The PNPLA3 rs738409 GG genotype is associated with poorer prognosis in 239 patients with autoimmune hepatitis

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Summary

Background: Fibrosis progression in autoimmune hepatitis can be attenuated by immunosuppressive treatment; however, some patients progress despite therapy. Single nucleotide polymorphisms (SNPs) such as PNPLA3-rs738409, TM6SF2-rs58542926 and MBOAT7-rs641738 are associated with non-alcoholic fatty liver disease and fibrosis progression, whereas a splice variant in HSD17B13-rs72613567:TA has been shown to be protective.

Aim: To analyse the impact of different SNPs on the long-term outcome of patients with autoimmune hepatitis.

Methods: We included 239 patients into this study who had been treated between 1983 and 2018 for autoimmune hepatitis. Genomic DNA was isolated from whole blood and SNPs were determined by PCR analysis. Liver biopsies were available for 215/239 patients (90%). Clinical and laboratory patient data were assessed by chart review.

Results: Mean age at baseline was 42.1 years with 74.1% being female. The median follow-up was 9.4 years (IQR 3.5-15.0), 11.7% of the patients (n = 28) died or required liver transplantation. In the Kaplan-Meier analysis of the combined endpoint time to liver transplantation or death, we observed that patients with the PNPLA3-rs738409 GG variant met more frequently the primary endpoint (P = 0.005). In Cox regression analysis PNPLA3-rs738409 GG as well as liver cirrhosis were identified as strong predictors for time to liver transplantation or death (HR 4.5 [CI 1.48-13.72], P = 0.008 and HR 9.24 [CI 2.11-40.44], P = 0.003, respectively). Neither steatosis, diabetes mellitus nor obesity were associated with outcome.

Conclusions: PNPLA3-rs738409 variant GG is a predictor for time to liver transplantation or death and may help to identify autoimmune hepatitis patients at risk for disease progression.

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INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease leading to liver fibrosis and cirrhosis. Imunosuppressive treatment can attenuate disease progression and improve survival. However, patients may develop significant liver fibrosis/cirrhosis over time, some even despite biochemical response to immunosuppressive therapy. Moreover, the increasing prevalence of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), which also occur in patients with AIH, may influence the clinical course of AIH resulting in increased mortality. Other risk factors for an unfavourable outcome of AIH are female gender, younger age and the presence of SLA antibodies. Genetic predispositions in the HLA haplotypes with risk-conferring or protective impact have also been proposed. In this context, several single nucleotide polymorphisms (SNPs) such as PNPLA3 rs738409, TM6SF2 rs58542926 and MBOAT7 rs641738 have been identified as risk factors for liver disease progression. On the other hand, the splice-variant rs72613567:TA in the HSD17B13 gene is associated with a reduced risk of both chronic liver disease and of progression from steatosis to steatohepatitis. None of these SNPs have been investigated in patients with AIH. The aim of this study was to analyse whether SNPs that have been established to influence the progression of various liver diseases including NAFLD and NASH also have an impact on the clinical course and outcome of patients with AIH.

METHODS

2.1 Patients

Patients with the diagnosis of AIH were prospectively recruited into our observational AIH cohort at Hannover Medical School between 2000 and 2014. All patients included into this study fulfilled the criteria for probable or definite AIH according to the simplified International Autoimmune Hepatitis Group (IAIHG) score system. After signed written informed consent, whole-blood samples treated with ethylenediaminetetraacetate (EDTA) were taken for genetic testing of 264 patients. Remaining DNA for additional genetic testing was available for 239 of 264 patients (90%) and those 239 patients were included into this study (Table 1; Figure S1). AIH was diagnosed as early as 1983 and patient long-term follow-up was analysed until 2018. All patients were Caucasians.

2.2 SNP genotyping

Whole blood samples treated with ethylenediaminetetraacetate (EDTA) were taken for DNA extraction. Genomic DNA was isolated from whole blood (NucleoSpin Blood L; Macherey Nagel) according to manufacturer’s protocol. SNPs were determined by PCR analysis using TaqMan® SNP assays (Table S1) and TaqMan® Genotyping Master Mix (Thermofisher). The following SNPs were analysed by allelic discrimination carried out on a Applied Biosystems 7300 Real-Time PCR System (Thermofisher): PNPLA3 rs738409 C>G, TM6SF2 rs58542926 C>T, MBOAT7 rs641738 C>T as well as a splice variant in HSD17B13 rs72613567:TA.

2.3 Clinical, laboratory and histologic data

Clinical and laboratory patient data were assessed by retrospective chart review at baseline, 1 year after treatment initiation and at the end of follow-up (median follow-up 9.4 years [IQR 3.5-15.0 years]). As laboratory data were collected over a 36-year period, data were normalised by using the upper limit of normal (ULN) or lower limit of normal (LLN), respectively, from the respective time of analysis. Liver biopsy was performed at the discretion of the treating
physician. Non-invasive fibrosis scores were calculated from the available laboratory values. Advanced fibrosis was defined by an aspartate aminotransferase to platelet ratio index (APRI) score ≥ 1\(^{16}\) or a FIB-4 score ≥ 2.67.\(^{17}\) Complete biochemical remission was defined as normalisation of aminotransferases, whereas partial remission was defined as decrease in aminotransferases below twice the ULN. Clinical endpoints (liver transplantation, death) were analysed throughout the follow-up period. Analysis of liver histology was performed at the time of biopsy by the local pathologist. Pathology reports were analysed to reveal the diagnosis of fibrosis (absence of fibrosis, fibrosis and cirrhosis) or steatosis. Steatosis was defined as present when >5% or described as at least “discrete” when specifically mentioned in the pathology report.\(^{18}\)

### 2.4 | Statistical analysis

Statistical analysis was performed using SPSS version 24 (SPSS Software Cor.) and StatXact\(^{15,18}\). Data are reported as means ± SD or estimated marginal means ± SEM. Two-sided P-values were assessed and considered to be of relevance for \(P < 0.05\). Detailed statistical methods can be found in the Supporting Information.

### 2.5 | Ethics

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the appropriate institutional review committee. Written consent was obtained from all patients included in the study.

### 3 | RESULTS

A total of 239 patients (177 females, 74.1%) with AIH were included into this study. At baseline, the mean age of the patients was 42.1 ± 16.9 years and the body mass index (BMI) was 24.3 ± 5.1 kg/m\(^2\). Half of the patients (\(n = 119, 49.8\)% had already received immunosuppressive therapy (Table 1; Figure S1). During follow-up, treatment was initiated in additional 114 patients, resulting in a total of 233/239 (97.5%) patients who received immunosuppressive therapy during the study period. Only six patients had never received any immunosuppressive medication for various reasons (mild disease course [\(n = 4\)], noncompliance [\(n = 1\)] and burnt out cirrhosis [\(n = 1\)]). During the study period, 28 patients (11.7%) died or required liver transplantation.

#### 3.1 | PNPLA3-rs738409 GG is associated with increased mortality

To determine, whether disease progression is associated with SNPs that are known to play a role in the progression of alcoholic or non-alcoholic steatohepatitis, we performed genotyping for PNPLA3-rs738409, TM6SF2-rs58542926, MBOAT7-rs641738 and HSD17B13-rs72613567TA. No substantial deviation from Hardy-Weinberg equilibrium was observed (Methods S1, Table S2). Next, we analysed available laboratory data and non-invasive fibrosis scores at the last visit of the follow-up period according to the different SNP genotypes. PNPLA3 was significantly associated with advanced liver disease. When comparing patients with homozygous ancestral (CC), heterozygous (GC) and homozygous variant (GG) of PNPLA3 (rs738409), we observed that patients with the GG genotype had based on clinical laboratory values advanced disease with impaired liver synthesis as determined by cholinesterase and prothrombin time expressed as quick value, as well as higher bilirubin levels and lower platelet counts (Table 2). In addition, significant liver fibrosis determined by non-invasive liver fibrosis scores (APRI score ≥ 1, FIB-4 score ≥ 2.67) was more frequently observed in patients with the PNPLA3-rs738409 GG variant compared with CG or CC patients (\(P < 0.01\), Table S3), despite similar disease duration of AIH in all three groups (12.47 ± 0.7, 12.16 ± 0.9 and 10.58 ± 2.5 years, \(P = 0.8\), Table 2). Neither TM6SF2, MBOAT7 nor HSD17B13 had an influence on clinically laboratory values or non-invasive fibrosis scores at the end-of follow-up (Tables S4-S6).

Comparing time to event (liver transplantation or death) curves between the three different PNPLA3-rs738409 genotypes, we observed that patients with the GG variant had a significantly higher risk for an event than patients with the CC or CG genotype (Figure 1A). In total 33.3% (4/12) patients with the GG variant underwent liver transplantation or died during the study period, compared to 10.2% (9/88) and 10.8% (15/139) with the CG and the CC genotype respectively (Table 3). Neither TM6SF2, MBOAT7 nor HSD17B13 variants had an impact on survival of AIH patients (Figure 1B-D). Even though homozygous HSD17B13 insertion TA:TA had no influence on the outcome, patients with the homozygous insertion were diagnosed at an older age (37.2 ± 1.5 and 39.3 ± 2.0 vs 48.3 ± 3.6 years, \(P = 0.02\), Table S6).

#### 3.2 | PNPLA3-rs738409 genotype but not steatosis is associated with outcome in patients with AIH

Next, we were interested whether factors associated with nonalcoholic fatty liver disease or nonalcoholic steatohepatitis had an influence on the clinical course of patients with AIH. Liver biopsies were available for 215 of 239 patients (90%). Steatosis in the biopsy was present in 34 of 215 patients (15.8%). At the end of follow-up, steatosis was not associated with liver inflammation, liver function or cumulative survival. Patients with steatosis on liver biopsy were characterised by higher BMI (25.0 kg/m\(^2\) vs 29.3 kg/m\(^2\), \(P < 0.001\)) at the end of follow-up (Figure S2; Table S7).

Univariate logistic regression analysis identified BMI ≥ 30 kg/m\(^2\) to be associated with steatosis in the liver biopsy (Table S8). TM6SF2-rs58542926 variants showed a strong trend towards association with steatosis (\(P = 0.05\) and 0.07 for CT and TT, respectively).
with an additive effect of each risk allele (OR 2.51 and 6.58, respectively). To test for confounders with respect to TM6SF2, we performed bivariate logistic regression analyses including all factors with a P-value <0.2. When adjusted for HSD17B13, BMI ≥ 30 kg/m² or age at diagnosis (≥40 years), we observed that the TM6SF2 variant was an independent risk factor for the presence of steatosis (Table S9). Homozygous presence of the TM6SF2 minor variant was associated with a higher risk for steatosis, however, this was not formally significant due to the low number of patients having the TT genotype (4/239).

In order to identify factors that were associated with time to liver transplantation or death we performed a univariate cox regression analysis and identified PNPLA3-rs738409 GG variant as a strong predictor in our cohort (HR 4.5 [1.48-13.72], P = 0.008, Table 3). Neither histologically confirmed steatosis (HR 1.4 [0.53-3.7]), diabetes mellitus (HR 2.29 [0.78-6.71]) nor BMI ≥ 30 kg/m² (HR 0.8 [0.24-2.62])

| TABLE 2 Characteristics of patients and laboratory data at end of follow-up according to PNPLA3 genotype |
|---------------------------------|-------------------------------------------------|---------------------------------|---------------------------------|------------------|
|                                | Homozygous ancestral (CC)                       | Heterozygous variant (CG)       | Homozygous variant (GG)         | P-value          |
| Number of patients             | 139                                             | 88                              | 12                              |                  |
| Age at last visit (y)          | 51.8 ± 1.4                                      | 52.9 ± 1.76                     | 46.4 ± 4.8                       | 0.4              |
| Age at diagnosis (y)           | 38.8 ± 1.5                                      | 40.2 ± 1.9                      | 35.4 ± 5.2                       | 0.6              |
| Duration of AIH (y)            | 12.47 ± 0.7                                     | 12.16 ± 0.9                     | 10.58 ± 2.5                      | 0.8              |
| Liver biopsy                   | N = 123                                         | N = 81                           | N = 11                           | 0.3*             |
| No fibrosis                    | 32 (26%)                                        | 25 (30.9%)                      | 9 (9.1%)                         |                  |
| Fibrosis                       | 56 (45.5%)                                      | 33 (40.7%)                      | 4 (36.4%)                        |                  |
| Cirrhosis                      | 35 (28.5%)                                      | 23 (28.4%)                      | 6 (54.5%)                        |                  |
| Liver biopsy                   |                                                 |                                 |                                 |                  |
| No steatosis                   | 101 (82.1%)                                     | 61 (75.3%)                      | 8 (72.7%)                        | 0.4*             |
| Steatosis                      | 22 (17.9%)                                      | 20 (24.7%)                      | 3 (27.3%)                        |                  |
| AST (>ULN)                     | 1.31 ± 0.14                                     | 1.45 ± 0.17                     | 2.25 ± 0.46                      | 0.1              |
| ALT (>ULN)                     | 1.41 ± 0.17                                     | 1.31 ± 0.22                     | 1.75 ± 0.59                      | 0.8              |
| GLDH (>ULN)                    | 1.89 ± 0.56                                     | 1.29 ± 0.8                      | 0.83 ± 1.9                       | 0.8              |
| AP (>ULN)                      | 0.89 ± 0.05                                      | 0.87 ± 0.07                     | 1.06 ± 0.18                      | 0.6              |
| gGT (>ULN)                     | 1.96 ± 0.25                                     | 1.81 ± 0.32                     | 2.38 ± 0.85                      | 0.8              |
| CHE (>LLN)                     | 1.56 ± 0.05                                     | 1.49 ± 0.06                     | 0.94 ± 0.17                      | 0.003            |
| Albumin (>LLN)                 | 1.09 ± 0.02                                     | 1.1 ± 0.02                      | 0.88 ± 0.06                      | 0.005            |
| Leukocytes (10⁷/µL)            | 6.7 ± 0.2                                       | 6.2 ± 0.3                       | 5.8 ± 0.8                        | 0.3              |
| Hemoglobin (g/dL)              | 13.2 ± 0.2                                      | 13.4 ± 0.2                      | 11.6 ± 0.5                       | 0.008            |
| Thrombocytes (×10⁹)            | 211 ± 8                                         | 190 ± 10                        | 121 ± 28                         | 0.005            |
| Prothrombin time (%)           | 92.5 ± 1.9                                      | 90.8 ± 2.4                      | 67 ± 6.3                         | 0.001            |
| IgG (g/L)                      | 14.45 ± 0.54                                    | 13.77 ± 0.66                    | 16.71 ± 2.09                     | 0.4              |
| APRI Score                     | 0.9 ± 0.14                                      | 1.35 ± 0.18                     | 2.67 ± 0.48                      | 0.001            |
| FIB-4 Score                    | 2.16 ± 0.29                                     | 3.09 ± 0.36                     | 5.65 ± 0.98                      | 0.001            |
| BMI at baseline (kg/m²)        | 24.7 ± 0.4                                      | 24.8 ± 0.6                      | 26.4 ± 1.5                       | 0.6              |
| BMI last visit (kg/m²)         | 25.7 ± 0.5                                      | 25.7 ± 0.6                      | 26.4 ± 1.6                       | 0.9              |
| Diabetes mellitus              | 20 (14.4%)                                      | 12 (13.6%)                      | 1 (8.3%)                         | 0.7*             |
| IDDM                            | 14 (10.1%)                                      | 7 (8.0%)                        | 1 (8.3%)                         | 0.2*             |
| Anti-SLA positive (n = 47)     | 29 (20.8%)                                      | 17 (19.3%)                      | 1 (8.3%)                         | 0.6*             |
| Treatment response             | N = 135                                         | N = 87                          | N = 11                           | 0.2*             |
| Complete remission             | 74 (54.8%)                                      | 38 (43.7%)                      | 3 (27.3%)                        |                  |
| Partial remission              | 40 (29.6%)                                      | 35 (40.2%)                      | 5 (45.5%)                        |                  |
| No remission                   | 21 (15.6%)                                      | 14 (16.7%)                      | 3 (27.3%)                        |                  |

Note: Data are presented as estimated marginal means ± SEM and respective P-value from ANOVA or absolute and relative frequencies and Chi-square test (#).
had an influence on outcome in patients with AIH. Importantly, the presence of steatosis in the first liver biopsy was evenly distributed between patients with the different PNPLA3 genotypes (Table 2). To exclude that the effect of PNPLA3-rs738409 GG is influenced by factors that are associated with progression or advanced liver disease, we performed bivariate Cox regression analysis with all factors with a P-value <0.2 in the univariate Cox regression analysis. Here, we could confirm that the effect of PNPLA3-rs738409 GG is independent of diabetes mellitus, liver cirrhosis, non-invasive fibrosis scores (APRI, FIB-4), type of AIH and treatment response (Table 4).

3.3 | APRI score can predict outcome of patients with AIH

To evaluate, whether fibrosis stage determined by histology or non-invasive liver fibrosis scores had an influence on the outcome in our cohort we analysed liver biopsies for presence of fibrosis and cirrhosis and observed that cirrhosis was a strong predictor for time to liver transplantation or death (HR 9.24 [2.11-40.44], P = 0.003), which is in line with our previous study. There was a trend towards time to liver transplantation or death in patients with liver fibrosis (HR 4.04 [0.89-18.48], P = 0.07, Table 3).

To evaluate whether outcome can also be predicted by non-invasive fibrosis scores, we calculated the APRI- and FIB-4 score. As half of the patients presented with acute AIH at the baseline visit and calculation of fibrosis scores during phases of acute hepatitis reveal misleading results, we chose a time point approximately 1 year after treatment initiation or 1 year after baseline for those patients where no treatment starting date was documented to avoid bias introduced by untreated hepatitis. We used these data for comparing non-invasive fibrosis scores. At this 1 year time point, only APRI score (≥1.0) was significantly associated with time to liver transplantation or death (HR 4.4 [1.67-11.5], P = 0.003, Table 3). Notably, the effect of APRI score (≥1.0) on time to liver transplantation or death was not confounded by PNPLA3-rs738409 GG genotype (Table 4).
Fibrosis progression varies between different types of liver disease but also according to age and gender. It has been shown that steatosis is not only a co-factor for the progression of chronic hepatitis C virus infection but also may influence the course of alcohol- and drug-induced liver disease. A small study of patients with AIH and coincident non-alcoholic fatty liver transplantation or death (univariate cox regression analysis)

| Table 3 | Factors associated with liver transplantation or death (univariate cox regression analysis) |
|---------|------------------------------------------------------------------------------------------|
| PNPLA3 rs738409 | | | | | |
| CC | 124 | 15 | 1 | | |
| CG | 79 | 9 | 0.8 | 0.36-1.89 | 0.6 |
| GG | 8 | 4 | 4.5 | 1.48-13.72 | 0.008 |
| Diabetes mellitus at baseline | | | | | |
| No diabetes | 191 | 24 | 1 | | |
| Diabetes | 20 | 4 | 2.29 | 0.78-6.71 | 0.1 |
| Steatosis in first biopsy | | | | | |
| No steatosis | 159 | 22 | 1 | | |
| Steatosis | 29 | 5 | 1.4 | 0.53-3.70 | 0.5 |
| BMI at baseline (kg/m²) | | | | | |
| <30 | 182 | 25 | | | |
| ≥30 | 29 | 3 | 0.8 | 0.24-2.62 | 0.7 |
| Gender | | | | | |
| Female | 158 | 19 | 1 | | |
| Male | 53 | 9 | 1.14 | 0.52-2.54 | 0.74 |
| Liver histology | | | | | |
| F0 | 57 | 1 | 1 | | |
| F1-4 | 87 | 6 | 4.04 | 0.89-18.48 | 0.07 |
| F5/6 | 44 | 20 | 9.24 | 2.11-40.44 | 0.003 |
| APRI score at 1 y | | | | | |
| <1 | 133 | 7 | 1 | | |
| ≥1 | 31 | 11 | 4.4 | 1.67-11.5 | 0.003 |
| FIB-4 score at 1 y | | | | | |
| <2.67 | 140 | 15 | 1 | | |
| ≥2.67 | 22 | 3 | 2.81 | 0.75-10.53 | 0.13 |
| FIB-4 score at 1 y | | | | | |
| >1.3 | 60 | 7 | 1 | | |
| ≤1.3 | 102 | 11 | 0.64 | 0.24-1.67 | 0.36 |
| AIH type | | | | | |
| 1 | 158 | 14 | 1 | | |
| 2 | 16 | 4 | 2.91 | 0.96-8.86 | 0.06 |
| 3 | 37 | 10 | 2.25 | 0.99-5.09 | 0.05 |
| Treatment response (N = 232) | N = 206 | N = 26 | | | |
| CR | 106 | 9 | 1 | | |
| PR | 72 | 8 | 1.11 | 0.43-2.89 | 0.83 |
| NR | 28 | 9 | 3.23 | 1.31-7.98 | 0.001 |
| Age at diagnosis (y) | | | | | |
| <40 | 104 | 19 | 1 | | |
| ≥40 | 107 | 9 | 0.72 | 0.32-1.62 | 0.4 |
TABLE 4 Factors associated with liver transplantation or death (bivariate cox regression analysis)

| Model | HR     | 95% CI       | P-value |
|-------|--------|--------------|---------|
| Model 1 |        |              |         |
| PNPLA3 CC/CG | 1     |              |         |
| PNPLA3 GG     | 5.06  | 1.73–14.79   | 0.003   |
| No diabetes mellitus | 1     |              |         |
| Diabetes mellitus | 2.41  | 0.82–7.11    | 0.1     |
| Model 2 |        |              |         |
| PNPLA3 CC/CG | 1     |              |         |
| PNPLA3 GG     | 3.41  | 1.16–10.04   | 0.03    |
| Liver histology |        |              |         |
| F0             | 1     |              |         |
| F1–4           | 4.09  | 0.49–34.15   | 0.2     |
| F5/6           | 19.18 | 2.56–143.89  | 0.004   |
| Model 3 |        |              |         |
| PNPLA3 CC/CG | 1     |              |         |
| PNPLA3 GG     | 5.73  | 1.22–27.04   | 0.03    |
| APRI score < 1 | 1     |              |         |
| APRI score ≥ 1 | 3.95  | 1.49–10.47   | 0.006   |
| Model 4 |        |              |         |
| PNPLA3 CC/CG | 1     |              |         |
| PNPLA3 GG     | 6.55  | 1.27–33.67   | 0.02    |
| FIB-4 score < 2.67 | 1     |              |         |
| FIB-4 score ≥ 2.67 | 1.99  | 0.49–8.11   | 0.3     |
| Model 5 |        |              |         |
| PNPLA3 CC/CG | 1     |              |         |
| PNPLA3 GG     | 5.96  | 2.01–17.65   | 0.001   |
| AIH type      |        |              |         |
| 1              | 1     |              |         |
| 2              | 3.11  | 1.02–9.51    | 0.047   |
| 3              | 2.55  | 1.11–5.84    | 0.03    |
| Model 6 |        |              |         |
| PNPLA3 CC/CG | 1     |              |         |
| PNPLA3 GG     | 5.65  | 1.93–16.58   | 0.002   |
| Treatment response |        |              |         |
| CR             | 1     |              |         |
| PR             | 1.11  | 0.43–2.89    | 0.8     |
| NR             | 3.33  | 1.35–8.21    | 0.009   |

liver disease showed an adverse outcome of patients with AIH and NASH but no impact of simple steatosis on the clinical outcome. In recent years, the influence of genetic variations on the course of liver diseases have been extensively studied especially in patients with NAFLD/NASH, alcoholic and viral hepatitis. In this context, our study investigated factors of disease progression in a cohort of patients with AIH and we observed several important findings: (a) the PNPLA3-rs738409 GG variant is associated with time to liver transplantation or death in AIH patients, (b) neither steatosis nor the TM6SF2-rs58542926 variant seem to have an impact on the outcome of AIH patients, but the TM6SF2-rs58542926 minor variant was associated with steatosis, (c) patients with the homozygous insertion of a splice variant within the HSD17B13-rs72613567 locus were diagnosed at an older age, and (d) noninvasive APRI score can predict outcome of patients with AIH.

Genome-wide association studies (GWAS) performed in the last decade revealed several genetic variants that are associated with liver disease severity and outcome. It has been shown that a SNP in the PNPLA3 locus (rs738409[G], encoding I148M) confers susceptibility for NAFLD and was strongly associated with increased hepatic fat levels as well as hepatic inflammation. The same locus was also associated with an increased risk for alcoholic liver disease and alcohol-related cirrhosis. Our study in a large single centre cohort demonstrated that the same variant in the PNPLA3 locus was also associated with disease progression, liver transplantation and death in patients with AIH. Unexpectedly, presence of steatosis in the liver biopsy did not differ between the different PNPLA3 genotypes in our study. In line with a previous study, the PNPLA3-rs738409 variant was not associated with an increased risk for metabolic syndrome; demonstrated by similar BMI across all PNPLA3-rs738409 genotypes as well as same frequency of patients with diabetes mellitus. These findings suggest that an additional mechanism than hepatic fat content might be responsible for the observed progression in liver disease in patients with AIH and the PNPLA3-rs738409 GG variant. One explanation could be the association of PNPLA3 with elevated ALT levels, which may also influence the progression of chronic liver disease. However, probably owing to our limited sample size in a rare disease and different treatment regimens, we did not observe a difference in ALT levels between the different PNPLA3-rs738409 genotypes in our study.

Another SNP that confers susceptibility to NAFLD is TM6SF2-rs58542926. Despite the relatively low number of patients with steatosis at baseline in our study (n = 34), we also observed that TM6SF2-rs58542926 was associated with steatosis in AIH patients. However, in contrast to a previous study, we did not observe an effect of TM6SF2-rs58542926 genotype on fibrosis progression. Owing to the low frequency of the risk allele for TM6SF2-rs58542926, only 36 of 239 patients in our study had at least one risk allele, limiting the possibility to study the effect on liver fibrosis progression of this SNP especially in a rare disease such as AIH.

A loss-of-function variant in HSD17B13-rs72613567:TA was associated with reduced ALT and AST levels in patients with alcoholic and non-alcoholic steatohepatitis and a reduced risk of progression from steatosis to NASH. Moreover, it has been shown that this variant is also protective of HCC development in patients with ALD. The precise function of HSD17B13 remains unknown, but it has been demonstrated that HSD17B13 is a hepatic retinol dehydrogenase, which is associated with histological features of NAFLD, but does not affect hepatocyte lipid content. In our study, the HSD17B13-rs72613567 genotype was neither associated with alterations in ALT levels nor outcome. A recent study on HSD17B13-rs72613567 analysed over...
100,000 patients with clinical liver disease to observe a difference of plasma ALT of 1.3 U/L in TA:TA homozygotes vs TT homozygotes. Given that ALT levels are affected by several factors including disease state, gender and co-medication and the low prevalence of AIH of 2-3 per 10,000 patients, it probably would be necessary to identify and test at least all German patients with AIH to demonstrate an effect of HSD17B13-rs72613567 genotype on ALT levels in this rare disease. Interestingly, in our study we observed that patients with the homozygous insertion of the splice variant in HSD17B13-rs72613567 were significantly older at diagnosis of AIH, suggesting either a delayed onset or a slower progression until clinical disease manifestation in these patients.

Previous studies on the course of AIH showed that presence of cirrhosis is associated with an unfavourable outcome. Similarly, in our study, histological evidence of cirrhosis was a strong predictor for time to liver transplantation or death. Liver biopsy is the gold standard for the determination of fibrosis stage, but a recent study showed that transient elastography can also be applied to determine the presence of cirrhosis in patients with AIH. To our knowledge, non-invasive fibrosis scores have not been systematically evaluated in patients with AIH. One study claimed that APRI score is not useful in patients with AIH. However, in our study, APRI score ≥ 1 as early as 1 year after baseline was predictive for death and transplantation, and could therefore be applied as an easy non-invasive fibrosis score in resource limited settings.

Our study had several limitations including the single centre design, the retrospective analysis of a prospectively recruited cohort as well as the low patient number in a rare disease such as AIH. The low patient number also limits potential subanalysis that would have been of interest. AIH type 2 and 3 is associated with disease progression independent of PNPLA3, however, as only 2/12 patients presented with the Type 2 or 3 AIH and PNPLA3-rs738409 GG genotype, additional effects were not possible to study. Similarly, we also tested a polygenic risk score including PNPLA3, TM6SF2 and MBOAT7 based a previous study and observed a trend towards reduced survival in patients with a higher amount of risk alleles (Log rank test, P = 0.065, data not shown). However, only three patients had four risk alleles, two patients had five risk alleles and none of the patients displayed all six risk alleles, therefore the results from the polygenic risk must be interpreted with caution and should be re-evaluated in a larger patient cohort.

In conclusion, testing AIH patients for the presence of PNPLA3-rs738409 variants may help to identify patients at risk for a more progressive course of disease. Although TM6SF2-rs58542926 genotype may predict steatosis, neither TM6SF2 nor steatosis are a risk factor for disease progression.

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Author contributions: YSM collected patient data, performed SNP genotyping and analysis, statistical data analysis and interpretation and wrote the manuscript. MMK collected patient data and performed a critical review of the manuscript. AG performed statistical data analysis and performed a critical review of the manuscript. SM processed patient samples and isolated genomic DNA. FM recruited patients and processed patient’s samples. MPM and AV initiated the prospective AIH cohort, recruited patients and performed a critical review of the manuscript. IM designed and supervised the study, performed data analysis and interpretation and wrote the manuscript. All authors approved the final version of the manuscript.

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SUPPORTING INFORMATION
Additional supporting information will be found online in the Supporting Information section.