



## Programmed cell death-1 *rs11568821* and interleukin-28B *rs12979860* polymorphisms in autoimmune hepatitis

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### ABSTRACT

**Background:** Autoimmune hepatitis (AIH) is a relatively rare chronic liver disease of unknown etiology. The genetic background affects susceptibility, clinical phenotype, and prognosis. The *programmed cell death-1 rs11568821* polymorphism (*PD1.3*) has been associated with susceptibility to autoimmune diseases. The *interleukin-28B (IL28B) rs12979860* polymorphism has been associated with steatosis, inflammation, and fibrosis in liver diseases.

**Aim:** Our aim was to investigate for the first time the incidence and clinical significance of *PD1.3* and *IL28B rs12979860* in AIH.

**Methods:** Two hundred patients with AIH were evaluated, while 100 healthy subjects were used as controls. Genotyping was performed with *in-house* allelic discrimination End-Point PCR.

**Results:** The SNP *PD1.3/A* was present in 36/200 (18%) AIH patients compared to 28/100 (28%) healthy controls ( $p = 0.065$ ). The AA/GA genotypes were not associated with the mode of presentation of AIH, the histological grade or stage, the presence of cirrhosis, risk of disease progression, response to treatment and survival. The *IL28B rs12979860* genotype distribution was CC 79/200 (39.5%), TT 36/200 (18%) and CT 85/200 (42.5%), in similar rates with healthy controls ( $p = 0.878$ ). Inflammatory activity and fibrosis stage did not differ between CC homozygotes and CT/TT carriers. LDL cholesterol was significantly higher in CC than CT/TT patients ( $P = 0.027$ ), though no differences was found regarding the presence of steatosis or steatohepatitis. On-treatment response to immunosuppressive treatment was not affected by the *IL28B rs12979860* polymorphism. However, CC homozygotes AIH patients achieved treatment withdrawal in significantly higher rates (OR 2.3, 95%CI: 1.1–4.7,  $P = 0.02$ ) irrespective of the presence of steatosis or steatohepatitis.

**Conclusions:** The *PD1.3* and *IL28B rs12979860* variants are unlikely to contribute to AIH susceptibility, disease presentation and prognosis. The *IL28B rs12979860* is not associated with the presence of concurrent steatosis or steatohepatitis. However, although on-treatment response rates to immunosuppression were not affected by the *IL28B rs12979860* polymorphism, AIH patients with CC homozygosity were more likely to achieve complete treatment withdrawal. This novel finding needs validation and further clarification from larger multicenter studies.

**Abbreviations:** AIH, Autoimmune hepatitis.; HLA, Human leukocyte antigen.; PD1, Programmed cell death-1.; ANA, Antinuclear antibodies.; SNP, Single nucleotide polymorphism.; SLE, Systemic lupus erythematosus.; IL28B, Interleukin 28B.; HCV, Hepatitis C virus.; NAFLD, Non-alcoholic fatty liver disease.; ULN, Upper limit of normal.; INR, International normalized ratio.; CR, Complete response.; IgG, Immunoglobulin class G.; MetS, Metabolic syndrome.; HDL, High density lipoprotein.; SMA, Smooth muscle antibodies.; Anti-LKM1, Liver kidney microsomal type-1 antibodies; Anti-LC1, Liver cytosol type-1 antibodies.; Anti-SLA/LP, Soluble liver antigen/liver pancreas antibodies.; PCR, Polymerase chain reaction.; SD, Standard deviation.; IQR, Interquartile range.; HWE, Hardy-weinberg equilibrium.; HCC, Hepatocellular carcinoma.; LDL, Low density lipoprotein.

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## 1. Introduction

Autoimmune hepatitis (AIH) is a relatively rare, chronic, progressive autoimmune liver disease characterized by polyclonal hyperglobulinemia, autoantibodies, interface hepatitis on liver histology and a favorable response to immunosuppressive treatment [1–5]. It affects all ages (about 30% above 60 years), both sexes (approximately 30% males) and all ethnic groups with an increasing prevalence [6–8].

Although AIH etiology remains undefined, it is widely accepted that genetic factors interplay with environmental triggers to generate a continual autoimmune attack against the liver [3,5,9,10]. To date the strongest genetic associations lay within the Human Leukocyte Antigen (HLA) class II region and include specific DRB1 alleles [11]. Since they exhibit important variations between different ethnic and age groups as well as geographic regions [6,11–14] the quest for additional contributing factors outside the major histocompatibility complex is appealing. A unifying theory proposes that the initial triggering event that is antigen selection is dictated by HLA II predisposition and subsequently polymorphisms in other immune regulatory and cytokine-producing genes may promote and perpetuate immune reactivity, loss of self-tolerance and inflammatory responses that affect the clinical phenotype [10,15,16].

*Programmed cell death-1 (PD1)* is a member of the B7-CD28 regulatory pathway [17–19]. It is expressed on the surface of activated CD4<sup>+</sup> and CD8<sup>+</sup> T-cells and following interaction with its ligands (PD-L1, PD-L2), it hinders T-cell proliferation by arrest in the G0-G1 phase and inhibits cytokine production, favoring self-tolerance [20–23]. *PD1* deficient mice (PD-1<sup>-/-</sup>) develop arthritis and lupus-like glomerulonephritis, autoimmune dilated cardiomyopathy and other types of organ specific autoimmunity depending on the mouse model examined [24–26]. Fatal AIH with characteristic infiltration of the liver parenchyma with autoreactive T-cells and production of anti-nuclear antibodies (ANA) developed in mice that are both PD-1<sup>-/-</sup> and Treg deficient [27]. In clinical practice, monoclonal antibodies that block the PD1-PD-L axis have resulted in a variety of immune-mediated side effects, including hepatic assault that is sometimes histologically indistinguishable from AIH [28–30]. It was found that a single nucleotide polymorphism (SNP) in the *PD1* gene (*rs11568821*, SNP G/A, *PD1.3*) could be a functional polymorphism [31]. The *PD1.3A* allele, which is found in the intron 4 enhancer region, changes the binding location of the RUNX1 transcription factor, resulting in abnormal protein expression and suggesting a mechanism for self-tolerance failure [32]. In this context, *PD1.3* has been associated with susceptibility to systemic lupus erythematosus (SLE) in Caucasian non-Spanish populations, rheumatoid arthritis and disease progression in multiple sclerosis [33–35].

*Interleukin 28B (IL28B)* is a member of the type III interferon family (INF- $\lambda$ ) that gained attention, when the genetic polymorphism *rs12979860* (SNP C/T) was strongly associated with response to treatment with pegylated-interferon and ribavirin in hepatic C virus (HCV) infection and even spontaneous viral clearance [36]. The same polymorphism has been associated with the lipid profile, insulin resistance and the presence of hepatic steatosis in patients with HCV infection [37–39]. The *IL28B* CC genotype was linked to higher total cholesterol, apolipoprotein B and low-density lipoprotein cholesterol, as well as lower triglyceride levels [37], reduced insulin resistance [39] and lower frequency of hepatic steatosis [38]. Besides, in patients with non-alcoholic fatty liver Disease (NAFLD), the *IL28B* CC genotype was associated with milder lobular inflammation [40]. To the best of our knowledge the role of the *IL28B* SNP in inflammatory response and liver damage has not been studied in autoimmune liver diseases. Also keeping in mind, the significant coincidence of NAFLD in AIH, reaching about 20% in certain populations and with impacts in disease prognosis [41, 42], the possibility that the *rs12979860* polymorphism might act as a disease modifier in AIH is appealing.

Accordingly, in the present study we aimed to investigate the prevalence and clinical significance of the *IL28B rs12979860* and the *PD1.3*

polymorphisms in patients with AIH.

## 2. Materials and methods

### 2.1. Study population

Two hundred patients of Greek descent with well-established AIH [1, 2] were included in the study. Clinical presentation was classified as insidious, when symptoms were vague and non-specific or abnormal biochemistry was discovered during a routine check-up. Acute presentation refers to episode of acute icteric hepatitis, with aminotransferases  $\geq 10$ 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 (INR)  $\geq 1.5$  but without any sign of hepatic encephalopathy and without chronic disease at the histological level [43,44]. One hundred age- and sex-matched healthy subjects served as controls.

AIH treatment algorithms and response to treatment were in accordance with the European [1] and the Hellenic Clinical Practice Guidelines [2] as well as our previous reports [6,44–46]. Based on our experience and previous publications [6,44–46], 152 (76%) patients received prednisolone in combination with mycophenolate mofetil, 26 (13%) received prednisolone with azathioprine and 17 (8.5%) received only prednisolone (3 due to current history of non-liver related neoplasia, 4 pediatric 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 because of personal reasons. Treatment response was defined as complete response (CR), when aminotransferases and immunoglobulin G (IgG) normalized, symptoms improved or disappeared, and liver histology (if performed) showed minimal or no inflammation.

Clinical and laboratory data, including metabolic parameters to assess for the presence of Metabolic Syndrome (MetS) 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) 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 treated for type 2 diabetes mellitus, (4) serum triglycerides  $>150$  mg/dL, and (5) high density lipoprotein (HDL)  $<40$  mg/dL for men and  $<50$  mg/dL for women [47].

### 2.2. Liver histology

Liver biopsies from 186 AIH patients at the time of diagnosis were available for analysis. Liver biopsies were assessed using the Knodell histologic/activity index score [48]. According to our previous publications [6,45,46,49], patients were divided into two groups according to inflammation: minimal-mild (score: 0–8) and moderate-severe (score: 9–18) and according to fibrosis: minimal/mild-moderate (score: 0–2) and severe fibrosis-cirrhosis (score: 3–4). All biopsies had detailed documentation of possible concurrent NAFLD findings (amount and location of steatosis, presence/absence of Malory's hyaline, hepatocyte ballooning, lobular inflammation, zone 3 fibrosis).

As we have reported previously, in patients without available liver biopsy, cirrhosis was established by findings from ultrasonography, and/or transient elastography, endoscopy or physical findings (ascites, hepatic encephalopathy) [50].

### 2.3. Autoantibodies

Smooth muscle antibodies (SMA), ANA, 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- $\mu$ m fresh frozen sections of in-house rodent kidney, liver, and stomach

tissue sections as described [3,5,51]. 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 [3,5,52].

#### 2.4. DNA extraction and quantification

Genomic DNA was extracted from whole blood samples (stored at  $-80^{\circ}\text{C}$ ) 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 to 280 nm absorbance levels (A260/A280).

#### 2.5. PD1.3 and IL28B rs12979860 SNP genotyping

PD1.3 and IL28B rs12979860 genotypes were determined by a TaqMan 5'-nuclease chemistry real-time polymerase chain reaction (PCR) method designed for amplification and detection of the specific polymorphism. Approximately 20 ng of each purified genomic DNA sample was amplified using TaqMan® Gene Expression Master Mix (Applied Biosystems, ABI, MA, USA) and specific custom-made primers and minor groove binder probes (TIB MolBiol, Germany). The sequences of the primers and probes used for the PCR assays are provided in Table 1. Quantitative PCR was carried out in a total volume of 15  $\mu\text{l}$ , containing 0.5  $\mu\text{M}$  specific forward and reverse primers and probes. Amplification and detection were performed in a Lightcycler® 96 Instrument (Roche Life Sciences, Bavaria, Germany), under the following conditions: 10 min at  $95^{\circ}\text{C}$ , 45 cycles: 10 s at  $95^{\circ}\text{C}$ , 30 s at  $60^{\circ}\text{C}$ , and 1 s at  $72^{\circ}\text{C}$ . Automated allele calling was performed by means of endpoint genotyping software (Roche Life Sciences, Bavaria, Germany) to identify the genotypes and simplify discrimination into homozygous and heterozygous samples. Validation experiments were carried out in duplicates and negative and positive controls were used in every experiment.

Confirmation of results for PD1.3 was made by restricted fragment length polymorphism PCR on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Waltham, MA, USA). 100 ng of genomic DNA was used at a final volume of 25  $\mu\text{l}$  with 1.5 mM of  $\text{MgCl}_2$ , 100  $\mu\text{M}$  of dNTPs and 5 Units of AmpliTaq Gold DNA polymerase (Applied Biosystems, Waltham, MA, USA) and 0.1  $\mu\text{M}$  of each of the specific primers (Forward: 5'-CCTCAATCCCTAAAGCCATGATCTG-3', Reverse: 5'-CAGGCAGGCACACATG-3'). The amplification was performed under the following conditions: initial denaturation step of 10 min at  $95^{\circ}\text{C}$ , followed by 35 cycles: 15 s at  $95^{\circ}\text{C}$ , 30 s at  $60^{\circ}\text{C}$ , 15 s at  $72^{\circ}\text{C}$  and

**Table 1**  
Oligonucleotides for genotyping of SNPs studied.

SNP	Primers	Sequences
PD1.3	Forward primer	5'-CCTCAATCCCTAAAGCCATGATCTG-3'
	Reverse primer	5'-CAGGCAGGCACACATG-3'
	Probe (G allele)	5'-FAM- ACCTGCGGTCTCC-3'
	Probe (A allele)	5'-VIC- ACCTGCAGTCTCC-3'
IL28B	Forward primer	5'-TGCCTGTCTGTACTGAACCA-3'
	Reverse primer	5'-GAGCGCGGAGTGCAATTC-3'
	Probe (C allele)	5'-VIC-AAGGCGCGAACCA-3'
	Probe (T allele)	5'-FAM-TCCCGAAGGCGTGA-3'

Abbreviations are same as in the text: SNP, single nucleotide polymorphism; PD1, programmed cell death-1; IL28B, interleukin 28B. \*The position of the SNP alleles in sequence of each probe is depicted with bold.

then another cycle 5 min at  $72^{\circ}\text{C}$  before termination. Ten microliters of the PCR product were digested (incubation for 60 min at  $37^{\circ}\text{C}$ ) with 0.5 Units of the restriction endonuclease PstI (Thermo Fischer Scientific, CA, USA). The digested products were electrophoresed on 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light. According to the allele present the AA homozygotes yielded bands of 130 bp and 50 bp, the GG wild type yielded one band of 180 bp, and the GA heterozygotes yielded three bands of 180 bp, 130 bp and 50 bp.

Confirmation of results for IL28B rs12979860 was made by direct sequencing. In details, a semi-nested PCR was designed for the amplification of a fragment of 228 bp. The reaction was carried out in a total volume of 50  $\mu\text{l}$  containing 1.5 mM  $\text{MgCl}_2$ , 0.2 mM of each dNTP, 0.4  $\mu\text{M}$  of each primer (Forward 5'-CCCCTAACCTCTGCACAGTC-3', Inner Reverse 5'-AGGCTCAGGTC AATCACAG-3', Outer Reverse 5'-AGG-GACCGCTACGTAAGTCA-3'), 2.5 U AmpliTaq Gold® DNA polymerase (ABI) with a buffer supplied by the manufacturer, using 5  $\mu\text{l}$  of genomic DNA in the first PCR or 2  $\mu\text{l}$  of product in the second PCR. The PCR conditions were as follows: denaturation at  $95^{\circ}\text{C}$  for 10 min, followed by 35 cycles of three steps holds ( $94^{\circ}\text{C}$  for 30 s,  $58^{\circ}\text{C}$  for 30 s, and  $72^{\circ}\text{C}$  for 30 s) before final extension at  $72^{\circ}\text{C}$  for 10 min. Validation of PCR amplicon size was performed by gel electrophoresis. Finally, bi-directional analysis sequencing of amplicons was performed using the Big-dye Terminator v3.1 Cycle sequencing Kit on an ABI 3730 DNA sequencer (ABI). Genotyping was based on the chromatographs of nucleotide bases [53].

All subjects provided written informed consent to participate in the study. The protocol was approved by the Ethical Committee of the Larissa University Hospital 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  $\pm$  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  $\chi^2$  analysis. Genotype and allele comparison data were analyzed by Pearson chi-square, Fisher's exact test, *t*-test, Mann-Whitney *U* test and Cox regression analysis where applicable. Two-sided *p* values  $< 0.05$  were considered significant.

### 3. Results

#### 3.1. General characteristics of AIH patients

The baseline characteristics of AIH patients (females 142/200, 71%) enrolled in the study are summarized in Table 2. Disease presentation was symptomatic in 135 (67.5%) patients, while 44 out of 200 (22%) patients had established cirrhosis at baseline, including 7 (16%) patients who had already experienced at least one episode of decompensation in the past. Concurrent presence of NAFLD was confirmed in 49 out of 186 (26.3%) patients with available liver biopsy [simple steatosis, 35/186 (18.8%); steatohepatitis, 14/186 (7.5%)]. At least one metabolic risk factor was present in 135 out of 200 (67.5%) patients with 29 (14.5%) fulfilling the criteria for the diagnosis of MetS (Table 2).

Other concurrent autoimmune diseases were present in 48.5% of patients (Table 2; Hashimoto thyroiditis, *n* = 37; Biermer's anemia, *n* = 16; multiple sclerosis, *n* = 12; inflammatory bowel disease, *n* = 7; rheumatoid arthritis, *n* = 5; Sjogren's syndrome, *n* = 7; celiac disease, *n* = 3; psoriasis, *n* = 3; SLE, *n* = 2; immune thrombocytopenia, *n* = 2; scleroderma, *n* = 1; retroperitoneal fibrosis, *n* = 1; giant cell vasculitis, *n* = 1).

On-treatment CR was attained in 176 out of 195 (90.3%) patients, in 3 (5) months after treatment initiation. At the time of this writing,

**Table 2**

Baseline demographic, clinical, laboratory and histological characteristics of AIH patients in total and according to *PD1.3* and *IL28B rs12979860* genotype (n = 200).

	PD1.3			P value	IL28B rs12979860		P value
	Total (n = 200)	GG (n = 164)	GA/AA (n = 36)		CC (n = 79)	CT/TT (n = 121)	
Age at disease onset (years)	44.6 ± 27	43.6 ± 17.6	49.4 ± 16.1	0.071	46.1 ± 17.4	43.6 ± 17.5	0.314
Female	142 (71%)	118 (72%)	24 (66.7%)	0.667	52 (65.8%)	90 (74.4%)	0.252
Time to diagnosis (months)	11 (56)	11 (58)	6.5 (34)	0.301	14.5 (59)	10 (51)	0.448
Disease duration till last follow-up (months)	124 (131)	128 (131)	109 (134)	0.787	117 (138)	130 (125)	0.674
Follow-up (months)	71 (93)	73 (94)	69 (99)	0.754	67 (81)	77 (101)	0.365
Type of presentation							
Insidious	119 (59.5%)	97 (59.1%)	22 (61.1%)	0.837	48 (60.8%)	71 (58.7%)	0.922
Acute	46 (23%)	39 (23.8%)	7 (19.4%)		17 (21.5%)	29 (24%)	
Acute severe	35 (17.5%)	28 (17.1%)	7 (19.4%)		14 (17.7%)	21 (17.4%)	
Presence of symptoms	135 (67.5%)	113 (68.9%)	22 (61.1%)	0.479	51 (64.6%)	84 (69.4%)	0.573
Concurrence of extra-hepatic autoimmune diseases	97 (48.5%)	89 (54.3%)	8 (22.2%)	0.001	37 (46.8%)	60 (49.6%)	0.814
AST (IU/L, ULN: 35)	133 (379)	133 (366)	132 (571)	0.890	136 (335)	125 (451)	0.569
ALT (IU/L, ULN: 40)	187 (515)	187 (478)	182 (603)	0.850	223 (467)	169 (544)	0.955
γ-GT (IU/L, ULN: 37)	83 (137)	83 (143)	91 (140)	0.639	88 (128)	82 (144)	0.824
ALP (IU/L, ULN: 120)	106 (81)	110 (81)	100 (81)	0.471	106 (76)	107 (88)	0.426
Bilirubin (mg/dL, ULN: 1.1)	1.04 (1.66)	1.03 (1.53)	1.19 (2.26)	0.947	1 (1.7)	1.1 (1.5)	0.665
Albumin (g/dL, normal range: 3.5–5.2)	4 ± 0.6	3.9 ± 0.6	4.1 ± 0.6	0.102	3.9 ± 0.6	4 ± 0.6	0.450
γ-globulin (g/dL, ULN: 3.5)	3.7 (1.2)	3.6 (1.2)	3.7 (0.8)	0.428	3.6 (1.1)	3.6 (1.2)	0.784
IgG (mg/dL, ULN: 1400)	1859 (1039)	1820 (1030)	1920 (910)	0.161	1803 (915)	1889 (977)	0.260
INR	1.06 (0.23)	1.07 (0.24)	1.04 (0.19)	0.901	1.04 (0.22)	1.07 (0.24)	0.251
Platelets (x10 <sup>3</sup> /mm <sup>3</sup> , normal range: 140–400)	214 (90)	208 (93)	220 (89)	0.595	215 (79)	210 (103)	0.996
Cholesterol (mg/dL)	185 (67)	188 (66)	172 (78)	0.196	196 (61)	180 (67)	0.083
HDL (mg/dL)	52 ± 20	54 ± 20	45 ± 20	0.019	53 ± 18	52 ± 21	0.638
LDL (mg/dL)	112 (49)	112 (48)	115 (67)	0.416	124 (52)	104 (46)	0.027
Triglycerides (mg/dL)	104 (66)	103 (68)	112 (56)	0.454	106 (64)	103 (70)	0.741
Positive ANA	123 (61.5%)	99 (60.4%)	24 (66.7%)	0.607	50 (63.3%)	73 (60.3%)	0.786
Positive SMA	186 (93%)	151 (92.1%)	35 (97.2%)	0.472	75 (94.9%)	111 (91.7%)	0.559
Positive anti-LKM	12 (6%)	10 (6.1%)	2 (5.6%)	1.000	5 (6.3%)	7 (5.8%)	1.000
Positive anti-SLA/LP	22 (11%)	19 (11.6%)	3 (8.3%)	0.771	6 (7.6%)	16 (13.2%)	0.311
Histology	n = 186	n = 153	n = 33		n = 74	n = 112	
Moderate or Severe Grade	114 (57%)	93 (60.8%)	21 (63.6%)	0.914	48 (64.9%)	66 (58.9%)	0.509
Severe Fibrosis or Cirrhosis	59 (29.5%)	50 (32.7%)	9 (27.3%)	0.690	26 (35.1%)	33 (29.5%)	0.514
Steatosis	35 (18.8%)	31 (20.3%)	4 (12.1%)	0.401	19 (25.7%)	16 (14.3%)	0.079
Steatohepatitis	14 (7%)	11 (7.2%)	3 (9.1%)	0.717	4 (5.4%)	10 (8.9%)	0.544
Presence of cirrhosis	44 (22%)	38 (23.2%)	6 (16.7%)	0.507	18 (22.8%)	26 (21.5%)	0.862
Decompensated cirrhosis	7 (3.5%)	7 (18.4%)	0 (0%)	0.568	4 (22.2%)	3 (11.5%)	0.419

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.

corticosteroids withdrawal was reached in 86 out of 176 (48.9%) patients who achieved on-treatment CR while complete withdrawal of immunosuppression according to the European and the Hellenic recommendations [1,2] was feasible in 46 out of 176 (26.1%) patients who achieved on-treatment CR. After treatment discontinuation, 34 out of 46 (73.9%) patients, maintained CR (Table 3).

Analysis of disease progression was performed in the 195 treated patients with AIH (42 cirrhotic and 153 non-cirrhotic at baseline). Five non-cirrhotic patients progressed to cirrhosis during follow-up (3.3%). Development of at least one episode of decompensation was observed in 9 out of 47 (19.1%) patients with cirrhosis either at baseline or during follow-up. Seven patients died due to liver-related cause, 1 patient

**Table 3**

Response to immunosuppressive treatment according to *IL28B rs12979860* genotype (n = 195).

	PD1.3			P value	IL28B rs12979860		P value
	Total (n = 195)	GG (n = 159)	GA/AA (n = 36)		CC (n = 77)	CT/TT (n = 118)	
Treatment schedule							
Prednisolone + Mycophenolate mofetil	152 (77.9%)	126 (79.2%)	26 (72.2%)	0.489	61 (79.2%)	91 (77.1%)	0.659
Prednisolone + Azathioprine	26 (13.3%)	19 (11.9%)	7 (19.4%)		11 (14.3%)	15 (12.7%)	
Prednisolone only	17 (8.7%)	14 (8.8%)	3 (8.3%)		5 (6.5%)	12 (10.2%)	
Treatment duration (months)	63 (77)	66 (69)	62 (74)	0.830	61 (46)	67 (81)	0.172
On-treatment complete response	176 (90.3%)	144 (90.6%)	32 (88.9%)	0.758	72 (93.5%)	104 (88.1%)	0.323
Time to achieve complete response (months)	3 (5)	3 (5)	3 (3)	0.891	2 (4)	3 (5)	0.426
Corticosteroids withdrawal in patients with on-treatment complete response	86/176 (48.9%)	70/144 (48.6%)	16/32 (52%)	1.000	31/72 (43.1%)	55/104 (52.9%)	0.259
Relapse during treatment after corticosteroids withdrawal	38/86 (44.2%)	32/70 (45.7%)	6/16 (37.5%)	0.751	14/31 (45.2%)	24/55 (43.6%)	1.000
Complete treatment withdrawal	46/176 (26.1%)	38/144 (26.4%)	8/32 (25%)	1.000	26/72 (36.1%)	20/104 (19.2%)	0.020
Maintenance of response after complete treatment withdrawal	34/46 (73.9%)	28/38 (73.7%)	6/8 (75%)	1.000	17/26 (65.4%)	17/20 (85%)	0.245

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



underwent orthotopic liver transplantation and 6 patients developed hepatocellular carcinoma (HCC). In total, disease progression with at least one of the above events was documented in 17 out of 195 (8.7%) treated patients.

### 3.2. *PD1 rs1156882 (PD1.3)*

The allele and genotype frequencies of *PD1.3* polymorphisms found among the disease and healthy control group are shown in Table 4. The genotype distribution of *PD1.3* in AIH patients (GG 82%, GA 17%, AA 1%) and controls (GG 72%, GA 26%, AA 2%) was similar to that predicted by the HWE model ( $P = 0.872$  and  $P = 0.844$ , respectively). No association was found regarding the alleles or genotypes (dominant or recessive models) of *PD1.3* SNP with the risk of AIH (Table 4).

*PD1.3* genotypes were not associated with epidemiological characteristics of the patients such as, age at disease onset and sex. Similarly, no correlation was found with the type of disease presentation or the clinical stage at diagnosis (presence of cirrhosis, decompensation). On liver histology, the grade of inflammation and the stage of fibrosis at diagnosis did not correlate with the presence of SNP *PD1.3/A*. On laboratory investigations, the only significant difference was the presence of higher HDL levels in GG genotype (Table 2;  $P = 0.019$ ). Interestingly, GG homozygotes suffered more frequently from concurrent extrahepatic autoimmune diseases (Table 2;  $P = 0.001$ ), but without predisposition for a particular autoimmune disease compared to GA/AA haplotypes (data not shown). The presence of autoantibodies (ANA, SMA, LKM, SLA/LP) was not affected either. Response to

**Table 4**  
Distribution of *PD1.3* and *IL28B rs12979860* genotypes in AIH patients and healthy controls.

	AIH patients (n = 200)	Controls (n = 100)	Model	P-value
<b><i>PD1.3</i></b>				
<b>(rs11568821)</b>				
Genotype				
GG	164 (82%)	72 (72%)	Codominant	0.132
GA	34 (17%)	26 (26%)		
AA	2 (1%)	2 (2%)		
Allele				
G	362 (90.5%)	170 (85%)		0.062
A	38 (9.5%)	30 (15%)		
Genotypes				
GG	164 (82%)	72 (72%)	Dominant	0.065
AA + GA	36 (18%)	28 (28%)		
Genotypes				
AA	2 (1%)	2 (2%)	Recessive	0.603
GG + GA	198 (99%)	98 (98%)		
Genotypes				
GG + AA	198 (99%)	98 (98%)	Over-dominant	0.603
GA	2 (1%)	2 (2%)		
<b><i>IL28B</i></b>				
<b>(rs12979860)</b>				
Genotype				
CC	79 (39.5%)	42 (42%)	Codominant	0.878
CT	85 (42.5%)	42 (42%)		
TT	36 (18%)	16 (16%)		
Allele				
C	243 (60.8%)	126 (63%)		0.656
T	157 (39.3%)	74 (37%)		
Genotypes				
CC	79 (39.5%)	42 (42%)	Dominant	0.771
TT + CT	121 (60.5%)	58 (58%)		
Genotypes				
TT	36 (18%)	16 (16%)	Recessive	0.787
CC + CT	164 (82%)	84 (84%)		
Genotypes				
CC + TT	115 (57.5%)	58 (58%)	Over-dominant	1.000
CT	85 (42.5%)	42 (42%)		

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

immunosuppressive treatment was similar between GG and GA/AA patients (Table 3). Finally, the *PD1.3* genotype did not affect the survival free of cirrhotic events (decompensation, liver transplantation, HCC) (HR = 2.27, 95%CI 0.29–17.7,  $P = 0.436$ ) and liver-related death/liver transplantation (HR = 1.65, 95%CI: 0.20–13.41,  $P = 0.640$ ) in treated patients with AIH.

### 3.3. *IL28B rs12979860*

The distribution of *IL28B rs12979860* genotypes in AIH patients (CC 39.5%, CT 42.5%, TT 18%) and controls (CC 42%, CT 42%, TT 16%) was in concordance with HWE ( $P = 0.123$  and  $P = 0.321$ , respectively). Neither the dominant nor recessive models used in the study showed a connection of the *IL28B rs12979860* SNP with the risk of AIH (Table 4).

This polymorphism was not associated with epidemiological characteristics of AIH patients, the mode of presentation of the disease, the clinical stage at diagnosis, levels of liver function tests,  $\gamma$ -globulin and IgG levels. Similarly, the grade of inflammatory activity and stage of fibrosis at the histological level at diagnosis did not differ between CC homozygotes and TT/CT carriers. However, low density lipoprotein (LDL) cholesterol was significantly higher in CC patients compared to those with CT/TT (Table 2;  $P = 0.027$ ), even though no differences was found regarding the presence of steatosis, steatohepatitis or MetS (Table 2).

On-treatment response was not affected by the *rs12979860* polymorphism. However, among patients with on-treatment CR [n = 176, 72/77 (93.5%) CC and 104/118 (88.1%) CT/CC patients], CC homozygotes achieved significantly higher treatment withdrawal rates compared to CT heterozygotes and TT homozygotes [26/72 (36.1%) in CC vs. 20/104 (19.2%) in CT/TT patients;  $P = 0.02$ ] (Table 3). This favorable response of CC homozygotes remained statistically significant even after adjustment for the presence of simple steatosis or steatohepatitis [OR = 2.3, 95%CI: 1.1–4.7,  $P = 0.02$ ]. Finally, the *rs12979860* genotype did not affect the survival free of cirrhotic events (decompensation, liver transplantation, HCC) (HR = 0.67, 95%CI 0.18–2.54,  $P = 0.559$ ) and liver-related death/liver transplantation (HR = 0.58, 95% CI: 0.12–2.91,  $P = 0.513$ ) in treated patients with AIH.

## 4. Discussion

To the best of our knowledge, this is the first molecular epidemiological study addressing the potential association between *PD1.3* and *IL28B rs12979860* polymorphisms and AIH.

AIH is a complex autoimmune disease whose pathogenetic mechanisms cannot be fully explained by a single pathway defect. T-cell dysregulation and breakdown of self-tolerance are key playmakers. *PD1* is involved in immune response regulation at several levels from thymic selection to lymphocyte activation and peripheral regulatory T cells differentiation [21,54,55]. Several studies have associated *PD1* polymorphisms with diverse autoimmune diseases [31,33–35,56,57] while increasing body of evidence in vivo and in vitro support the relation between disruption of the PD1/PDL1/PDL2 axis and autoimmunity [17, 19,22,23,27–30]. It has also been shown that *PD1* and its ligands are expressed on lymphocytes that infiltrate the liver at portal and sinusoidal areas and this phenomenon was restricted to patients with autoimmune liver diseases and not in healthy controls [58]. Other studies have associated the presence of putative antibodies against *PD1* in AIH patients, with possible implications in treatment response [59,60].

However, we did not find any association between *PD1.3* and susceptibility to AIH for patients of Greek descent. Furthermore, we tested for correlation with clinical aspects of the disease such as, mode of presentation, severity, risk of progression, treatment response and prognosis, but no correlations were detected. Similar investigations in SLE patients, found no consistent link between *PD1.3* and the risk of SLE development [61–63]. In a recent meta-analysis in SLE patients [64], there was a link between *PD1.3* polymorphism and SLE risk in

Caucasians and Mexicans, but not in African American and Asian patients. Additionally, there is a spatial heterogeneity in the frequency of *PD1.3A* allele across Europe, with the frequency decreasing from northern to southern Europe [63]. This may reflect ethnic differences or point to a possible interaction between this polymorphism and an environmental factor. With these findings in mind, it is reasonable to assume that the contribution of genetic variants to the risk for diseases will vary depending on the population investigated, as well as on the etiology and pathogenesis of different diseases. Further research of *PD1.3* polymorphism in diverse diseases and populations is needed [65]. Another possibility is that other *PD1* polymorphisms that were not investigated in the present study could be responsible for AIH susceptibility.

The *IL28B rs12979860* polymorphism has been implicated in inflammatory response in patients with HCV and NAFLD. Indeed, the *rs12979860* CC genotype, which has been linked to favorable response to antiviral treatment with pegylated interferon and ribavirin, was associated with more pronounced liver inflammation and biochemical activity [66,67]. The effect on inflammation extends to NAFLD patients as well [40]. The proposed mechanism involves increased *IL28B* transcriptional activity in CC homozygotes that upregulates interferon-stimulated genes including pro-inflammatory cytokines and leads to increased liver inflammation [68–70]. However, our results demonstrated that the CC genotype was not associated with inflammatory activity of the disease either at the biochemical or at the histological level. Besides, no relation was found regarding the clinical manifestations and prognosis of the disease.

Metabolic associations of the *rs12979860* CC genotype include a correlation with less hepatic steatosis and lower insulin resistance in HCV patients, again through the effect of upregulated interferon-stimulated genes [39,40,67]. Our results showed an association of significantly higher LDL cholesterol levels in AIH patients with the *IL28B* CC genotype similarly to previous studies [37], while no association was found between the presence of steatosis or steatohepatitis and *IL28B* genotypes indicating that the effect of *IL28B* polymorphisms may have impact only in HCV patients as has also been shown in a previous study in NAFLD [71]. Quite interestingly, however, CC homozygotes patients with AIH achieved significantly higher rates of treatment withdrawal after on-treatment CR. This novel finding of the association of *IL28B rs12979860* genotype with the potential of treatment discontinuation in autoimmune liver diseases needs confirmation and further clarification in a larger group of AIH patients in an attempt to see whether this specific polymorphism could identify a subgroup of AIH patients who are candidates for complete treatment cessation after on-treatment CR.

## 5. Conclusion

In conclusion, this is the first report to explore a possible relation between *PD1.3* and *IL28B rs12979860* polymorphisms in a homogenous cohort of AIH patients. Both polymorphisms do not seem to contribute to AIH susceptibility, disease manifestations, treatment response rates and prognosis. However, even though on-treatment response rates to immunosuppression were not affected by the *IL28B rs12979860* polymorphism, CC homozygotes AIH patients were more likely to achieve complete treatment withdrawal suggesting its use as a surrogate marker for deciding treatment cessation in AIH treated patients who are in CR on-treatment.

## Submission declaration and verification

This study has not been published previously and is not under consideration for publication elsewhere. All authors and responsible authorities approved its publication. If accepted, it will not be published elsewhere in the same form in English or in any other language.

## Author contributions

Nikolaos Gatselis: Conceptualization, Formal Analysis, Investigation, Writing - review & editing, Visualization. Kalliopi Azariadis: Data curation, Formal analysis, Investigation, Writing - original draft. Aggeliki Lyberopoulou: Formal analysis, Investigation. George N. Dalekos: Conceptualization, Writing - review & editing, Visualization.

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## Declaration of competing interest

The authors have nothing to declare.

## References

- [1] European association for the study of the, EASL clinical practice Guidelines: autoimmune hepatitis, *J. Hepatol.* 63 (2015) 971–1004. <https://doi.org/10.1016/j.jhep.2015.06.030>.
- [2] G.N. Dalekos, J. Koskinas, G.V. Papatheodoridis, Hellenic association for the study of the liver clinical practice Guidelines: autoimmune hepatitis, *Ann. Gastroenterol.* 32 (2019) 1–23. <https://doi.org/10.20524/aog.2018.0330>.
- [3] N.K. Gatselis, K. Zachou, G.K. Koukoulis, G.N. Dalekos, Autoimmune hepatitis, one disease with many faces: etiopathogenetic, clinico-laboratory and histological characteristics, *World J. Gastroenterol.* 21 (2015) 60–83. <https://doi.org/10.3748/wjg.v21.i1.60>.
- [4] C.L. Mack, D. Adams, D.N. Assis, N. Kerker, M.P. Manns, M.J. Mayo, J.M. Vierling, M. Alsawas, M.H. Murad, A.J. Czaja, Diagnosis and management of autoimmune hepatitis in adults and children: 2019 practice guidance and Guidelines from the American association for the study of liver diseases, *Hepatology* 72 (2020) 671–722. <https://doi.org/10.1002/hep.31065>.
- [5] K. Zachou, P. Muratori, G.K. Koukoulis, A. Granito, N. Gatselis, A. Fabbri, G. N. Dalekos, L. Muratori, Review article: autoimmune hepatitis – current management and challenges, *Aliment. Pharmacol. Ther.* 38 (2013) 887–913. <https://doi.org/10.1111/apt.12470>.
- [6] G.N. Dalekos, K. Azariadis, V. Lygoura, P. Arvaniti, S. Gampeta, N.K. Gatselis, Autoimmune hepatitis in patients aged 70 years or older: disease characteristics, treatment response and outcome, *Liver, Bar Int.* 41 (2021) 1592–1599. <https://doi.org/10.1111/liv.14900>.
- [7] L. Gronbaek, H. Vilstrup, P. Jepsen, Autoimmune hepatitis in Denmark: incidence, prevalence, prognosis, and causes of death. A nationwide registry-based cohort study, *J. Hepatol.* 60 (2014) 612–617. <https://doi.org/10.1016/j.jhep.2013.10.020>.
- [8] L. Grønbaek, H. Otete, L. Ban, C. Crooks, T. Card, P. Jepsen, J. West, Incidence, prevalence and mortality of autoimmune hepatitis in England 1997–2015. A population-based cohort study, *Liver Int.* 40 (2020) 1634–1644. <https://doi.org/10.1111/liv.14480>.
- [9] K. Zachou, P. Arvaniti, A. Lyberopoulou, G.N. Dalekos, Impact of genetic and environmental factors on autoimmune hepatitis, *J. Transl. Autoimmun.* 4 (2021) 100125. <https://doi.org/10.1016/j.jtauto.2021.100125>.
- [10] A. Floreani, P. Restrepo-Jimenez, M.F. Secchi, S. De Martin, P.S.C. Leung, E. Krawitt, C.L. Bowlus, M.E. Gershwin, J.M. Anaya, Etiopathogenesis of autoimmune hepatitis, *J. Autoimmun.* 95 (2018) 133–143. <https://doi.org/10.1016/j.jaut.2018.10.020>.
- [11] Y.S. de Boer, N.M. van Gerven, A. Zwieters, B.J. Verwer, B. van Hoek, K.J. van Erpecum, U. Beuers, H.R. van Buuren, J.P. Drenth, J.W. den Ouden, R.C. Verdonk, G.H. Koek, J.T. Brouwer, M.M. Guichelaar, J.M. Vrolijk, G. Kraal, C.J. Mulder, C. M. van Nieuwkerk, J. Fischer, T. Berg, F. Stickel, C. Sarrazin, C. Schramm, A. W. Lohse, C. Weiler-Normann, M.M. Lerch, M. Nauck, H. Volzke, G. Homuth, E. Bloemena, H.W. Verspaget, V. Kumar, A. Zernakova, C. Wijmenga, L. Franke, G. Bouma, G. Dutch Autoimmune Hepatitis Study, S. LifeLines Cohort, P. Study of Health in, Genome-wide association study identifies variants associated with autoimmune hepatitis type 1, *Gastroenterology* 147 (2014) 443–452, e445. <http://doi.org/10.1053/j.gastro.2014.04.022>.
- [12] P. Muratori, A.J. Czaja, L. Muratori, G. Pappas, S. Maccariello, F. Cassani, A. Granito, R. Ferrari, V. Mantovani, M. Lenzi, F.B. Bianchi, Genetic distinctions between autoimmune hepatitis in Italy and North America, *World J. Gastroenterol.* 11 (2005) 1862–1866. <https://doi.org/10.3748/wjg.v11.i12.1862>.
- [13] N.M. van Gerven, Y.S. de Boer, A. Zwieters, B.J. Verwer, J.P. Drenth, B. van Hoek, K. J. van Erpecum, U. Beuers, H.R. van Buuren, J.W. den Ouden, R.C. Verdonk, G. H. Koek, J.T. Brouwer, M.M. Guichelaar, J.M. Vrolijk, M.J. Coenraad, G. Kraal, C. J. Mulder, C.M. van Nieuwkerk, E. Bloemena, H.W. Verspaget, V. Kumar, A. Zernakova, C. Wijmenga, L. Franke, G. Bouma, G. Dutch Autoimmune Hepatitis Study, HLA-DRB1\*03:01 and HLA-DRB1\*04:01 modify the presentation and outcome in autoimmune hepatitis type-1, *Gene Immun.* 16 (2015) 247–252. <https://doi.org/10.1038/gene.2014.82>.

- [14] A. Takahashi, T. Arinaga-Hino, H. Ohira, T. Torimura, M. Zeniya, M. Abe, K. Yoshizawa, A. Takaki, Y. Suzuki, J.H. Kang, N. Nakamoto, T. Fujisawa, K. Yonemoto, A. Tanaka, H. Takikawa, J. Autoimmune hepatitis study group-subgroup of the intractable hepato-biliary disease study group in, autoimmune hepatitis in Japan: trends in a nationwide survey, *J. Gastroenterol.* 52 (2017) 631–640. <https://doi.org/10.1007/s00535-016-1267-0>.
- [15] A.J. Czaja, Examining pathogenic concepts of autoimmune hepatitis for cues to future investigations and interventions, *World J. Gastroenterol.* 25 (2019) 6579–6606. <https://doi.org/10.3748/wjg.v25.i45.6579>.
- [16] M.P. Manns, A.W. Lohse, D. Vergani, Autoimmune hepatitis—Update, *J. Hepatol.* 62 (2015) S100–S111, 2015. <https://doi.org/10.1016/j.jhep.2015.03.005>.
- [17] V.A. Boussiotis, Molecular and biochemical aspects of the PD-1 checkpoint pathway, *N. Engl. J. Med.* 375 (2016) 1767–1778. <https://doi.org/10.1056/NEJMr1514296>.
- [18] A.H. Sharpe, G.J. Freeman, The B7-CD28 superfamily, *Nat. Rev. Immunol.* 2 (2002) 116–126. <https://doi.org/10.1038/nri727>.
- [19] M.R. Zamani, S. Aslani, A. Salmaninejad, M.R. Javan, N. Rezaei, PD-1/PD-L and autoimmunity: a growing relationship, *Cell. Immunol.* 310 (2016) 27–41. <https://doi.org/10.1016/j.cellimm.2016.09.009>.
- [20] Y. Agata, A. Kawasaki, H. Nishimura, Y. Ishida, T. Tsubata, H. Yagita, T. Honjo, Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes, *Int. Immunol.* 8 (1996) 765–772. <https://doi.org/10.1093/intimm/8.5.765>.
- [21] X. Chen, D. Fosco, D.E. Kline, L. Meng, S. Nishi, P.A. Savage, J. Kline, PD-1 regulates extrathymic regulatory T-cell differentiation, *Eur. J. Immunol.* 44 (2014) 2603–2616. <https://doi.org/10.1002/eji.201344423>.
- [22] G.J. Freeman, A.J. Long, Y. Iwai, K. Bourque, T. Chernova, H. Nishimura, L.J. Fitz, N. Malenkovich, T. Okazaki, M.C. Byrne, H.F. Horton, L. Fouser, B. Carter, V. Ling, M.R. Bowman, B.M. Carreno, M. Collins, C.R. Wood, T. Honjo, Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation, *J. Exp. Med.* 192 (2000) 1027–1034. <https://doi.org/10.1084/jem.192.7.1027>.
- [23] Y. Latchman, C.R. Wood, T. Chernova, D. Chaudhary, M. Borde, I. Chernova, Y. Iwai, A.J. Long, J.A. Brown, R. Nunes, E.A. Greenfield, K. Bourque, V. A. Boussiotis, L.L. Carter, B.M. Carreno, N. Malenkovich, H. Nishimura, T. Okazaki, T. Honjo, A.H. Sharpe, G.J. Freeman, PD-L2 is a second ligand for PD-1 and inhibits T cell activation, *Nat. Immunol.* 2 (2001) 261–268. <https://doi.org/10.1038/85330>.
- [24] H. Nishimura, M. Nose, H. Hiai, N. Minato, T. Honjo, Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor, *Immunity* 11 (1999) 141–151. [https://doi.org/10.1016/s1074-7613\(00\)80089-8](https://doi.org/10.1016/s1074-7613(00)80089-8).
- [25] T. Okazaki, Y. Tanaka, R. Nishio, T. Mitsuiye, A. Mizoguchi, J. Wang, M. Ishida, H. Hiai, A. Matsumori, N. Minato, T. Honjo, Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice, *Nat. Med.* 9 (2003) 1477–1483. <https://doi.org/10.1038/nm955>.
- [26] T. Yoshida, F. Jiang, T. Honjo, T. Okazaki, PD-1 deficiency reveals various tissue-specific autoimmunity by H-2b and dose-dependent requirement of H-2g7 for diabetes in NOD mice, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 3533–3538. <https://doi.org/10.1073/pnas.0710951105>.
- [27] M. Kido, N. Watanabe, T. Okazaki, T. Akamatsu, J. Tanaka, K. Saga, A. Nishio, T. Honjo, T. Chiba, Fatal autoimmune hepatitis induced by concurrent loss of naturally arising regulatory T cells and PD-1-mediated signaling, *Gastroenterology* 135 (2008) 1333–1343. <https://doi.org/10.1053/j.gastro.2008.06.042>.
- [28] E. De Martin, J.M. Michot, B. Papouin, S. Champiat, C. Mateus, O. Lambotte, B. Roche, T.M. Antonini, A. Coilly, S. Laghouati, C. Robert, A. Marabelle, C. Guettier, D. Samuel, Characterization of liver injury induced by cancer immunotherapy using immune checkpoint inhibitors, *J. Hepatol.* 68 (2018) 1181–1190. <https://doi.org/10.1016/j.jhep.2018.01.033>.
- [29] J. Haanen, F. Carbone, C. Robert, K.M. Kerr, S. Peters, J. Larkin, K. Jordan, E. G. Committee, Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, *Ann. Oncol.* 28 (2017) iv119–iv142. <https://doi.org/10.1093/annonc/mdx225>.
- [30] F. Martins, L. Sofiya, G.P. Sykiotis, F. Lamine, M. Maillard, M. Fraga, K. Shabafrouz, C. Ribí, A. Cairolí, Y. Guex-Crosier, T. Kuntzer, O. Michielin, S. Peters, G. Coukos, F. Spertini, J.A. Thompson, M. Obeid, Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance, *Nat. Rev. Clin. Oncol.* 16 (2019) 563–580. <https://doi.org/10.1038/s41571-019-0218-0>.
- [31] L. Prokunina, L. Padyukov, A. Bennet, U. de Faire, B. Wiman, J. Prince, L. Alfredsson, L. Klareskog, M. Alarcon-Riquelme, Association of the PD-1.3A allele of the PDCD1 gene in patients with rheumatoid arthritis negative for rheumatoid factor and the shared epitope, *Arthritis Rheum.* 50 (2004) 1770–1773. <https://doi.org/10.1002/art.20280>.
- [32] M. Mahmoudi, A. Rezaeiamesh, A. Salmaninejad, S. Harsini, S. Poursani, T. Bahrami, F. Tahghighi, V. Ziaee, N. Rezaei, PDCD1 single nucleotide genes polymorphisms confer susceptibility to juvenile-onset systemic lupus erythematosus, *Autoimmunity* 48 (2015) 488–493. <https://doi.org/10.3109/08916934.2015.1058370>.
- [33] L. Prokunina, C. Castillejo-Lopez, F. Oberg, I. Gunnarsson, L. Berg, V. Magnusson, A.J. Brookes, D. Tentler, H. Kristjansdottir, G. Gronald, A.I. Bolstad, E. Svenungsson, I. Lundberg, G. Sturfelt, A. Jonsson, L. Truedsson, G. Lima, J. Alcocer-Varela, R. Jonsson, U.B. Gyllenstein, J.B. Harley, D. Alarcon-Segovia, K. Steinsson, M.E. Alarcon-Riquelme, A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans, *Nat. Genet.* 32 (2002) 666–669. <https://doi.org/10.1038/ng1020>.
- [34] E.K. Kong, L. Prokunina-Olsson, W.H. Wong, C.S. Lau, T.M. Chan, M. Alarcon-Riquelme, Y.L. Lau, A new haplotype of PDCD1 is associated with rheumatoid arthritis in Hong Kong Chinese, *Arthritis Rheum.* 52 (2005) 1058–1062. <https://doi.org/10.1002/art.20966>.
- [35] A. Kroner, M. Mehling, B. Hemmer, P. Rieckmann, K.V. Toyka, M. Maurer, H. Wiendl, A PD-1 polymorphism is associated with disease progression in multiple sclerosis, *Ann. Neurol.* 58 (2005) 50–57. <https://doi.org/10.1002/ana.20514>.
- [36] D.L. Thomas, C.L. Thio, M.P. Martin, Y. Qi, D. Ge, C. O’Huigin, J. Kidd, K. Kidd, S. I. Khakoo, G. Alexander, J.J. Goedert, G.D. Kirk, S.M. Donfield, H.R. Rosen, L. H. Tobler, M.P. Busch, J.G. McHutchison, J.B. Goldstein, M. Carrington, Genetic variation in IL28B and spontaneous clearance of hepatitis C virus, *Nature* 461 (2009) 798–801. <https://doi.org/10.1038/nature08463>.
- [37] J.H. Li, X.Q. Lao, H.L. Tillmann, J. Rowell, K. Patel, A. Thompson, S. Suchindran, A.J. Muir, J.R. Guyton, S.D. Gardner, J.G. McHutchison, J.J. McCarthy, Interferon-lambda genotype and low serum low-density lipoprotein cholesterol levels in patients with chronic hepatitis C infection, *Hepatology* 51 (2010) 1904–1911. <https://doi.org/10.1002/hep.23592>.
- [38] H.L. Tillmann, K. Patel, A.J. Muir, C.D. Guy, J.H. Li, X.Q. Lao, A. Thompson, P. J. Clark, S.D. Gardner, J.G. McHutchison, J.J. McCarthy, Beneficial IL28B genotype associated with lower frequency of hepatic steatosis in patients with chronic hepatitis C, *J. Hepatol.* 55 (2011) 1195–1200. <https://doi.org/10.1016/j.jhep.2011.03.015>.
- [39] A.F. Stattermayer, K. Rutter, S. Beinhardt, T.M. Scherzer, A. Stadlmayr, H. Hofer, F. Wrba, P. Steindl-Munda, M. Krebs, C. Datz, M. Trauner, P. Ferenci, Association of the IL28B genotype with insulin resistance in patients with chronic hepatitis C, *J. Hepatol.* 57 (2012) 492–498. <https://doi.org/10.1016/j.jhep.2012.04.036>.
- [40] S. Petta, S. Grimaudo, C. Camma, D. Cabibi, V. Di Marco, G. Licata, R.M. Pipitone, A. Craxi, IL28B and PNPLA3 polymorphisms affect histological liver damage in patients with non-alcoholic fatty liver disease, *J. Hepatol.* 56 (2012) 1356–1362. <https://doi.org/10.1016/j.jhep.2012.01.007>.
- [41] G.N. Dalekos, N.K. Gatselis, K. Zachou, G.K. Koukoulis, NAFLD and autoimmune hepatitis: do not judge a book by its cover, *Eur. J. Intern. Med.* 75 (2020) 1–9. <https://doi.org/10.1016/j.ejim.2020.02.001>.
- [42] A. Takahashi, T. Arinaga-Hino, H. Ohira, K. Abe, T. Torimura, M. Zeniya, M. Abe, K. Yoshizawa, A. Takaki, Y. Suzuki, J.H. Kang, N. Nakamoto, T. Fujisawa, A. Tanaka, H. Takikawa, A.I.H.S.G. Japan, Non-alcoholic fatty liver disease in patients with autoimmune hepatitis, *JGH Open* 2 (2018) 54–58. <https://doi.org/10.1002/jgh3.12046>.
- [43] G.N. Dalekos, N.K. Gatselis, K. Zachou, Acute severe autoimmune hepatitis: corticosteroids or liver transplantation? *Liver Transplant.* 25 (2019) 1588–1589. <https://doi.org/10.1002/lt.25615>.
- [44] K. Zachou, P. Arvaniti, K. Azariadis, V. Lygoura, N.K. Gatselis, A. Lyberopoulou, G. K. Koukoulis, G.N. Dalekos, Prompt initiation of high-dose i.v. corticosteroids seems to prevent progression to liver failure in patients with original acute severe autoimmune hepatitis, *Hepatol. Res.* 49 (2019) 96–104. <https://doi.org/10.1111/hpr.13252>.
- [45] K. Zachou, N.K. Gatselis, P. Arvaniti, S. Gabeta, E.I. Rigopoulou, G.K. Koukoulis, G.N. Dalekos, A real-world study focused on the long-term efficacy of mycophenolate mofetil as first-line treatment of autoimmune hepatitis, *Immunol. Pharmacol. Ther.* 43 (2016) 1035–1047. <https://doi.org/10.1111/apt.13584>.
- [46] K. Zachou, N. Gatselis, G. Papadamou, E.I. Rigopoulou, G.N. Dalekos, Mycophenolate for the treatment of autoimmune hepatitis: prospective assessment of its efficacy and safety for induction and maintenance of remission in a large cohort of treatment-naïve patients, *J. Hepatol.* 55 (2011) 636–646. <https://doi.org/10.1016/j.jhep.2010.12.032>.
- [47] K.G. Alberti, R.H. Eckel, S.M. Grundy, P.Z. Zimmet, J.I. Cleeman, K.A. Donato, J. C. Fruchart, W.P. James, C.M. Loria, S.C. Smith Jr., E. International Diabetes Federation Task Force on Prevention, L. National Heart, I. Blood, A. American Heart, F. World Heart, S. International Atherosclerosis, O. International Association for the Study of, Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity, *Circulation* 120 (2009) 1640–1645. <https://doi.org/10.1161/CIRCULATIONAHA.109.192644>.
- [48] R.G. Knodell, K.G. Ishak, W.C. Black, T.S. Chen, R. Craig, N. Kaplowitz, T. W. Kiernan, J. Wollman, Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis, *Hepatology* 1 (1981) 431–435. <https://doi.org/10.1002/hep.1840010511>.
- [49] N.K. Gatselis, A.G. Vakrakou, K. Zachou, T. Androutsakos, K. Azariadis, G. Hatzis, M.N. Manoussakis, G.N. Dalekos, Decreased serum DNase1-activity in patients with autoimmune liver diseases, *Autoimmunity* 50 (2017) 125–132. <https://doi.org/10.1080/08916934.2017.1279610>.
- [50] N.K. Gatselis, K. Zachou, G. Giannoulis, S. Gabeta, G.L. Norman, G.N. Dalekos, Serum cartilage oligomeric matrix protein and golgi protein-73: new diagnostic and predictive tools for liver fibrosis and hepatocellular cancer? *Cancers* 13 (2021). <https://doi.org/10.3390/cancers13143510>.
- [51] G.N. Dalekos, K. Zachou, C. Liaskos, N. Gatselis, Autoantibodies and defined target autoantigens in autoimmune hepatitis: an overview, *Eur. J. Intern. Med.* 13 (2002) 293–303. [https://doi.org/10.1016/s0953-6205\(02\)00089-4](https://doi.org/10.1016/s0953-6205(02)00089-4).
- [52] K. Zachou, S. Gampeta, N.K. Gatselis, K. Oikonomou, J. Goulis, M.N. Manoussakis, Y. Renaudineau, D.P. Bogdanos, G.N. Dalekos, Anti-SLA/LP alone or in combination with anti-Ro52 and fine specificity of anti-Ro52 antibodies in patients with autoimmune hepatitis, *Liver Int.* 35 (2015) 660–672. <https://doi.org/10.1111/liv.12658>.

- [53] N.K. Gatselis, A. Soulier, S. Chevaliez, K. Patel, A.J. Muir, J.-M. Pawlowsky, Utility of serum samples for IL28B genotyping: a valuable source of genomic DNA, *J. Hepatol.* 58 (2013) S187.
- [54] J.A. Brown, D.M. Dorfman, F.R. Ma, E.L. Sullivan, O. Munoz, C.R. Wood, E. A. Greenfield, G.J. Freeman, Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production, *J. Immunol.* 170 (2003) 1257–1266. <https://doi.org/10.4049/jimmunol.170.3.1257>.
- [55] M.E. Keir, M.J. Butte, G.J. Freeman, A.H. Sharpe, PD-1 and its ligands in tolerance and immunity, *Annu. Rev. Immunol.* 26 (2008) 677–704. <https://doi.org/10.1146/annurev.immunol.26.021607.090331>.
- [56] F. Fathi, E. Sadeghi, N. Lotfi, H. Hafezi, M. Ahmadi, S. Mozafarpour, H. Motedayyeh, Effects of the programmed cell death 1 (PDCD1) polymorphisms in susceptibility to systemic lupus erythematosus, *Int. J. Immunogenet.* 47 (2020) 57–64. <https://doi.org/10.1111/iji.12456>.
- [57] Y.H. Lee, S.C. Bae, J.H. Kim, G.G. Song, Meta-analysis of genetic polymorphisms in programmed cell death 1. Associations with rheumatoid arthritis, ankylosing spondylitis, and type 1 diabetes susceptibility, *Z. Rheumatol.* 74 (2015) 230–239. <https://doi.org/10.1007/s00393-014-1415-y>.
- [58] N. Mataka, K. Kikuchi, T. Kawai, M. Higashiyama, Y. Okada, C. Kurihara, R. Hokari, A. Kawaguchi, S. Nagao, T. Kondo, K. Itoh, H. Miyakawa, S. Miura, Expression of PD-1, PD-L1, and PD-L2 in the liver in autoimmune liver diseases, *Am. J. Gastroenterol.* 102 (2007) 302–312. <https://doi.org/10.1111/j.1572-0241.2006.00948.x>.
- [59] K. Matsumoto, Y. Miyake, H. Matsushita, A. Ohnishi, F. Ikeda, H. Shiraha, A. Takaki, K. Nouse, K. Yamamoto, Anti-programmed cell death-1 antibody as a new serological marker for type 1 autoimmune hepatitis, *J. Gastroenterol. Hepatol.* 29 (2014) 110–115. <https://doi.org/10.1111/jgh.12340>.
- [60] Y. Miyake, K. Yamamoto, H. Matsushita, M. Abe, A. Takahashi, T. Umemura, A. Tanaka, M. Nakamura, Y. Nakamoto, Y. Ueno, T. Saibara, H. Takikawa, K. Yoshizawa, H. Ohira, M. Zeniya, M. Onji, H. Tsubouchi, J. Intractable Hepato-Biliary Disease Study Group of, Multicenter validation study of anti-programmed cell death-1 antibody as a serological marker for type 1 autoimmune hepatitis, *Hepatol. Res.* 44 (2014) 1299–1307. <https://doi.org/10.1111/hepr.12305>.
- [61] M. Mostowska, M. Wudarski, H. Chwalinska-Sadowska, P.P. Jagodzinski, The programmed cell death 1 gene 7209 C>T polymorphism is associated with the risk of systemic lupus erythematosus in the Polish population, *Clin. Exp. Rheumatol.* 26 (2008) 457–460.
- [62] K.H. Chua, L.H. Lian, X.J. Sim, T.E. Cheah, T.P. Lau, Association between PDCD1 gene polymorphisms and risk of systemic lupus erythematosus in three main ethnic groups of the Malaysian population, *Int. J. Mol. Sci.* 16 (2015) 9794–9803. <https://doi.org/10.3390/ijms16059794>.
- [63] I. Ferreiros-Vidal, J.J. Gomez-Reino, F. Barros, A. Carracedo, P. Carreira, F. Gonzalez-Escribano, M. Liz, J. Martin, J. Ordi, J.L. Vicario, A. Gonzalez, Association of PDCD1 with susceptibility to systemic lupus erythematosus: evidence of population-specific effects, *Arthritis Rheum.* 50 (2004) 2590–2597. <https://doi.org/10.1002/art.20436>.
- [64] J. Gao, N. Gai, L. Wang, K. Liu, X.H. Liu, L.T. Wei, T. Tian, S.L. Li, Y. Zheng, Y. J. Deng, Z.J. Dai, R.G. Fu, Meta-analysis of programmed cell death 1 polymorphisms with systemic lupus erythematosus risk, *Oncotarget* 8 (2017) 36885–36897. <https://doi.org/10.18632/oncotarget.16378>.
- [65] S. Bayram, H. Akkiz, Y. Ulger, A. Bekar, E. Akgollu, S. Yildirim, Lack of an association of programmed cell death-1 PD1.3 polymorphism with risk of hepatocellular carcinoma susceptibility in Turkish population: a case-control study, *Gene* 511 (2012) 308–313. <https://doi.org/10.1016/j.gene.2012.09.119>.
- [66] M. Noureddin, E.C. Wright, H.J. Alter, S. Clark, E. Thomas, R. Chen, X. Zhao, C. Conry-Cantilena, D.E. Kleiner, T.J. Liang, M.G. Ghany, Association of IL28B genotype with fibrosis progression and clinical outcomes in patients with chronic hepatitis C: a longitudinal analysis, *Hepatology* 58 (2013) 1548–1557. <https://doi.org/10.1002/hep.26506>.
- [67] J.A. Agundez, E. Garcia-Martin, M.L. Maestro, F. Cuenca, C. Martinez, L. Ortega, M. Carballo, M. Vidaurreta, M. Agreda, G. Diaz-Zelaya, A. Suarez, M. Diaz-Rubio, J. M. Ladero, Relation of IL28B gene polymorphism with biochemical and histological features in hepatitis C virus-induced liver disease, *PLoS One* 7 (2012), e37998. <https://doi.org/10.1371/journal.pone.0037998>.
- [68] V. Suppiah, M. Moldovan, G. Ahlenstiel, T. Berg, M. Weltman, M.L. Abate, M. Bassendine, U. Spengler, G.J. Dore, E. Powell, S. Riordan, D. Sheridan, A. Smedile, V. Fragomeli, T. Muller, M. Bahlo, G.J. Stewart, D.R. Booth, J. George, IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy, *Nat. Genet.* 41 (2009) 1100–1104. <https://doi.org/10.1038/ng.447>.
- [69] M. Honda, A. Sakai, T. Yamashita, Y. Nakamoto, E. Mizukoshi, Y. Sakai, T. Yamashita, M. Nakamura, T. Shirasaki, K. Horimoto, Y. Tanaka, K. Tokunaga, M. Mizokami, S. Kaneko, G. Hokuiriku Liver Study, Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C, *Gastroenterology* 139 (2010) 499–509. <https://doi.org/10.1053/j.gastro.2010.04.049>.
- [70] Y. Tanaka, N. Nishida, M. Sugiyama, M. Kurosaki, K. Matsuura, N. Sakamoto, M. Nakagawa, M. Korenaga, K. Hino, S. Hige, Y. Ito, E. Mita, E. Tanaka, S. Mochida, Y. Murawaki, M. Honda, A. Sakai, Y. Hiasa, S. Nishiguchi, A. Koike, I. Sakaida, M. Imamura, K. Ito, K. Yano, N. Masaki, F. Sugauchi, N. Izumi, K. Tokunaga, M. Mizokami, Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C, *Nat. Genet.* 41 (2009) 1105–1109. <https://doi.org/10.1038/ng.449>.
- [71] M.E. Garrett, M.F. Abdelmalek, A. Ashley-Koch, M.A. Hauser, C.A. Moylan, H. Pang, A.M. Diehl, H.L. Tillmann, IL28B rs12979860 is not associated with histologic features of NAFLD in a cohort of Caucasian North American patients, *J. Hepatol.* 58 (2013) 402–403. <https://doi.org/10.1016/j.jhep.2012.09.035>.