Journal of Translational Autoimmunity 9 (2024) 100243

Contents lists available at ScienceDirect

Journal of Translational Autoimmunity

journal homepage: www.sciencedirect.com/journal/journal-of-translational-autoimmunity

PNPLA3 I148 M genetic variant in autoimmune hepatitis characterises advanced disease at diagnosis and reduced survival free of cirrhotic events and liver-related mortality

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ARTICLE INFO

Handling editor: Y Renaudineau

Keywords:
Autoimmune hepatitis
PNPLA3 I148 M
Polymorphisms
Biomarker
Liver-related mortality

ABSTRACT

Background: Autoimmune hepatitis (AIH) is a relatively rare autoimmune disease with a strong genetic background. The patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) *I148 M* (*rs7*38409 C/G) variant has been associated with hepatic inflammation and fibrosis in chronic hepatic diseases beyond metabolic dysfunction-associated steatotic liver disease (MASLD).
Aim: Our aim was to investigate the significance of *PNPLA3 I148 M* variant in AIH.
Method: Two hundred AIH patients, followed in our centre, were evaluated while 100 healthy subjects served as controls. Genotyping was performed with allelic discrimination end-point polymerase chain reaction (PCR).
Results: The *I148 M* variant was present in 95/200 (47.5 %) AIH patients compared to 47/100 (47 %) healthy controls ($p = 1.000$). Patients with GG/CG genotypes were more likely to present with decompensated cirrhosis at diagnosis (GG/CG 6.3 % vs. CC 1 %, $p = 0.039$). Comorbidity with cardiometabolic risk factors and concurrence of MASLD was similar across genotypes. Simple steatosis was present in 37/186 (19.9 %) and steatohepatitis in 14/186 (7.5 %) patients with available liver biopsy without correlation with *PNPLA3* genotype. Fibrosis stage and grade of inflammation were not correlated with any genotype. Response to treatment was also independent of the presence of the *I148 M* variant, even though a longer time was needed to achieve complete biochemical response in those carrying the GG/CG genotypes ($p = 0.07$). On Kaplan Meier analysis homozygosity for the G allele corelated with reduced survival free of decompensation ($p = 0.006$), cirrhotic events (decompensation, liver transplantation, hepatocellular carcinoma; $p = 0.001$) and liver-related death or liver transplantation ($p = 0.011$) in treated patients.
Conclusions: The *PNPLA3 I148 M* variant in AIH patients is associated with increased risk of advanced disease at diagnosis and reduced survival free of cirrhotic events and liver-related death or liver transplantation, regardless of the presence of MASLD. This signifies a potential role for the *PNPLA3 I148 M* variant as a new AIH biomarker allowing to identify patients at increased risk of disease progression.

Abbreviations: AIH, Autoimmune hepatitis.; IgG, Immunoglobulin class G.; HLA, Human leukocyte antigen.; PNPLA3, patatin-like phospholipase domain-containing protein 3.; SNP, Single nucleotide polymorphism.; MASLD, Metabolic dysfunction-associated steatotic liver disease.; MASH, metabolic dysfunction-associated steatohepatitis.; ALD, Alcohol related liver disease.; HWE, Hardy–Weinberg equilibrium; HCC, Hepatocellular carcinoma.; HCV, Hepatis C virus.; ULN, Upper limit of normal.; MetS, Metabolic syndrome.; BMI, Body mass index.; HDL, High density lipoprotein.; Anti-LKM1, Liver kidney microsomal type-1 antibodies; Anti-LC1, Liver cytosol type-1 antibodies.; Anti-SLA/LP, Soluble liver antigen/liver pancreas antibodies.; qPCR, Quantitative polymerase chain reaction.; SD, Standard deviation.; IQR, Interquartile range.; CBR, Complete biochemical response.

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https://doi.org/10.1016/j.jtauto.2024.100243
Received 27 April 2024; Received in revised form 22 May 2024; Accepted 2 June 2024
Available online 6 June 2024
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1. Introduction

Autoimmune hepatitis (AIH) is a relatively rare chronic liver disease that affects both sexes and all ages [1–3]. Clinical presentation has a wide range from absence of symptoms to acute hepatitis, rarely with fulminant hepatic failure [4–6]. The diagnosis relies on a combination of clinical, laboratory and supporting histological findings that include polyclonal hyperglobulinemia with preferential immunoglobulin G (IgG) increase, circulating non-organ specific autoantibodies and interface hepatitis [1,7–9].

The etiology of AIH remains obscure, and the phenotype of the disease exhibits significant variations between different ethnicities, age groups and geographic regions [2,10]. To date, no single pathophysiological hypothesis can comprehensively explain the entire spectrum of the disease. A unifying theory suggests that the initial triggering event, involving antigen selection, is influenced by human leukocyte antigen (HLA) II predisposition. Subsequently, polymorphisms in other immune regulatory and cytokine-producing genes including epigenetics may promote and perpetuate immune reactivity, loss of self-tolerance and inflammatory responses [11–16].

The patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) *I148 M* polymorphism (*rs7*38409 C/G, single nucleotide mutation of isoleucine to methionine) is an established genetic modifier of metabolic dysfunction-associated steatotic liver disease (MASLD) [17–19]. The prevalence of the mutated G allele ranges between 40 and 70 % among patients with MASLD, showing a strong association with race, being more common in Hispanics than Caucasians [17,20]. The exact mechanism promoting steatosis is currently under investigation. Factors such as diminished lipase activity [21], amplified acyltransferase activity [22], and increased expression of the enzyme on the surface of lipid droplets due to decreased proteolytic disintegration [23] have been implicated in this process. On the other hand, the occurrence of MASLD and metabolic dysfunction-associated steatohepatitis (MASH), or the components of metabolic syndrome (MetS), are as frequent in patients with AIH as in the general population [24–27]. Notably, the coexistence of MASH in patients with AIH appears to indicate a more severe liver disease at diagnosis [25,27].

Investigations concerning the role of *PNPLA3 I148 M* beyond MASLD have linked this polymorphism to an increased risk of cirrhosis in alcohol related liver disease (ALD) and hepatocellular carcinoma (HCC) in ALD-related cirrhosis [28,29] as well as among patients with HCV [30,31]. As a result, the mechanisms connecting *PNPLA3 I148 M* with inflammation and fibrosis driving disease progression, have gained great scientific interest [28,31–39]. Accordingly, the present study aimed to investigate the prevalence and clinical significance of the *PNPLA3 I148 M* variant in a large cohort of Greek AIH patients as up to the present only one study has evaluated this topic [39].

2. Materials and methods

2.1. Study population

Two hundred consecutive Greek patients with well-established AIH [40,41] were included in the study. Clinical presentation was categorized as insidious, when symptoms were vague and non-specific or abnormal biochemistry was discovered during a routine check-up. Acute presentation referred to episode of acute icteric hepatitis, with aminotransferases ≥10 x the upper limit of normal (ULN) plus clinically evident jaundice. Acute severe AIH was defined, according to our previous publications, as an acute symptomatic presentation of newly diagnosed acute hepatitis with international normalized ratio ≥1.5 but without any sign of hepatic encephalopathy and without chronic disease at the histological level [5,42]. One hundred age- and sex-matched healthy subjects served as controls.

The treatment algorithms followed in our AIH patients adhered to the European [7] and the Hellenic Clinical Practice Guidelines [8] as well as our previous reports [43–46]. In total, 152/195 (77.9 %) patients received prednisolone in combination with mycophenolate mofetil, 26/195 (13.3 %) prednisolone with azathioprine and 17/195 (8.7 %) only prednisolone (3 due to current history of non-liver-related neoplasia, 4 paediatric patients whose parents denied immunomodulating agents, 1 because of lethal acute liver failure and 9 because of personal choice). Two patients were not eligible for treatment due to burn-out compensated cirrhosis and 3 declined treatment due to personal reasons.

Treatment response was assessed according to the updated response criteria and endpoints in AIH by the International AIH Group [47]. Specifically, non-response within 4 weeks of treatment initiation was defined as a <50 % decrease of transaminases, and complete biochemical response was defined as the normalization of transaminases and IgG below the ULN no later than 6 months after treatment initiation [47]. Data regarding maintenance of response at 12 months and at the end of follow-up were are also assessed.

Clinical and laboratory data, including metabolic parameters to assess the presence of cardiometabolic risk factors, were available for all patients. The presence of MetS was documented in patients fulfilling at least 3 out of 5 of the following criteria: (1) body mass index (BMI) ≥25 kg/m^2 or waist circumference >94 cm for men and >80 cm for women, (2) arterial pressure >130/85 mmHg or treated for hypertension, (3) fasting glucose >100 mg/dL or HbA1c >5.7 % or type 2 diabetes or treatment for type 2 diabetes, (4) serum triglycerides >150 mg/dL, and (5) high density lipoprotein (HDL) <40 mg/dL for men and <50 mg/dL for women or lipid lowering treatment [48]. MASLD was defined according to the 2023 consensus statement [46] requiring the presence of hepatic steatosis on imaging or biopsy with at least one cardiometabolic risk factor, in the absence of other causes of steatosis [48]. Patients with alcohol excess (140–350 g/week and 210–420 g/week for females and males, respectively) were excluded from the study.

2.2. Liver histology

Liver biopsies from 186 AIH patients at the time of diagnosis were available for analysis. Biopsies were assessed using the Knodell histologic/activity index score [49]. Consistent with our previous publications [2,50], patients were categorized into two groups based on inflammation: minimal-mild (score: 0–8) and moderate-severe (score: 9–18), and based on fibrosis: minimal/mild-moderate (score: 0–2) and severe fibrosis-cirrhosis (score: 3–4). All biopsies had detailed documentation of possible concurrent MASLD findings, including the amount and location of steatosis, presence/absence of Malory's hyaline, hepatocyte ballooning, lobular inflammation and zone 3 fibrosis. As previously reported, in patients without available liver biopsy, cirrhosis was established by findings from ultrasonography, and/or transient elastography, endoscopy or physical findings of portal hypertension [2,51].

2.3. Autoantibodies

Smooth muscle antibodies, antinuclear antibodies, antibodies against liver kidney microsomal type-1 (anti-LKM1), and against liver cytosol type-1 (anti-LC1) were initially detected by indirect immunofluorescence on 5-μm fresh frozen sections of in-house rodent kidney, liver, and stomach tissue sections as described [1,52]. Anti-LC1, anti-LKM1, and antibodies against soluble liver antigen/liver pancreas (anti-SLA/LP) were additionally evaluated by immunoblotting using rat liver microsomal or cytosolic extracts. Commercially available enzyme-linked immunosorbent assays using recombinant formiminotransferase cyclodeaminase (Euroimmun Medizinische Labordiagnostika, Lubeck, Germany), SLA/LP/tRNP (Ser) Sec (Inova Diagnostics, San Diego, CA, USA) and cytochrome P450 2D6 (Inova) were used also for anti-LC1, anti-SLA/LP and anti-LKM1 determination respectively, according to the manufacturer's instructions [52,53].

2.4. DNA extraction and quantification

Genomic DNA was extracted from whole blood samples (stored at −80 °C) by binding to a silica-based membrane using the QIAamp Blood mini purification kit (QIAGEN, Hilden, Germany). Quantification of the isolated product was determined by measuring the absorbance at 260 nm in a UV-VIS Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA purity was also determined by calculating the ratio of 260 nm–280 nm absorbance levels (A260/A280).

2.5. PNPLA3 rs738409 SNP genotyping

PNPLA3 rs738409 genotype was determined by a TaqMan® SNP Genotyping Assay (Applied Biosystems, ABI, MA, USA) with pre-determined primers and minor groove binder (MGB) FAM and VIC probes designed for amplification and detection of the specific polymorphism (5′-AGGCCTTGGTATGTTCCTGCTTCAT[**C/G**]CCCTTCTA-CAGTGGCCTTATCCCTC-3′). The missense mutation that codes for the amino acid change *I148 M* is depicted with bold characters. The transcript accession is NM_025225.2 under the GRCh38 Assembly.

Quantitative polymerase chain reaction (qPCR) was carried out in a total volume of 10 μl containing approximately 10 ng of each purified genomic DNA sample. Amplification and detection were performed in a LightCycler® 96 Instrument (Roche Life Sciences, Bavaria, Germany) under the following conditions: 10 min at 95 °C, 40 cycles: 15 s at 95 °C, 60 s at 60 °C. Automated allele calling was performed by means of endpoint genotyping. The endpoint signal intensities of the two reporter dyes were used by the LightCycler® 96 Application Software (Roche Life Sciences, Bavaria, Germany) to identify the genotypes and simplify discrimination into homozygous and heterozygous samples. For each experiment a scatter plot was created, and samples were visualized in groups based on the intensity distribution of the two dyes.

All subjects provided written informed consent to participate in the study. The protocol was approved by the University of Thessaly Medical School Ethics Committee (July 03, 2015, reference number 2742). in accordance with the protocol and principles of the 1975 Declaration of Helsinki.

2.6. Statistical analysis

Normality of the distribution of variables was assessed by Kolmogorov-Smirnov test. Normally distributed values are expressed as mean ± standard deviation (SD), while non-normally distributed as median [interquartile range (IQR)]. The Hardy-Weinberg equilibrium (HWE) was tested for both SNPs for patients with AIH by comparing observed and expected frequencies of genotypes using χ^2 analysis. Genotype and allele comparison data were analyzed by Pearson chi-square, Fisher's exact test, *t*-test, Mann-Whitney *U* test, Cox-regression analysis and Kaplan-Meier analysis where applicable. Two-sided P values < 0.05 were considered significant.

3. Results

3.1. PNPLA3 genotypes distribution

The distribution of *PNPLA3* genotype in AIH patients (CC 52.5 %, CG 36.5 %, GG 11 %) and controls (CC 53 %, CG 38 %, GG 9 %) was in concordance with the Hardy–Weinberg equilibrium (HWE) (P = 0.248 and P = 0.847, respectively). Neither the dominant nor the recessive models used in the study showed a connection of the *PNPLA3 I148M* polymorphism with the risk of AIH (Table 1).

3.2. Characteristics of AIH patients

The baseline characteristics of AIH patients enrolled in the study are summarized in Table 2. Female patients comprised 71 % of the study group (142/200), with a mean age at disease onset of 44.6 ± 17 years old and a median (IQR) follow-up of 71 (93) months. Disease presentation was symptomatic in 135/200 (67.5 %), with 44/200 (22 %) of the patients being cirrhotic at diagnosis and 7/200 (3.5 %) having experienced at least one episode of decompensation by that time. *PNPLA3* genotypes did not show associations with the epidemiological characteristics of AIH patients, the mode of disease presentation, liver function tests, γ-globulin and IgG levels (Table 2). Concerning the clinical stage at diagnosis, individuals carrying the polymorphism (GG/CG genotypes) were more likely to present with decompensation at the time of diagnosis (Table 2; P = 0.039), even though rates of cirrhosis at diagnosis were similar between groups. Similarly, the histological grade of AIH inflammatory activity and stage of fibrosis at the time of diagnosis did not differ between and GG/CG carriers and CC homozygotes (Table 2).

Table 1
Distribution of PNPLA3 I148 M genotypes in AIH patients and healthy controls.

	AIH patients (n = 200)	Controls (n = 100)	Model	P-value
PNPLA3 I148 M				
Genotype				
CC	105 (52.5 %)	53 (53 %)	Codominant	0.860
CG	73 (36.5 %)	38 (38 %)		
GG	22 (11 %)	9 (9 %)		
Allele				
C	283 (70.8 %)	144 (72 %)		0.824
G	117 (29.2 %)	56 (28 %)		
Genotypes				
CC	105 (52.5 %)	53 (53 %)	Dominant	1.000
GG + CG	95 (47.5 %)	47 (47 %)		
Genotypes				
GG	22 (11 %)	9 (9 %)	Recessive	0.737
CC + CG	178 (89 %)	91 (91 %)		
Genotypes				
CC + GG	127 (63.5 %)	62 (62 %)	Over-dominant	0.899
CG	73 (36.5 %)	38 (38 %)		

Abbreviations are same as in the text. n, number of patients in each group.

At least one cardiometabolic risk factor was present in 158/200 (79 %) patients with 29/200 (14.5 %) fulfilling the criteria for MetS. The most common cardiometabolic comorbidity was increased BMI and/or increased waist circumference (117/200, 58.5 %), followed by low HDL or lipid lowering treatment (79/200, 39.5 %), hypertension (56/200, 28 %), hypertriglyceridemia (50/200, 25 %) and type 2 diabetes (32/200, 16 %). Hepatic steatosis was detected in 37/186 (19.9 %) and steatohepatitis in 7.5 % (14/186) of patients with available liver biopsy. Concurrence of MASLD was confirmed in 69/200 (34.5 %) of patients. The prevalence of cardiometabolic comorbidities and MASLD was comparable across groups, irrespective of the *PNPLA3 I148 M* genotype. Moreover, the presence of the polymorphism was not correlated with histologically documented steatosis or steatohepatitis (Table 2).

3.3. Response to treatment

Complete biochemical response (CBR) at 6 months, 12 months, and at the end of follow-up was achieved in 135/194 (69.6 %), 147/192 (76.6 %), and 176/195 (90.3 %) patients, respectively. One patient with follow-up less than 6 months and two patients with follow-up less than 12 months were excluded from the respective analyses. The median time for achieving CBR was 3 (5) months with a significant trend for a longer time among those carrying the GG/CG genotypes (p = 0.07; Table 3). As of the current status, 86 out of 176 (48.9 %) patients who achieved CBR were able to discontinue corticosteroids. Also, during this writing, complete withdrawal of immunosuppression, following the European and Hellenic recommendations [7,8], was successfully accomplished in 46/176 (26.1 %) patients with a median treatment duration of 63 (77)

Table 2
Baseline demographic, clinical, laboratory and histological characteristics of AIH patients in total and according to *PNPLA3 I148 M* genotype (n = 200).

	Total (n = 200)	PNPLA3 I148 M		
		GG/CG (n = 95)	CC (n = 105)	P value
Age at disease onset (years)	44.6 ± 17	45.3 ± 16	44 ± 18	0.602
Female	142 (71 %)	71 (74.7 %)	71 (67.6 %)	0.341
Time to diagnosis (months)	11 (56)	15 (59)	8 (46)	0.398
Disease duration till last follow-up (months)	124 (131)	127 (122)	118 (137)	0.856
Follow-up (months)	71 (93)	69 (91)	77 (94)	0.876
Type of presentation				
Insidious	119 (59.5 %)	60 (63.2 %)	59 (56.2 %)	0.424
Acute	46 (23 %)	18 (18.9 %)	28 (26.7 %)	
Acute severe	35 (17.5 %)	17 (17.9 %)	18 (17.1 %)	
Presence of symptoms	135 (67.5 %)	63 (66.3 %)	72 (68.6 %)	0.850
Extra-hepatic autoimmune diseases	97 (48.5 %)	47(49.5 %)	50 (47.6 %)	0.904
BMI (kg/m^2)	26.2 (±4.6)	26.5 (±4.8)	25.9 (±4.5)	0.382
BMI≥25m^2 or waist circumference > 94 cm (♂)/> 80 cm (♀)	117 (58.5 %)	56 (58.9 %)	61 (58.1 %)	0. 903
Hypertension	56 (28 %)	31 (32.6 %)	25 (23.8 %)	0.219
Diabetes type 2 or impaired glucose tolerance	32 (16 %)	17 (17.9 %)	15 (14.3 %)	0.616
Serum triglycerides ≥ 150 mg/dL or under lipid lowering treatment	50 (25 %)	21 (22.1 %)	29 (27.6 %)	0.462
HDL ≤40 (♂)/≤ 50 mg/dL (♀) or under lipid lowering treatment	76 (38 %)	40 (43 %)	36 (36.4 %)	0.427
Presence of at least one cardiometabolic risk factor	158 (79 %)	77 (81.1 %)	81 (77.1 %)	0.614
Metabolic syndrome	29 (14.5 %)	13 (13.7 %)	16 (15.2 %)	0.912
AST (IU/L, ULN: 35)	124 (371)	109 (376)	137 (406)	0.658
ALT (IU/L, ULN: 40)	174 (500)	153 (530)	206 (487)	0.606
γ-GT (IU/L, ULN: 37)	83 (137)	89 (123)	81 (146)	0.689
ALP (IU/L, ULN: 120)	106 (81)	114 (74)	100 (87)	0.295
Bilirubin (mg/dL, ULN: 1.1)	1.04 (1.66)	1.06 (1.59)	1.02 (2.08)	0.910
Albumin (g/dL, normal range: 3.5–5.2)	4 ± 0.6	3.9 (±0.61)	4 (±0.6)	0.821
γ-globulin (g/dL, ULN: 3.5)	3.6 (1.1)	3.5 (1.2)	3.7 (1.01)	0.177
IgG (mg/dL, ULN: 1500)	1859 (1039)	1869 (1005)	1880 (983)	0.383
INR	1.06 (0.23)	1.06 (0.23)	1.06 (0.24)	0.280
Platelets (x10^3/mm^3, normal range: 140–400)	214 (90)	203 (96)	218 (92)	0.340
Cholesterol (mg/dL)	185 (67)	183 (72)	186 (65)	0.733
HDL (mg/dL)	52 ± 20	53 (±21)	51 (±18)	0.355
LDL (mg/dL)	112 (49)	113 (45)	110 (52)	0.469
Triglycerides (mg/dL)	104 (66)	102 (57)	106 (62)	0.375
Positive ANA	123 (61.5 %)	59 (62.1 %)	64 (61 %)	0.983
Positive SMA	186 (93 %)	86 (90.5 %)	100 (95.2 %)	0.305
Positive anti-LKM	12 (6 %)	6 (6.3 %)	6 (5.7 %)	1.000
Positive anti-SLA/LP	22 (11 %)	7 (7.4 %)	15 (14.3 %)	0.182
Histology	n = 186	n = 92	n = 94	
Moderate or Severe Grade	114 (61.3 %)	55 (59.8 %)	59 (62.8 %)	0.789
Severe Fibrosis or Cirrhosis	59 (31.7 %)	31 (33.7 %)	28 (29.8 %)	0.678
Steatosis	37 (19.9 %)	18 (19.6 %)	19 (20.2 %)	1.000
Steatohepatitis	14 (7.5 %)	9 (9.8 %)	5 (5.3 %)	0.381
Presence of cirrhosis	44 (22 %)	23 (24.2 %)	21 (20 %)	0.584
Decompensated cirrhosis	7 (3.5 %)	6/95 (6.3 %)	1/105 (1 %)	0.039
MASLD	69 (34.5 %)	34 (35.8 %)	35 (33.3 %)	0.507

Normally distributed values are expressed as mean ± standard deviation (SD), while non-normally distributed as median [interquartile range (IQR)]. Abbreviations are same as in the text. n, number of patients in each group; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; INR, international normalized ratio; LDL, low density lipoprotein; ANA, antinuclear antibodies; SMA, smooth muscle antibodies.

Table 3
Response to immunosuppressive treatment according to *PNPLA3 I148 M* genotype (n = 195).

		PNPLA3		
	Total (n = 195)	GG/CG (n = 92)	CC (n = 103)	P value
Treatment schedule				
Prednisolone + Mycophenolate mofetil	152 (77.9 %)	75 (81.2 %)	77 (74.8 %)	0.473
Prednisolone + Azathioprine	26 (13.3 %)	11 (12 %)	15 (14.6 %)	
Prednisolone only	17 (8.7 %)	6 (6.5 %)	11 (10.7 %)	
No-response at 4 weeks	n = 176 36 (20.5 %)	n = 83 19 (22.9 %)	n = 93 17 (18.3 %)	0.569
CBR at 6 months	n = 194* 135 (69.6 %)	n = 92 63 (68.5 %)	n = 102 72 (70.6 %)	0.871
CBR at 12 months	n = 192** 147 (76.6 %)	n = 90 69 (76.7 %)	n = 102 78 (76.5 %)	1.000
CBR at end of follow-up	n = 195 176 (90.3 %)	n = 92 83 (90.2 %)	n = 103 93 (90.3 %)	1.000
Time to achieve complete response (months)	3 (5)	3 (5)	2 (3)	0.071
Treatment duration (months)	63 (77)	65.6 (79)	63 (65)	0.753
Corticosteroids withdrawal	86/176 (48.9 %)	43/83 (51.8 %)	47/93 (50.5 %)	0.986
Complete treatment withdrawal	46/176 (26.1 %)	22/83 (26.5 %)	24/93 (25.8 %)	1.000
Maintenance of response after complete treatment withdrawal	34/46 (73.9 %)	16/22 (72.7 %)	18/24 (75 %)	1.000

Abbreviations are same as in the text. n, number of patients in each group. *One patient with follow-up less than 6 months was excluded from the analysis; **Three patients with follow-up less than 12 months were excluded from the analysis.

months. Following treatment discontinuation, 34/46 (73.9 %) patients maintained biochemical remission (Table 3). Response to immunosuppressive treatment was not affected by the *PNPLA3 I148 M* polymorphism (Table 3).

3.4. Disease progression and outcome

Disease progression was evaluated in 195 patients who received immunosuppression. Within this group, 153 (78.5 %) were non-cirrhotic, 36 (18.5 %) had compensated cirrhosis and 6 (3 %) presented with decompensated cirrhosis at the time of diagnosis. In the group of 153 non-cirrhotic patients at baseline, 5 (3.3 %) progressed to cirrhosis during a median (IQR) follow-up period of 71 (93) months. Among the 41 compensated cirrhotic patients either at baseline or during follow-up, 8 (19.5 %) experienced decompensation till the end of the study. Seven patients died due to liver-related causes, 1 patient underwent orthotopic liver transplantation and 6 patients developed HCC. In total, disease progression, defined by at least one of the above events, was documented in 17 out of 195 (8.7 %) treated patients. All events (decompensation of cirrhosis, development of HCC, liver related death, liver transplantation) occurred in patients with either cirrhosis at baseline or in those who developed cirrhosis during follow-up, apart from one patient with acute severe AIH who died because of acute liver failure within one month of disease presentation. In treated patients, homozygosity for the G allele did not corelate with survival free of cirrhosis (Fig. 1A), but was associated with reduced survival free of decompensation (Fig. 1B, P = 0.006), cirrhotic events (decompensation, liver transplantation, HCC) (Fig. 1C, P = 0.001) and liver-related death or liver transplantation (Fig. 1D, P = 0.011).

Sub-analysis of 41 compensated cirrhotic patients either at baseline or during follow-up revealed that patients who experienced a cirrhotic event during follow-up did not differ from those who did not in terms of sex (P = 0.694), age at disease onset (P = 0.424), type of immunosuppression (P = 0.964), presence of MASLD (P = 0.719) or MetS (P = 0.167). Response to treatment did not differ between these two groups of patients, regarding CBR at 6mo (P = 1.000), CBR at 12mo (P = 0.247) and CBR at the end of follow-up (P = 0.316). Multivariate Cox-regression analyses showed that the GG genotype was associated with higher risk of cirrhotic events during follow-up [hazard ratio (HR) = 5.21, 95 % confidence interval (CI): 1.33–20.41, P = 0.018] even after adjustment for age at disease onset, sex, co-incidence of MASLD and MetS.

4. Discussion

Our study explored the significance of *PNPLA3 I148 M* variant in a well characterised cohort of AIH, yielding interesting results: (1) AIH patients carrying the G allele were more likely to present with decompensated cirrhosis at the time of diagnosis, (2) time for achieving CBR tended to be longer, though not significant, in those carrying the GG/CG genotypes and (3) *PNPLA3 I148 M* GG homozygotes are at increased risk for decompensation, cirrhotic events, death or liver transplantation. Notably, the effect of the variant was unrelated to the presence of hepatic steatosis or steatohepatitis on liver biopsy, as well as the coincidence of cardiometabolic factors and MASLD. These findings suggests that mechanisms beyond hepatic steatosis contribute to the progression of liver fibrosis.

The impact of genetic background on the course of liver disease has gained a lot of attention in recent years. *PNPLA3 I148 M* has emerged as a potent genetic modifier of MASLD, influencing both susceptibility and disease prognosis [17–20]. Interestingly, a number of studies have

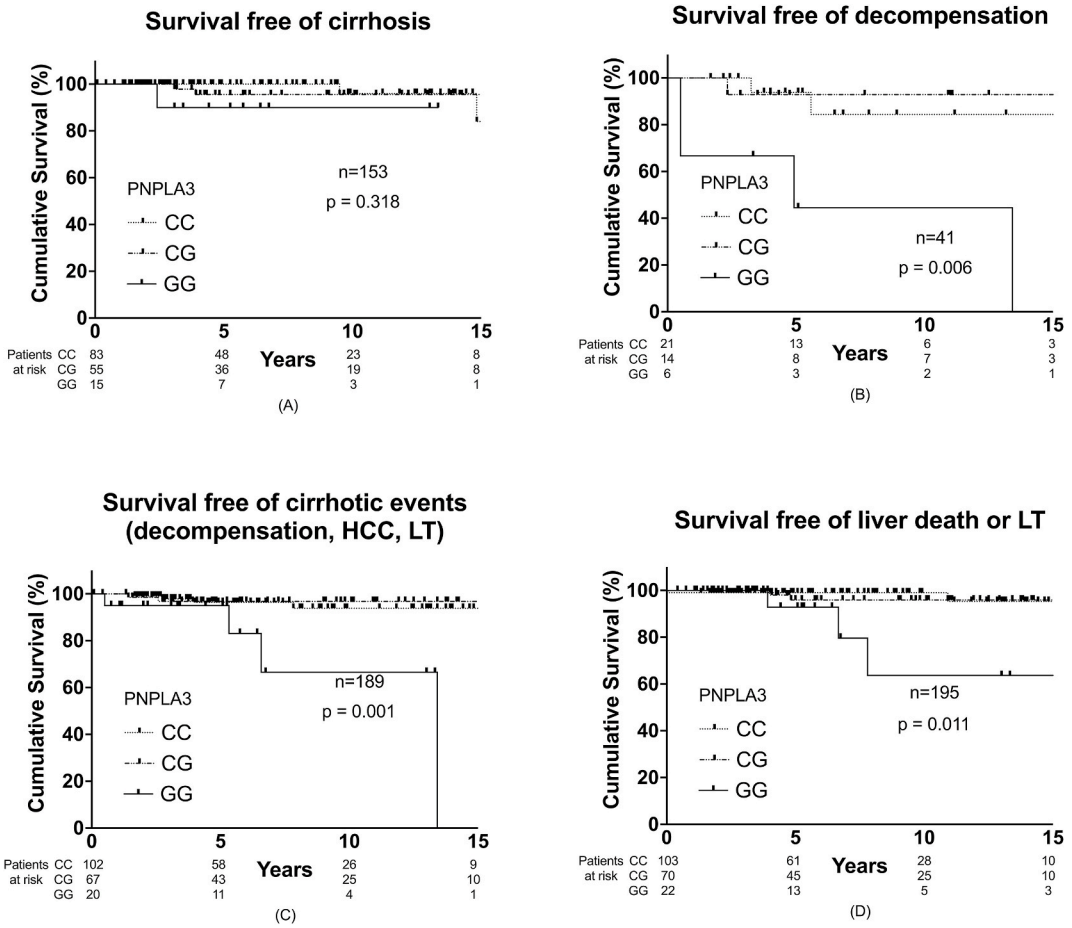

Fig. 1. Kaplan-Meier curves to display cumulative survival, stratified by PNPLA3 I148 M genotype of treated AIH patients: (A) free of cirrhosis development (P = 0.318); (B) free of decompensation development (P = 0.006); (C) free of cirrhotic events development (P = 0.001); (D) free of liver related death or liver transplantation (P = 0.011).

shown that the impact of *PNPLA3 I148 M* expands beyond steatosis and over a broader range of liver diseases [28–39].

The presence of the *PNPLA3 I148 M* variant is associated with an increased risk of advanced liver fibrosis and cirrhosis in patients with chronic liver diseases, including chronic hepatitis B and chronic hepatitis C, autoimmune liver disease and hemochromatosis [31,39,54,55]. Both genome-wide association studies and large meta-analyses have confirmed that *PNPLA3 I148 M* variant is linked to nearly a four-fold risk of alcohol related liver cirrhosis [28,56]. Furthermore, this effect extends to an increased risk of HCC in the same patient population [29,57].

Studies dedicated to the role of *PNPLA3 I148 M* solely in autoimmune liver disease are limited. One study, which explored the impact of the variant in primary sclerosing cholangitis (PSC), revealed reduced survival in male PSC patients with severe disease and bile duct stenosis [38]. Consistent with our findings, a recent similar sized cohort of AIH patients demonstrated that GG homozygotes had clinically more advanced disease, as evidenced by laboratory markers and non-invasive liver fibrosis scores, and experienced worse survival free of liver-related death or transplantation, irrespective of the presence of steatosis [39]. These findings highlight the importance of *PNPLA3* in liver disease beyond its associations with steatosis, emphasizing the need to explore its role in the context of liver fibrosis.

The answer might be on the effect of the variant on hepatic stellate cells (HSCs). Initial studies indicate that HSCs carrying the *PNPLA3 I148 M* variant undergo metabolic and phenotypic changes, transitioning from a quiescent state to a highly activated state [36,37]. Bruschi et al. [58] provided evidence that *PNPLA3 I148 M* acts as a positive modulator of activated human HSCs, affecting secretion of proinflammatory cytokines, proliferation, and migration. This study provides a molecular mechanism explaining the heightened risk of severe progressive disease observed in patients with the *I148 M* variant [58]. The effect is likely mediated through down-regulation of PPARγ transcriptional activity, leading to reduced liver X Receptor signalling that promotes enhanced expression of proinflammatory mediators (CCL2, CCL5, and IL-8), proliferation, migration, and eventually myofibroblast phenotypic changes [58].

Moreover, *PNPLA3* is implicated in hepatic inflammation. Experimental models have demonstrated that *PNPLA3 I148 M* is transcriptionally activated by NF-kB to enhance tumour necrosis factor-alpha expression through the ER stress IRE-1a/JNK/v-Jun pathway in Hep2G2 cells treated long-term with palmitic acid [59]. The role of the JNK/c-Jun signalling pathway in oxidate stress leading to hepatocellular injury has been stressed in various studies in relation to steatosis [60–62]. These pathogenetic mechanisms may account for the adverse effect of the *PNPLA3 I148 M* G allele on long-term prognosis, even though this polymorphism did not influence treatment response or other factors such as the co-incidence of MASLD. Our results were further enhanced after multivariate analysis which showed that GG genotype was associated with 5-fold risk of events (cirrhosis decompensation, liver transplantation, HCC) during follow-up even after adjustment for other risk factors and especially co-incidence of MASLD or MetS. However, further elucidation is needed to understand the effect of *PNPLA3 I148 M* on other resident non-parenchymal cells and the dynamic interactions between them in the natural history of liver fibrosis.

5. Conclusion

In conclusion, our study establishes an association between the *PNPLA3 I148 M* variant and disease severity, as well as reduced survival in AIH patients, regardless of the presence of MASLD. This signifies a potential role for the *PNPLA3 I148 M* variant as a novel biomarker for AIH, enabling the identification of patients at increased risk of disease progression.

CRediT authorship contribution statement

Kalliopi Azariadis: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Nikolaos K. Gatselis:** Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Aggeliki Lyberopoulou:** Validation, Software, Methodology, Investigation, Formal analysis. **Pinelopi Arvaniti:** Writing – original draft, Validation, Software, Formal analysis, Data curation. **Kalliopi Zachou:** Validation, Supervision, Methodology, Data curation. **Stella Gabeta:** Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **George N. Dalekos:** Writing – review & editing, Validation, Supervision, Project administration, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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