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**ORIGINAL ARTICLE**

# HLA-DRB1*03:01 and HLA-DRB1*04:01 modify the presentation and outcome in autoimmune hepatitis type-1

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The classical human leukocyte antigen *(HLA)-DRB1*03:01* and *HLA-DRB1*04:01* alleles are established autoimmune hepatitis (AIH) risk alleles. To study the immune-modifying effect of these alleles, we imputed the genotypes from genome-wide association data in 649 Dutch AIH type-1 patients. We therefore compared the international AIH group (IAIHG) diagnostic scores as well as the underlying clinical characteristics between patients positive and negative for these HLA alleles. Seventy-five percent of the AIH patients were *HLA-DRB1*03:01/HLA-DRB1*04:01* positive. *HLA-DRB1*03:01/HLA-DRB1*04:01*-positive patients had a higher median IAIHG score than *HLA-DRB1*03:01/HLA-DRB1*04:01*-negative patients ($P < 0.001$). We did not observe associations between HLA alleles and alanine transaminase levels (*HLA-DRB1*03:01*: $P = 0.2$; *HLA-DRB1*04:01*; $P = 0.5$); however, *HLA-DRB1*03:01* was independently associated with higher immunoglobulin G levels ($P = 0.04$). The *HLA-DRB1*04:01* allele was independently associated with presentation at older age ($P = 0.03$) and a female predominance ($P = 0.04$). *HLA-DRB1*03:01*-positive patients received immunosuppressive medication and liver transplantation. In conclusion, the *HLA-DRB1*03:01* and *HLA-DRB1*04:01* alleles are both independently associated with the aggregate diagnostic IAIHG score in type-1 AIH patients, but are not essential for AIH development. *HLA-DRB1*03:01* is the strongest genetic modifier of disease severity in AIH.

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## INTRODUCTION

Autoimmune hepatitis (AIH) is an uncommon autoimmune liver disease of unknown etiology.[1,2] The disease has a prevalence of 17–18.3 per 100 000 and is characterised by chronic destructive inflammation within the liver parenchyma, elevated serum immunoglobulin G levels and the presence of serum autoantibodies.[1–4] AIH type-1 is characterized by antinuclear antibodies (ANA), smooth muscle antibodies (SMA) and soluble liver antigen/liver pancreas antibodies, and occurs predominantly in adult women, whereas the rare AIH type-2 occurs predominantly in children and is associated with liver kidney microsomal-1 antibodies.[1,2] In up to 10% of patients, a clinical overlap is seen with primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC).[5,6] Based on these clinical characteristics, the international AIH group (IAIHG) developed two scoring systems as tools to aid in the diagnosis of AIH.[3,7] Several small gene association studies associated the HLA class-II alleles *HLA-DRB1*03:01* and *HLA-DRB1*04:01* at the HLA-DRB1 locus with AIH susceptibility, but were unable to identify reliable associations outside the HLA region.[8–15] Despite a shared amino-acid motif in the binding groove between these respective HLA proteins, the *HLA-DRB1*03:01* and *HLA-DRB1*04:01* alleles were shown to have contrasting associations with disease severity and outcome of AIH.[8,9,16] Collectively, these alleles share the HLA-DRB1 locus on chromosome 6, which means that homozygosity for one allele is mutually exclusive for the other allele. Through a hypothesis-free genome-wide association study we established the strong association of *HLA-DRB1*03:01* and *HLA-DRB1*04:01* alleles with AIH type-1 susceptibility in addition to two non HLA loci in a large cohort of Caucasian patients and controls.[17] Using this patient cohort, we here aimed to take these findings a step further and refine the role and significance of *HLA-DRB1*03:01* and *HLA-DRB1*04:01* alleles in the clinical presentation IAIHG score and outcome of type-1 AIH in a large cohort of patients.

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## RESULTS

### Cohort characteristics

The included AIH cohort consisted of 145 males and 504 (78%) females with a mean age at diagnosis of 48 years (s.d. ± 18). The median revised IAIHG diagnostic score was 18 (interquartile range: 15–20). Evidence for a clinical overlap syndrome with PBC (AIH-PBC) and PSC (AIH-PSC) was found in 57 (9%) patients and 44 (7%) patients, respectively.

### *HLA-DRB1*03:01*, *HLA-DRB1*04:01* and IAIHG score

The combined allele frequency of *HLA-DRB1*03:01* (32%) and *HLA-DRB1*04:01* (15%) was less than half of the total number of the *HLA-DRB1* alleles in the cohort. However, a total of 75% ($n = 484$) of AIH type-1 patients was positive for one or two of these alleles. Forty-three (9%) of these patients were compound heterozygote for *HLA-DRB1*03:01* and *HLA-DRB1*04:01*. The 165 (25%) *HLA-DRB1*03:01* and *HLA-DRB1*04:01*-negative patients had significantly lower median IAIHG scores than *HLA-DRB1*03:01/HLA-DRB1*04:01*-positive patients (16.2 interquartile range: 12.9–19 vs 18.3 interquartile range: 15.6–20.8; $P < 0.001$; Figure 1). All characteristics are summarized in Table 1.

### Association of HLA with clinical features

The *HLA-DRB1*03:01* allele was associated with the IAIHG score at diagnosis ($P = 0.01$) and showed an independent additive association with an IAIHG score ⩾18 after correction for multiple testing ($P_{Holm} < 0.001$; Table 2). The *HLA-DRB1*03:01* allele showed an additive association with earlier age of onset ($P = 0.01$) and male gender ($P = 0.01$). However, when tested in a model together with *HLA-DRB1*04:01*, this significance disappeared ($P_{Holm} = 0.4$ and $P_{Holm} = 0.4$, respectively; Table 2). Median immunoglobulin G levels, but not alanine transaminase levels, were associated with the *HLA-DRB1*03:01* allele ($P < 0.001$ and $P = 0.2$, respectively). Autoantibody positivity for ANA and SMA was equally distributed among the *HLA-DRB1*03:01* genotypes. *HLA-DRB1*03:01*-positive AIH patients more often had one or more concomitant autoimmune diseases than those without *HLA-DRB1*03:01* ($P = 0.03$), but there was no difference between homo- and heterozygotes (27 vs 27%). *HLA-DRB1*03:01*-positive patients less often had manifestations of AIH-PBC overlap syndrome ($P = 0.01$). However, after correction for multiple testing in a multivariate regression model, both the presence of concomitant autoimmune disease and PBC overlap syndrome were no longer statistically significant ($P_{Holm} = 0.06$ and $P_{Holm} = 0.3$, respectively; Table 2). All characteristics are summarized in Supplementary table 1.

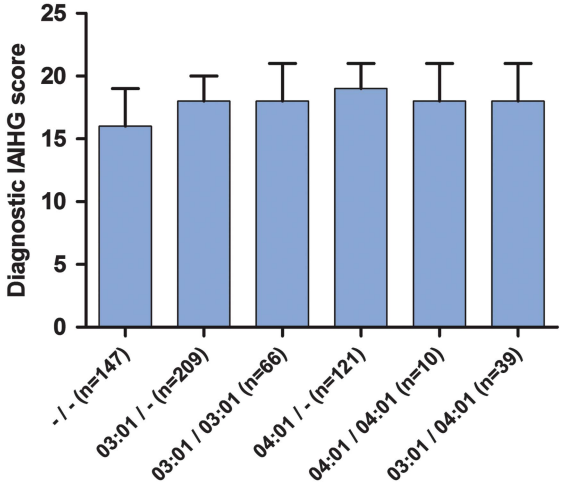


**Figure 1.** Median IAIHG diagnostic score for the *HLA-DRB1* genotypes. Bars represent median scores with interquartile range (IQR) of the international autoimmune hepatitis group (IAIHG) diagnostic score for the *HLA-DRB1*03:01* and *HLA-DRB1*04:01* genotypes.

The *HLA-DRB1*04:01* allele was associated a with higher IAIHG score at diagnosis ($P = 0.01$) and showed an independent additive association with an IAIHG score ⩾18 ($P_{Holm} = 0.005$; Table 2). In contrast to *HLA-DRB1*03:01, HLA-DRB1*04:01* was independently associated with later onset of disease ($P_{Holm} = 0.004$) and female predominance ($P_{Holm} = 0.005$). No associations of *HLA-DRB1*04:01* genotypes with immunoglobulin G and alanine transaminase levels were found. The frequencies of positivity for ANA and SMA, as well as the presence of one or more concomitant autoimmune diseases were similarly distributed among the *HLA-DRB1*04:01* genotypes. In addition, we could not identify a significant relation between *HLA-DRB1*04:01* and the occurrence of AIH-PSC and AIH-PBC. All characteristics are summarized in Supplementary table 2.

### HLA-DRB1 genotypes and clinical course

Although *HLA-DRB1*03:01* was not associated with cirrhosis ($P = 0.7$), it showed an independent additive relation with fibrosis at diagnosis ($P = 0.02$). However, this association did not remain statistically significant after correction for multiple testing in a multivariate regression model (odds ratio 1.4, 95% confidence interval: 1.1–1.9, $P_{Holm} = 0.1$; Table 2). Cirrhosis and fibrosis were similarly distributed among the *HLA-DRB1*04:01* genotypes. Patients with the *HLA-DRB1*03:01* allele were more likely to receive medication for AIH ($P < 0.001$) in contrast to the *HLA-DRB1*04:01* allele ($P < 0.001$). However, in a multivariate regression model incorporating both *HLA-DRB1*03:01* and *HLA-DRB1*04:01*, only *HLA-DRB1*03:01* retained statistical significance ($P_{Holm} < 0.001$; Table 2). Evidence of hepatocellular carcinoma was only observed in three patients (0.5%), who all were *HLA-DRB1*03:01* negative. Liver transplantation had only been performed in 12 patients (2%), and 4 additional patients (1%) were on the waiting list. *HLA-DRB1*03:01*-positive patients underwent a liver transplantation and were on the waiting list more often than *HLA-DRB1*03:01*-negative patients (4% vs 1%, $P = 0.02$; Supplementary table 1). In contrast, no patients with *HLA-DRB1*04:01* were on the waiting list or had undergone liver transplantation (Supplementary table 2).

## DISCUSSION

In this large multicenter study, we show that *HLA-DRB1*03:01* and *HLA-DRB1*04:01* alleles are independently associated with a higher overall aggregate IAIHG score at diagnosis. Both alleles are thus not only risk factors for AIH type-1, but also have a clear relation to the separate clinical characteristics that define this disease.

Exogenous antigens are primarily presented on HLA class-II molecules by professional antigen-presenting cells to CD4 T-cells. So-called molecular mimicry, in which an immune response is mounted against a pathogenic exogenous antigen, which is similar to an endogenous (self) antigen, has been suggested as a plausible mechanism for the development autoimmune disease.[18–20] Previous studies showed that *HLA-DRB1*03:01*-positive patients have more severe laboratory abnormalities.[21] Although we observed a strong independent association of *HLA-DRB1*03:01* with higher serum levels of the adaptive immune system serum marker immunoglobulin G, we did not identify significant associations between the HLA alleles and alanine transaminase levels. Similarly, *HLA-DRB1*03:01* and *HLA-DRB1*04:01*-negative patient were less likely to be ANA or SMA antibody positive, but we did not identify a significant association between the individual *HLA-DRB1*03:01* and *HLA-DRB1*04:01* genotypes and these autoantibodies. Associations between HLA

**Table 1.** Patient characteristics in *HLA-DRB1*03:01* and/or *HLA-DRB1*04:01*-positive and -negative AIH patients

| *Features* | *HLA-DRB1*03:01 and HLA-DRB1*04:01 negative* N = *165* | *HLA-DRB1*03:01 and/or HLA-DRB1*04:01 positive* N = *484* | N | P-*value* |
|---|---|---|---|---|
| IAIHG score median (IQR) | 16.2 (12.9–19) | 18.3 (15.6–20.8) | 592 | < 0.001 |
| Gender (female); *n* (%) | 128 (78) | 373 (77) | 646 | 0.4 |
| Age; year median (IQR) | 45.8 (27.4–60.8) | 45.6 (29.9–59) | 614 | 0.9 |
| ALT; IU $l^{-1}$ median (IQR) | 260 (97–655) | 349 (114–846) | 572 | 0.08 |
| IgG; g $l^{-1}$ median (IQR) | 17.2 (13.8–25) | 22.5 (17–31.2) | 477 | 0.001 |
| ALP; IU $l^{-1}$ median (range) | 151 (113–227) | 142 (98–226) | 570 | 0.2 |
| SMA pos; *n* (%) | 65 (52) | 233 (61) | 508 | 0.04 |
| ANA pos; *n* (%) | 81 (60) | 284 (70) | 540 | 0.08 |
| Fibrosis (biopsy); *n* (%) | 76 (58) | 260 (65) | 530 | 0.07 |
| Cirrhosis (biopsy); *n* (%) | 19 (14) | 43 (11) | 520 | 0.17 |
| Concomitant autoimmune disease; *n* (%) | 22 (15) | 119 (27) | 612 | 0.002 |
| PBC overlap; *n* (%) | 22 (13) | 35 (7) | 649 | 0.02 |
| PSC overlap; *n* (%) | 9 (6) | 35 (7) | 649 | 0.3 |
| HCC; *n* (%) | 2 (1) | 1 (0.2) | 649 | 0.16 |
| Liver transplantation; *n* (%) | 3 (2) | 9 (2) | 649 | 0.6 |
| Liver transplantation and waiting list; *n* (%) | 3 (2) | 13 (3) | 649 | 0.4 |
| Treatment naive; *n* (%) | 51 (31) | 72 (15) | 604 | < 0.001 |

Abbreviations: ALP, Alkaline phosphatase; ANA, antinuclear antibodies; HCC, Hepatocellular carcinoma; IAIHG, international autoimmune hepatitis group; IgG, immunoglobulin G; PBC, Primary biliary cirrhosis; PSC, Primary sclerosing cholangitis; SMA, smooth muscle antibodies; ALT, alanine transaminase.

**Table 2.** Uni- and multivariate analysis of *HLA-DRB1*03:01* and *HLA-DRB1*04:01* and clinical characteristics in AIH type-1 patients

| *Features* | *HLA-DRB1*03:01* | | | | | *HLA-DRB1*04:01* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Multivariate*[a] | | | | | *Multivariate*[a] | | | |
| | *Univariate* P | *Beta/OR* | *95%-CI* | P | $P_{Holm}$[b] | *Univariate* P | *Beta/OR* | *95%-CI* | P | $P_{Holm}$[b] |
| IAIHG score | 0.01 | | | | | 0.01 | | | | |
| IAIHG score ⩾ 18 | 0.08 | 1.7 | 1.3–2.2 | < 0.001 | | 0.02 | 1.7 | 1.2–2.5 | 0.005 | |
| Age[c] | 0.01 | −1.8 | | 0.1 | 0.4 | 0.01 | 4.6 | | 0.004 | 0.03 |
| Female[d] | 0.01 | 0.8 | 0.6–1.1 | 0.2 | 0.4 | 0.001 | 2.0 | 1.2–3.3 | 0.005 | 0.03 |
| IgG | < 0.001 | 2.3 | | 0.006 | 0.04 | 0.5 | 1.4 | | 0.2 | 1 |
| Concomitant autoimmune disease | 0.01 | 1.5 | 1.1–2.0 | 0.009 | 0.06 | 0.8 | 1.3 | 0.9–2.0 | 0.2 | 1 |
| PBC overlap | 0.01 | 0.7 | 0.4–1.1 | 0.1 | 0.3 | 0.9 | 0.7 | 0.4–1.3 | 0.2 | 0.8 |
| PSC overlap | 0.2 | 1.2 | 0.7–1.9 | 0.6 | 0.6 | 0.4 | 0.9 | 0.4–2.0 | 0.8 | 1 |
| Fibrosis (biopsy) | 0.02 | 1.4 | 1.1–1.9 | 0.02 | 0.1 | 0.2 | 1.0 | 0.7–1.4 | 0.9 | 0.9 |
| Treatment naive | < 0.001 | 0.5 | 0.3–0.7 | < 0.001 | < 0.001 | < 0.001 | 1.0 | 0.6–1.7 | 0.9 | 1 |
| Liver transplantation and waiting list[e] | 0.07 | 1.9 | 1.0–3.8 | 0.07 | 0.4 | 0.05 | - | - | - | |

Abbreviations: CI, confidence interval; IAIHG, international autoimmune hepatitis group; IgG, immunoglobulin G; OR, odds ratio; PBC, Primary biliary cirrhosis. [a]Logistic/linear regression with *HLA-DRB1*03:01*, *HLA-DRB1*04:01*, sex and age as covariates. [b]*P*-value after Holm-Bonferroni correction for multiple testing. [c]Age not included in multivariate model. [d]Sex not included in multivariate model. [e]*HLA-DRB1*04:01* not included in multivariate model.

alleles and autoantibodies have mainly been described for pathogenic antibodies, such as the association of myasthenia gravis with *HLA-DRB1*03:01* and the association of anti-citrullinated protein antibodies (ACPA) with the shared epitopes at the *HLA-DRB1*01*, *HLA-DRB1*04* and *HLA-DRB1*10* alleles in rheumatoid arthritis.[22–24] In addition, Ferucci *et al.*[25] recently reported that *HLA-DRB1*03:01* and anti-dsDNA are associated in Alaskan native AIH patients. Similarly, Ma *et al.*[26] identified an association between *HLA-DRB*03:01* and the presence of soluble liver antigen antibodies, which are in turn associated with disease severity. In contrast, the ANA and SMA antibodies that classify type-1 AIH are neither pathogenic nor organ-specific, and thus may constitute an epiphenomenon in AIH. Our findings are consistent with the findings by Czaja *et al.*[8] reaffirming that ANA and SMA are useful in the diagnosis of AIH type-1 but do not represent a specific pathogenic process.

So far, the basis for the strong female predisposition to type-1 AIH remains unclear. Age-related differences in genetic susceptibilities and clinical phenotypes are evident in white North American and Northern European patients.[27] In line with previous studies, we found that the *HLA-DRB1*04:01* allele is associated with presentation at older age and is more often seen in female patients.[9,16,28] We did not find an independent association between *HLA-DRB1*03:01* and these traits. Our results thus implicate *HLA-DRB1*04:01* as an age-related risk factor for type-1 AIH in females. This suggests that change in hormone status and increasing age may lead to the occurrence of antigens that are effectively presented and induce an immune response by *HLA-DRB1*04:01* but not *HLA-DRB1*03:01*.

In a recent study by Liu *et al.*[29] in a large cohort of PBC patients, no association was found for the *HLA-DRB1*03:01* and *HLA-DRB1*04:01* alleles investigated in this study. Accordingly, our data

suggest that *HLA-DRB1*03:01*-positive AIH patients are less likely to have manifestations of an overlap syndrome with PBC. However, some of the clinical traits investigated in this study, including the presence of AIH-PBC and AIH-PSC overlap, only constitute small groups in this *HLA-DRB1*03:01* and *HLA-DRB1*04:01*-enriched AIH study population. This may explain the lack of association with *HLA-DRB1*03:01* between AIH and AIH-PSC patients, which in previous genetic studies was found to be a strong susceptibility risk factor in PSC.[29,30]

In contrast to the study by Czaja *et al.*,[8] we did not find a clear-cut association of *HLA-DRB1*04:01* with the presence of concomitant autoimmune disease. Instead, we identified an independent relation between *HLA-DRB1*03:01* and the presence of one or more concomitant autoimmune diseases. *HLA-DRB1*03:01* also showed an independent, additive relation with the presence of fibrosis at clinical presentation, suggesting a gene-dosing effect with more severe disease in homozygotes relative to heterozygotes. It should be noted that these results did not remain significant after stringent correction for multiple testing. However, *HLA-DRB1*03:01*-positive AIH patients more often received immunosuppressive medication and were more likely to need a liver transplantation.

Previous studies have shown that patients with *HLA-DRB1*03:01* and/or *HLA-DRB1*04:01* can have variable severity of disease and treatment outcomes. AIH Patients with *HLA-DRB1*03:01* and/or *HLA-DRB1*04:01* alleles had a greater frequency of treatment failure during corticosteroid treatment, and *HLA-DRB1*03:01* accounted for the observed association for poor outcome.[8,16] In our study, $< 3\%$ of the included patients were either transplanted or considered for liver transplantation, but none of these were *HLA-DRB1*04:01*-positive. As these data were retrospectively collected from the registries of all three liver transplant centers in the Netherlands, it is unlikely that any cases were missed.

In this study, we found clear, independent associations between the *HLA-DRB1*03:01* and *HLA-DRB1*04:01* alleles and the IAIHG score. Given the strong relation of the *HLA-DRB1*03:01* and *HLA-DRB1*04:01* alleles with overall disease susceptibility in the Dutch population[17] and the association with the primary end point, we aimed to describe the effect of the *HLA-DRB1*03:01* and *HLA-DRB1*04:01* alleles on the individual clinical variables in this cohort. Although this is the largest investigated cohort to date, this study has a relatively low power to identify associations with several clinical traits when stringent correction for multiple testing is applied. It should be noted that this study was performed in a cohort of Caucasian type-1 AIH patients with mostly adult onset of the disease, whereas several previous studies in different populations have identified *HLA-DRB1*13:01, HLA-DRB1*04:04, HLA-DRB1*04:05* and *HLA-DRB1*07* as HLA susceptibility alleles for both AIH type-1 and 2. These alleles were not found to be associated with AIH type-1 in the Netherlands, which shows that the susceptibility profile of AIH differs between populations and disease phenotype. Despite this, our observations reaffirm the likelihood that disease severity and treatment response in type-1 AIH have a genetic basis and that it may be possible to define genetic indices of prognosis. In clinical practice, the assessment of *HLA-DRB1* alleles can help to identify individuals who may have an unfavorable prognosis. This study emphasizes the importance of genetic factors in occurrence, clinical expression and behavior of AIH.

We conclude that the type-1 AIH risk alleles *HLA-DRB1*03:01* and *HLA-DRB1*04:01* are predictors of clinical presentation of type-1 AIH. In addition, the *HLA-DRB1*03:01* allele modifies disease severity in AIH.

## PATIENTS AND METHODS

### Patient population

The cases for the discovery set were identified by the Dutch AIH Study Group consortium (http://www.autoimmuunhepatitis.nl), as described previously.[17] Between 2008 and 2012, we recruited 743 patients with a clinical diagnosis of AIH. Twenty-four patients with AIH type-2 were excluded. An additional 70 patients were excluded owing to stringent quality control measures, including control for population homogeneity.[17,31] Prior to the start of the study, institutional review board approval to carry out the study was obtained in all participating centers. All participants provided written informed consent.

### Data collection

AIH patients were identified by treating physicians and by searching the database for international classification of diseases codes in eight academic and 23 general hospitals in the Netherlands. The search was performed in local diagnostic registers in the departments of gastroenterology and hepatology as well as internal medicine. Clinical and biochemical parameters were assessed in all patients to exclude other etiologies such as alcoholic hepatitis, drug-induced liver disease and metabolic disorders. Viral hepatitis was excluded by serological testing. If performed, liver biopsy was used to establish diagnosis and presence of fibrosis and cirrhosis. If available, we recorded manifestations of overlap syndromes with PBC and PSC in the context of AIH as noted by the treating physician. For PBC, these criteria consisted of antimitochondrial antibody titers higher than 1:80 and typical histological findings, whereas manifestations of PSC were recorded in case of typical histological and radiological findings. The presence of other concomitant autoimmune disorders, including type-1 diabetes mellitus, celiac disease, Hashimoto's disease, Sjögren syndrome, ulcerative colitis and Crohn's disease, was separately ascertained if noted in the medical records. Diagnostic scores were determined according to the revised original IAIHG criteria.[3] In this study, results from *HLA-DRB1*03:01* and *HLA-DRB1*04:01* genotyping were not incorporated in the IAIHG score. The median IAIHG scores between *HLA-DRB1*03:01/HLA-DRB1*04:01*-positive patients and *HLA-DRB1*03:01* and *HLA-DRB1*04:01*-negative patients was the primary end point of the study. The clinical variables that constitute the IAIHG score as well as outcome variables (Table 1) were selected as secondary end points for further association analysis with the *HLA-DRB1*03:01* and *HLA-DRB1*04:01* alleles. Available data on induction and maintenance therapy, as initiated and recorded by the treating physician, were retrospectively collected from the patient hospital records. Similarly, both clinical response to induction therapy and the occurrence of a relapse after treatment withdrawal were scored as assessed by the treating physician. Liver-related complications such as liver transplantation and/or the development of a hepatocellular carcinoma were extracted from the records from the eight academic centers.

### Genotyping and quality control

Genomic DNA was obtained from peripheral blood mononuclear cells. The genotyping, quality control and imputation of the MHC region in the AIH samples is describes elsewhere.[17] Briefly, cases were genotyped using the Illumina CytoSNP 12.0 platform (containing 300 739 single-nucleotide polymorphisms). Ethnic outliers and cases with a call rate of $< 99\%$ were excluded.[31] After quality control, 649 AIH type-1 patients were included for further analysis. Classic HLA genotypes were imputed using the SNP2HLA imputation tool (http://www.broadinstitute.org/mpg/snp2hla/) on available single-nucleotide polymorphisms in the MHC region (20–40 mb on chromosome 6).[32]

### Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS 22.0, Armonk, NY, USA: IBM Corp.). Continuous variables were described as means with their s.d. in case of Gaussian distribution or median with interquartile range in cause of non-Gaussian distribution. Summary statistics for categorical variables were expressed as numbers with percentages. Depending on Gaussian or non-Gaussian distribution, parametric (*t*-test and analysis of variance) and nonparametric (Mann–Whitney *U* and Jonckheere-Terpstra) tests were used to test for statistical significance of the differences between groups. Logarithmic transformation on continuous variables was applied when appropriate. We used $X^2$- or Fisher's exact test to compare proportional difference between groups. Two-sided *P*-values $< 0.05$ were considered statistically significant. Significant associations between HLA alleles and the clinical and outcome variables, were tested for independence in multivariate logistic or linear regression model with the other investigated HLA-allele, age and sex as forced selected covariates. We generated a dichotomous variable from the ordinal IAIHG score with a cutoff at the overall median value ($\geqslant 18$) to

perform multivariate analysis on this variable. Results with a *P*-value $< 0.05$ after Holm–Bonferroni correction for multiple testing were considered statistically significant.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## AUTHOR CONTRIBUTIONS

CM and GB had the original idea. YdB performed analysis and wrote the manuscript. NvG coordinated and contributed to the characterization of individuals and collection of clinical data and samples. VK and LF performed the analysis. AZh performed the imputation and analysis of the MHC region. BV, BvH, KvE, UB, HvB, MC, JD, JdO, RV, GK, JB, MG, JV, CvN, WN, EB and HV characterized individuals and collected clinical data and samples. YdB, BV, AnZ and GK contributed to the design of the study. CW, LF and GB designed the study. YdB, NvG and GB wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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## APPENDIX

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