HLA DRB1 Alleles Discriminate the Manifestation of Autoimmune Hepatitis as Type 1 or Type 2 in North Indian Population

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Background: **Autoimmune hepatitis is a polygenic disorder of unknown etiology, where genetic factors affect the occurrence and clinical phenotype of the disease. It has been reported as a rare disease entity in the Indian subcontinent. This study was undertaken to investigate the association of HLA alleles with autoimmune hepatitis type 1 and type 2 in north Indian population and to analyze if distinct human leukocyte antigen (HLA) alleles help in characterization of the subtypes of autoimmune hepatitis.** ***Methods:*** **Sixty-eight patients with autoimmune hepatitis and 128 healthy controls were recruited in the study. Out of 68 patients, 55 were diagnosed with autoimmune hepatitis type 1 and 13 with autoimmune hepatitis type 2. The patients and the controls were typed for HLA class II alleles by PCR-SSP method.** ***Results:*** **HLA DRB1*04 and DRB1*08 were found to be significantly associated with autoimmune hepatitis type 1 in north Indian population. It was also observed that DRB1*04, DRB1*13 were significantly associated with pediatric autoimmune hepatitis type 1 and DRB1*08 was significantly associated with adult autoimmune hepatitis type 1. DRB1*14 was significantly associated with autoimmune hepatitis type 2.** ***Conclusion:*** **The study indicates that autoimmune hepatitis in north Indian population is associated with HLA alleles that may help to discriminate the subtypes as autoimmune hepatitis type 1 and type 2. The study also highlights the ethnic variations in the Indian subcontinent in context to the genetic association of HLA with autoimmune diseases. (J Clin Exp Hepatol 2014;4:14–18)**

Autoimmune hepatitis (AIH) is an organ specific, chronic inflammatory disease of unknown etiology characterized by presence of circulating autoantibodies, hypergammaglobulinemia, necroinflammatory changes on hepatic histology and response to immunosuppressive therapy.[1,2] The cause of the disease is unknown, but there is a presumed loss of self-tolerance after repeated exposure to foreign antigens that resemble self-antigens. It follows a fluctuating course, shows a female preponderance and can affect children and adults of any age, sex or ethnic group.[3,4] AIH has a global occurrence as it has been described in African Americans, native Alaskans, Arabs, Asians, Europeans, Iranians, South Americans and subcontinental Indians.[5,6] The frequency of AIH among patients with chronic liver disease in North Americans is between 11 and 23%.[7]

AIH has been classified into different types based on serum autoantibody profile. Type 1 AIH is characterized by presence of antinuclear antibody (ANA), anti smooth muscle antibody (ASMA) or both and constitutes more than 80% of the cases. Type 2 AIH is characterized by presence of anti liver kidney microsomal (LKM) and/or anti-liver cytosol (LC1) antibodies.[8] Type 2 AIH is less common and tends to be more severe than AIH type 1. It shares most of the features with AIH-1, but occurs more commonly in children.[9,10]

AIH reflects a complex interaction between triggering factors, autoantigens, genetic predisposition and immune regulatory networks. It is a polygenic disease, as several genes lead to genetic predisposition in different population. There are several candidate genes of potential interest, but genes of HLA region have been strongly implicated in susceptibility to AIH and other autoimmune diseases.[11,12] The major histocompatibility complex (MHC) that codes for HLA occupies a 4-megabase (Mb) segment of chromosome 6p21.3 and the genes encoded therein exhibit a very high

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Abbreviations: AIH: autoimmune hepatitis; ANA: antinuclear antibody; ASMA: anti smooth muscle antibody; LKM: liver kidney microsomal; HLA: human leukocyte antigen; MHC: major histocompatibility complex; Mb: megabase; AASLD: American Association for the Study of the Liver; IAHG: International Autoimmune Hepatitis Group; IIF: indirect immuno florescence; PCR-SSP: polymerase chain reaction-sequence specific primers; OR: odds ratio; CI: confidence interval; RR: relative risk
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degree of polymorphism. Thus, there are currently approximately 1000 different HLA alleles[13] which have been described in different populations in association with AIH type 1 and AIH type 2. Among white northern Europeans and Americans, there is a well-recognized association between increased susceptibility to AIH and inheritance of DR3 and DR4.[14]

The present study was undertaken to study the association of HLA alleles in patients with AIH type 1 and AIH type 2 from north Indian population.

METHODS

Sixty-eight patients attending the Hepatology clinic and Paediatric Gastroenterology Clinic, at the Post Graduate Institute of Medical Education and Research, Chandigarh were included in the study after an informed consent. Detailed written information about this study and its prospects was given to the adult patients and guardians of the pediatric patients in the language of their preference. After the signatory expressed his willingness, a written informed consent was obtained. The medical records of the patients were reviewed and pertinent information of each patient, including history as well as symptoms (pruritus, fatigue), clinical findings (jaundice, cirrhosis) and data from laboratory and other diagnostic investigations were obtained at initial visit and at follow-ups after initiation of therapy. The diagnosis of the patients was made on the basis of the criteria and scoring system codified by AASLD (American Association for the Study of the Liver) and IAHG (International Autoimmune Hepatitis Group)[15,16] respectively. The Institute ethics committee approved the study.

For genetic analysis, age and sex matched 128 voluntary healthy controls were also included in the study. The controls were unrelated to patients and were from same ethnic group. The control population was negative for autoimmune markers and had no history of any chronic illness. A written informed consent was obtained from these individuals in a similar way as for the patients.

Sample Collection

Five ml of peripheral venous blood sample was obtained from patients and the controls; 3 ml in EDTA vial for DNA extraction and 2 ml in plain vial for serum separation.

Autoantibodies

AMA, ASMA, ANA, LKM were detected in the serum of patients by in house indirect immuno florescence (IIF) on rat tissue sections (kidney, liver and stomach). Positivity was defined by titer of ≥1/80. LKM positivity was further confirmed by LKM1 ELISA (Varelisa, Phadia, USA) and line immunoassay of liver profile (AMA-2, LKM1, LC1, SLA and F actin) procured from Dtek Germany.

Human Leukocyte Antigen Typing

The genomic DNA was extracted from EDTA blood sample using commercially available DNA extraction kit (Axygen Biosciences, USA) and was thereafter quantified on calibrated spectrophotometer at 260 nm. Sixty patients and 128 controls were typed for HLA class II loci DRB1 and DQB1 by polymerase chain reaction-sequence specific primers (PCR-SSP) using low resolution HLA typing kits[17] (Innotrain, Germany).

Radial Immunodiffusion Assay

The quantitative determination of IgG was done in sera of patients by radial immunodiffusion assay (Bindarid, Binding site USA).

Statistics

Quantitative data were expressed as mean, range and frequency. The groups of AIH type 1 and type 2 were individually analyzed with reference to controls. Data were analyzed by Chi square test with Yates's correction, odds ratio (OR) with 95% confidence interval (CI), relative risk (RR), Spearman's rank correlation and logistic regression where applicable. Two sided *P* values less than 0.05 were considered significant. The analysis was done by SPSS software (Statistical Package for Social Sciences. Version 10.0, Chicago, USA).

RESULTS

Sixty-eight patients diagnosed with AIH were included in the study and were dichotomized into two groups: Group I (patients with AIH type 1) and group II (patients with AIH type 2). 55 (80.9%) patients were included in group I and 13 (19.1%) in group II. In group I out of 55, 35 (63.6%) patients were adult and 20 (36.4%) were pediatric patients. In group II, 5 (38.5%) patients were adult and 8 (61.5%) were pediatric.

All the patients were scored according to the IAHG criteria. All but one patient had scored above 15 confirming definitive hepatitis, only one patient had scored 14 leading to diagnosis of probable hepatitis. Most of them gained points for female gender, absence of AMA and drug abuse, presence of ANA/SMA or LKM in titer of more than 1:80, raised IgG levels etc. The age range, mean age, sex ratio and IgG levels of the patients in all the groups are summarized in Table 1.

The HLA association studies were done in all the subgroups separately and also in AIH type 1 and AIH type 2 groups and the frequencies of HLA alleles obtained are cited in Table 2. The association studies in patients with AIH type I showed that DRB1*04 (P = 0.008, OR = 3.292, CI = 1.370–7.913) and DRB1*08 (P = 0.027, OR = 10.5, CI = 1.183–33.54) were significantly associated with the disease. DRB1*13, DRB1*14 and

Table 1 Patients' Characteristics.

Patients' characteristic	AIH-1 (group I) *n* = 55		AIH-2 (group II) n = 13	
	Pediatric patients (*n* = 20)	Adult patients (*n* = 35)	Pediatric patients (*n* = 8)	Adult patients (*n* = 5)
Age range	2–13	17–62	3–15	20–60
Mean age	7.4 ± 3.13	41.14 ± 12.75	8.25 ± 3.99	34.25 ± 18.19
Sex ratio (M/F)	1.2/1	1/1.3	1/1	3/1
Patients with IgG levels above normal	15	31	6	3

DRB4 were found at higher frequency in the patient group in comparison to the controls. The high-risk haplotypes in this patients group were DRB1*07-DRB1*08 and DRB1*04-DRB4. HLA association analysis was also done on dividing the patients with AIH type 1 into two groups: pediatric and adult. The analysis showed that in pediatric group DRB1*04 (P = 0.004 OR = 5.727, CI = 1.892–17.333) and DRB1*13 (P = 0.020, OR = 3.371, CI = 1.261–9.012) were significantly associated with the disease and DRB1*13-DQB1*06 was the high-risk haplotype present in this disease group. DRB1*03 and DQB1*02 were not significantly associated with the pediatric AIH type 1 group but were increased in comparison to the controls (40% Vs 22.7%, 45% Vs 35.3% respectively).

In adult group DRB1*08 (P = 0.005, OR = 10.5, CI = 1.942–56.76) was significantly associated with the disease and DRB1*07-DRB1*08 was the high-risk haplotype present. HLA analysis of AIH type II revealed that DRB1*14 (P = 0.047, OR = 3.5 CI = 1.060–12.132) was significantly associated with the disease in this population and DRB*14-DRB1*13 and DRB1*14-DQB1*05 were the high-risk haplotypes for this group.

DISCUSSION

This report describes the HLA alleles associated with AIH type 1 and AIH type 2 in north Indian population. HLA DRB1*08 and DRB1*04 were significantly associated with AIH type 1 in the population and DRB1*14 was associated with AIH type 2 in the north Indian population. Different susceptibility alleles of HLA DR locus have been described in different populations. We emphasize that the scores for DR3, DR4 in the AASLD guidelines are relevant only for Caucasoid population and require correction for different ethnic populations. In white Europeans and North American patients with AIH type 1 genetic susceptibility is associated with the presence of DRB1*0301, DRB3 and DRB1*04.[18,19] In Argentine adults and children DRB1*0405 and DRB1*13 have been described as the susceptibility alleles respectively.[20] In our study DRB1*04 and DRB1*13 were associated with pediatric AIH only. In Brazilian population, DRB1*13 and DRB1*03 have been described to be associated with AIH,[21] contrary to this we have previously reported the association of DRB1*03 with PBC/AIH overlap in north Indian population.[22] DRB1*15 has been described as the protective allele in the Caucasoid population for AIH,[23] but presence of this allele is high in the north Indian population irrespective of the disease association as evident from frequencies cited in Table 2, further highlighting the ethnic variations in susceptibility and protective alleles.

Table 2 Genotype Frequencies of Alleles of DRB1 and DQB1 in Group I (AIH Type 1 Patients) and II (AIH Type 2 Patients) and Group III (Controls).

HLA allele	AIH-I (group I)			AIH-II (group II) (*n* = 13)	Controls group III (*n* = 128)
	Pediatric + adult patients (*n* = 55)	Pediatric patients (*n* = 20)	Adult patients (*n* = 35)		
DRB1*01	1.8	0	2.9	0	5.5
DRB1*15	38.2	25	45.7	46.2	46.1
DRB1*03	25.5	40	17.1	38.5	22.7
DRB1*04	23.6	35	17.1	0	8.6
DRB1*07	16.4	15	17.1	0	21.1
DRB1*08	9.1	0	14.3	0	1.6
DRB1*09	0	0	0	0	6.3
DRB1*10	9.1	5	11.4	15.4	13.3
DRB1*11	12.7	15	11.4	38.5	18
DRB1*12	0	0	0	15.4	2.3
DRB1*13	30.9	45	22.9	0	19.3
DRB1*14	18.2	10	22.9	38.5	14.6
DRB3	60	65	57.1	76.9	66.1
DRB4	43.6	45	42.9	15.4	40.6
DRB5	41.8	35	45.7	38.5	40.6
DQB1*02	34.5	45	28.6	38.5	35.3
DQB1*03	43.6	45	42.9	53.8	32.6
DQB1*04	5.5	0	8.6	0	1.6
DQB1*05	30.9	30	31.4	53.8	43.9
DQB1*06	50.0	57.9	45.7	30.8	50.8

Several studies from Asian countries like Thailand, Korea, and Japan have also reported different HLA alleles to be associated with the disease in the respective populations. DRB1*0405 and DRB1*0401 have been reported to be the associated alleles in the Japanese and Korean patients, whereas studies from Thai population have reported DRB1*03 to be associated with the disease.[24–27] Studies from western India have reported increase in frequency of DRB1*13, DRB1*03, DRB1*15, DRB1*14 alleles in patient group in comparison to the controls.[28,29] Contrary to this, our study has found that DRB1*08 and DRB1*04 to be associated with the disease. On subdividing the AIH type 1 patient group into pediatric and adult it was found that DRB1*04 was associated with the pediatric population and DRB1*08 was associated with the adult population. Hence, it can be said that both DRB1*04 and DRB1*08 genetically predispose the population to the disease, but presence of DRB1*04 leads to early manifestation of the disease and DRB1*08 leads to diseased state in the adults. The difference in this study and the western reports highlights the fact that India is a large subcontinent and the ethnic variations in the northern, western, southern regions are quite conspicuous as previously described by the Indian genetic consortium.[30]

There is a paucity of studies on type 2 AIH. In a study by Bittencourt et al, patients with AIH type 2 had significantly higher frequency of DRB1*07, DRB4 and DQB1*02 compared to controls.[31] In a Canadian study, DQB1*0201 was found to be primarily responsible for the increase in the susceptibility to AIH type 2.[32] In another study, American and German patients with AIH type 2 had significantly higher frequency of DRB1*07, DRB1*15 and DQB1*06[33] compared to controls. In our cohort, patients with AIH type 2 were found to be significantly associated with DRB1*14. Further subgrouping of the patients into type 2a and type 2b was not done due to the small number of patients in this group. There can be two limiting factors that remain in the HLA association studies in AIH type 2 groups, which are prominent in the above-mentioned studies as well as in our study; these include the limited number of patients that can be recruited in the study as the disease is so rare and the other being the false sero-negative status of HCV in these patients due to less sensitive immunoblots that are commonly used. Rather a more sensitive technique like quantitative copy number of the virus should be assessed in these patients by real time PCR that may bring out the subtle differences in genetic susceptibility between AIH type 2a and type 2b.

This is the first report from north India on genetic basis of AIH type 1 and type 2 that clearly brings out the differences in HLA basis of this disease from those reported in other countries. The study paves path for future research on understanding the effect of HLA on disease modulation and similar studies may help in benefitting the predisposed individuals with early therapy.

CONCLUSION

Thus HLA DRB1 alleles were found to be differently associated with AIH type 1 and type 2. DRB1*08, DRB1*04 were associated with AIH type 1 and DRB1*14 was associated with AIH type 2. Multi centric studies with large cohorts will further help to stratify the disease phenotype by HLA alleles as well as validate these findings.

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CONFLICTS OF INTEREST

All authors have none to declare.

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