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# Association Between HLA Class II Molecules and Autoimmune Hepatitis Type 1 in Thai Patients

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## ABSTRACT

***Background:*** Current knowledge suggests that pathogenesis of autoimmune hepatitis (AIH) may be environmental triggering e.g. viral, or drugs in a genetically susceptible host. Defining the genetic basis of the disease is a major challenges to expand the understanding of the disease.

***Aim:*** To investigate the association between AIH type I and HLA class II alleles in Thai patients.

***Patients and Methods:*** We analyzed data of 56 AIH patients and collected blood for Human leucocyte Antigen (HLA) class II analysis in 42 patients who have attended Siriraj Hepatitis Clinic between January 1990 to October 2003. Data included clinical presentations, laboratory results, and liver histology. Serological typing and class II genotyping were performed using the polymerase chain reaction (PCR-SSOP) methods.

***Results:*** The HLA DRB1*0301, and DQA1*0101 were significantly associated with AIH patients when compared to controls; [OR = 3.92 (1.18-13.30), $\chi^2$ = 5.28, p value 0.021, OR = 2.31 (1.13-4.73), $\chi^2$ = 5.43, p value 0.019, respectively]. When considering only 18 patients with "definite" AIH, only HLA DRB1*0301 was significantly associated with the disease (OR = 5.22, 95%CI = 1.28-20.92, $\chi^2$ = 5.87, p value 0.015)

***Conclusion:*** DQA1*0101 and, particularly HLA DRB1*0301 were significantly associated with AIH in Thailand.

**Key words :** HLA class II, autoimmune, hepatitis type 1

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## BACKGROUND

Autoimmune chronic hepatitis (AIH) is a liver disease characterized by piecemeal necrosis, hypergammaglobulinemia and high serum autoantibody1. The importance of the association between HLA antigens and various autoimmune diseases have been recognized and extensively studied. There are 2 proposed mechanisms that explain the importance of HLA antigens to autoimmune diseases. First, HLA antigen is only a genetic marker for non-HLA gene-associated disease susceptibility which is located within the HLA gene region. For example, congenital adrenal hyperplasia is a caused by a defective mutation in the 21OH hydroxylase mapped in the class III region of the HLA gene complex. Second, specific amino acid sequences of polymorphic HLA antigens have an important role in the immunoresponse to disease onset. For example, polymorphic HLA-DRB1 and/or DQB1 molecules are believed to influence the development of several autoimmune disease, including rheumatoid arthritis[10,11], diabetes mellitus type 1[12,13], celiac disease[14], and pemphigus vulgaris[15]. Specific HLA class II antigens have also been suggested to affect the susceptibility and resistance to autoimmune disease.

AIH has a strong genetic predisposition that may affect susceptibility, clinical features, and treatment outcome[2-7]. This disease has global distribution, and differences in the genetic risk factors may influence its occurrence and behavior in differences ethnic groups and geographic regions[2].

In Caucasian, HLA-B8[8] and -DR3[9] have been found to associate with the disease. In Asia, report form Japan found association between AIH and HLA-DR4[4,25]. However, there is no data in Thailand. The objectives of this study is to determine the association between various HLA Class II and AIH in Thailand.

## PATIENTS AND METHODS

### Patients

Among >2000 patients who have been followed up in Hepatitis Clinic, Siriraj Hospital during January 1990 to October 2003, 56 patients were diagnosed as AIH. Diagnosis of AIH was based on International Autoimmune Hepatitis Group Scoring System to classified patients as definite or probable AIH[16]. We excluded other causes of chronic hepatitis e.g. chronic hepatitis B or C, alcoholic hepatitis, drug-induced hepatitis.

Control group consisted of 100 healthy subjects who were unrelated to the patients.

### Methods

**HLA DNA typing** A sample of 10 ml clotted blood was obtained from the patients. Genomic DNAs were isolated and analyzed by PCR-SSOP (Polymerase Chain Reaction and hybridization with Sequence Specific Oligonucleotide Probes)[26]

**Serological Tests** HBsAg, anti-HCV, serum autoantibodies, including antinuclear antibody (ANA), and antismoothmuscle antibody (ASMA) were analyzed all patients. Titer of ≥1:80 were considered positive for ANA, ASMA.

**Statistic Analysis** Data were analyzed SPSS Version 11. Categorial variables were calculated using $\chi^2$ test and presented as Odd Ratio and 95% confidence interval. P value of <0.05 was considered statistically significant.

## RESULTS

Of the 56 patients with the diagnosis of AIH, 6 were excluded due to the features of overlap syndrome. Eventually 50 patients were AIH type 1. Forty three (86%) were female, with a ratio of F: M of 6:1. Mean age of patients was 62 years (range 29-85 years). In the Figure 1 showed distribution of age at presentation of AIH that compared to other studies. Seventeen patients (34%) presented with acute hepatitis, 22 patients (44%) had cirrhosis presented with fatigue, anorexia, jaundice and complications of cirrhosis i.e. ascites and esophageal varices. Eleven patients (22%) were asymptomatic and discovered during routine check up (Figure 2). Laboratory values at presentation were shown in Table 1. Forty eight patients (96%) were positive for ANA whereas ASMA was positive in 28%. Liver biopsy was done in 22 patients (44%). Histological findings were all compatible with AIH. All viral hepatitis markers were negative.

### HLA Antigens

Genotyping for HLA class II alleles was available and performed in 37 of 50 AIH patients whose blood samples could be obtained. The frequency of HLA class II antigens in AIH patients compared to those in healthy individuals is shown in Table 2. HLA-DR3

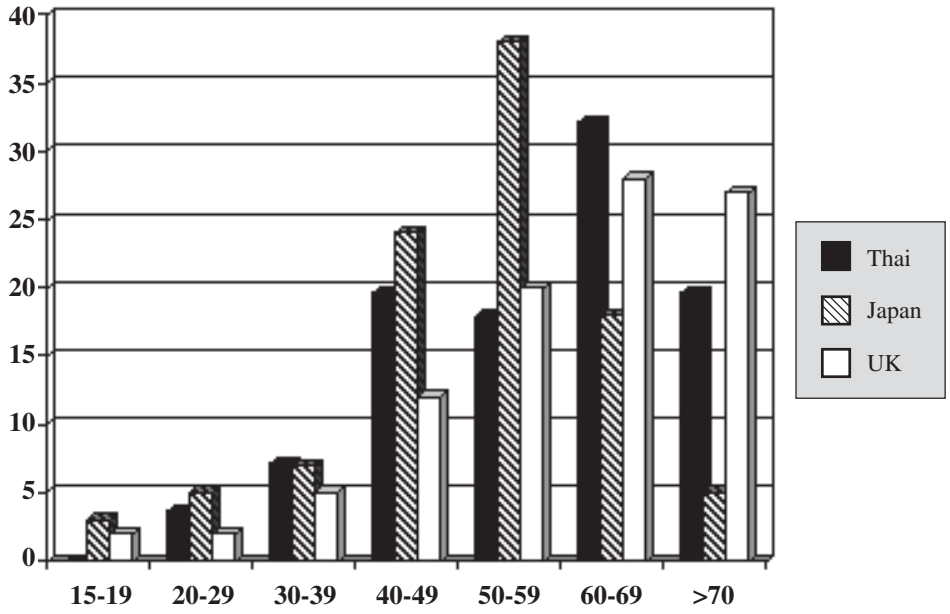


**Figure 1** Distribution of age at presentation of AIH in this study, compared to AIH in Japanese and Caucasians.

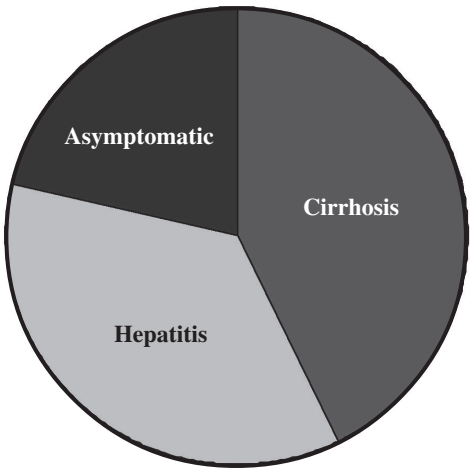


**Figure 2** Clinical presentation of 50 patients with AIH

was significantly associated with AIH.

## Genotype of HLA-DRB1

The results of HLA-DNA typing for DRB1 alleles are summarized in table 3. Only DRB1*0301 allele was significantly increased in AIH type1 compared to controls.

## Genotype of HLA-DQA1

The results of HLA-DNA typing for DQA1 gene are shown in Table 4. The incidence of DQA1*0101 allele was significantly higher in the AIH patients.

**Table 1** Laboratory findings of 50 AIH patients

| Laboratory Values | Mean ± SD | Range |
|---|---|---|
| AST (IU/L) | 398 ± 405 | 21-1788 |
| ALT (IU/L) | 316 ± 341 | 16-1779 |
| Albumin (g/dL) | 3.33 ± 0.72 | 2.1-4.7 |
| Globulin (g/dL) | 5.4 ± 1.2 | 3.0-9.9 |
| Alkaline phosphatase (IU/L) | 158 ± 66 | 38-292 |
| Total bilirubin(mg/dL) | 6.5 ± 8 | 0.2-36.2 |

## Genotype of HLA-DQB1

The results of HLA-DQB1 gene are shown in Table 5. No any DQB1 alleles were significantly associated with AIH.

When comparing only 18 patients with çdefiniteé AIH and control group, only HLA DRB1*0301 was significantly associated with the disease (OR = 5.22, 95%CI = 1.28-20.92, $\chi^2 = 5.87$, p value 0.015)

## DISCUSSION

In the present study, we studied 50 patients with AIH who have attended Siriraj Hospital during the 13-year-period. The predominantly female patients (F:M 6:1) in our study is higher than data from Caucasians[16,18]. Our mean age of presentation was 62 years. Approximately one-third of the patients presented with

**Table 2** Frequency of HLA-DR antigens in patients with AIH and controls

| HLA antigens | AIH 74 alleles (%) | Controls 200 alleles (%) | OR | 95% CI | $\chi^2$ | Corrected *p* |
|---|---|---|---|---|---|---|
| DR2 | 20 (27.03) | 38 (19) | 1.58 | 0.81-3.07 | 1.63 | NS |
| ***DR3*** | ***8 (10.81)*** | ***6 (3)*** | ***3.92*** | ***1.18-13.3*** | ***5.28*** | ***0.02*** |
| DR4 | 7 (9.46) | 28 (14) | 0.64 | 0.24-1.63 | 0.63 | NS |
| DR52 | 31 (41.89) | 62 (31) | 1.60 | 0.89-2.89 | 2.39 | NS |
| DR7 | 7 (9.46) | 24 (12) | 0.53 | 0.2-1.82 | 1.06 | NS |
| DR9 | 7 (9.46) | 21 (10.5) | 0.62 | 0.17-1.55 | 0.50 | NS |

*p values are given only where significant at <5% level.*
*NS: non-significant*

**Table 3** HLA-DNA typing for DRB1

| DRB1 allele | AIH 74 alleles (%) | Healthy controls 200 alleles (%) | OR | 95% CI | $\chi^2$ | Corrected *p* |
|---|---|---|---|---|---|---|
| 1501 | 4 (5.40) | 13 (7.5) | 0.82 | 0.22-2.83 | 0.00 | NS |
| 1502 | 13 (17.56) | 18 (9) | 2.15 | 0.93-4.96 | 3.14 | NS |
| 1602 | 3 (4.05) | 7 (3.5) | 1.16 | 0.23-5.18 | 0.02 | NS |
| ***0301*** | ***8 (10.81)*** | ***6 (3)*** | ***3.92*** | ***1.18-13.30*** | ***5.28*** | ***0.02*** |
| 0405 | 7 (9.46) | 11 (5.5) | 1.80 | 0.60-5.26 | 0.81 | NS |
| 1101 | 4 (5.40) | 9 (4.5) | 1.21 | 0.30-4.49 | 0.00 | NS |
| 1202 | 13 (17.56) | 41 (20.5) | 0.83 | 0.39-1.73 | 0.14 | NS |
| 1301 | 1 (1.35) | 0 | undefined | limitted | limitted | NS |
| 1302 | 1 (1.35) | 6 (3) | 0.44 | 0.02-3.8 | 0.11 | NS |
| 1312 | 1 (1.35) | 0 | undefined | limitted | limitted | NS |
| 1401 | 5 (6.75) | 6 (3) | 2.34 | 0.60-9.02 | 1.12 | NS |
| 1404 | 3 (4.05) | 2 (1) | 4.18 | 0.56-36.6 | 1.37 | NS |
| 0701 | 5 (6.75) | 24 (12) | 0.53 | 0.17-1.55 | 1.06 | NS |
| 0803 | 1 (1.35) | 7 (3.5) | 0.38 | 0.02-3.13 | 0.29 | NS |
| 0901 | 5 (6.75) | 21 (10.5) | 0.62 | 0.20-1.82 | 0.50 | NS |

*p values are given only where significant at <5% level.*
*NS: non-significant*

**Table 4** HLA-DNA typing of DQA1

| HLA antigens | AIH 74 alleles (%) | Controls 200 alleles (%) | OR | 95% CI | $\chi^2$ | Corrected *p* |
|---|---|---|---|---|---|---|
| 0101 | 19 (25.67) | 26 (13) | 2.31 | 1.13-4.73 | 5.43 | 0.019 |
| 0102 | 12 (16.21) | 33 (16.5) | 0.98 | 0.45-2.12 | 0.02 | NS |
| 0103 | 2 (2.70) | 8 (4) | 0.67 | 0.1-3.5 | 0.00 | NS |
| 0201 | 5 (6.75) | 24 (12) | 0.53 | 0.17-1.55 | 1.06 | NS |
| 0301 | 12 (16.21) | 49 (24.5) | 0.60 | 0.28-1.25 | 1.69 | NS |
| 0501 | 13 (17.56) | 23 (11.5) | 1.64 | 0.73-3.64 | 1.25 | NS |
| 0601 | 11 (14.86) | 37 (18.5) | 0.77 | 0.35-1.68 | 0.60 | NS |

*p values are given only where significant at <5% level.*
*NS: non-significant*

**Table 5** HLA-DNA typing of DQBI

| HLA antigens | AIH 74 alleles (%) | Controls 200 alleles (%) | OR | 95% CI | $\chi 2$ | Corrected *p* |
|---|---|---|---|---|---|---|
| 0201 | 13 (17.56) | 18 (9) | 2.15 | 0.93-4.96 | 3.14 | NS |
| 0301 | 16 (21.62) | 55 (27.5) | 0.73 | 0.37-1.43 | 0.69 | NS |
| 0302 | 1 (1.35) | 16 (8) | 0.16 | 0.01-1.16 | 3.04 | NS |
| 0303 | 5 (6.75) | 33 (16.5) | 0.37 | 0.12-1.04 | 3.52 | NS |
| 0401 | 6 (8.11) | 11 (5.5) | 1.52 | 0.48-4.65 | 0.26 | NS |
| 0501 | 11 (14.86) | 14 (7) | 2.32 | 0.93-5.78 | 3.14 | NS |
| 0502 | 13 (17.56) | 25 (12.5) | 1.49 | 0.67-3.27 | 0.78 | NS |
| 0503 | 2 (2.70) | 5 (2.5) | 1.08 | 0.14-6.48 | 0.11 | NS |
| 0601 | 4 (5.40) | 15 (7.5) | 0.70 | 0.19-2.37 | 0.11 | NS |
| 0602 | 1 (1.35) | 5 (2.5) | 0.53 | 0.02-4.82 | 0.01 | NS |
| 0603 | 1 (1.35) | 1 (0.5) | 2.73 | 0-101.13 | 0.00 | NS |
| 0605 | 1 (1.35) | 2 (1) | 1.36 | limitted | 0.16 | NS |

*p values are given only where significant at 5% level.*
*NS: non-significant*

acute hepatitis and over than 40% presented with cirrhosis. Mean age of patients with cirrhosis was 70 years, compared to 56 years in the hepatitis group. These figures are not different from data from Western contries[19].

Using PCR-SSOP based class II DNA typing, we found that HLA DRB1*0301 and DQA1*0101 alleles were significantly associated with Thai patients with AIH type 1 compared to healthy individuals. When analyzing according to the International Autoimmune Hepatitis Group scoring system as definite or probable AIH[16,17], only HLA DRB1*0301 remains significantly associated with definite AIH with OR 5.22. Our finding is compatible to the data in most studies from Western which found the association with HLA DRB1*0301 and DRB1*0401[23,24].

In Caucasians, a dual association of HLA-DR3 and -DR4 has been found in patients with DM type 1 as well as with AIH. Recently, molecular analyses to demonstrate HLA-linked susceptibility or resistance to some autoimmune diseases have become possible by the advent of PCR technique.

Studies from Western indicated that HLA DR3 (DRB1*0301) and -DR4 (DRB1*0401) are independent risk factors influencing disease expression and behavior as well as susceptibility to disease. In Caucasian type 1 AIH, DRB1*0301 individuals are younger6 and have a higher rate of treatment failure6, high rate of relapse after drug withdrawal20 and more requirement for liver transplantation (OLT)[21,22]. In contrast, DRB1*0401 individuals are older, frequently have concurrent autoimmune diseases but respond better to corticosteroids than individuals with DRB1 *0301[5,6]. Thus, Thai AIH patients who are more likely to associate with HLA DRB1*0301 might not have favorably outcomes. Further study should be carried to confirm this result.

We believe that using HLA analysis to predict the natural course of the disease would be beneficial by alerting physician for more aggressive treatments in order to delay disease progression to cirrhosis and need for OLT.

Etiologic agent that may trigger the onset of disease in AIH patients has not been identified. It has been proposed that the initial triggers are organ specific agents e.g. viral infection of the liver or exposure to hepatotoxic agents e.g. drugs, with ensuing autoimmune response and influenced by the presence of certain HLA susceptibilities, especially HLA-DR, causing the presentation of organ specific autoantigens or processed fragments of foreign antigens (from virus gene products) that have a molecular mimicry with organ specific autoantigen to the T cells. A high prevalence of antibody against asialogycoprotein receptor has recently been described in AIH patients, suggesting that the asialogycoprotein receptor may be an autoantigen targeted by the antibody specificity seen in AIH. HLA-DR antigen plays an important role in presenting the human asialogycoprotein receptor to the T cells as an initial immune response in AIH.

Although type 1 AIH is considerably less common than DM type 1 or RA, it may serve as a useful model for other immune diseases. The emergence of high technologies will significantly enhance our ability to study the interactions between genes and both disease expression and behavior. In the future, functional studies and genome scanning may help us to diagnose, predict severity, and guide treatments of AIH. Under these circumstances, genetic studies may be the most practical, low risk investigation to help us understanding the pathogenesis of type I AIH and many other autoimmune diseases.

## CONCLUSION

HLA DRB1*0301, and DQA1*0101 were significantly associated with AIH in Thailand. Considering only definite AIH, HLA DRB1*0301 was the only HLA associated with AIH.

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