Autoantibody and Human Leukocyte Antigen Profiles in Children With Autoimmune Liver Disease and Their First-Degree Relatives

*Pengyun Wang, ||Haibin Su, *James Underhill, *Laura J. Blackmore, *Maria Serena Longhi, †Tassos Grammatikopoulos, ‡Elizabeth Veronica Okokon, §Edward T. Davies, *Diego Vergani, †Giorgina Mieli-Vergani, and *Yun Ma

ABSTRACT

Objective: Familial clustering of juvenile autoimmune liver disease (AILD), including autoimmune hepatitis and autoimmune sclerosing cholangitis (ASC), is rare, despite a high prevalence of autoimmune disorders in AILD families.

Methods: To investigate this discrepancy, we measured autoantibodies diagnostic for AILD, anti-nuclear, anti-smooth muscle, anti-liver kidney microsomal type 1, anti-liver cytosol type 1, and anti-soluble liver antigen antibodies, and human leukocyte antigen profiles in 31 patients and 65 of their first-degree relatives (FDR). The autoantibody profile was compared with that of 42 healthy subjects (HS).

Results: Autoantibodies were detected in 71% (22/31) patients. Anti-nuclear antibody or anti-smooth muscle antibody were present in 4/65 FDR (6.2%). HS were negative for all autoantibodies. The frequencies of homozygous HLA *DRB1∗0301* (DR3) genes and haplotype A1-B8-DR3 were higher in the patients (25% and 43%) than in FDR (9% and 27%) and HS (0% and 16%). The frequencies of disease-protective genes DR4 and/or DR15 were lower in the patients (25%) than in FDR (42%) and HS (42%). Only 1 family contained 2 patients with AILD, 1 with ASC and 1 with primary sclerosing cholangitis. Both patients possessed A1-B8-DR3 genes, the ASC being homozygous and the primary sclerosing cholangitis heterozygous. Six FDR had nonhepatic autoimmune disorders, none being autoantibody positive.

Conclusions: Homozygosity for DR3 plays a major role in the predisposition to juvenile AILD. Diagnostic autoantibodies for AILD are rare among patients' FDR and not linked to clinical manifestation of AILD.

Key Words: autoantibodies, autoimmunity, hepatitis, human study, humoral immunity

(*JPGN* 2014;58: 457–462)

Immunological changes characteristic of autoimmune disease, that is, increased levels of immunoglobulin, circulating autoantibodies, and activated T lymphocytes, can be detected in genetically predisposed individuals, such as first-degree relatives (FDR) of patients with autoimmune disease. In some FDR, these findings herald the onset of autoimmunity and can be present years before clinical symptoms or detectable abnormalities of target-organ function (1–3). Multiple autoantibodies are present in the siblings of patients with type 1 diabetes mellitus (T1D) (4–6), and in the FDR of patients with systemic sclerosis (SS) (7) and primary biliary cirrhosis (PBC) (8). High titres of autoantibodies diagnostic for type 1 autoimmune hepatitis (AIH-1), for example, anti-nuclear antibody (ANA) and smooth muscle antibody (SMA), have been reported in the healthy members of 1 family, in which 3 of 7 children had AIH, 2 of them possessing the disease risk alleles HLA DR3/DR7 (9). Whether the presence of autoantibodies in this unique AIH family was a sporadic phenomenon or it is a common event in the families with a history of AIH is unknown. We have, therefore, investigated the presence of autoantibodies and the HLA profile in a large number of families of children with autoimmune liver disease (AILD), including AIH-1, AIH-2, and autoimmune sclerosing cholangitis (ASC) (10). Besides ANA and SMA, we have measured 3 other diagnostic antibodies, namely, anti-soluble liver antigen (anti-SLA), targeting *o*-phosphoseryl-tRNA:selenocysteinyl-tRNA synthase (SepSecS) (11), anti-liver cytosol type 1 (anti-LC1), targeting formimino-transferase cyclodeaminase (FTCD), and anti-liver kidney microsomal type 1 (anti-LKM1), targeting cytochrome P4502D6 (CYP2D6). These 3 autoantibodies, which are highly specific for autoimmune liver disease and whose titres are associated with disease severity (12–14), have never been investigated in the FDR of affected individuals. A link between autoantibody positivity, HLA profile, and presence of clinical evidence of autoimmunity was sought.

Received and accepted November 9, 2013.

From the *Institute of Liver Studies, the †Paediatric Liver, GI & Nutrition Centre, the ‡Clinical Biochemistry, the §Clinical Immunology, King's College London School of Medicine at King's College Hospital, Denmark Hill, London, UK, and the ||Department of Transplantation, Beijing 302 Hospital, Xisihuanzhonglu, Beijing, China.

Address correspondence and reprint requests to Dr Yun Ma, Institute of Liver Studies, King's College Hospital, Denmark Hill, London SE5 9RS, UK (e-mail: yun.ma@kcl.ac.uk).

P.W. and H.S. share first authorship. D.V., G.M.V., and Y.M. participated equally in this study.

This article has been developed as a Journal CME Activity by NASPGHAN. Visit *http://www.naspghan.org/wmspage.cfm?parm1=742* to view instructions, documentation, and the complete necessary steps to receive CME credit for reading this article.

P.W. was supported by the PhD studentship from the Medical Research Council (MRC), UK; H.S. was supported by a scholarship from Beijing 302 Hospital, China; L.J.B. was supported by the King's College Hospital Foundation Trust and is presently supported by the Clinical Training Fellowship from the Wellcome Trust, UK; M.S.L. was supported by the Roger Dobson Fund, King's College Hospital Charity, UK, when this project was started and is presently supported by the Clinician Scientist Fellowship from MRC.

The authors report no conflicts of interest.

DOI: 10.1097/MPG.0000000000000245

METHODS

Patients

Thirty-one patients with childhood onset AILD (14 AIH-1, 7 AIH-2 and 10 ASC) were recruited between 2007 and 2010 according to the following criteria: diagnosis of AILD, treated or untreated; availability of FDR; informed consent for the family study. To establish whether these 31 patients were representative of the overall childhood AILD population, their HLA profiles were compared with those of a reference set of 149 patients with juvenile AILD diagnosed in our center.

The diagnosis of AIH complied with the criteria of the International AIH Group (15,14). ASC was diagnosed if patients also had evidence of cholangiopathy on imaging (10). All of the patients had histological features of interface hepatitis at presentation. Twenty-three patients were studied while undergoing immunosuppressive therapy with prednisolone 2.5 to 5 mg/day with or without azathioprine 1–2 $mg \cdot kg^{-1} \cdot day^{-1}$; and 8 patients were investigated at diagnosis before starting treatment. In patients with ASC, in addition to the above immunosuppressive treatment, ursodeoxycholic acid (UDCA) was given at a dose of 15 to 20 $mg \cdot kg^{-1} \cdot day^{-1}$. Ten patients were in full remission (defined as normal amino transferase [AST] and immunoglobulin G [IgG] levels, titre of ANA and SMA 1:20, anti-LKM1 negative) (16) and 21 had active disease (defined as ANA and/or SMA >1:20 or positive anti-LKM1, with or without elevated AST and/or IgG levels) (16). Patients' demographics, biochemical data, and family history of autoimmune disease are shown in Table 1.

Sixty-five FDR from the families of these 31 patients were studied, including 51 parents (28 mothers and 23 fathers) and 14 siblings. Nine FDR had autoimmune disorders, only 1 being liver related (primary sclerosing cholangitis [PSC] in an older brother): 2 had T1D (1 father and 1 mother), 1 had T1D and autoimmune thyroiditis (mother), 3 had autoimmune thyroiditis (all mothers), 1 had rheumatoid arthritis (RA, father), and 1 had celiac disease ([CD], a sister). FDR demographics, biochemical data, and family history of autoimmune disease are shown in Table 2.

Forty-two HS served as controls (37 females, median age 36 years, range 22–54) (Table 2). A history of autoimmune disease within the extended family (ie, including grandparents, uncles, aunts, and cousins) was sought for patients and HS and was present in 22 of the 44 patients, but in none of the 42 HS.

Written consent was obtained for each subject, including children $\geq$16 years of age and parents/guardians for younger children. This study was approved by the ethics committee of King's College Hospital, London.

Detection of Autoantibodies

The presence of circulating ANA, SMA, anti-LKM1, and anti-LC1 in patients, their FDR, and HS was investigated by indirect immunofluorescence (IIF) as described previously (17,18). An in-house radioligand assay (RLA) (12) was used to detect anti-SLA antibodies. All sera testing positive for anti-SLA by RLA were also tested by commercial Western blot, where prokaryotically expressed recombinant SLA/LP was used as antigen and analyzed by the EUROLineScan Programme (Euroimmun, Groβ Grönau, Germany).

HLA Typing

HLA genotyping was performed in patients and family members, for whom a sufficient number of cells was available, using polymerase chain reaction/sequence-specific primers (SSP), according to the manufacturer's instructions using the HLA SSP typing kit (ROSE Europe GmbH, Frankfurt, Germany), and compared with the HLA of 149 patients with childhood onset AILD in our database, for whom the complete autoantibody profile was available (63 [42%] with AIH-1, 35 [24%] with AIH-2 and 51 [34%] with ASC).

Statistical Analysis

The χ^2 and the 1-tailed Fisher exact tests were used to compare autoantibody and HLA frequencies in different groups. A P value of <0.05 was considered significant and a P value between 0.05 and 0.15 was considered a trend.

RESULTS

ANA, SMA, Anti-LC1, and Anti-LKM1 Autoantibodies

Autoantibodies were detected in 71% (22/31) of the patients with AILD. In the 10 patients with AIH-1 and active disease, ANA were present in 7 (70%) and SMA in 8 (80%), double positivity for ANA/SMA was found in 5 patients (50%), and double negativity in none. The median titre of ANA was 1/80 (range 1/40–1/320) and of SMA was 1/160 (range 1/40–1/2560). Among the 4 AIH-1 patients

TABLE 1. Demographic and biochemical data of patients with autoimmune liver disease (AILD)

	Patients with AILD (n = 31)		
	AIH-1 n = 14	AIH-2 n = 7	ASC n = 10
Family history of autoimmune disease, yes/no	7/7	1/6	6/4
Age, y	14 (7–27)	9 (5–13)	14 (9–18)
Sex, F/M	7/7	5/2	2/8
Active disease/remission	10/4	5/2	6/4
Total bilirubin (3–20 μmol/L)	16 (5–239)	10 (6–102)	10 (4–256)
AST (10–50 IU/L)	82 (26–991)	61 (21–657)	54 (19–2716)
IgG (6.34–18.11 g/L)	20.7 (8.7–68.6)	12.6 (6.0–19.7)	16.9 (10.2–53.4)
Ethnicity	13/14 white	White	White

AIH = autoimmune hepatitis; ANA = anti-nuclear antibodies; anti-LC1 = anti-liver cytosol antibody type 1; anti-LKM-1 = anti-liver kidney microsomal antibody type 1; anti-SLA; anti-soluble liver antigen antibody; ASC = autoimmune sclerosing cholangitis; AST = aspartate aminotransferase; IgG = immunoglobulin G; neg = negative; RLA = radioligand assay; SMA = anti-smooth muscle antibodies. All values are presented as median and range.

TABLE 2. Demographic and biochemical data of patients' FDR and healthy subjects

	FDR n = 65			HS n = 42
	Mothers n = 28	Fathers n = 23	Siblings n = 14	
Autoimmune disease (%)	5 (17.9)	2 (8.7)	2 (14.3)	None
Age, y	44 (24–53)	47 (38–58)	13 (5–24)	36 (22–54)
Sex, F/M	28/0	0/23	6/8	37/5
AST, 10–50 IU/L	Normal	Normal	Normal	Normal
Ethnicity	27/28 white	White	White	39/42 white

AST = aspartate aminotransferase; FDR = first-degree relatives; HS = healthy subjects.

in remission, ANA were present in 2 (50%), with an immunofluorescence titre of 1/20. SMA was not detected in these 4 patients.

In the 6 patients with ASC and active disease, ANAs were present in 4 (67%), SMAs in 2 (33%), and 1 patient was double positive. The titres of ANA were 1/20, 1/20, 1/80, and 1/160, respectively; the titres of SMA were 1/40 and 1/80. Of the 4 patients with ASC in remission, none were positive for ANA and SMA.

Of the 5 patients with AIH-2 and active disease, all were positive for autoantibodies: anti-LKM1 were present in 4 (80%), with a median titre of 1/80 (range 1/20–1/2560); anti-LC1s were present in 1 (ELISA). Anti-LKM1 was not present in 2 patients in remission.

Among 65 FDR, the frequency of positivity for either ANA or SMA was 6.2% (4/65). Of the 4 FDR positive for autoantibodies, 2 mothers of patients with ASC (P23 and P29) and 1 sister of a patient with AIH-2 (P20) were positive for ANA, whereas 1 father of a patient (P5) with AIH-1 was positive for SMA. Their autoantibody and HLA profiles are summarized in Table 3. The ANA immunofluorescence pattern in both the patients and FDR was homogenous. None of the FDR were positive for anti-LC1 or anti-LKM1.

All of the 42 HS were negative for ANA, SMA, anti-LC1, and anti-LKM1 antibodies. The frequency of ANA or SMA positivity in FDR was significantly lower than in the patients with AILD (4/65 vs 22/31, $P < 0.0000001$) and tended to be higher than in HS ($P = 0.13$).

Anti-SLA/SepSecS Antibodies

Detection of anti-SLA/SepSecS antibodies by both RLA and commercial immunoblot was performed in all 31 patients, 65 FDR, and 42 HS. Anti-SLA was positive by RLA in 8 of the 31 (26%) patients with AILD, in one of whom it was also positive by immunoblot. The frequency of anti-SLA was 29% (4/14) in AIH-1, 10% (1/10) in AIH-2, and 30% (3/10) in ASC. All 8 patients positive for anti-SLA by RLA had active disease. Anti-SLA antibodies were not detected in FDR or HS.

Frequency of HLA Class I and II Alleles in Patients, Their FDR, and HS

The frequencies of HLA class I and II alleles conferring susceptibility or resistance to AILD were determined in 28 of the 31 patients, 45 of the 65 FDR from 19 families, and 31 of the 42 HS. HLA class I *A* alleles were assessed in 42 FDR and *B* alleles in 41 FDR.

The comparison between the HLA profile of the 28 patients, the 45 FDR, and the 31 autoantibody tested HS with the reference set of 149 patients with AILD is shown in Table 4. The frequency of all alleles, apart from homozygous HLA *DRB1∗3* (*DR3*), was similar between the reference set and the AILD study group. The frequency of homozygous *DR3* in the reference set was lower than in the study group ($P = 0.03$).

The frequency of HLA *A∗01* in the patients (46%) was higher than in HS (23%, $P = 0.05$), it tended to be higher in FDR (41%) than in HS ($P = 0.1$). The frequency of homozygous HLA *A∗01* in the patients (18%) was slightly higher than in FDR (12%), and higher than in HS (3%, $P = 0.06$). The frequency of HLA *B∗08* was similar in the patients (46%) and FDR (41%), both being higher than in HS (16%, $P = 0.01$ and $P = 0.02$, respectively). The frequency of homozygous *B∗08* was slightly higher in the patients than in FDR (14% vs 5%, $P =$ NS), but significantly higher than in HS (0%, $P = 0.03$).

The patients with AILD (68%) and FDR (56%) were more frequently positive for HLA *DRB1∗0301* (*DR3*) than HS (23%, $P = 0.0005$ and $P = 0.004$, respectively). The frequency of homozygous *DR3* was significantly higher in the patients (25%) than in FDR (9%, $P = 0.05$) and HS (0/31, $P = 0.004$).

The frequency of HLA *DRB1∗0701* (DR7) and *DRB1∗1301* (DR13) was similar among patients, FDR, and HS. Owing to the small sample size, the frequency of the 2 genes *DRB1∗0401* and *DRB1∗1501,* reported to confer resistance to childhood AIH (10), was analyzed together. The frequency of these genes was slightly lower in the patients (25%) than in FDR (42%) and HS (42%), but the difference was not statistically significant.

The frequency of other genes (*DR2*, *DR5*, *DR6*, *DR8*, and *DR10*), which have not been defined as disease predisposition or protection genes, was also analyzed together. The frequency of these genes was similar in the patients (5%) and FDR (0/45), both being significantly lower than in HS (29%, $P = 0.009$ and $P = 0.0001$, respectively).

In the patients with AILD, the frequency of the HLA *A1-B8-DR3* haplotype tended to be higher than in FDR (43% vs 27%, $P = 0.15$), being significantly higher than in HS (16%, $P = 0.02$). Seven of 19 families for which HLA data were available for both patients and FDR contained at least 1 FDR with autoimmune disease, but only 1 family had 2 brothers with liver-related autoimmune disease: patient number 25, diagnosed as having ASC in childhood, had a brother with PSC diagnosed in adult life. At the time of investigation, patient number 25, his brother with PSC, sister, and parents were well, with normal liver function tests and negative autoantibodies. Although patient number 25, his brother with PSC, and parents were all *A1-B8-DR3* positive, only the 2 brothers with autoimmune liver disease were homozygous for disease predisposing alleles, patient number 25 being homozygous for *A1-B8-DR3* and his brother for *A1* and *DR3* (Table 3). None of the 6 FDR with nonliver autoimmune diseases was homozygous for *DR3* or for the haplotype *A1-B8-DR3*.

TABLE 3. Autoantibody and HLA data within the families of 10 patients whose FDR were positive for AABs and/or had autoimmune diseases

Patient no.		AABs	Autoimmune disease	HLA class I A B	HLA class II DRB1	
Families with FDR positive for AABs with or without autoimmune disease						
5	Patient*	SMA 1/160	AIH-1	1, 12	44, 44	*DR7/DR14*
5	Father	SMA 1/40	No	nd	nd	nd
20	Patient	neg**	AIH-2	1, 1	8, 8	*DR3/DR3*
20	Mother	neg	No	10, 19	16, 16	*DR1/DR4*
20	Sister-1	ANA 1/80	No	10, 19	8, 16	*DR3/DR13*
20	Sister-2	neg	No	10, 19	8, 16	*DR3/DR13*
23	Patient	ANA 1/160	ASC and colitis	nd	nd	nd
23	Mother	AND 1/40	Autoimmune thyroiditis	nd	nd	nd
29	Patient	Neg	ASC	1, 1	8, 37	*DR3/DR3*
29	Mother	ANA 1/40	No	1,1	8, 8	*DR3/DR3*
29	Father	neg	No	1, 3	7, 37	*DR3/DR5*
29	Brother	neg	No	1, 1	8, 37	*DR3/DR3*
29	Sister	neg	Celiac disease	1, 3	7, 8	*DR3/DR5*
Families with FDR negative for AABs but with autoimmune disease						
3	Patient	Neg	AIH-1	nd	nd	*DR3/DR15*
3	Mother	Neg	Thyroiditis	2, 19	8, 18	*DR3/DR15*
3	Father	Neg	No	2, 3	27, 40	*DR3/DR3*
7	Patient	ANA 1/20	AIH-1	2, 2	46, 46	*DR5/DR5*
7	Father	Neg	Rheumatoid arthritis	3, 29	7, 40	*DR4/DR7*
10	Patient	ANA pos	AIH-1	2, 32	51, 53	*DR13/DR13*
10	Mother	Neg	Thyroiditis and T1D	2, 9	5, 21	*DR3/DR13*
12	Patient*	SMA 1/320	AIH-1	2, 2	18, 35	*DR3/DR11*
12	Mother	Neg	Thyroiditis	1, 24	44, 44	*DR7/DR14*
12	Father	Neg	No	1, 2	7, 18	*DR5/DR7*
16	Patient	Neg	AIH-2	2, 26	8, 51	*DR3/DR4*
16	Mother	Neg	T1D	2, 3	5, 7	*DR4/DR15*
16	Father	Neg	No	2, 3	7, 40	*DR4/DR15*
16	Brother	Neg	No	3, 10	7, 8	*DR3/DR15*
16	Sister	Neg	No	2, 3	nd	*DR4/DR15*
25	Patient	Neg	ASC	1, 1	8, 8	*DR3/DR3*
25	Mother	Neg	No	1, 2	8, 14	*DR3/DR3*
25	Father	Neg	No	1, 3	8, 18	*DR3/DR6*
25	Brother	Neg	PSC	1, 1	8, 14	*DR3/DR3*
25	Sister	Neg	No	nd	nd	*DR3/?*

AABs = autoantibodies; AIH = autoimmune hepatitis; ANA = anti-nuclear antibodies; ASC = autoimmune sclerosing cholangitis; nd = not done; PSC = primary sclerosing cholangitis; SMA = anti-smooth muscle antibodies; T1D = type 1 diabetes mellitus.

* Patients studied before receiving treatment.

** Positive for anti-liver kidney microsomal antibody type 1 (LKM-1) and ANA at diagnosis.

DISCUSSION

The high prevalence of autoimmune disorders in family members of the patients with AIH or ASC, in contrast to the rare familial clustering of liver autoimmune pathology, has prompted us to perform this study, in which autoimmune serology associated with juvenile autoimmune liver disease was investigated in the patients and their FDR, and correlated with the presence of liver or nonliver autoimmune disorders and HLA profiles.

Positivity for autoantibodies associated with juvenile AILD was found in 4 (6.2%) of the patients' FDR, 2 of whom had nonliver autoimmune disorders (thyroiditis in a mother and celiac disease in a sister), but in none of the healthy controls. The only antibodies detected in FDR, nowever, were ANA and/or SMA, the least liver specific of the autoantibodies present in AIH and ASC. No FDR was positive for anti-LKM or anti-SLA, antibodies strongly associated with liver autoimmune disease, confirming their diagnostic specificity.

In primary biliary cirrhosis, an autoimmune disorder affecting adults, the disease-specific anti-mitochondrial antibodies are found in one-fifth of patients' FDR (19) and predict the disease several years before its clinical onset (20). In other autoimmune disorders, such as diabetes and systemic lupus erythematosus, it is the presence of multiple disease-associated autoantibodies that heralds the onset of clinical disease. Only multiple autoantibodies to islet cells (ICA), insulin, GAD65, IA-2, and IA-2β predict T1D in relatives (21,22).

Nonliver autoimmune diseases were present in some 12% of our patients' FDR, although a family history of autoimmune disorders, including second- and third-degree relatives, was present in 50%, a frequency similar to the 40% previously reported in juvenile AILD (10,23). In contrast, clustering of autoimmune liver disease was found in only 1 of the families studied, confirming the rarity of familial occurrence of juvenile autoimmune liver disease, only 5 family clusters of AIH or ASC having been reported to date (9,24–27).

TABLE 4. Frequencies (%) of HLA class I and II alleles in patients with AILD, their first-degree relatives and healthy subjects compared with an in-house AILD reference group

HLA alleles Allotypes	AILD reference group n = 149 n, %	*P* value reference group vs HS	AILD study group n = 28 n, %	*P* value study group vs HS	FDR n = 45 n, %	*P* value FDR vs HS	HS n = 31 n, %
A∗01	73 (49)	0.007	13 (46)	0.05	17/42 (41)	0.1	7 (23)
A∗01/A∗01	14 (9)	NS	5 (18)	0.06	5/42 (12)	NS	1 (3)
B∗08	79 (53)	0.00018	13 (46)	0.01	17/41 (41)	0.02	5 (16)
B∗08/B∗08	14 (9)	0.086	4 (14)	0.03	2/41 (5)	NS	0 (0)
DRB1∗3	78 (52)	0.0025	19 (68)	0.0005	25 (56)	0.004	7 (23)
DRB1∗3/B1∗3	15 (10)*	0.07	7 (25)†	0.004	4 (9)	NS	0 (0)
DRB1∗07	44 (30)	NS	5 (18)	NS	15 (33)	NS	7 (23)
DRB1∗13	34 (23)	NS	4 (14)	NS	5 (13)	NS	4 (13)
DRB1∗04, -∗15	42 (28)	NS	7 (25)	NS	19 (42)	NS	13 (42)
DRB1∗02, -∗05, -∗06, -∗08, -∗10	12 (8)	0.0009	1 (5)	0.009	0 (0)	0.0001	9 (29)
HLA haplotypes							
A1-B8-DR3	47 (32)	0.08	12 (43)‡	0.02	12 (27)	NS	5 (16)

AILD = autoimmune liver disease; FDR = first-degree relatives; HLA = human leukocyte antigen; HS = healthy subjects; NS = not significant.
* Significantly lower in the reference group than in the AILD study group, $P = 0.03$.
† Significantly higher in the AILD study group than in FDR, $P = 0.05$.
‡ Tended to be higher in the AILD study group than in FDR, $P = 0.15$.

The question arises why the occurrence of juvenile AILD is rare within families, despite up to 50% of family members having other autoimmune diseases, such as diabetes, thyroiditis, rheumatoid arthritis, and celiac disease. Analysis of FDR HLA data may answer this question. Despite the relatively small number of subjects tested, it is of interest that FDR, although possessing the disease susceptibility alleles *A1*, *B8*, or *DR3* at a frequency similar to that of patients and higher than that of HS, had a lower frequency of homozygosity for these genes than did patients. It has been suggested that homozygous alleles have a gene dose effect on disease occurrence and outcome (28–30). Indeed, the frequency of homozygous alleles *B8* and *DR3* was higher in the patients with AILD than in their FDR and HS. In addition, the frequency of the haplotype *A1-B8-DR3*, which is known to be associated with the development of autoimmunity (31), was lower in FDR than in the patients. The juvenile AILD protecting genes *DR4* and *DR15* (32,33) are more frequent in FDR than in the patients. Although this difference does not reach statistical significance, probably because of the small number of subjects typed, this protective HLA profile may partially explain the rarity of liver-centered autoimmunity in the FDR of patients with AIH or ASC.

The pathogenesis of AIH and ASC is multifactorial, involving the engagement of effector mechanisms targeting liver autoantigens, probably triggered by an external agent (34,35). This autoimmune attack is facilitated by impaired immunoregulatory control (36,37). Our data confirm the importance of HLA genes in the predisposition to juvenile AILD, but also stress the special role of the HLA class II products encoded by *DR3*, because the presence of autoimmune liver disease is strongly associated with homozygosity for this allele. These results should be further confirmed in a multicenter study including a larger number of the patients with AILD and their families.

Acknowledgment: We thank Dr Maria Mytilinaiou for technical assistance on anti-SLA and anti-LC1 antibodies testing.

REFERENCES

1. Riley WJ, Maclaren NK, Krischer J, et al. A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. *N Engl J Med* 1990;323:1167–72.
2. Rose NR, Bona C. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol Today* 1993;14:426–30.
3. Kulmala P, Savola K, Reijonen H, et al. Genetic markers, humoral autoimmunity, and prediction of type 1 diabetes in siblings of affected children. Childhood Diabetes in Finland Study Group. *Diabetes* 2000;49:48–58.
4. Parkkola A, Härkönen T, Ryhänen SJ, et al. Finnish Pediatric Diabetes Register. Extended family history of autoimmune diseases and phenotype and genotype of children with newly diagnosed type 1 diabetes. *Eur J Endocrinol* 2013;169:171–8.
5. Lambert AP, Gillespie KM, Thomson G, et al. Absolute risk of childhood-onset type 1 diabetes defined by human leukocyte antigen class II genotype: a population-based study in the United Kingdom. *J Clin Endocr Metab* 2004;89:4037–43.
6. Achenbach P, Warncke K, Reiter J, et al. Type 1 diabetes risk assessment: improvement by follow-up measurements in young islet autoantibody-positive relatives. *Diabetologia* 2006;49:2969–76.
7. Arora-Singh RK, Assassi S, del Junco DJ, et al. Autoimmune diseases and autoantibodies in the first degree relatives of patients with systemic sclerosis. *J Autoimmun* 2010;35:52–7.
8. Lazaridis KN, Juran BD, Boe GM, et al. Increased prevalence of antimitochondrial antibodies in first-degree relatives of patients with primary biliary cirrhosis. *Hepatology* 2007;46:785–92.
9. Hodges S, Lobo-Yeo A, Donaldson P, et al. Autoimmune chronic active hepatitis in a family. *Gut* 1991;32:299–302.
10. Gregorio GV, Portmann B, Karani J, et al. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. *Hepatology* 2001;33:544–53.
11. Araiso Y, Palioura S, Ishitani R, et al. Structural insights into RNA-dependent eukaryal and archaeal selenocysteine formation. *Nucleic Acids Res* 2008;36:1187–99.
12. Ma Y, Okamoto M, Thomas MG, et al. Antibodies to conformational epitopes of soluble liver antigen define a severe form of autoimmune liver disease. *Hepatology* 2002;35:658–64.
13. Johanet C, Ballot E. Auto-antibodies in autoimmune hepatitis: anti-soluble liver antigen (SLA). *Clin Res Hepatol Gastroenterol* 2012;36:244–6.
14. Hennes EM, Zeniya M, Czaja AJ, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008;48:169–76.
15. Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999;31:929–38.
16. Mieli-Vergani G, Heller S, Jara P, et al. Autoimmune hepatitis. *J Pediatr Gastroenterol Nutr* 2009;49:158–64.

17. Smith MG, Williams R, Walker G, et al. Hepatic disorders associated with liver-kidney microsomal antibodies. *BMJ* 1974;2:80–4.
18. Vergani D, Alvarez F, Bianchi FB, et al. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. *J Hepatol* 2004; 41:677–83.
19. Zografos TA, Gatselis N, Zachou K, et al. Primary biliary cirrhosis-specific autoantibodies in first degree relatives of Greek primary biliary cirrhosis patients. *World J Gastroenterol* 2012;18:4721–8.
20. Metcalf JV, Mitchison HC, Palmer JM, et al. Natural history of early primary biliary cirrhosis. *Lancet* 1996;348:1399–402.
21. Maclaren N, Lan M, Coutant R, et al. Only multiple autoantibodies to islet cells (ICA), insulin, GAD65, IA-2 and IA-2beta predict immune-mediated (Type 1) diabetes in relatives. *J Autoimmun* 1999;12:279–87.
22. Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 2003;349:1526–33.
23. Gregorio GV, Portmann B, Mowat AP, et al. A 12-year-old girl with antimitochondrial antibody-positive autoimmune hepatitis. *J Hepatol* 1997;27:751–4.
24. Findor JA, Sorda JA, Daruich JR, et al. Familial association in autoimmune liver disease. *Medicina* 2002;62:241–4.
25. Ohira H, Shinzawa J, Suzuki T, et al. Two sister cases of autoimmune hepatitis. *Fukushima J Med Sci* 1998;44:113–20.
26. Nobili V, Devito R, Dall'oglio L, et al. Autoimmune sclerosing cholangitis in two sisters. *Eur J Pediatr* 2008;167:107–8.
27. Yoshida O, Abe M, Furukawa S, et al. A familial case of autoimmune hepatitis. *Internal Med* 2009;48:315–9.
28. Brown JH, Jardetzky T, Saper MA, et al. A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature* 1988;332:845–50.
29. Doherty DG, Penzotti JE, Koelle DM, et al. Structural basis of specificity and degeneracy of T cell recognition: pluriallelic restriction of T cell responses to a peptide antigen involves both specific and promiscuous interactions between the T cell receptor, peptide, and HLA-DR. *J Immunol* 1998;161:3527–35.
30. Turesson C, Schaid DJ, Weyand CM, et al. The impact of HLA-DRB1 genes on extra-articular disease manifestations in rheumatoid arthritis. *Arthritis Res Ther* 2005;7:R1386–93.
31. Krawitt EL, Kilby AE, Albertini RJ, et al. Immunogenetic studies of autoimmune chronic active hepatitis: HLA, immunoglobulin allotypes and autoantibodies. *Hepatology* 1987;7:1305–10.
32. Pando M, Larriba J, Fernandez GC, et al. Pediatric and adult forms of type I autoimmune hepatitis in Argentina: evidence for differential genetic predisposition. *Hepatology* 1999;30:1374–80.
33. Donaldson PT. Immunogenetics in liver disease. *Bailliere's Clin Gastroenterol* 1996;10:533–49.
34. Sathyabama S, Khan N, Agrewala JN. Friendly pathogens: prevent or provoke autoimmunity. *Crit Rev Microbiol* 2013.
35. Vergani D, Mieli-Vergani G. Cutting edge issues in autoimmune hepatitis. *Clin Rev Allergy Immunol* 2012;42:309–21.
36. Longhi MS, Ma Y, Bogdanos DP, et al. Impairment of CD4(+)CD25(+) regulatory T-cells in autoimmune liver disease. *J Hepatol* 2004;41:31–7.
37. Longhi MS, Ma Y, Mieli-Vergani G, et al. Regulatory T cells in autoimmune hepatitis. *J Hepatol* 2012;57:932–3.