTCTGTGCAGAT3' (Tm 60℃, complementary to codons 193-200 in the 5'end of exon 4) gave rise to a 796 base pair (bp) fragment.

**PCR-SSP**[24,25] Each PCR reaction mixture contained 2-4 allele- or group-specific DRB1 primers and the internal positive control primer pair in a 5-fold lower concentration. The PCR reaction mixtures (13 μL) consisted of 100 ng genomic DNA in 2 μL, PCR buffer [50 mmol·$L^{-1}$ KCl/1.5 mmol·L-1 $MgCl_2$/10 mmol·$L^{-1}$ Tris-Cl, pH 8.3/0.001% (w/v) gelatin], 200 μmol·L-1 of each dATP, dCTP, dGTP and dTTP, 1 μmol·$L^{-1}$ of the allele- and group-specific DRB primers, 0.2 μmol·$L^{-1}$ of Ampli Taq (diluted 1 to 10 in 1×PCR buffer, Shanghai Branch, Canadian Sangon Bioengineer Co.). PCR amplifications were carried out in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus Instruments). DNAs were amplified by 30 three-temperature cycles in 1 h and 20 min. Each cycle consisted of denaturation at 94℃ for 20 s, annealing at 65℃ for 50 s and extension at 72℃ for 20 s.

**Visualization of amplification** Absence or presence of PCR products was visualized by agarose gel electrophoresis. After addition of 2.5μL loading buffer [300 mL·$L^{-1}$% (v/v) gllycerol stained with bromophenol blue and xylene cyanol], the PCR reaction mixtures were loaded in 3 mm wide slost in 10 g·$L^{-1}$ ME agarose gels pre-stained with ethidium bromide (0.5 mg·$L^{-1}$ gel). Gels were run for 25-30 min at 7-8 V·$cm^{-1}$, then were examined under UV illumination and documented by photography.

### *Statistical analysis*

Data were analyzed by the standard statistical procedure of $\chi^2$ contingency table analysis with Yates' correction. Corrected *P* values were calculated using the Bonferroni inequality method. Relative risk was obtained by the cross-product ratio of the entries in the 2×2 table. A level of $P<0.05$ was considered as statistically significant.

## RESULTS

Allelic frequencies of HLA-DRB1 are shown in Table 1. HLA-DRB1 typing by PCR-SSP showed that DRB1 *04, which encodes DR4 antigen, had a significantly increased frequency among patients with AIH versus healthy control (46.9% *vs* 20.8%; relative risk = 3.35, $P = 0.014$). No other allele was significantly associated with autoimmune hepatiti s. DRB1 *04 has 11 subtypes officially assigned as DRB1 *0401-0411. As shown in Table 2, there was a trend of an increase in gene frequency of DRB1 *0405 in patients with AIH versus healthy controls (21.9% *vs* 6.3%, $P = 0.04$, $P_c = 0.08$).

The "shared motif hypothesis" suggests that many susceptibility-associated alleles encode a conserved epitope spanning the third allelic hypervariable region of the DRâ molecule, which may form the molecular basis of susceptibility to autoimmune diseases such as rheumatoid arthritis. The sequence over this third allelic hypervariable region, which spans amino acid 70-74 of the DRβ chain, are QKRAA and QRRAA. QKRAA is encoded by allele DRB1 *0401, QRRAA by DRB1 *0404, DRB1 *0405, DRB1 *0408 and DRB1 *0410. Our analysis indicates a significant increase in the alleles encoding QRRAA from the third hyperpolymorphic region of DR4 in the patients with AIH (13 cases or 86.7% of 15 DRB1 *04 positive patients *vs* 4 cases or 40.0% of 10 DRB1 *04 positive controls, $P = 0.016$, $P_c = 0.028$, RR = 9.75).

**Table 1** Allelic frequencies of DRB1 in autoimmune hepatitis

| DRB1 allele | DR Ag | Autoimmune hepatitis % ($n$ = 32) | Healthy control % ($n$ = 48) | RR | $P$ value | Corrected $P$ value |
|---|---|---|---|---|---|---|
| DRB1*01 | DR1 | 0.0 | 4.2 | | | |
| DRB1*15,*16 | DR2 | 37.5 | 35.4 | | | |
| DRB1*03 | DR3 | 15.6 | 14.6 | | | |
| DRB1*04 | DR4 | 46.9 | 20.8 | 3.35 | 0.014 | 0.026 |
| DRB1*11,*12 | DR5 | 12.5 | 29.2 | | | |
| DRB1*13,*14 | DR6 | 9.4 | 12.5 | | | |
| DRB1*07 | DR7 | 3.1 | 4.2 | | | |
| DRB1*08 | DR8 | 9.4 | 10.4 | | | |
| DRB1*09 | DR9 | 34.4 | 37.5 | | | |
| DRB1*10 | DR10 | 0 | 2.1 | | | |

*P* values are given only where significant at <0.05 level.

**Table 2** Allelic frequencies of DRB1*04 subtypes in autoimmune hepatitis

| Allele | Autoimmune hepatitis % ($n$ = 32) | Healthy control % ($n$ = 48) | RR | $P$ value | Corrected $P$ value |
|---|---|---|---|---|---|
| DR4 | 46.9 (15) | 20.8 (10) | 3.4 | 0.014 | 0.026 |
| DRB1*0401 | 3.1 (1) | 4.2 (2) | | | |
| DRB1*0402 | 0 | 0 | | | |
| DRB1*0403 | 3.1 (1) | 4.2 (2) | | | |
| DRB1*0404 | 12.5 (4) | 2.1 (1) | | | |
| DRB1*0405 | 21.9 (7) | 6.3 (3) | 4.2 | 0.04 | 0.08 |
| DRB1*0406 | 3.1 (1) | 4.2 (2) | | | |
| DRB1*0407 | 0 | 0 | | | |
| DRB1*0408 | 0 | 0 | | | |
| DRB1*0409 | 0 | 0 | | | |
| DRB1*0410 | 6.2 (2) | 0 | | | |
| DRB1*0411 | 0 | 0 | | | |

*P* values are given only where significant at <0.05 level.

## DISCUSSION

Autoimmune hepatitis (AIH) is a disease of unknown etiology but is presumed to have a basis in aberrant autoreactivity[26,27]. It is characterized by hypergammaglobulinaemia due mainly to selective elevation of serum IgG, a wide range of circulating tissue autoant ibodies, a picture of periportal (interface) hepatitis on liver biopsy with a predominantly lymphoplasmacytic necroinflammatory infiltrate without cholangiolitic or other changes normally associated with liver diseases of other aetiologies, and usually a notable response to immunosuppressive therapy[1,23,28,29]. Diagnosis is based on the combination of these features together with careful exclusion of all other possible causes of liver disease. Corticosteriods remain as the standard therapy for patients with severe autoimmune hepatitis but the treatment is not universally effective[30]. Novel drugs with potent immunosuppressive and cytoprotetive functions must be evaluated[31,32]. Liver transplanta tion has been an extremely successful treatment option for the decompensated patients[33], but better drug regimens are needed to conserve this limited resource.

The association of AIH with inheritance of the HLA-DR3 allotype in Caucasians has long been recognized. The secondary association with DR4 (in DR3 negative patients) has been more recently defined[34-39]. DR3 tends to occur more frequently in younger patients with severe disease whereas DR4 is mostly associated with an older age at presentation and with generally milder disease that is easier to control[11-15].Using HLA-DR typing by PCR-SSP, we found that DRB1*04 (encoding DR4) was presented in 46.9% of the AIH group versus 20.8% of healthy control individuals,

giving a relative risk of 3.35. No other allele was significantly associated with autoimmune hepatitis in Chinese. In white patients, a dual association of HLA-DR3 and -DR4 has been found in patients with autoimmune hepatitis. The discrepancy in HLA association between Chinese and white patients with AIH is probably caused by the racial or disease differences in the distribution of HLA alleles. Other genetic predispositions or environmental factor responsible for the pathogenesis of these autoimmune diseases may vary among ethnic groups. Our results are similar to the reports from Japan, where relationship between the susceptibility of AIH and the presence of HLA-DR4 had been firmly demonstrated[14-16]. In our study, there was a trend of an increase in gene frequency of DRB1*0405 increased in patients with AIH versus healthy controls, but the difference between patients and controls was not statistically significant, probably due to the small number of patients. "The shared epitope hypothesis" suggests that many alleles encode a similar amino acid sequence in the critical portion of the antigen-binding groove of the HLA DR molecule. The presence of shared third allelic hypervariable region epitopes QKRAA and QRRAA at position 70-74 of the HLA-DRâ chain was analyzed in our study. Unlike in the Caucasian population, the DRB1*0401 allele, which possesses the QKRAA sequence, was very uncommon in the Chinese subjects. There was a significant increase in the percentage of patients who possess the QRRAA sequence. This was accounted for mainly by the DRB1*0405 allele, with the rest being DRB1*0404 allele.

Autoimmune hepatitis is thought to be triggered by various environmental factors in individuals rendered susceptibility by a distinct and genetic background[40]. The immune response is initiated in human when the T-cell receptor recognizes foreign or self-peptide fragments that can be bound to self-HLA molecules encoded by the major histocompatibility complex genes on the short arm of the sixth chromosome. Differences in immune responsiveness among individuals are considered to be caused by allelic variations in HLA antigens with autoimmune diseases associated with specific HLA antigens. DR4 may regulate immune responsiveness to some autoimmune hepatitis, but not all. Other genetic polymorphism of HLA, such as HLA-DR6, -DQ, -C4[41-45], may also play an important role in the pathogenesis of AIH in the white patients, but there has been no data in Chinese yet. Autoimmune promoter genes outside the HLA, such as CTLA-4 (cytotoxic T-lymphocyte antigen-4)[46], T cell repertoire[47,48], cytokine gene[49,50], were investigated in white patients with AIH. So the genetic background of AIH may be very complex, but the clarity of this genetic predisposition and its clinical consequences will render defining susceptible populations and seeking novel therapeutic interventions.

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