Association between HLA Class II Alleles and Autoimmune Hepatitis Type 1 in Thai Patients

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Objective: *To investigate the association between HLA class II alleles and autoimmune hepatitis (AIH) type I in Thai patients.*
Material and Method: *The clinical data of 50 autoimmune hepatitis patients type 1 (AIH) at Siriraj hepatitis clinic were analysed, 37 of whom were tested for HLA class II genotyping using polymerase chain reaction and sequence-specific oligonucleotide technique (PCR-SSO).*
Results: *AIH is an uncommon chronic hepatitis in Thailand with females predominant. The HLA DRB1*0301, and DQA1*0101 were significantly associated with AIH patients when compared to controls; (OR = 3.92 [1.18-13.30], p 0.021, OR = 2.31 [1.13-4.73], p 0.019, respectively). When 18 patients with "definite" AIH were analysed, only HLA DRB1*0301 was still significantly associated with AIH (OR = 5.22, 95%CI = 1.28-20.92, p 0.015)*
Conclusion: *HLA genotyping has shown that HLA DRB1*0301 and HLA DQA1*0101 were significantly associated with AIH.*

Keywords: *Autoimmune hepatitis type 1, HLA class II alleles*

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Autoimmune chronic hepatitis type 1 (AIH) is a chronic liver disease characterized by piecemeal necrosis, hypergammaglobulinemia and high serum autoantibody[1]. Epidemiologically, AIH is more common in the western European population with an incidence of about 1 per 100,000 population/year. A study from Japan estimated that AIH accounted for less than 5% of all cases of chronic hepatitis. Recently, Nishioka et al. compared the incidence of AIH with primary biliary and primary sclerosing cholangitis from England, France, Austria, and Japan. The estimated incidence per 100,000/year of AIH was 0.1-0.2 in England, 0.12 in France, 1.2 in Austria, and only 0.015-0.08 in Japan[2]. There were very few data of AIH elsewhere in Asian countries and generally AIH is considered rare in this region. AIH has a strong genetic predisposition that may affect susceptibility, clinical features, and treatment outcome[3-8].

Studies in Western Europe have found that AIH is associated with alleles of HLA complex class II and this association has regional variations that may cause difference in clinical manifestation and disease outcome. In Europeans and Caucasoid Americans, AIH is significantly associated with the HLA A1-B8-DR3 haplotype[7-11]. For those with DR3-negative AIH, a significant number of patients are associated with HLA DR4. HLA A1-B8-DR3 associated AIH presented at a younger age and responded more poorly to corticosteroid treatment as compared with HLA DR4-positive patients[11]. Studies in Northern European and North American white patients with AIH have shown the strongest association with alleles DRB1*0301 (DR3) and DRB1*0401(DR4). Overall about 80-85% of AIH in Caucasoid adults carried HLA DR3, DR4, or both

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markers[3]. Studies from Japan did not show any association of AIH and HLA A1-B8-DR3 which was also rare in the general population. By contrast, HLA DR4 was found significantly associated with AIH in Japan and accounted for about 90%. AIH in Japan is found in elderly patients who have mild inflammatory activity and a low frequency of treatment failure which resembles HLA DR4-positive patients in the United States. HLA genotyping has revealed that DRB1*0405 is the principle susceptibility allele[12-13]. A study from India of 20 patients with AIH revealed association with DRBI*01XX, DRB1*14XX, DRB1*15XX, and DRB1*07XX at DRB1 loci different from western patients[14]. There is no data regarding AIH and HLA class II association elsewhere in Asian countries[15,16] including Thailand. The objective of this study was to determine the association between various HLA Class II and AIH in Thailand.

Material and Method

Patients

Of more than 2000 patients with chronic hepatitis regularly monitored at Siriraj Hepatitis Clinic, Siriraj Hospital during January 1990 to October 2004, 56 patients were diagnosed as AIH (definite or probable) based on the International Autoimmune Hepatitis Group Scoring System[17,18]. The control group for HLA analysis consisted of 100 healthy subjects who were unrelated to the patients.

HLA Typing

HLA-DRB1, -DQA1, -DQB1 typings were performed by polymerase chain reaction using a sequence-specific oligonucleotide technique (PCR-SSO). The protocol, primers, and probes were used as previously described[19]. In brief, genomic DNA from peripheral blood cells was isolated by using a modified guanidine hydrochloric acid extraction method. The polymerase chain reaction amplification of sample DNA was carried out using 3 sets of generic primers (DRB, primers DRBAMP-A and DRBAMP-B; DQA, primers DQAAMP-A and DQAAMP-B; and DQB; primers DQBAMP-A and DQBAMP-B). For analysis of certain alleles, the DNA was amplified using 3 sets of group-specific primers (DR2, primers DRBAMP-2 and DRBAMP-B; DR4, primers DRBAMP-4 and DRBAMP-B; DR52-DRB1, primers DRBAMP3 and DRBAMP-B). Amplified DNA was hybridized with non-radioactive (DIG-ddUTP)-labelled oligonucleotide probes in a dot blot-type assay. The reaction was detected using anti-digoxigenin alkaline phosphatase conjugate by the chemiluminescent method. For generic typing of DRB1, DQA1 and DQB1 alleles, 26, 17 and 25 probes were used for hybridization and an additional 8, 14, and 27 probes were used for hybridization after group-specific amplification of HLA-DR2, -DR4, and -DR52.

Statistical analysis

Quantitative data were summarized as mean and standard deviation. To test the difference in alleles between two groups, a chi-test test was applied. Odds ratio and its 95% confidence interval (CI) were also reported.

A 2-sided p-value of less than 0.05 was considered statistically significant. All statistical data analyses were performed using SPSS Version 11.

Results

Of the 56 patients with the diagnosis of AIH, 6 were excluded due to the features of overlapping syndrome. Finally there were 50 patients with AIH (18 definite, 32 probable). Forty three (86%) were female with mean age of 62 years old (range 29-85 years). Seventeen patients (34%) presented with acute hepatitis, 22 patients (44%) had evidence of cirrhosis at presentation (fatigue, anorexia, jaundice and complications of cirrhosis i.e. ascites and oesophageal varices). Eleven patients (22%) were asymptomatic and were discovered during routine check up. Laboratory values at presentation are shown in Table 1. Forty eight patients (96%), and 14 (28%) were positive for antinuclear antibody and anti-smooth muscle antibody, respectively. Liver biopsy was done in 22 patients (44%) and all had histological findings compatible with AIH.

Blood samples were obtained for HLA class II alleles genotyping in 37 of 50 patients. The frequency of HLA class II antigens in AIH patients compared to healthy individuals is shown in Table 2. HLA-DR3 is significantly associated with AIH.

The results of HLA-DNA typing for DRB1 alleles are summarized in Table 3. Only the DRB1*0301

Table 1. Laboratory findings of 50 AIH type 1 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 ± 0.7	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

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

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

p-values are given only where significant at < 5% level
NS: not significant

Table 3. HLA-DNA typing for DRB1

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

p-values are given only where significant at < 5% level
NS: not significant

Table 4. HLA-DNA typing of DQA1

Allele DQA1*	AIH 74 alleles (%)	Controls 200 alleles (%)	OR	95%CI	Corrected p
0101	19 (25.67)	26 (13)	2.31	1.13-4.73	0.019
0102	12 (16.21)	33 (16.5)	0.98	0.45-2.12	NS
0103	2 (2.70)	8 (4)	0.67	0.1-3.5	NS
0201	5 (6.75)	24 (12)	0.53	0.17-1.55	NS
0301	12 (16.21)	49 (24.5)	0.60	0.28-1.25	NS
0501	13 (17.56)	23 (11.5)	1.64	0.73-3.64	NS
0601	11 (14.86)	37 (18.5)	0.77	0.35-1.68	NS

p-values are given only where significant at < 5% level
NS: not significant

Table 5. HLA-DNA typing of DQB1

Allele DQB1*	AIH 74 alleles (%)	Controls 200 alleles (%)	OR	95%CI	Corrected P
0201	13 (17.56)	18 (9)	2.15	0.93-4.96	NS
0301	16 (21.62)	55 (27.5)	0.73	0.37-1.43	NS
0302	1 (1.35)	16 (8)	0.16	0.01-1.16	NS
0303	5 (6.75)	33 (16.5)	0.37	0.12-1.04	NS
0401	6 (8.11)	11 (5.5)	1.52	0.48-4.65	NS
0501	11 (14.86)	14 (7)	2.32	0.93-5.78	NS
0502	13 (17.56)	25 (12.5)	1.49	0.67-3.27	NS
0503	2 (2.70)	5 (2.5)	1.08	0.14-6.48	NS
0601	4 (5.40)	15 (7.5)	0.70	0.19-2.37	NS
0602	1 (1.35)	5 (2.5)	0.53	0.02-4.82	NS
0603	1 (1.35)	1 (0.5)	2.73	0-101.13	NS
0605	1 (1.35)	2 (1)	1.36	limited	NS

p-values are given only where significant at < 5% level
NS: not significant

allele is significantly increased in AIH compared to the controls. Table 4 shows HLA-DNA typing for the DQA1 gene. The incidence of the DQA1*0101 allele is significantly higher in the AIH patients. For the HLA-DQB1 gene, there were no DQB1 alleles significantly associated with AIH as shown in Table 5.

When only 18 patients with "definite" AIH were analyzed, only HLA DRB1*0301 was still significantly associated. (OR=5.22, 95%CI=1.28-20.92, p = 0.015)

Discussion

The prevalence of AIH in our study was low. Of about 2000 cases of chronic hepatitis, there were only 50 patients diagnosed with AIH during 14 years. This finding is similar to reports from Japan, Taiwan, and India[2,15-16] where AIH is also uncommon. This contrasts with data from western European studies where the estimated prevalence is 11-20% of all cases of chronic liver disease. The majority of the patients in our study were in their fifth to sixth decades with a mean age of 62 years and there was a female preponderance (F: M 6:1). Studies in northern European Caucasians, found that elderly patients were not uncommon and the mean age of patients from a study from Japan was 50.8 years[17,18,20]. The presentation of AIH in Thailand is similar to western European patients where approximately one-third presented with acute hepatitis and over 40% presented with evidence of cirrhosis[20,21].

The low prevalence in our population may be attributed to possible genetic or geographic variation. In Caucasians, a dual association of HLA-DR3 and -DR4 was also found in patients with DM type 1[22]. Studies have indicated that HLA DR3 (DRB1*0301) and -DR4 (DRB1*0401) are independent risk factors influencing disease expression and behaviour as well as susceptibility to autoimmune diseases including AIH. In Caucasian AIH, DRB1*0301 individuals are younger[11] and have a higher rate of treatment failure[7], a high rate of relapse after drug withdrawal[23] and more requirement for liver transplantation (OLT)[11,24]. In contrast, DRB1*0401 individuals are older and frequently have concurrent autoimmune diseases, but respond better to corticosteroid than individuals with DRB1*0301[6,7]. The significant association between HLA DR3, DR4 and AIH in Caucasians leads to incorporation of HLA DR3, DR4 as a criteria for diagnosis of AIH[17,18].

In our study using PCR-SSO based HLA class II DNA typing, we found that HLA DRB1*0301 and DQA1*0101 alleles are significantly associated with AIH compared to healthy individuals. When only "definite" AIH according to the International Autoimmune Hepatitis Group scoring system[17,18] was analyzed, only HLA DRB1*0301 was still significantly associated with AIH (OR 5.22). This association with HLA DRB1*0301 and DQA1*0101 is different from previous reports from Japan and India[12-14]. The finding of an association between the HLA DRB*0301 allele and AIH is similar to data from western Europeans and Caucasoid Americans[11,25]. However, the prevalence of HLA DRB1*0301 positive AIH in Thai patients is only about 11%, much less than in studies from the

west. The association between HLA DQA*0101 and AIH was not previously been recognized. This association cannot be observed when probable AIH is excluded. The clinical significance and usefulness of HLA genotyping for diagnosis and prediction of disease outcomes in Thai patients requires further study.

Conclusion

AIH is an uncommon disease in Thailand, more common in older females and usually presents with cirrhosis or acute hepatitis. AIH in Thailand is significantly associated with the HLA DRB1*0301, and DQA1*0101 alleles. However, when definite AIH was analyzed, HLA DRB1*0301 was the only HLA associated with AIH.

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ความสัมพันธ์ระหว่าง HLA class II alleles และ autoimmune hepatitis type 1 ในผู้ป่วยชาวไทย

ทวีศักดิ์ แทนวันดี, สุเทพ วนิชผล, ศศิจิต เวชแพศย์, ศิวะพร ไชยนุวัติ, วัชรศักดิ์ โชติยะปุตตะ

วัตถุประสงค์: *เพื่อศึกษาความสัมพันธ์ระหว่าง HLA class II alleles และ autoimmune hepatitis type 1 (AIH) ในผู้ป่วยชาวไทย*

วัสดุและวิธีการ: *ได้รวบรวมผู้ป่วย AIH 50 รายที่มารับการรักษาที่คลินิกโรคตับ โรงพยาบาลศิริราช โดยมีผู้ป่วย 37 ราย ที่สามารถตรวจ HLA class II genotyping ด้วยวิธี polymerase chain reaction sequence-specific oligonucleotide technique (PCR-SSO).*

ผลการศึกษา: *จากการศึกษาพบว่า AIH เป็นโรคตับเรื้อรังที่พบได้น้อยในประเทศไทยพบมากในเพศหญิง การศึกษา HLA พบว่า HLA DRB1*0301 และ DQA1*0101 มีความสัมพันธ์กับ AIH ในประเทศไทยอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุม (OR = 3.92 [1.18-13.30], p 0.021, OR = 2.31 [1.13-4.73], p 0.019 ตามลำดับ) เมื่อวิเคราะห์เฉพาะผู้ป่วยที่เป็น "definite" AIH 18 รายพบว่าเฉพาะ HLA DRB1*0301 ที่ยังมีความสัมพันธ์กับ AIH (OR = 5.22, 95%CI = 1.28-20.92, p 0.015)*

สรุป: *HLA DRB1*0301 และ HLA DQA1*0101 พบมีความสัมพันธ์กับ AIH อย่างมีนัยสำคัญ*