Association of HLA DRB1 Allele Profile with Pediatric Autoimmune Liver Disease in India

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Objectives: **The aim of this study is to study the association of human leukocyte antigen (HLA) DRB1 alleles with treatment response in Indian children with autoimmune liver disease (AILD).** ***Methods:*** **HLA DRB1 alleles of 71 Indian children with pediatric AILD (pAILD) were analyzed along with 25 genetically confirmed patients with Wilson disease as controls. After 1 year of therapy, all those who failed to normalize aspartate & alanine transferase (AST/ ALT) (below 1.5 times of upper limit of normal) and/or failed to normalize IgG levels, or who had >2 relapses (AST/ALT levels >1.5 times of upper limit of normal) while on treatment, were labeled as difficult to treat (DTT).** ***Results:*** **HLA DRB1*3 was found to be significantly associated with AIH type 1 (46.2% vs. 4% in controls; *P* corrected = 0.011). Majority of the patients [55 (77.5%)] had chronic liver disease at presentation, with 42 (59.2%) having portal hypertension and 17 (23.9%) having ascites. Out of the 71 with pAILD, 19 (26.8%) were DTT. HLA DRB1*14 was found to be independently associated with DTT cases (36.8% vs. 9.6%, OR 5.87, 95% CI 1.07–32.09, *P* = 0.041). Other factors independently associated with DTT were presence of autoimmune sclerosing cholangitis (OR 8.57, *P* = 0.008) and high-risk varices (OR 7.55, *P* = 0.016), improving the correctness of classification of the model from 73.2% to 84.5%.** ***Conclusion:*** **HLA DRB1*14 is independently associated with treatment response in pAILD and HLA DRB1*3 is associated with AIH type 1. HLA DRB1 alleles may thus provide supportive information for diagnosis and prognosis of AILD.** **(J Clin Exp Hepatol 2023;13:397–403)**

Pediatric autoimmune liver disease (pAILD) is a group of chronic progressive inflammatory liver disorders that comprise autoimmune hepatitis (AIH) type 1, AIH type 2, autoimmune sclerosing cholangitis (AISC), and seronegative AIH.[1] Although the disease etiology is unknown, genetic and environmental factors are implicated in its pathogenesis.[2] Many studies have shown the association of AIH with human leukocyte antigen (HLA) genes located with the major histocompatibility complex (MHC) region on the short arm of chromosome 6.[3] In Europe and North America, susceptibility to adult AIH type 1 is conferred by HLA DRB1*3:01 and HLA DRB1*4:01,[4–6] while in Japan, HLA DRB1*4:01 and HLA DRB1*15:01 are implicated[7,8] and association of DRB1*4:04, 4:05 and 16:02 has been reported by studies from Latin America.[9] From the Indian subcontinent, association of HLA DRB1*14:01, 13:01 and 3:01 with adult AILD has been reported.[10–12] In pAILD, the alleles implicated are HLA DRB1*3:01, 13:01, and 7:01 in Caucasian children,[13–15] while DRB1*13:01, 3:01, and 7:01 are associated with Latin American children with AILD[16–19] and HLA DRB1*13:01 with middle eastern children.[20] Adult AILD studies from Japan have found HLA DRB1*13:02 and DRB1*4:05 to be associated with advanced disease and cirrhosis and HLA DRB1*15:01 to be associated with hepatocellular carcinoma.[8,21] HLA DRB1*3:01 was associated with lower rates of sustained remission and higher levels of transaminases at baseline in European studies.[22,23]

There are no studies on association of HLA DRB1 alleles with pAILD from the Indian subcontinent and neither on association with treatment response. Thus, this study was conducted in order to search HLA DRB1 alleles associated with pediatric AILD and its response to treatment among Indian children, the underlying hypothesis being that HLA DRB1 alleles are associated with treatment response in children with ALD.

METHODS

This was a single-center study carried out in the Department of Pediatric Hepatology of a tertiary care hospital over the course of 2 years from March 2020 to Feb 2022. All patients less than 18 years of age who attended the inpatient or outpatient clinic during the study period either previously or currently diagnosed as probable/definite AIH on the basis of simplified diagnostic criteria as

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Abbreviations: AIH: Autoimmune Hepatitis; AILD: autoimmune liver disease; AISC: autoimmune sclerosing cholangitis; DTT: difficult to treat; ETT: easy to treat; HLA: human leukocyte antigen
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proposed by International Autoimmune Hepatitis Group (IAIHG) 2008 were part of the study.[24] Only those patients whose complete records were available were also included in the study. Definite (≥7) and probable (≥6) autoimmune hepatitis was defined as per simplified diagnostic score.[24] Patients with probable AIH were further subjected to the original revised IAIHG scoring system.[25] Those with a pretreatment original AIH score of >15 or a posttreatment score >17 were included in the study.[25] This was done to ensure that all the patients included the study were patients with AIH. Certain criteria in the original revised IAIHG score[25] are redundant in children, and these were not considered while calculating the score. Ethical approval for the study was obtained from Institutional Review Board (IEC/2020/75/MA 12).

Types of AIH were defined on the basis of autoantibodies. Antinuclear antibody (ANA), antismooth muscle antigen antibody (ASMA), antiliver kidney microsome type 1 antibody (antiLKM1), and perinuclear anti neutrophil cytoplasmic antibody (pANCA) were performed in all patients. Those negative for ANA, ASMA, and anti LKM1 with or without pANCA positive were in addition screened with antisoluble liver antigen (anti-SLA) and antiliver cytosol-specific antibody type 1 (anti LC1). Those patients with definite AIH with pANCA positive or high GGT or ultrasound evidence of abnormal biliary tract underwent magnetic resonance cholangiopancreatography (MRCP). AISC was considered as the diagnosis if findings on MRCP or liver biopsy were suggestive of sclerosing cholangitis.[26] Patients without autoantibody positivity (ANA, ASMA, anti-LKM1, LC1, SLA, and pANCA), but with the diagnosis of AIH as mentioned before were classified as seronegative AIH. For ANA and ASMA, titers of >1:20 and for anti-LKM1 >1:10 were considered to be positive. Patients with history of poor compliance with treatment within the first 12 months of starting therapy were excluded from the group. Ninety-five children with AILD who attended the outdoor and indoor services of the hospital were initially considered for inclusion; however, 24 were excluded due to noncompliance and 71 were finally included in the study. Apart from these 71 pAILD cases, 25 unrelated genetically proven patients with Wilson disease were taken as controls.

Management Protocol and Treatment Response

Guidelines prescribed by American Association for the Study of Liver Diseases[27] were followed for management of the patients. Patients were started on prednisolone at 2 mg/kg for 4 weeks and azathioprine at 2 mg/kg (when total bilirubin was less than 5 mg/dl). Response to treatment was monitored every 4–8 weeks by performing liver function test and hemogram, and dose of azathioprine was increased up to 2.5 mg/kg in case of persistently high AST/ALT. AISC cases were treated with AIH regimen along with ursodeoxycholic acid (dose 15–20 mg/kg/day) and biliary radiological interventions (dilation with or without stenting) if needed. After 1 year of starting therapy, if AST/ALT failed to decrease below 1.5 times of normal and/or IgG failed to normalize, or those patients who had> 2 relapses (defined as increase in AST/ALT >1.5 times of normal) over any time period, while on adequate immunosuppression, were categorized as difficult to treat (DTT). The patients who normalized their AST/ALT to within 1.5 times upper limit of normal and IgG levels after 1 year of receiving standard treatment were categorized as easy to treat (responders).

HLA DRB1 Allele Identification

All patients underwent HLA DRB1 allele typing by Luminex Polymerase Chain Reaction Sequence Specific Primer method. Genotyping was done by specially trained laboratory personnel who was unaware of the original diagnosis. Further details of HLA allele typing, patient evaluation, and management protocol are available as supplementary material (SM-1).

Statistical Analysis

Statistical analysis was performed using SPSS version 22 (IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA). The categorical data were analyzed using chi-square or Fischer tests and continuous data were compared using Mann Whitney test or Kruskal Wallis test. Allele frequency was estimated by the presence of each HLA DRB1 allele in patients. *P* values were subjected to Bonferroni correction by multiplication by the number of different alleles observed. Univariate and multivariate binomial logistic regression analysis was used to identify independent factors associated with DTT patients. Further details are available in SM-1.

RESULTS

Of the 95 cases of pAILD patients who attended the outdoor and indoor services of the hospital during the study period, 71 were included and 24 excluded (SM-2). Twenty patients were enrolled prospectively, while data of 51 patients were analyzed retrospectively. Moreover, 25 unrelated genetically confirmed patients with Wilson disease were taken as controls. Of the 71 cases of pAILD, 55 patients (77.4%) were definite AIH and 16 (22.6%) were probable AIH. All of the 16 had either had original IAIHG pretreatment score of >15 or post treatment score >17. These baseline characteristics of the study cohort are depicted in SM-3. The clinical and laboratory parameters are depicted in SM-4.

Outcome of Patients

Of the 71 included AILD patients, 52 (73.2%) were classified as responders and 19 (26.8%) were DTT. All cases were started on prednisolone and azathioprine, and this

was continued in 64 (90.1%) patients, whereas due to inadequate response, four (5.6%) patients were shifted to prednisolone and MMF and four (5.6%) patients were further shifted to prednisolone with tacrolimus. As shown in SM-5, relapse was seen in 11 (15.5%) patients at a median time of 7 months (range 2.5–29.5 months) of starting therapy and of these six responded to increase in prednisolone dose alone, three required increase in both prednisolone and azathioprine, and two required second-line immunosuppressant, i.e., MMF. Of the 19 patients in the DTT group, despite showing an initial reduction in AST/ALT and IgG did not achieve normal levels, despite a year of therapy. DTT group had three (15.8%) patients of AIH type 1, four (21.2%) patients of AIH type 2, ten (52.6%) patients of AISC, and two (10.5%) seronegative AIH. As is evident from Supplementary Table 3, AISC patients were 7 times more likely to be DTT than non-AISC (OR, 7.14; 95% CI, 2.12–23.76; P = 0.001). Hundred percent of the DTT patients had chronic liver disease at the time of diagnosis versus 69.2% of responders' patients (OR, 1.52; 95% CI, 1.26–1.85; P = 0.006). Other features of advanced liver disease like ascites (47.4% vs. 15.4%; OR, 4.950; 95%, CI 1.53–16.01; P = 0.005), and portal hypertension (84.2% vs. 50%; OR, 5.33; 95% CI 1.38–20.52, P value = 0.009) were significantly associated with DTT patients. Presence of varices was also found to be associated with DTT patients (OR, 5.28; 95% CI, 1.64–17.04; P = 0.006) as was the presence of high-risk varices (OR, 6.9; 95% CI, 1.99–23.81; P = 0.002). In the laboratory parameters, higher Ishaks fibrosis score (4 vs. 2, P = 0.011) and fibroscan (30.4 kPa vs. 19.4 kPa, P = 0.007) were significantly associated with DTT AILD (SM-6).

HLA DRB1 Allele Frequency in Overall Cohort

As depicted in Table 1, a total of 11 different HLA DRB1 alleles were present in the patient and control group. The frequency of HLA DRB1*3 was increased in patients of pAILD compared with controls (31% vs. 4%; OR, 7.5; 95% CI 1.08–52.8; P = 0.006); however, on applying the Bonferroni correction, the P corrected reduced to 0.066, suggesting no statistically significant association. The same happened with the frequency of HLA DRB1*10 allele, which was less in pAILD as compared with controls; however, significance was lost on applying Bonferroni correction (4% vs. 28%, P corrected = 0.066). HLA DRB1*7 frequency was 32.4% in patients versus 56% in controls, however, was not statistically significant (P corrected = 0.407) after Bonferroni correction. The most frequent allele among pAILD patients was HLA DRB1*13 (33.8%); however, its frequency in controls was also high (16%), with no significant difference between the two groups, suggestive that the overall prevalence of this allele in the population may be high. Similarly, HLA DRB1*15 frequency was 32.4% in patients versus 48% in controls, making it the second most frequent allele; however, there was no statistically significant difference. On comparing allele frequencies across types of pAILD with controls, it was found that there was significant difference in the frequencies of HLA DRB1*3 across the groups as shown in Figure 1. AIH type 1 was found to have significantly increased frequency of HLA DRB1*3 when compared with controls (46.2% vs. 4%, P corrected = 0.011). Although frequency of HLA DRB1*3 was also higher in seronegative AIH versus controls (37.5% vs. 4%), it did not attain statistical significance.

Table 1 HLA DRB1 Allele Frequency in AILD Patients Versus Controls.

HLA DRB1 allele	AILD patients (N = 71)	Controls (N = 25)	P value	P corrected
HLA DRB1*1	2 (2.8%)	0	0.396	1
HLA DRB1*3	22 (31%)	1 (4%)	0.006	0.066
HLA DRB1*4	7 (9.9%)	2 (8%)	0.784	1
HLA DRB1*7	23 (32.4%)	14 (56%)	0.037	0.407
HLA DRB1*10	4 (5.6%)	7 (28%)	0.006	0.066
HLA DRB1*11	4 (5.6%)	2 (8%)	0.674	1
HLA DRB1*12	3 (4.2%)	1 (4%)	0.961	1
HLA DRB1*13	24 (33.8%)	4 (16%)	0.092	1
HLA DRB1*14	12 (16.9%)	2 (8%)	0.278	1
HLA DRB1*15	23 (32.4%)	12 (48%)	0.163	1
HLA DRB1*16	4 (5.6%)	0	0.225	1

Abbreviations: AILD, autoimmune liver disease; HLA, human leukocyte antigen; OR, odds ratio.

HLA DRB1 combination allele analysis revealed HLA DRB1*7-HLA DRB1*13 to be most frequent in pAILD patients (12.7% in patients vs. 4% in controls; P = 0.446). HLA DRB1*7-HLA DRB1*10 had a frequency of 2.8% in patients versus 16% in control; P value 0.038, suggestive of a protective effective of this allele combination for AIH. As depicted in SM-7, no other significant difference in allele combination frequency was found in pAILD patients and controls.

Allele Frequencies in DTT Patients

When allele frequencies were studied in the responders group versus DTT group, it was found that HLA DRB1*7 (36.5%) and HLA DRB1*15 (34.6%) were the most frequent alleles in responders' patients, in contrast to HLA DRB1*13 (36.8%), which was the most frequent allele in DTT patients. HLA DRB1*14 was 9.6% in responders and 36.8% in DTT with pre correction P = 0.012; however, on Bonferroni correction, this significance was lost (P corrected = 0.130). No significant difference in allele frequencies was found in responders versus DTT patients as depicted in Table 2. Allele combinations of HLA DRB1*7- HLA DRB1*13 and HLA DRB1*3- HLA

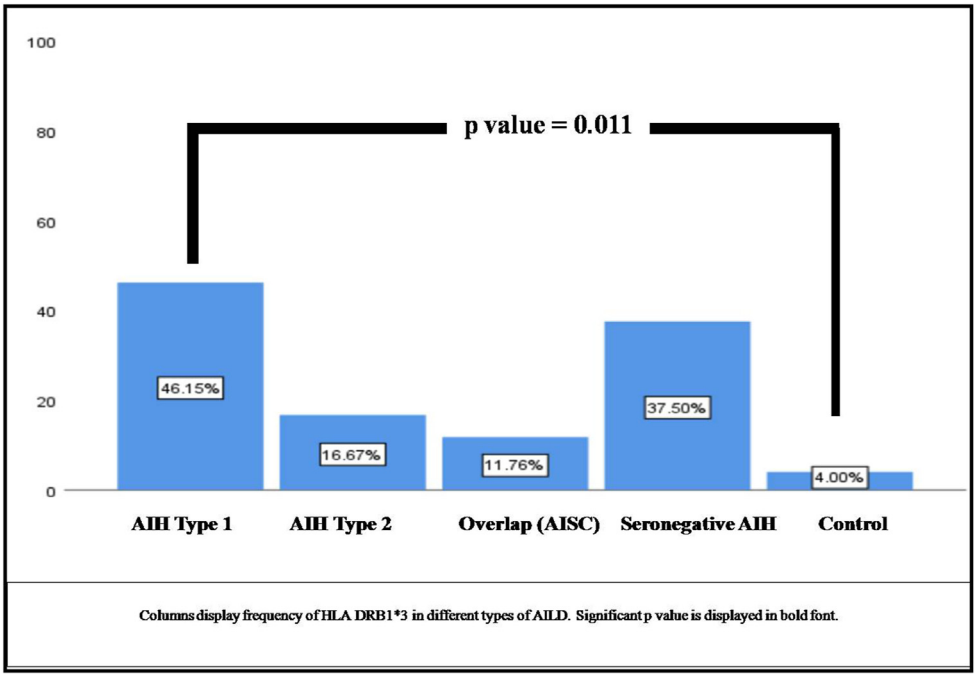

Figure 1 Frequency of HLA DRB1*3 by AILD type. Abbreviations: AIH, autoimmune hepatitis; AILD, autoimmune liver disease; AISC, autoimmune sclerosing cholangitis.

DRB1*13, and HLA DRB1*13-HLA DRB1*13 were most frequent in the DTT group. HLA DRB1*7- HLADRB1*15 was the most frequent allele combination in the responders group (13.5%).

Baseline Characteristics across HLA DRB1 Alleles

There was no significant difference in the age of presentation, gender, AIH type, or portal hypertension among the different HLA DRB1 alleles (SM-8). The laboratory parameters at baseline also did not show any significant difference across the allele types. As shown in SM-9, the highest PELD score and liver stiffness scores were seen with HLA DRB1*13 and median Ishaks fibrosis score in HLA DRB1*11(4, range 1–6). However, there was no significant difference across the alleles.

Predictors of Difficult to Treat Autoimmune Liver Disease

A logistic regression was performed to ascertain the effects of AIH overlap (AISC), presence of ascites, high-risk varies, and presence of HLA DRB1*14 as these parameters were

Table 2 HLA DRB1 Allele Frequency in Easy to Treat Versus Difficult to Treat AIH.

HLA DRB1 allele	Easy to treat (responders) (N = 52)	Difficult to treat (N = 19)	*P* value	*P* corrected
HLA DRB1*1	1 (1.9%)	1 (5.3%)	0.466	1
HLA DRB1*3	16 (30.8%)	6 (31.6%)	1.0	1
HLA DRB1*4	5 (9.6%)	2 (10.5%)	1	1
HLA DRB1*7	19 (36.5%)	4 (21.1%)	0.263	1
HLA DRB1*10	4 (7.7%)	0 (0%)	0.568	1
HLA DRB1*11	2 (3.8%)	2 (10.5%)	0.289	1
HLA DRB1*12	3 (5.8%)	0	0.559	1
HLA DRB1*13	17 (32.7%)	7 (36.8%)	0.782	1
HLA DRB1*14	5 (9.6%)	7 (36.8%)	0.012	0.13
HLA DRB1*15	18 (34.6%)	5 (26.3%)	0.578	1
HLA DRB1*16	4 (7.7%)	0	0.568	1

Abbreviations: AIH, autoimmune hepatitis; HLA, human leukocyte antigen.

the most significantly associated with DTT on univariate analysis. HLADRB1*14 was used in the model since it was the only allele significantly associated with DTT before Bonferroni correction. The model was statistically significant and explained 48.4% (Nagelkerke R^2) of variance in treatment response and improved the classification from 73.2% to 84.5% of cases. AIH overlap was 8.5 times more likely to be DTT as compared to other AILD (OR, 8.57; 95% CI, 1.77–41.50, P = 0.008). HLA DRB1*14 was independently predictive of DTT (OR, 5.87; 95% CI, 1.07–32.09; P = 0.041). This suggests HLA DRB1*14 is independently associated with treatment response. Other criterion that was found to be significant was high-risk varices (OR 7.55, 95% CI 1.46–38.93, P = 0.016) (Table 3).

DISCUSSION

This is the first study to look at HLA DRB1 association with treatment response in Indian pAILD. Patients were initially diagnosed as AIH based on the simplified IAIHG diagnostic score.[24] Those with a score of 6 (probable AIH) were started on treatment and then reassessed for the sake of inclusion in this study by the original revised IAIHG score.[25] This ensured that all patients included in the study were genuine AIH patients.

The proportion of AIH type1, AIH type 2, AISC, and seronegative AIH patients was similar to what has been previously described.[27–31] The analysis was patient based, meaning that the allele in a patient was considered present, irrespective of its zygosity. The advantage of this is that it does not underestimate the diagnostic value of an allele in case of heterozygosity; however, its disadvantage is that it leads to overlapping of "N."

HLA DRB1 Alleles in DTT Patients

HLA DRB1*14 was found to be independently associated with DTT patients (36.8% vs. 9.6%; OR, 5.87; 95% CI, 1.07–32.09; P = 0.041). While HLA DRB1*14 has been previously reported to be significantly associated with adult AIH patients from the Indian subcontinent,[10–12] this is the first study to report its association with treatment response in pAILD. The association of HLA DRB1*14 with DTT AILD and AILD itself has not been reported outside the Indian subcontinent, suggesting that this association may be unique to this region. Studies on adults with AILD from Europe and South East Asia have implicated HLA DRB1*13 and HLA DRB1*3 with severe disease[21,22] as has a study on children with AILD from Europe.[16] In this study, HLA DRB1*13(36.8% vs. 26.3%, P corrected = 1) and HLA DRB1*3 (30% vs. 31.6%, P corrected = 1) were not found to be associated with treatment response. This is in concordance with other studies on pAILD patients[13,14] suggesting that influence of genes varies across age and ethnic groups. AISC and the presence of high-risk varices at presentation were found to be independently associated with DTT pAILD apart from HLA DRB1*14. This suggests that it is the type of AIH and stage of liver disease at the time of initiation of treatment and presence of HLA DRB1*14 that determines response. The present study had high proportions of advanced liver disease, which reflects late referral pattern for these patients to a tertiary care liver transplant center like ours. Better referral systems in place would definitely help these patients.

HLA DRB1 Alleles in AILD

The difference inHLA DRB1 allele frequencies between AIH type 1 was compared with controls (46.2% vs. 4%) with a P corrected = 0.011 suggesting a significant association of HLA DRB1*3 with AIH type 1. Seronegative AIH also showed HLA DRB1*3 in 37.5% cases versus 4% controls; however, the difference was not statistically significant (P corrected = 0.09). This is in consonance with previously published literature on pAILD from Europe[13–15] and adult literature from India[10] where HLA DRB1*3 has been found to be significantly associated with AILD. However, studies from other non-European countries failed to find a similar association.[20] Other alleles like HLA DRB1*13 and HLA DRB1*7 which have been found to significantly associated with pAILD in European and Latin American children[13–17] were not found to associated with AILD in our cohort. This suggests that the association of HLA DRB1*3 with AIH type 1 may be present in the subcontinent and may extend across age and ethnic groups. Among all the HLA

Table 3 Logistic Regression Analysis for Independent Risk Factors of Difficult to Treat AILD.

Parameter	Responders (N = 52)	DTT (N = 19)	OR (95% CI)	P value
AISC	7 (13.5%)	10 (52.6%)	8.57 (1.77–41.05)	0.008
High risk varices	5 (9.6%)	9(47.4%)	7.55 (1.46–38.93)	0.016
HLA DRB1*14	5 (9.6%)	7 (36.8%)	5.87 (1.07–32.09)	0.041
Presence of ascites	8 (15.4%)	9 (47.4%)	4.28 (0.88–20.83)	0.071

Abbreviations: AILD, autoimmune liver disease; AISC, autoimmune sclerosing cholangitis; DTT, difficult to treat; ETT, easy to treat; HLA, human leukocyte antigen; OR, odds ratio.

DRB1 alleles, HLA DRB1*3 showed greatest variation in its frequency between pAILD patients and controls (31% vs. 4%), however statistically insignificant after applying the Bonferroni correction (*P* corrected = 0.066).

This study's main limitation was single center nature and subsequently restricted sample size (with limited number of control subjects), but the size is comparable with pediatric cohorts[13] described till date. Limited genotyping as well as low-resolution typing of HLA DRB1 alleles was done instead of high-resolution typing due to logistic issues. Although high-resolution typing depicting Field 2 digits also may well have eroded the allele differences found in this study. To the best of our knowledge, this is the only pAILD study from the Indian subcontinent, which describes the association with HLA typing. Due to retrospective nature of the study, magnetic resonance cholangiopancreatography (MRCP) was performed only in selected cases, which may have led to under detection of AISC, although two previous studies from India have reported overall lower proportions of AISC than the present study AISC.[28,31]

Thus, to conclude, advanced liver disease and presence of HLA DRB1* 14 were independent predictors of DTT patients. While HLA DRB1*14 cannot be said to be the sole determining factor of treatment response, as revealed by the logistic regression analysis, it is an independent predictor along with advanced liver disease. HLA DRB1*3 may have potential in identifying Indian children with AIH Type 1. This paves the way for larger multicentric population specific studies to address the inclusion of HLA DRB1 allele typing in AIH diagnostic and prognostic scores, specific for Indian children. Currently, there are only limited single-center studies describing the natural course and outcome of Indian children with pAILD. An Indian pAILD registry is needed to describe the clinical course, outcome, response to treatment, and association with HLA genes in Indian children with pAILD, so that results can be generalized to the entire population. We, thus, propose the establishment of an Indian pAILD consortium for a collaborative effort throughout the country to study the clinical course, outcome, and HLA alleles associated with pAILD patients of Indian ethnicity.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

SA, RK, AM, and VS conceptualized the research paper. AM collected all data which was validated by VS and BBL. NT performed the HLA DRB1 allele typing. AM and VS prepared the first draft. All authors contributed to the critical revision of the final manuscript for important intellectual content, approved the final version, and agreed to be accountable for all aspects of work. SA is the corresponding author of this manuscript and takes the responsibility of coordinating the work from its inception to publication and can be approached for access to raw data.

CONFLICTS OF INTEREST

All authors declare no conflict of interest.

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SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jceh.2023.01.001.