



OPEN ACCESS

EDITED BY

Jules Russick,
Commissariat à l'Energie Atomique et aux
Energies Alternatives (CEA), France

REVIEWED BY

Ashwin Ajith,
Augusta University, United States
Manuel Muro,
Virgen de la Arrixaca University
Hospital, Spain
Hani S. Mousa,
University of Cambridge, United Kingdom
Sabrine Dhouioui,
Tunis El Manar University, Tunisia

*CORRESPONDENCE

Miglianti Michela

✉ m.miglianti420@gmail.com

Mocci Stefano

✉ stefano.mocci.9@gmail.com

Littera Roberto

✉ roby.litter@gmail.com

Giglio Sabrina

✉ sabrinar.giglio@unica.it

Perra Andrea

✉ andrea.perra@unica.it

†These authors have contributed
equally to this work and share
first authorship

RECEIVED 28 February 2025

ACCEPTED 08 July 2025

PUBLISHED 29 July 2025

CITATION

Miglianti M, Mocci S, Littera R, Serra G,
Balestieri C, Conti M, Pes F, Deidda S,
Lorrai M, Mereu C, Murgia M, Sanna C,
Mascia A, Sedda F, Duś-Ilnicka I, Cipri S,
Carta MG, Lai S, Giuressi E, Melis M, Zolfino T,
Giglio S, Perra S and Chessa L (2025)
The role of HLA-G in primary biliary
cholangitis and response to therapy.
Front. Immunol. 16:1585535.
doi: 10.3389/fimmu.2025.1585535

COPYRIGHT

© 2025 Miglianti, Mocci, Littera, Serra,
Balestieri, Conti, Pes, Deidda, Lorrai, Mereu,
Murgia, Sanna, Mascia, Sedda, Duś-Ilnicka,
Cipri, Carta, Lai, Giuressi, Melis, Zolfino,
Perra and Chessa. This is an open-access
article distributed under the terms of the
[Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/).
The use, distribution or reproduction in other
forums is permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication in
this journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted
which does not comply with these terms.

The role of HLA-G in primary biliary cholangitis and response to therapy

Michela Miglianti^{1†*}, Stefano Mocci^{2,3†*}, Roberto Littera^{4,5†*},
Giancarlo Serra¹, Cinzia Balestieri⁶, Maria Conti⁶,
Francesco Pes⁶, Silvia Deidda⁷, Michela Lorrai²,
Caterina Mereu², Michela Murgia², Celeste Sanna²,
Alessia Mascia⁸, Francesca Sedda⁸, Irena Duś-Ilnicka⁹,
Selene Cipri⁴, Mauro Giovanni Carta¹, Sara Lai⁵, Erika Giuressi⁵,
Maurizio Melis⁴, Teresa Zolfino⁶, Sabrina Giglio^{2,3,5*},
Andrea Perra^{4,8*} and Luchino Chessa^{1,10}

¹Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy, ²Medical Genetics, Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy, ³Centre for Research University Services (CeSAR, Centro Servizi di Ateneo per la Ricerca), University of Cagliari, Cagliari, Italy, ⁴AART-ODV (Association for the Advancement of Research Transplantation), Cagliari, Italy, ⁵Medical Genetics, R. Binaghi Hospital, ASL Cagliari, Cagliari, Italy, ⁶Gastroenterology Unit, ARNAS Brotzu, Cagliari, Italy, ⁷Pneumology Unit, R. Binaghi Hospital, ASSL Cagliari, Cagliari, Italy, ⁸Oncology and Molecular Pathology Unit, Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy, ⁹Department of Oral Pathology, Wrocław Medical University, Wrocław, Poland, ¹⁰Liver Unit, Department of Internal Medicine, University Hospital of Cagliari, Cagliari, Italy

Introduction: Primary biliary cholangitis (PBC) is a rare autoimmune liver disease involving bile duct damage and fibrosis. This study explores the role of HLA-G, an immunomodulatory molecule crucial for immune tolerance, in PBC pathogenesis and treatment.

Methods: A cohort of 166 PBC patients from Sardinia was compared to 180 healthy controls and 205 autoimmune hepatitis type 1 (AIH-1) patients. Plasma soluble HLA-G (sHLA-G) levels, *HLA-G* alleles, and *3'UTR* haplotypes were analyzed alongside clinical data, including therapy response to ursodeoxycholic acid.

Results: The *UTR-1* haplotype was significantly more frequent in PBC patients than in controls (48.2% vs 34.3%, $P = 0.0018$). The extended haplotype *HLA-G*01:01:01:08/UTR-1* was also strongly associated with PBC (23.2% vs 12.5% in controls, $P = 0.008$; 23.2% vs 6.6% in AIH-1, $P = 2.6 \times 10^{-9}$). PBC patients exhibited lower sHLA-G levels compared to controls and AIH-1 (9.1 U/mL vs 24.03 U/mL and 13.9 U/mL, respectively). Among *UTR-1* carriers, sHLA-G levels were particularly reduced in PBC patients. The *HLA-G*01:01:01:08/UTR-1*

haplotype correlated with the lowest sHLA-G levels and poorer therapy response (60% vs 24.1%, $P = 0.0001$).

Discussion: These findings suggest HLA-G variants, especially *HLA-G*01:01:01:08/UTR-1*, as potential biomarkers for PBC prognosis and treatment outcomes.

KEYWORDS

HLA-G, primary biliary cholangitis, ursodeoxycholic acid, Sardinia (Italy), autoimmune diseases

1 Introduction

Primary biliary cholangitis (PBC) is a significant but relatively uncommon disease that primarily affects women. It is characterized as an autoimmune cholestatic liver disease, featuring distinct attributes such as cholestasis, serologic responsiveness to antimitochondrial antibodies (AMA) or antinuclear antibody (ANA) reactivity, combined, when it is necessary, with histological indications of chronic non-suppurative, granulomatous, lymphocytic small bile duct cholangitis (1–3). PBC is considered a rare disease, with a global incidence and prevalence estimated at 3 and 21.05 cases per 100,000, respectively. In Europe, the estimated incidence and prevalence are 2.57 and 25 cases per 100,000 (4). Similarly, Italy reported a point prevalence of 27.90 cases per 100,000 and an annual incidence of 5.3 cases per 100,000 inhabitants (5).

PBC significantly impacts patients in two main ways progression to end-stage liver disease (e.g., cirrhosis, liver transplantation) and symptomatic manifestations.

These symptoms include cholestatic pruritus, sicca complex, cognitive symptoms, symptoms of social and emotional dysfunction, sleep disturbance and depression, abdominal discomfort and fatigue which may be impact on the patients' quality of life (6, 7). However, the exact aetiology and pathogenesis of autoimmune diseases is not fully understood. Its pathogenesis likely involves both innate and adaptive immune responses, likely influenced by environmental factors interacting with immunogenetic and epigenetic backgrounds. This interaction leads to chronic immune-mediated biliary epithelial injury, causing

cholestasis, ductopenia, hepatocyte injury, and eventual biliary fibrosis and cirrhosis (8–10).

Histologically, liver tissue in PBC shows damage to biliary epithelial cells (BECs) in relation to T cell, B cell, macrophage, eosinophil, and natural killer (NK) cell infiltration in the portal area (11). Another important aspect is the clear evidence of genetic susceptibility as indicated by family and twin studies. Among monozygotic twins, there is a 60% concordance rate, and the sibling relative risk is estimated to be 10.5 (12–15). In this context, the Human Leukocyte Antigen G (*HLA-G*) emerges as ideal candidate for delving into these pathologies. Specifically, this non-classical HLA-class I molecule actively contributes to the immunomodulatory functions mediated by NK cells (16, 17).

Indeed, it is a crucial component of the tolerogenic system (18). Influenced by genetic variability and environmental stimuli (19, 20), *HLA-G* expression is largely regulated by polymorphic sites in the 3' untranslated region (*UTR*), impacting different plasma levels of *HLA-G* (sHLA-G) (21–24).

For instance, *HLA-G UTR-1* haplotype is known to produce the highest levels of soluble *HLA-G* (sHLA-G) (25). Globally, three main *HLA-G* alleles are associated with *UTR-1*: *HLA-G*01:01:01:01/UTR-1* is the haplotype with the highest frequency (0.2277%), followed by haplotype *HLA-G*01:01:01:08/UTR-1* (0.0204%), and the least frequent haplotype *HLA-G*01:01:01:09/UTR-1* (0.0033%) (26).

Originally identified in placental cells, *HLA-G* expression extends to various non-fetal tissues (27, 28). Its immunosuppressive functions have been implicated in inflammation, infections, transplantation tolerance, and cancer where it has been demonstrated to facilitate immune evasion and tumor progression (18, 29–32). Notably, *HLA-G*'s involvement in liver-related conditions, such as chronic hepatitis B and C, underscored its role in liver homeostasis and injury response, with implications for fibrosis and hepatocellular carcinoma prognosis (33–37). Regarding autoimmune liver diseases, studies have demonstrated that type 1 autoimmune hepatitis (AIH-1) patients exhibit significantly lower levels of sHLA-G compared to the control group. Additionally, lower sHLA-G levels have been observed in AIH-1 patients with more severe disease (38). These findings are consistent with other

Abbreviations: AIH-1, Type 1 Autoimmune Hepatitis; ALP, Alkaline Phosphatase; AMA, Antimitochondrial Antibodies; ANA, Antinuclear Antibody; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; BECs, Biliary Epithelial Cells; CI, Confidence Interval; EASL, European Association for the Study of the Liver; GGT, Gamma Glutamyl Transferase; HbsAg, Hepatitis B Surface Antigen; HLA, Human Leukocyte Antigen; IgG, Immunoglobulin G; IgM, Immunoglobulin M; NGS, Next-Generation Sequencing; NK, Natural Killer (cell); PBC, Primary Biliary Cholangitis; sHLA-G, Soluble *HLA-G*; UDCA, Ursodeoxycholic Acid; ULN, Upper Limit of Normal; UTR, Untranslated Region.

autoimmune diseases like rheumatoid arthritis (38, 39), multiple sclerosis (40) and systemic lupus erythematosus (41). While *HLA* gene system has undergone comprehensive examination in the context of PBC (42), with in-depth investigations into polymorphisms conducted through large-scale studies (43). The findings from these studies indicate that susceptibility to PBC in white ethnic populations is associated with *HLA DRB1*08* alleles. Conversely, a study focused on the Italian population indicates that the *DRB1*11* allele has a protective effect against PBC (44).

However, the potential immunomodulatory role of *HLA-G* expression and its role in PBC, have not been investigated yet. In this context, Sardinia, being a genetic isolate, possesses homogeneous and lowly polymorphic genetic characteristics, leading to a higher prevalence of autoimmune diseases (e.g., type 1 diabetes, multiple sclerosis) compared to other populations (45, 46). This unique genetic landscape offers an opportunity for studying genetic associations in rare autoimmune diseases. The current research focuses on elucidating the function of *HLA-G* molecules in PBC, driven by their anti-inflammatory effects in autoimmune conditions. We investigate the role of *HLA-G* in PBC by examining both genetic variability and the phenotypic expression of soluble *HLA-G* and their impact on a group of PBC patients and their therapy, comparing it to a control group and another autoimmune liver disease (AIH-1) from the same geographic area (Sardinia, Italy).

2 Materials and methods

2.1 Patients and controls selection

A cohort of 166 Sardinian PBC patients was compared to a panel of 180 individuals from Sardinia, going back at least two generations of family history, has been utilized as healthy control population; they have been enrolled from the regional bone marrow registry to reflect the genetic background of Sardinian population (47). This population group accurately reflects the genetic profiles and male-to-female ratio of the population in the central-south geographical areas from where the PBC patients were recruited. The cohort of PBC patients was also compared to a panel of 205 AIH-1 patients originating from central-southern Sardinia who were referred to the same outpatient clinic. This comparison aimed to ensure the highest homogeneity in terms of genetic characteristics. The *HLA-G* alleles and 3'UTR haplotypes of the patients and the healthy controls were compared. Patients' and controls' plasma sHLA-G levels were measured, and the results were categorized based on the several *HLA-G* 3'UTR haplotypes that have been linked to affecting *HLA-G* expression (30). Finally, sHLA-G levels were compared among the three extended haplotypes presenting the *HLA-G UTR-1* haplotype (*HLA-G*01:01:01:01/UTR-1*, *HLA-G*01:01:01:08/UTR-1*, and *HLA-G*01:01:01:09/UTR-1*).

The biochemical response to the therapy was assessed either using qualitative definitions based on discrete binary variables or through quantitative scoring systems computed from continuous

parameters, as described in the EASL Clinical Practice Guideline (48).

In particular, at the Liver Unit of University Hospital of Cagliari, the criteria for selecting patients with an inadequate response involve those who, after 12 months of therapy, present ALP levels $\geq 1.5 \times$ ULN (Paris-II criteria). sHLA-G levels were measured concurrently with the assessment of the biochemical response.

2.2 Ethics statement

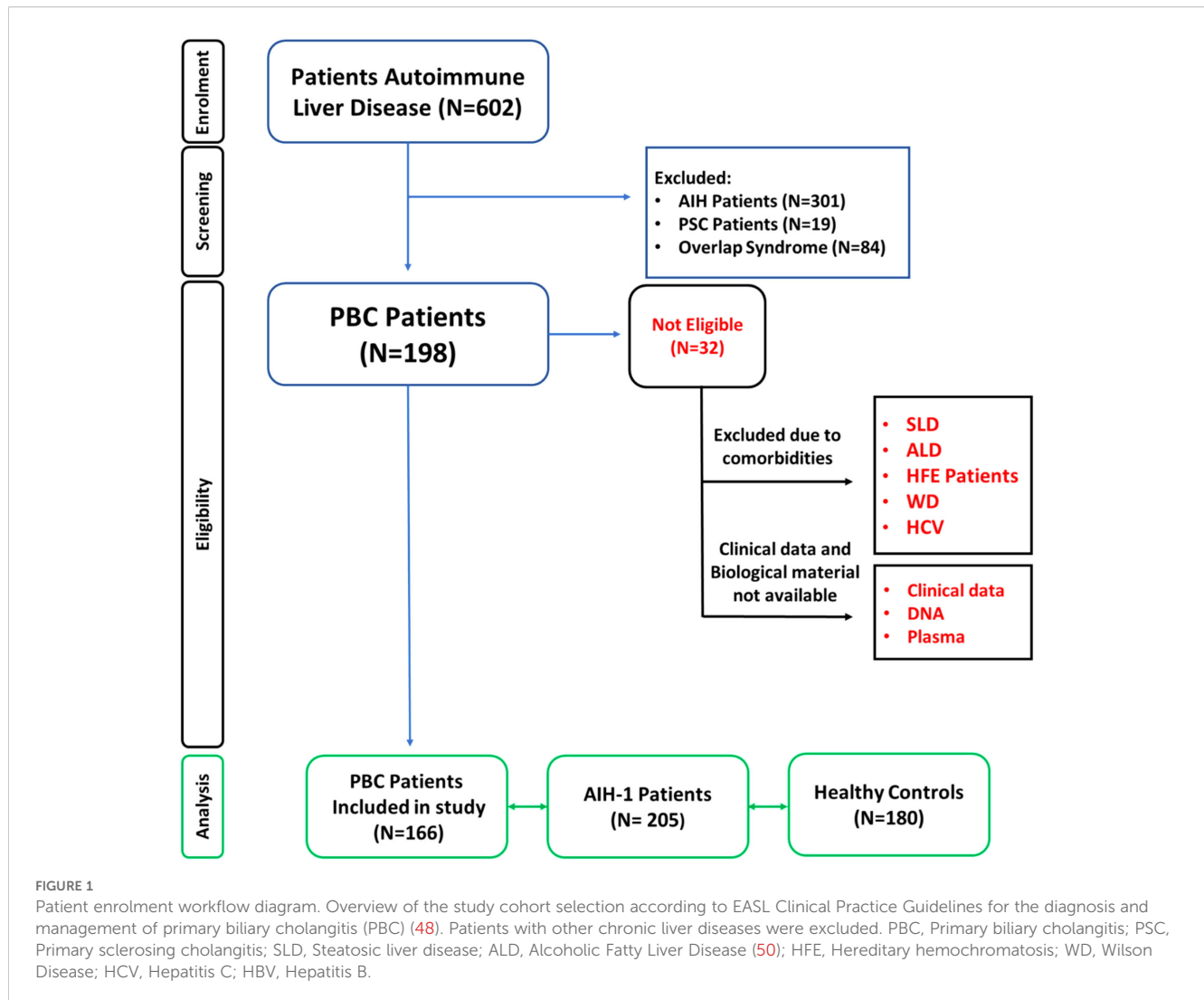
Written informed consent was obtained from each patient or healthy subject included in the study, following the institutional and national ethical standards of the local human research committee. The study protocol, including informed consent procedures, conforms to the ethical guidelines of the Declaration of Helsinki and was approved by the responsible ethics committee (Ethics Committee of the Cagliari University Hospital; date of approval: January 23, 2014; protocol number NP/2014/456).

2.3 DNA extraction and HLA typing

Genomic DNA was extracted from peripheral blood mononuclear cells according to standard methods. Patients and controls were typed at high-resolution for the alleles at the *HLA-A*, *-B*, *-C* *-DR* loci and other 13 *HLA* loci (including *HLA-G*) using a Next-Generation Sequencing (NGS) method. AlloSeq Tx17 (CareDx). *HLA-G* 3'UTR were sequenced using V3 flow cells for 300 cycle paired-end in a MiSeq platform (Illumina). The *HLA-G* gene region extending from positions -1550 to 3404, relative to the start codon, was analyzed using long-range PCR (49). The automatically generated FASTQ files were then processed with the MiSeq Reporter v2.6 for alignment and variant calling, and VariantStudio Software v3.0 for variant classification (Illumina, Netherlands).

2.4 Soluble HLA-G plasma quantification

Plasma samples were obtained from 166 PBC patients, 180 controls and 205 AIH patients at the time of enrolment. Levels of sHLA-G were determined by the sHLA-G ELISA assay kit (Exbio, Prague, Czech Republic) according to the manufacturer's instructions. This kit detects both shedding *HLA-G-1* and soluble *HLA-G5* molecules. Briefly, plasma samples were immediately frozen after separation and stored at -80°C until use. Fifty μl of each sample were diluted 1:80 in the plasma-specific buffer prior to running the *HLA-G* assay. A six-point calibration curve was obtained using the human native *HLA-G* protein supplied with the kit. A microplate reader with a 450 nm filter was used to measure the optical density at the end of the reaction. The limit of sensitivity was 0.6 U/ml. All samples were assayed in duplicate.



2.5 Statistical analysis

Descriptive statistics for clinical and biochemical characteristics of PBC patients were reported as means with standard deviations (SD) for continuous variables, and as proportions for categorical variables. For all variables, 95% confidence intervals (CI) were provided. Group comparisons were conducted using the Student's t-test for continuous data and Fisher's exact test for categorical data, depending on the data distribution and sample size. Associations between *HLA-G* alleles or *HLA-G* 3'UTR haplotypes and different groups (PBC vs. healthy controls or AIH patients) were assessed using Fisher's exact test. In particular, to correct for multiple comparisons involving *HLA-G* 3'UTR haplotypes, P-values were adjusted using the Bonferroni method, and only those with adjusted P-values (P_c) below 0.05 were considered as statistically significant.

Plasma concentrations of soluble HLA-G across PBC, AIH, and healthy control groups were summarized using means and 95% CIs. Moreover, violin plots were used to visualize the distribution of values, incorporating boxplots to display medians and interquartile ranges. Analyses also included stratification by UTR-1 haplotype

status. Differences between groups were tested using the Student's t-test, along with corresponding 95% CIs. All statistical analyses were carried out using R (version 4.3.2; R Core Team, 2023) (<https://www.R-project.org/>).

2.6 Patient selective algorithm

From 2014 to 2023 a cohort of 166 outpatients with PBC were enrolled from central-southern Sardinia who were referred to the Liver Unit of University Hospital of Cagliari. The diagnosis was made in accordance with EASL Clinical Practice Guidelines for the diagnosis and management of patients with primary biliary cholangitis (e.g., elevation of ALP, AMA at a titre >1:40, specific ANA immunofluorescence, or histopathological features of PBC when biopsy is performed) (49).

Patients with autoimmune hepatitis, overlap syndrome, and primary sclerosing cholangitis were excluded from the study to avoid bias. Additionally, individuals with chronic liver disorders caused by drug or alcohol abuse, fatty liver diseases, and metabolic

TABLE 1 Clinical, biochemical and therapeutic characteristics of PBC patients.

Characteristic	Total patients (166)	Non/partial responders (63)	Responders (103)	P value	OR (95% CI) or χ^2 - χ^2_1 [§]
Gender: n (%)					
Female	147 (0.886)	55 (0.873)	92 (0.893)	0.803	0.82 (0.28 - 2.51)
Male	19 (0.114)	8 (0.127)	11 (0.107)	0.803	1.22 (0.40 - 3.55)
Clinical characteristics, mean \pm SD					
Age (yr)	67.8 \pm 12.9	66.2 \pm 5.7	68.8 \pm 14.1	0.165	-2.6 (-6.3; 1.1)
Age at diagnosis (yr)	55.8 \pm 7.1	52.9 \pm 4.9	57.6 \pm 8.2	6.0·10⁻⁵	-4.7 (-7.0; -2.4)
AST level (IU/L)	25.9 \pm 8.7	29.8 \pm 9.2	23.51 \pm 7.8	5.2·10⁻⁶	6.3 (3.7; 8.9)
ALT level (IU/L)	25.8 \pm 12	33.2 \pm 14.1	21.3 \pm 10.7	5.7·10⁻⁹	11.9 (8.1; 15.7)
GGT level (IU/L)*	61.0 \pm 56.3	91.2 \pm 33.9	42.5 \pm 32.1	1.2·10⁻¹⁵	48.7 (38.3; 59.1)
ALP level (IU/L)^	115.9 \pm 57	152.7 \pm 21.2	93.4 \pm 37.4	< 2.2·10⁻¹⁶	59.3 (49.1; 69.5)
Bilirubin (mg/dL)	2.5 \pm 2.1	0.83 \pm 0.3	3.52 \pm 2.7	4.6·10⁻¹³	-2.69 (-3.36; -2.02)
Albumin (g/dL)	3.9 \pm 0.4	3.87 \pm 0.5	3.92 \pm 0.3	0.421	-0.05 (-0.17; 0.07)
γ -globulin (g/dL)	1.01 \pm 0.03	1.39 \pm 0.08	0.77 \pm 0.02	< 2.2·10⁻¹⁶	0.62 (0.60; 0.64)
PT-INR	1.2 \pm 0.4	1.06 \pm 0.12	1.29 \pm 0.30	3.3·10⁻⁸	-0.23 (-0.31; -0.15)
Platelets (x10 ³ /ul)	241 \pm 83	239 \pm 122	243 \pm 95	0.814	-4 (-37; 29)
Autoimmune diseases, n (%)	58 (0.349)	24 (0.381)	34 (0.330)	0.508	1.25 (0.61 - 2.52)
Immunological characteristics n (%)					
ANA positivity	84 (0.506)	32 (0.508)	52 (0.505)	1	1.01 (0.52 - 1.99)
AMA positivity	123 (0.741)	44 (0.698)	79 (0.767)	0.364	0.71 (0.33 - 1.52)
sHLA-G U/mL, mean (95% CI)	9.10 \pm 6.20	8.67 \pm 7.11	13.81 \pm 12.36	0.003	-5.14 (-8.51; -1.77)
Therapeutic characteristics, n (%)					
Ursodeoxycholic acid therapy	165 (0.994)	63 (100)	102 (0.990)	1	> 0.016
Obeticholic acid therapy	13 (0.078)	13 (0.206)	0 (0)	1.4·10⁻⁶	> 5.903

[§]Mean differences (for continuous variables): χ^2 (non-responders)- χ^2_1 (responders).

[^]In our laboratory center the normal levels for GGT and ALP were in a range between 10–45 IU/L and 40–95 IU/L respectively, P-value calculated comparing responders vs non-responders. ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; GGT, Gamma-Glutamyl Transferase; PT-INR: Prothrombin Time - International Normalized Ratio; AMA, Antimitochondrial Antibodies; ANA, Antinuclear Antibodies; sHLA-G, Soluble Human Leukocyte Antigen G; OR, Odds Ratio; CI, Confidence Interval. Bold values indicate statistically significant results ($P < 0.05$ or $P_c < 0.05$).

or genetic disorders were excluded. Patients enrolled in the study tested negative for hepatitis B surface antigen (HbsAg), anti-hepatitis A virus IgM, anti-hepatitis C virus IgG antibody and anti-hepatitis D virus IgG antibody (Figure 1).

2.7 Clinical

The clinical, immunological and therapeutic characteristics of 166 PBC patients are shown in Table 1. The mean age at diagnosis was 55.8 \pm 7.1 years and 147 patients (89%) were females. Most of the patients (74%) had high titres of anti-mitochondrial antibodies (AMA), detected either alone or in combination with antinuclear antibodies (ANA) in approximately half of the patients (51%). More than one-third of the patients (35%) presented one or more associated autoimmune diseases, including Hashimoto's

thyroiditis, Sjogren syndrome, type 1 diabetes, rheumatoid arthritis and other autoimmune diseases.

At the latest clinical follow-up, the mean alkaline phosphatase (ALP) level was 115.9 \pm 57 IU/L and the mean gamma glutamyl transferase (GGT) level was 61 \pm 56.3 IU/L. In relation to cytolytic markers, the mean AST and ALT levels were 25.9 \pm 8.7 IU/L and 25.8 \pm 12 IU/L, respectively. Finally, the mean level of soluble HLA-G (sHLA-G) was 9.1 (3.5 – 22.3) U/mL (Table 1).

Subsequently, we assessed whether the presence of additional autoimmune conditions influenced sHLA-G levels in PBC patients. A subgroup analysis comparing patients with ($n = 58$) and without ($n = 108$) coexisting autoimmune diseases showed no statistically significant difference in sHLA-G concentrations [mean (95% CI): 10.14 (8.15 – 11.73) U/mL vs 8.14 (7.05 – 9.22) U/mL respectively; median (IQR): 8.08 (3.70 to 13.87) U/mL vs 6.28 (2.34 to 10.21) U/mL respectively; $P = 0.117$] (Supplementary Figure S2).

Finally, exploring the therapeutic approaches, nearly all of the patients (99%) had been treated with ursodeoxycholic acid, either alone or in combination with obeticholic acid (8%). One patient had discontinued UDCA therapy prior to sampling due to voluntary withdrawal. However, this individual showed no clinical flares or biochemical worsening during intermediate follow-up and maintained a stable disease course over 12 months. Therefore, the patient was included in the analysis and classified as a responder according to standard biochemical response criteria.

Instead, more than one-third of the patients (38%) were non-responders or partial responders to therapy. Among the laboratory markers, ALP and GGT were the most discordant parameters between the two patient groups [152.7 ± 21.2 IU/L vs 93.4 ± 37.4 IU/L; $x_2 - x_1 = 59.3$ (49.1; 69.5) IU/L; $P < 2.2 \times 10^{-16}$ and 91.2 ± 33.9 IU/L vs 42.5 ± 32.1 IU/L; $x_2 - x_1 = 48.7$ (38.3; 59.1) IU/L; $P = 1.2 \times 10^{-15}$, respectively] (Table 1).

Poor or partial responder patients exhibited clinical manifestations at a significantly younger age compared to the responder patient group 52.9 ± 4.9 yr vs 57.6 ± 8.2 yr respectively; $x_2 - x_1 = -4.7$ (-7.0; -2.4) yr; $P = 6.0 \times 10^{-5}$. It is also interesting to observe that the sHLA-G levels in these patients were on average lower compared to those who responded positively to UDCA therapy [8.67 ± 7.11 U/mL vs 13.81 ± 12.36 U/mL respectively; $x_2 - x_1 = -5.14$ (-8.51; -1.77) U/mL; $P = 0.003$] (Table 1).

3 Results

3.1 Genetic analysis

3.1.1 Comparison of HLA-G 3'UTR haplotype frequencies between PBC patients and controls

Table 2 shows 3'UTR haplotypes frequencies among 166 PBC patients and 180 healthy controls. The most prevalent haplotypes observed in both groups were: *UTR-1*, *UTR-2*, *UTR-5*, *UTR-3* and

UTR-7 [160/332 (48.2%), 36/332 (10.8%), 49/332 (14.8%), 35/332 (10.5%) and 32/332 (9.6%) in PBC patients vs 123/360 (34.3%), 92/360 (25.5%), 58/360 (15.9%), 30/360 (8.1%) and 24/360 (6.7%) in controls].

Interestingly, the *UTR-1* haplotype showed a significantly higher frequency in PBC patients than in controls [160/332 (48.2%) vs 123/360 (34.3%) respectively; OR = 1.79 (95% CI 1.32 – 2.44); $P = 2.0 \times 10^{-4}$; $P_c = 0.0018$].

On the other hand, the *UTR-2* haplotype was significantly less frequent in PBC patients than in control population [36/332 (10.8%) vs 92/360 (25.5%); OR = 0.35 (95% CI 0.23 – 0.54); $P = 4.9 \times 10^{-7}$; $P_c = 4.4 \times 10^{-6}$]. When comparing the frequencies of other *UTRs* between the PBC patient group and the control group, no statistical difference was observed.

3.1.2 Comparison of HLA-G alleles frequencies between PBC patients and controls

The evaluation of *HLA-G* alleles and 3'UTR haplotype frequencies between 166 PBC patients and 180 healthy controls has been detailed in Table 3. Analysis of the extended haplotypes (*HLA-G* alleles and 3'UTR haplotypes) revealed some notable differences in frequencies between the patients and the healthy controls. The most frequent extended haplotypes in both groups were *HLA-G*01:01:01:01/UTR-1*, *HLA-G*01:01:01:08/UTR-1*, *HLA-G*01:03:01:02/UTR-5*, *HLA-G*01:01:03:03/UTR-7* and *HLA-G*01:01:02:01/UTR-2* [78/332 (23.5%), 77/332 (23.2%), 47/332 (14.2%), 32/332 (9.6%) and 18/332 (5.4%) in PBC patients vs 77/360 (21.4%), 45/360 (12.5%), 55/360 (15.3%), 24/360 (6.7%) and 43/360 (11.9%) in controls]. The most relevant difference was observed in the frequencies of extended haplotype *HLA-G*01:01:01:08/UTR-1* that was significantly more present in PBC patients than in controls [77/332 (23.2%) vs 45/360 (12.5%); OR = 2.11 (95% CI 1.41 – 3.16); $P = 3.0 \times 10^{-4}$; $P_c = 0.008$]. In contrast, the extended haplotype *HLA-G*01:01:02:01/UTR-2* exhibited a significantly lower frequency in patients compared to the controls; however, this association did not remain statistically significant after

TABLE 2 Haplotype frequencies observed at the *HLA-G* 3' UTR polymorphic sites (14bp Ins/Del, 3003C/T, 3010C/G, 3027A/C, 3035C/T, 3142C/G, 3187A/G, 3196C/G) in healthy controls and PBC patients.

HLA-G 3'UTR	180 Controls		166 PBC		Controls vs patients		
Haplotypes	2N=360	%	2N=332	%	P	OR (95% CI)	P _c
<i>UTR-1</i> (DelTGCCCGC)	123	0.3429	160	0.482	2.0–10⁻⁴	1.792 (1.320 – 2.435)	0.0018
<i>UTR-2</i> (InsTCCCGAG)	92	0.2548	36	0.108	4.9–10⁻⁷	0.354 (0.233 – 0.539)	4.4–10⁻⁶
<i>UTR-5</i> (InsTCCTGAC)	58	0.1595	49	0.148	0.674	0.902 (0.596 – 1.363)	1
<i>UTR-3</i> (DelTCCCGAC)	30	0.081	35	0.105	0.362	1.296 (0.777 – 2.164)	1
<i>UTR-7</i> (InsTCATGAC)	24	0.0667	32	0.096	0.165	1.493 (0.860 – 2.593)	1
<i>UTR-4</i> (DelCGCCAC)	23	0.0619	18	0.054	0.631	0.840 (0.445 – 1.586)	1
<i>UTR-18</i> (DelTGCCAC)	4	0.0167	0	0	0.031	0 (0.000 – 0.914)	0.279
<i>UTR-10</i> (DelTCCCGAG)	4	0.0119	1	0.003	0.375	0.269 (0.030 – 2.418)	1
<i>UTR-6</i> (DelTGCCAC)	2	0.0048	1	0.003	1	0.541 (0.049 – 5.992)	1

% = allele frequencies expressed as decimals. OR, Odds Ratio; CI, Confidence Interval. Bold values indicate statistically significant results ($P < 0.05$ or $P_c < 0.05$).

TABLE 3 Extended haplotypes (*HLA-G* alleles and 3'UTR haplotypes) frequencies in population controls and PBC patients.

Extended haplotypes		180 Controls		166 PBC patients		Controls vs Patients		
Alleles	3'UTR haplotypes	2N = 360	%	2N = 332	%	P value	OR (95% CI)	P _c
G*01:01:01:01	UTR-1	77	0.214	78	0.235	0.524	1.129 (0.789 – 1.614)	1
G*01:03:01:02	UTR-5	55	0.153	47	0.142	0.748	0.915 (0.600 – 1.394)	1
G*01:01:02:01	UTR-2	43	0.119	18	0.054	0.003	0.423 (0.239 – 0.749)	0.075
G*01:01:03:03	UTR-7	24	0.067	32	0.096	0.165	1.493 (0.860 – 2.593)	1
G*01:01:01:08	UTR-1	45	0.125	77	0.232	3.0–10⁻⁴	2.114 (1.412 – 3.163)	0.008
G*01:01:22:01	UTR-2	22	0.061	8	0.024	0.024	0.279 (0.167 – 0.864)	0.600
G*01:04:01:01	UTR-3	20	0.056	24	0.072	0.436	1.325 (0.718 – 2.446)	1
G*01:01:01:05	UTR-4	18	0.050	16	0.048	1	0.962 (0.482 – 1.919)	1
G*01:06:01:01	UTR-2	13	0.036	3	0.009	0.021	0.243 (0.069 – 0.862)	0.525
G*01:05N	UTR-2	7	0.019	4	0.012	0.549	0.615 (0.178 – 2.120)	1
G*01:04:04	UTR-3	6	0.017	11	0.033	0.219	2.022 (0.739 – 5.530)	1
G*01:01:01:04	UTR-18	4	0.011	0	0.000	0.125	0 (0.000 – 1.638)	1
G*01:04:01:01	UTR-10	4	0.011	1	0.003	0.375	0.269 (0.030 – 2.418)	1
G*01:06:01:02	UTR-2	3	0.008	1	0.003	0.625	0.360 (0.037 – 3.473)	1
G*01:01:01:06	UTR-4	3	0.008	1	0.003	0.625	0.360 (0.037 – 3.473)	1
G*01:04:01:02	UTR-3	3	0.008	0	0.000	0.250	0 (0.000 – 2.620)	1
G*01:03:01:01	UTR-5	3	0.008	2	0.006	1	0.721 (0.120 – 4.343)	1
G*01:01:01:01	UTR-4	2	0.006	1	0.003	1	0.541 (0.049 – 5.992)	1
G*01:01:01:04	UTR-6	2	0.006	1	0.003	1	0.541 (0.049 – 5.992)	1
G*01:01:01:06	UTR-2	2	0.006	1	0.003	1	0.541 (0.049 – 5.992)	1
G*01:06:02:02	UTR-2	1	0.003	0	0.000	1	0 (0.000 – 42.290)	1
G*01:01:01:01	UTR-3	1	0.003	0	0.000	1	0 (0.000 – 42.290)	1
G*01:02:02	UTR-2	1	0.003	0	0.000	1	0 (0.000 – 42.290)	1
G*01:01:01:09	UTR-1	1	0.003	5	0.015	0.110	5.489 (0.638 – 47.231)	1
G*01:01:01:08	UTR-2	0	0.000	1	0.003	0.480	> 0.028	1

% = allele frequencies expressed as decimals. OR, Odds Ratio; CI, Confidence Interval. Bold values indicate statistically significant results (P < 0.05 or P_c < 0.05).

correction for multiple testing [18/332 (5.4%) vs 43/360 (11.9%); OR = 0.42 (95% CI 0.24 – 0.75); P = 0.003; P_c=0.075]. The prevalence of the extended haplotype *HLA-G*01:03:01:02/UTR-5* was approximately similar in PBC patients and controls [47/332 (14.2%) vs 55/360 (15.3%); OR = 0.92 (95% CI 0.60 – 1.39); P = 0.748; P_c= 1.00]. The occurrences of additional extended haplotypes were below 3%, giving a statistical assessment of the disparities between the two groups not achievable.

3.1.3 Comparison of HLA-G 3'UTR haplotype frequencies between PBC and AIH patients

The *UTR-1* haplotype was the most prevalent among both PBC and AIH patients, with frequencies of 48.2% (160 out of 332) and 40.2% (165 out of 410) respectively (Table 4). Although a statistical significance was not reached [OR = 1.38 (95% CI 1.03 – 1.85);

P=0.031; P_c=0.248]. Similarly, *UTR-5* haplotype showed a higher prevalence in PBC patients compared to those with AIH (14.8% (49/332) and 8.5% (35/410) respectively). However, once more, no significant difference was observed between the two groups [OR = 1.86 (95% CI 1.17 – 2.94); P=0.010; P_c = 0.080]. Conversely, the *UTR-2* haplotype is significantly less represented in PBC patients compared to those with AIH [10.8% (36/332) and 25.6% (105/410) respectively, OR = 0.35 (95% CI 0.23 – 0.53); P = 2.9 x 10⁻⁷; P_c = 2.3x10⁻⁶].

3.1.4 Comparison of HLA-G alleles frequencies between PBC and AIH patients

Table 5 outlines the comparison of *HLA-G* alleles and 3'UTR haplotype frequencies between 166 PBC patients and 205 AIH patients. In this comparison as well, the most significant difference

TABLE 4 Haplotype frequencies observed at the *HLA-G 3'UTR* polymorphic sites (14bp Ins/Del, 3003C/T, 3010CG, 3027A/C, 3035C/T, 3142C/G, 3187A/G, 3196C/G) in PBC and AIH type 1 patients.

HLA-G 3'UTR haplotypes	166 PBC patients 2N=332		205 AIH patients 2N=410		PBC Patients vs AIH Patients		
	2N	%	2N	%	P	OR (95% CI)	P _c
<i>UTR-1 (DelTGCCCCG)</i>	160	0.482	165	0.402	0.031	1.381 (1.031 – 1.850)	0.248
<i>UTR-5 (InsTCCTGAC)</i>	49	0.148	35	0.085	0.010	1.855 (1.171 – 2.940)	0.080
<i>UTR-2 (InsTCCCGAG)</i>	36	0.108	105	0.256	2.9–10⁻⁷	0.353 (0.234 – 0.533)	2.3–10⁻⁶
<i>UTR-3 (DelTCCCGAC)</i>	35	0.105	30	0.073	0.150	1.493 (0.896 – 2.488)	1
<i>UTR-7 (InsTCATGAC)</i>	32	0.096	25	0.061	0.095	1.643 (0.953 – 2.832)	0.760
<i>UTR-4 (DelCGCCAC)</i>	18	0.054	35	0.085	0.115	0.614 (0.341 – 1.106)	0.920
<i>UTR-10 (DelTCCCGAG)</i>	1	0.003	5	0.012	0.232	0.245 (0.028 – 2.105)	1
<i>UTR-6 (DelTGCCAC)</i>	1	0.003	10	0.024	0.028	0.121 (0.015 – 0.949)	0.224

2N = number of HLA-G 3'UTR haplotypes. % = allele frequencies expressed as decimals. OR, Odds Ratio; CI, Confidence Interval. Bold values indicate statistically significant results (P < 0.05 or P_c < 0.05).

TABLE 5 Extended haplotypes (HLA-G alleles and 3'UTR haplotypes) frequencies in PBC and AIH type 1 patients.

Extended haplotypes		166 PBC patients, 2N = 332		205 AIH patients, 2N = 410		PBC Patients vs AIH Patients		
Alleles	3'UTR haplotypes	2N	f (%)	2N	f (%)	P value	OR* (95% CI)	P _c
<i>HLA-G*01:01:01:01</i>	<i>UTR-1</i>	78	0.235	138	0.337	0.003	0.605 (0.437 – 0.839)	0.066
<i>HLA-G*01:01:01:08</i>	<i>UTR-1</i>	77	0.232	27	0.066	1.2–10⁻¹⁰	4.275 (2.641 – 7.098)	2.6–10⁻⁹
<i>HLA-G*01:03:01:02</i>	<i>UTR-5</i>	47	0.142	35	0.085	0.018	1.767 (1.111 – 2.810)	0.396
<i>HLA-G*01:01:03:03</i>	<i>UTR-7</i>	32	0.096	25	0.061	0.095	1.643 (0.953 – 2.832)	1
<i>HLA-G*01:04:01:01</i>	<i>UTR-3</i>	24	0.072	26	0.063	0.661	1.151 (0.648 – 2.045)	1
<i>HLA-G*01:01:02:01</i>	<i>UTR-2</i>	18	0.054	65	0.159	6.1–10⁻⁶	0.304 (0.177 – 0.524)	1.3–10⁻⁴
<i>HLA-G*01:01:01:05</i>	<i>UTR-4</i>	16	0.048	35	0.085	0.057	0.542 (0.295 – 0.999)	1
<i>HLA-G*01:04:04</i>	<i>UTR-3</i>	11	0.033	4	0.010	0.034	3.478 (1.097 – 11.025)	0.748
<i>HLA-G*01:01:22:01</i>	<i>UTR-2</i>	8	0.024	5	0.012	0.266	2.000 (0.648 – 6.172)	1
<i>HLA-G*01:01:01:09</i>	<i>UTR-1</i>	5	0.015	0	0.000	0.018	> 1.139	0.396
<i>HLA-G*01:05N</i>	<i>UTR-2</i>	4	0.012	10	0.024	0.282	0.488 (0.152 – 1.570)	1
<i>HLA-G*01:06:01:01</i>	<i>UTR-2</i>	3	0.009	15	0.037	0.016	0.240 (0.069 – 0.837)	0.352
<i>HLA-G*01:03:01:01</i>	<i>UTR-5</i>	2	0.006	0	0.000	0.200	> 0.232	1
<i>HLA-G*01:04:01:01</i>	<i>UTR-10</i>	1	0.003	2	0.005	1	0.616 (0.056 – 6.872)	1
<i>HLA-G*01:06:01:02</i>	<i>UTR-2</i>	1	0.003	3	0.007	0.632	0.410 (0.042 – 3.959)	1
<i>HLA-G*01:01:01:06</i>	<i>UTR-4</i>	1	0.003	0	0.000	0.447	> 0.032	1
<i>HLA-G*01:01:01:01</i>	<i>UTR-4</i>	1	0.003	0	0.000	0.447	> 0.032	1
<i>HLA-G*01:01:01:04</i>	<i>UTR-6</i>	1	0.003	10	0.024	0.028	0.121 (0.015 – 0.949)	0.616
<i>HLA-G*01:01:01:06</i>	<i>UTR-2</i>	1	0.003	1	0.002	1	1.236 (0.077 – 19.830)	1
<i>HLA-G*01:06:02:02</i>	<i>UTR-2</i>	1	0.000	2	0.005	0.505	0 (0.000 – 6.575)	1
<i>HLA-G*01:04:01:01</i>	<i>UTR-2</i>	0	0.000	4	0.010	0.132	0 (0.000 – 1.866)	1
<i>HLA-G*01:01:22:01</i>	<i>UTR-10</i>	0	0.000	3	0.007	0.257	0 (0.000 – 2.986)	1

2N = number of extended haplotypes. % = allele frequencies expressed as decimals. OR, Odds Ratio; CI, Confidence Interval. Bold values indicate statistically significant results (P < 0.05 or P_c < 0.05).

in frequencies was observed in the extended haplotype *HLA-G*01:01:01:08/UTR-1*, which exhibited a notably higher presence in PBC patients compared to AIH patients [77/332 (23.2%) vs 27/410 (6.6%); OR=4.28 (95% CI 2.64 – 7.10); $P = 1.2 \times 10^{-10}$; $P_c = 2.6 \times 10^{-9}$]. In contrast, *HLA-G*01:01:01:01/UTR-1* was statistically significantly less present in PBC than in AIH patients [78/332 (23.5%) vs 138/410 (33.7%); OR=0.61 (95% CI 0.44 – 0.84); $P = 0.003$]; however, the association did not remain statistically significant after correction for multiple testing ($P_c = 0.066$). Among the most frequent extended haplotypes (>3%), stands out the low frequency of *HLA-G*01:01:02:01/UTR-2* in PBC compared to those in AIH-1 patients [18/332 (5.4%) vs 65/410 (15.9%); OR = 0.30 (95% CI 0.18 – 0.52); $P = 6.1 \times 10^{-6}$; $P_c = 1.3 \times 10^{-4}$]. Another interesting observation is the increased frequency of two extended haplotypes, *HLA-G*01:03:01:02/UTR-5* and *HLA-G*01:01:03:03/UTR-7*, in PBC patients compared to AIH-1 patients. However, only *HLA-G*01:03:01:02/UTR-5* showed a statistically significant difference at the nominal level [47/332 (14.2%) vs 35/410 (8.5%); OR = 1.77 (95% CI 1.11–2.81); $P = 0.018$], which did not remain significant after correction for multiple testing ($P_c = 0.396$). The *HLA-G*01:01:03:03/UTR-7* haplotype, also showed a higher frequency in PBC patients, though without reaching statistical significance [32/332 (9.6%) vs 25/410 (6.1%); OR = 1.64 (95% CI 0.95–2.83); $P = 0.095$; $P_c = 1.00$]. Frequencies of other extended haplotypes were less than 3%, showing no significant differences between the two groups (PBC and AIH-1 patients).

3.2 Soluble HLA-G dosage

3.2.1 Comparison sHLA-G between PBC and population control group

Soluble HLA-G (sHLA-G) levels were measured in patients and healthy controls at the time of enrolment. The levels of sHLA-G were significantly lower in PBC patients than in controls [mean (95% CI): 9.10 (8.15 – 10.05) U/mL vs 24.03 (19.08 – 28.98) U/mL respectively; $P < 0.0001$; median (IQR): 7.98 (3.73 to 11.24) U/mL vs 20.73 (12.64 to 25.80) U/mL respectively] (Figure 2A). In addition, both patients and controls were divided according to the *HLA-G* 3'UTR haplotype. The only significant finding relates to the *UTR-1* haplotype. Specifically, the expression of sHLA-G was significantly lower in PBC patients compared to controls [mean (95% CI): 10.20 (8.85 – 11.55) U/mL vs 26.19 (19.00 – 33.38) U/mL respectively; $P < 0.0001$; median (IQR): 8.05 (4.81 to 13.82) U/mL vs 22.46 (12.78 to 27.54) U/mL respectively] (Figure 2B). To further explore these findings, we considered *HLA-G* alleles which are in linkage disequilibrium within the extended haplotype containing *UTR-1* (*HLA-G*01:01:01:08*, *HLA-G*01:01:01:01* and *HLA-G*01:01:01:09*) (26). The analysis indicated that patients carrying the *HLA-G*01:01:01:08* allele exhibited significantly lower levels of sHLA-G [mean (95% CI): 6.92 (5.57 – 8.27) U/mL; median (IQR): 5.29 (2.79 to 10.12) U/mL] compared to those with the *HLA-G*01:01:01:01* [mean (95% CI): 13.50 (11.10 – 15.89) U/mL; median (IQR): 10.13 (7.75 to 16.58) U/mL] and *HLA-G*01:01:01:09* [mean (95% CI):

15.36 (7.67 – 23.05) U/mL; median (IQR): 13.58 (12.58 to 21.58) U/mL] alleles [$P < 0.0001$ and $P = 0.036$ respectively] (Figure 2C).

3.2.2 Comparison sHLA-G between PBC and AIH-1 patients

We then aimed to verify if there were differences in the sHLA-G levels among patients with PBC and AIH. Comparing those two groups of patients we found significantly lower sHLA-G levels in PBC than in AIH patients [mean (95% CI): 9.10 (8.15 – 10.05) U/mL vs 13.9 (11.6 – 17.4) U/mL respectively; $P < 0.0001$; median (IQR): 7.98 (3.73 to 11.24) U/mL vs 10.52 (6.32 to 24.09) U/mL respectively] (Figure 3A). Following the previous analysis, we categorized the patients based on their *HLA-G* 3'UTR haplotypes. Once again, the only significant difference observed was associated with the *UTR-1* haplotype. Specifically, within the PBC patient group, lower levels of sHLA-G were found compared to the AIH patients [mean (95% CI): 10.20 (8.85 – 11.55) U/mL vs 11.2 (9.0 – 14.8) U/mL respectively; $P = 0.012$; median (IQR): 8.05 (4.81 to 13.82) U/mL vs 9.63 (7.47 to 12.86) U/mL respectively] (Figure 3B). Moreover, comparing PBC and AIH patients that do not carry *UTR-1*, no significant difference in HLA-G levels was found.

3.2.3 Soluble HLA-G dosage and therapy response

Another crucial aspect we aimed to explore is the correlation between sHLA-G levels and patients' response to therapy. We divided the patients into two groups based on their therapy response status (responder vs. non-responder), following the criteria described above. The analysis revealed a substantial difference between the two patient groups. Specifically, among PBC patients with an inadequate therapy response, we observed lower sHLA-G levels compared to those who responded positively to the UDCA therapy [mean (95% CI): 8.67 (6.50 – 10.08) U/mL vs 13.81 (10.65 – 15.48) U/mL respectively; $P = 0.003$; median (IQR): 9.38 (3.16 to 12.33) U/mL vs 14.35 (10.78 to 19.38) U/mL respectively] (Figure 4A). Moreover, to support these findings, we considered patients with ALP levels $> 1.5 \times \text{ULN}$ as indicative of an altered status (no responders). Figure 4B illustrates a significant difference between the two groups. Patients with ALP values above the threshold exhibited lower sHLA-G levels [mean (95% CI): 7.26 (2.7 – 29.1) U/mL vs 15.01 (6.53 – 59.53) U/mL respectively; $P = 0.0004$; median (IQR): 9.58 (3.54 to 12.39) U/mL vs 16.25 (10.78 to 22.29) U/mL respectively]. Notably, these sHLA-G levels, alongside those observed in patients carrying the *HLA-G*01:01:01:08/UTR-1* extended haplotype, were among the lowest measured in our entire cohort (Supplementary Figure S1).

Finally, we sought to examine the presence of the *HLA-G*01:01:01:08/UTR-1* extended haplotype concerning therapy response and ALP levels. As summarized in Table 6, the presence of this allele is notably higher in patients with a worse therapy response [60% vs 24.1% respectively; $P = 0.0001$], as well as in patients with ALP levels $> 1.5 \times \text{ULN}$ [51.3% vs 30.1% respectively; $P = 0.029$].

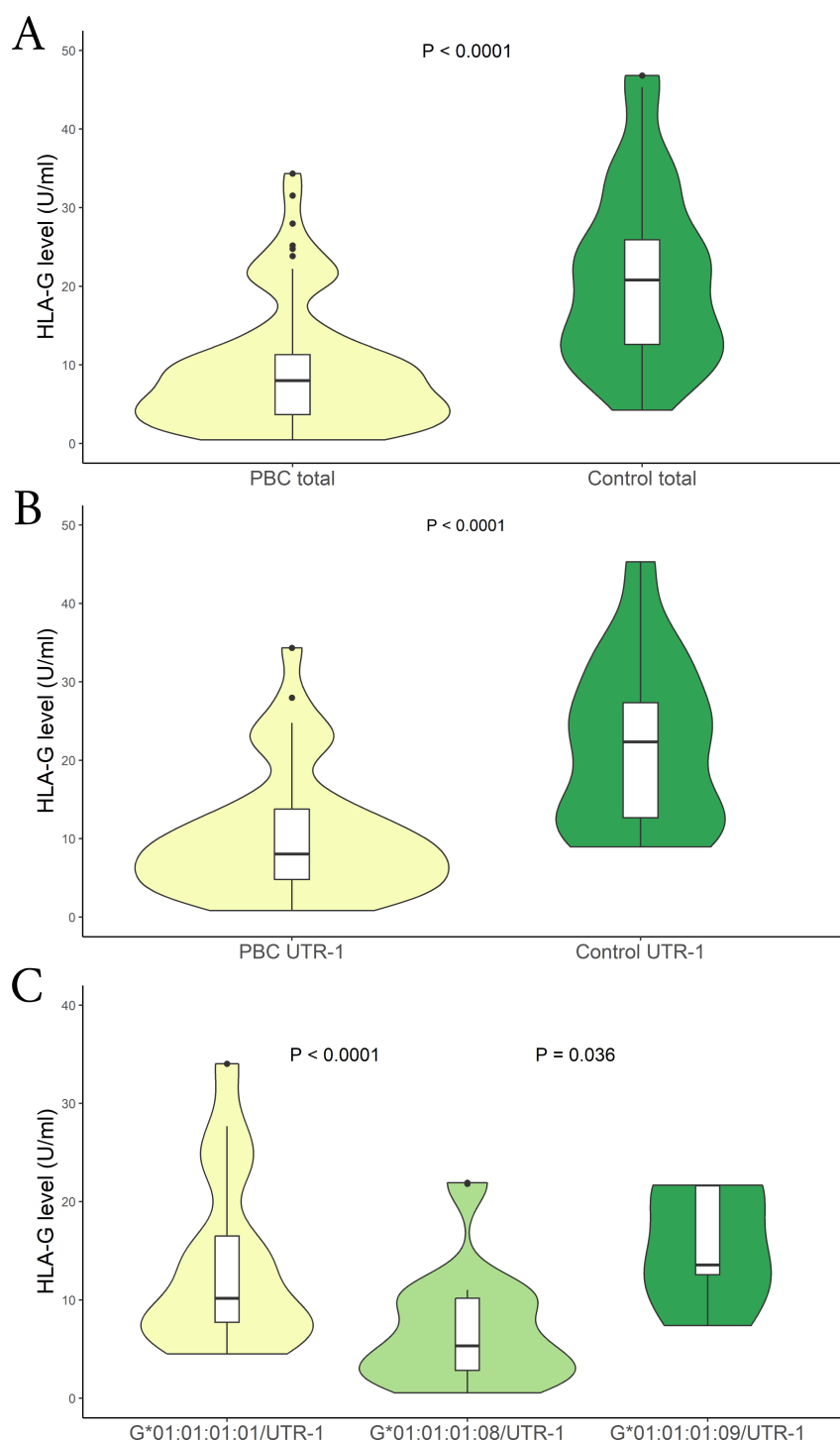


FIGURE 2

(A) Soluble HLA-G plasma levels (U/mL) were compared between in PBC patients (yellow) and control population (green). The P values reported was computed using the Student's t test. The boxplot is included in the violin to assess the median and interquartile range. (B) Soluble HLA-G plasma levels (U/mL) were compared between in PBC patients (yellow) and controls who carried the HLA-G UTR-1 haplotype (green). The P values reported was computed using the Student's t test. The boxplot is included in the violin to assess the median and interquartile range. (C) Soluble HLA-G plasma levels (U/mL) were compared between in PBC patients who carried the HLA-G*01:01:01:08/UTR-1 (light-green), G*01:01:01:01/UTR-1 (yellow) and G*01:01:01:09/UTR-1 (green) alleles combined with UTR-1. The P values reported was computed using the Student's t test. The boxplot is included in the violin to assess the median and interquartile range.

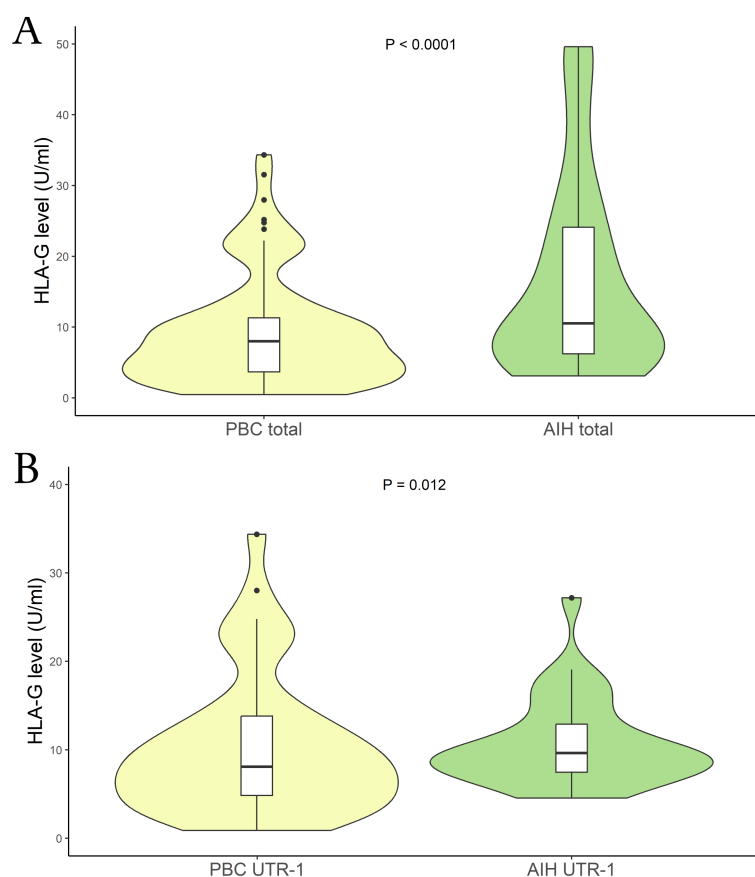


FIGURE 3

(A) Soluble HLA-G plasma levels (U/mL) were compared between in PBC (yellow) and AIH patients (light-green). The P values reported was computed using the Student's t test. The boxplot is included in the violin to assess the median and interquartile range. (B) Soluble HLA-G plasma levels (U/mL) were compared between PBC (yellow) and AIH patients who carried the HLA-G UTR-1 haplotype (light-green). The P values reported was computed using the Student's t test. The boxplot is included in the violin to assess the median and interquartile range.

4 Discussion

The Sardinian population and its genetic makeup provide a valuable research opportunity for investigating complex diseases such as primary biliary cholangitis (PBC) (51). This genetic background enables significant insights even with small sample sizes, particularly in rare autoimmune diseases. Although several studies have examined the role of HLA-G in autoimmunity (24, 38–40), no previous work has specifically investigated the genetic and molecular profile of HLA-G in PBC. Previous GWAS studies have suggested a potential correlation between HLA-G and PBC, highlighting the involvement of immune system pathways in the disease's pathogenesis (52).

Our study is the first to identify a potential correlation between HLA-G genetic variability, sHLA-G levels, and PBC pathogenesis. We found that the *UTR-1* haplotype, which is known to stabilize mRNA and produce higher levels of sHLA-G (33), was significantly more frequent in PBC patients compared to controls (48.2% vs 34.3%). However, this frequency was not significantly different between PBC and AIH-1 patients.

Despite the expected association between *UTR-1* and higher sHLA-G levels, PBC patients showed significantly lower sHLA-G concentrations than both controls and AIH-1 patients (9.1 vs 24.03

vs 13.9 U/mL, respectively). This discrepancy was especially pronounced in PBC patients carrying the *UTR-1* haplotype, suggesting that disease-specific mechanisms may override the expected genetic influence on sHLA-G expression.

HLA-G exists in both membrane-bound and soluble forms. Soluble isoforms (HLA-G5 to -G7) arise from alternative splicing, while HLA-G1 can be cleaved into soluble form by metalloproteases (MMPs) such as MMP-2 (53, 54). Both isoforms play immunomodulatory roles. This lower level of sHLA-G, particularly in comparison to healthy populations, is consistent with findings in other autoimmune diseases such as systemic sclerosis (SSc), rheumatoid arthritis, juvenile idiopathic arthritis, and systemic lupus erythematosus, suggest that low sHLA-G expression correlates with high disease activity (55). For instance, a recent study by Favoino et al. reported that patients with lower sHLA-G levels experienced more severe SSc disease (55).

The causes behind the low sHLA-G levels in PBC remain unclear. One possibility is reduced cleavage of membrane-bound HLA-G1 by MMP. Dysregulation of MMP activity in cholestatic liver injury have been discovered; experimental models of bile duct ligation show that MMP-2 and MMP-9 activity increases early and remains elevated (56). Nonetheless, higher MMP activity would predict increased sHLA-G levels, which we did not observe. A

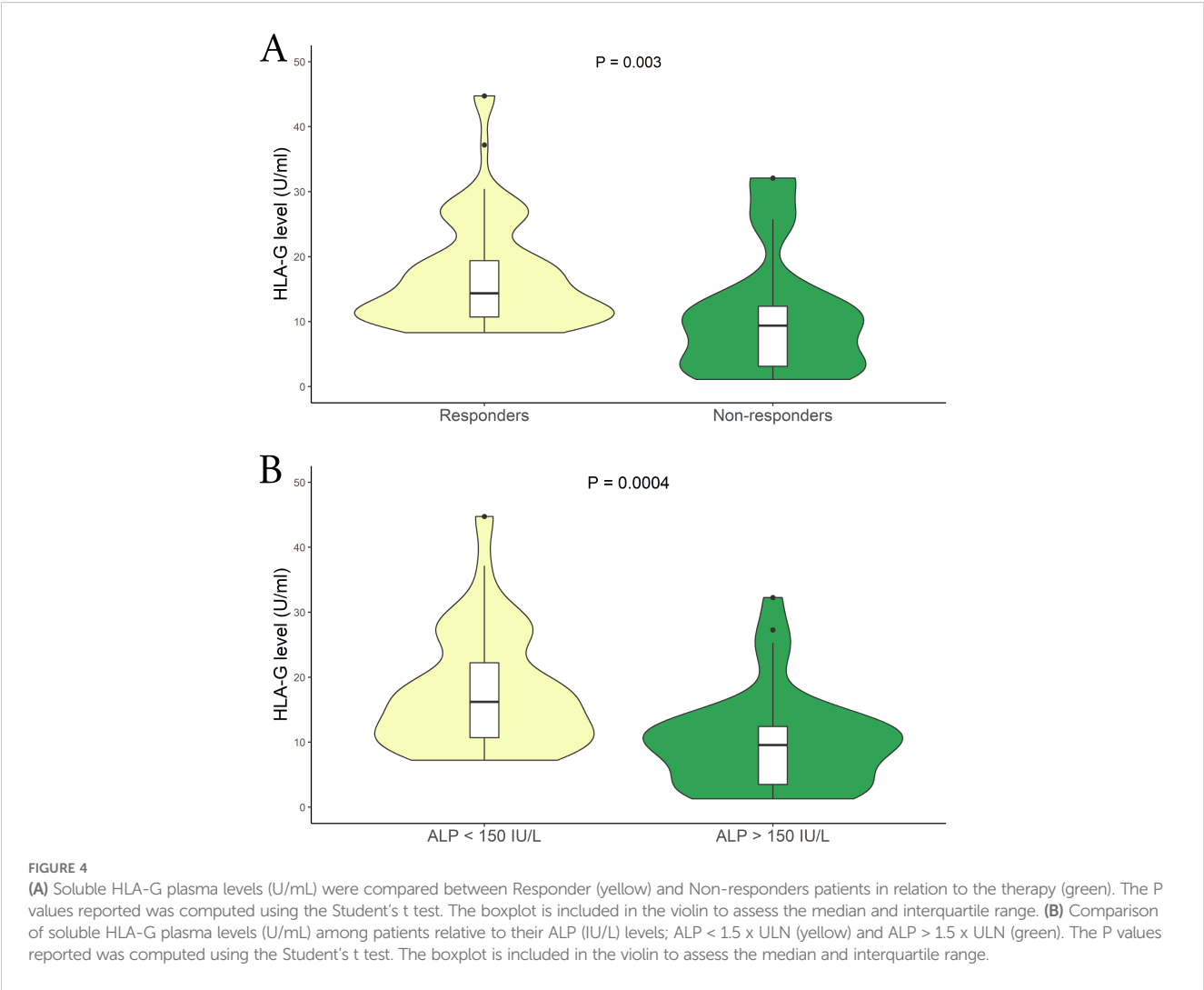


TABLE 6 Patients stratified after 1 year of UDCA therapy, based on response and serological parameters ALP and GGT.

Clinical characteristics	Categories	G*01:01:01:08/UTR-1 presence (%)	P value	OR (95% CI)
Therapy response	No Responders	60.00	1.0–10⁻⁴	4.65 (2.03 – 10.98)
	Responders	24.14		
ALP	ALP < 1.5 x ULN	30.11	0.029	0.41 (0.18 – 0.95)
	ALP > 1.5 x ULN	51.28		
GGT	GGT < 1 x ULN	33.33	0.334	0.67 (0.30 – 1.52)
	GGT > 1 x ULN	42.86		

ALP, Alkaline Phosphatase; GGT, Gamma-Glutamyl Transferase; OR, Odds Ratio; CI, Confidence Interval. Bold values indicate statistically significant results (P < 0.05).

possible explanation is the presence of functional polymorphisms in MMP genes that affect their activity (57), potentially modulating sHLA-G shedding at an individual level.

Another involves post-transcriptional regulation, including the influence of miRNAs such as *miR-148a* known to downregulate sHLA-G in the placenta of patients with intrahepatic cholestasis of pregnancy (ICP) and negatively correlates with serum total bile acid

levels (58). Given that PBC shares immunological and cholestatic features with ICP, it is plausible that similar miRNA-mediated downregulation could contribute to the unexpectedly low sHLA-G levels observed in our PBC cohort, despite the presence of the *UTR-1* haplotype associated with higher HLA-G expression.

Moreover, the role of membrane-bound HLA-G in the liver microenvironment also warrants attention. In AIH-1 and HCV-

infected livers, hepatic HLA-G expressions have been linked to histological severity and fibrosis (36, 38) possibly reflecting a mechanism of immune modulation or cellular self-protection. Although immunohistochemical data on HLA-G expression in liver tissue are currently lacking for PBC, it is plausible that individual variability in membranous HLA-G expression among hepatic immune cells could contribute to the differences in sHLA-G levels observed in plasma. Furthermore, the reduced presence of sHLA-G may reflect impaired proteolytic shedding, potentially resulting in local retention of membrane-bound HLA-G. Such retention could enhance localized immunomodulatory activity, possibly promoting an environment of sustained immune dysregulation and contributing to the increased histopathological severity observed in affected liver tissue.

Notably, in Sardinian population, the *HLA-G UTR-1* haplotype is associated with three *HLA-G* alleles: *HLA-G*01:01:01:01*, *HLA-G*01:01:01:09* and *HLA-G*01:01:01:08*, which was significantly more frequent in PBC patients compared to controls and AIH patients (0.232% vs 0.125% vs 0.066%). This allele, combined with *UTR-1*, was associated with significantly lower sHLA-G levels compared to other *UTR-1* haplotypes (*HLA-G*01:01:01:01/UTR-1* and *HLA-G*01:01:01:09/UTR-1*; $P < 0.0001$ and $P = 0.036$ respectively) (Figure 2C).

Despite encoding the full-length, functional HLA-G1 isoform, its lower soluble levels suggest that specific extended haplotypes may influence HLA-G regulation through mechanisms that extend beyond simple transcript abundance, potentially affecting post-transcriptional processes such as membrane expression dynamics or proteolytic cleavage efficiency.

The combination of high frequency and low sHLA-G levels in *HLA-G*01:01:01:08/UTR-1* carriers support its role as a genetic risk factor for PBC. Intriguingly, this haplotype was also associated with poorer response to UDCA therapy. Patients who had a good response to 12 months of UDCA therapy exhibited significantly higher sHLA-G levels compared to those with inadequate responses. Elevated alkaline phosphatase (ALP) levels ($> 1.5 \times \text{ULN}$), which indicate poorly controlled disease or progression, were inversely proportional to sHLA-G levels. Other studies have identified clear differences in both “immune” and “senescence” phenotypes between low-risk and high-risk PBC patients from the early stages of the disease. The low-risk disease appears to be characterized by cholestatic bile damage without any immune-mediated injury. Indeed, these low-risk PBC patients are well responsive to the detoxifying effects of UDCA (59). In contrast, high-risk disease is characterized by T-cell activation and apoptosis, leading to continuous bile duct damage which is not treatable by UDCA alone (59).

This distinction may relate to our findings; indeed, the *HLA-G*01:01:01:08/UTR-1* extended haplotype could help identify patients with poorer therapy responses, associated with different immune phenotypes exhibiting enhanced T-cell activity (60% vs 24.1%; $P < 1.0 \times 10^{-4}$). It is well-known that HLA-G molecules are involved in the inhibition of NK cell activity, CD4⁺ T lymphocyte responses, and dendritic cell maturation, as well as in the apoptosis

of CD8⁺ cytotoxic T cells and the development of regulatory T cells (60).

In PBC, autoreactive CD4⁺ and CD8⁺ T cells contribute to bile duct damage via recognition of mitochondrial antigens such as the E2 subunit of the pyruvate dehydrogenase complex (61). Thus, reduced sHLA-G may fail to control autoreactive T cells, perpetuating inflammation. Indeed, HLA-G has been shown to induce apoptosis in CD8⁺ T cells and to reduce the proliferation of CD4⁺ T cells and B cells (62).

Our data suggest that *HLA-G*01:01:01:08/UTR-1* may therefore support the idea that it could dysregulate the inflammatory and immune response, resulting in an increased susceptibility to the disease and its severity. Currently, no therapies specifically target HLA-G for the treatment of autoimmune and autoinflammatory diseases. However, research in experimental models, such as uveitis, has shown that increasing HLA-G levels can significantly improve clinical manifestations, indicating its potential as a therapeutic target (63).

An additional consideration is the presence of other autoimmune diseases in our cohort. PBC can frequently coexist with concomitant extrahepatic autoimmune diseases (64), potentially confounding immunological analyses and limiting the biological specificity of the cohort.

In our population, most patients with autoimmune comorbidities were affected by Hashimoto's thyroiditis (HT), one of the most common organ-specific autoimmune diseases in Sardinia (65). However, studies investigating the association between sHLA-G levels and HT remain limited and often report conflicting results (66, 67), making it unlikely that HT alone significantly influenced the findings.

Importantly, as shown in Supplementary Figure S2, the comparison of sHLA-G levels between patients with and without additional autoimmune conditions did not reveal any statistically significant differences. In addition, previous research has indicated that the presence of extrahepatic autoimmune diseases does not appear to negatively impact the long-term clinical outcomes of PBC (64). One additional aspect to consider is the absence of immunohistochemical data on liver tissue, which prevents the direct evaluation of HLA-G expression in infiltrating immune cells. Future studies integrating tissue-level analyses will be crucial to validate and further explore the mechanisms proposed in this work.

Prior research has thoroughly examined the role of HLA-G in Sardinian patients with AIH-1, showing a significant correlation between lower sHLA-G levels and increased disease severity (38). This suggests that, in addition to its role in AIH-1, HLA-G may also play a significant role in PBC. However, the mechanisms by which the *HLA-G*01:01:01:08/UTR-1* haplotype and low sHLA-G levels influence disease onset, therapy response, and progression require further exploration. In conclusion, further studies in diverse populations are essential to validate these findings.

The levels of sHLA-G, particularly those observed in individuals carrying the *HLA-G*01:01:01:08/UTR-1* haplotype, could serve as biomarkers for disease severity and therapy response in PBC. This

could lead to more personalized treatment strategies, tailoring therapies according to genetic risk profiles.

Data availability statement

The datasets presented in this study can be found in online repositories: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1191844>.

Ethics statement

The studies involving humans were approved by Ethics Committee of the Cagliari University Hospital; date of approval: January 23, 2014; protocol number NP/2014/456. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

MMi: Conceptualization, Writing – original draft. SM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. RL: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. GS: Writing – review & editing. CB: Writing – review & editing. MCo: Writing – review & editing. FP: Writing – review & editing. SD: Writing – review & editing. ML: Writing – review & editing. CM: Writing – review & editing. MMu: Writing – review & editing. CS: Writing – review & editing. AM: Writing – review & editing. FS: Writing – review & editing. ID-I: Writing – review & editing. MCa: Writing – review & editing. SL: Writing – review & editing. EG: Writing – review & editing. SC: Writing – review & editing. MMe: Writing – review & editing. Methodology, Software. TZ: Writing – review & editing. SG: Writing – original draft. AP: Writing – original draft. LC: Conceptualization, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. The research leading to

these results has received funding from the European Union -NextGenerationEU through the Italian Ministry of University and Research under PNRR -M4C2-I1.3 Project PE_00000019 “HEAL ITALIA” to AP, CUP F53C22000750006 University of Cagliari. This research activity is related to the spoke 1 and 4 of the project. The views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them.

Acknowledgments

This work is dedicated to the memory of RL, a dear friend and esteemed colleague, whose recent passing has left a profound void. His scientific insight, intellectual generosity, and unwavering support were a guiding force and an essential reference throughout the development of this study. His legacy continues to inspire our work and lives on in the values he instilled in all of us. This research was made possible through the collaboration of the volunteers of the non-profit organization “Associazione per l'Avanzamento della Ricerca sui Trapianti (AART-ODV), supported by “Fondazione di Sardegna”, grant #40974 -(2024.0015).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2025.1585535/full#supplementary-material>

References

- Beuers U, Gershwin ME, Gish RG, Invernizzi P, Jones DEJ, Lindor K, et al. Changing nomenclature for PBC: from 'cirrhosis' to 'cholangitis'. *Clinics Res Hepatol Gastroenterol*. (2015) 39:e57–9. doi: 10.1016/j.clinre.2015.08.001
- Selmi C, Bowlus CL, Gershwin ME. Primary biliary cirrhosis. *Lancet (London England)*. (2011) 377:1600–9. doi: 10.1016/S0140-6736(10)61965-4
- Hirschfield GM, Gershwin ME. The immunobiology and pathophysiology of primary biliary cirrhosis. *Annu Rev Pathol*. (2013) 8:303–30. doi: 10.1146/annurev-pathol-020712-164014
- Gazda J, Drazilova S, Janicko M, Jarcuska P. The epidemiology of primary biliary cholangitis in European countries: A systematic review and meta-analysis. *Can J Gastroenterol Hepatol*. (2021) 2021:9151525. doi: 10.1155/2021/9151525
- Marziani M, Bassanelli C, Ripellino C, Urbinati D, Alvaro D. Epidemiology of primary biliary cholangitis in Italy: Evidence from a real-world database. *Digest Liver Dis: Off J Ital Soc Gastroenterol Ital Assoc Study Liver*. (2019) 51:724–9. doi: 10.1016/j.dld.2018.11.008
- Mells GF, Pells G, Newton JL, Bathgate AJ, Burroughs AK, Heneghan MA, et al. Impact of primary biliary cirrhosis on perceived quality of life: the UK-PBC national study. *Hepatol (Baltimore Md)*. (2013) 58:273–83. doi: 10.1002/hep.26365
- Dyson JK, Wilkinson N, Jopson L, Mells G, Bathgate A, Heneghan MA, et al. The inter-relationship of symptom severity and quality of life in 2055 patients with primary biliary cholangitis. *Aliment Pharmacol Ther*. (2016) 44:1039–50. doi: 10.1111/apt.13794
- Juran BD, Lazaridis KN. Environmental factors in primary biliary cirrhosis. *Semin Liver Dis*. (2014) 34:265–72. doi: 10.1055/s-0034-1383726
- Webb GJ, Hirschfield GM. Using GWAS to identify genetic predisposition in hepatic autoimmunity. *J Autoimmun*. (2016) 66:25–39. doi: 10.1016/j.jaut.2015.08.016
- Bianchi I, Carbone M, Lleo A, Invernizzi P. Genetics and epigenetics of primary biliary cirrhosis. *Semin Liver Dis*. (2014) 34:255–64. doi: 10.1055/s-0034-1383725
- Reshetnyak VI. Primary biliary cirrhosis: Clinical and laboratory criteria for its diagnosis. *World J Gastroenterol*. (2015) 21:7683–708. doi: 10.3748/wjg.v21.i25.7683
- Jansen PLM, Ghallab A, Vartak N, Reif R, Schaap FG, Hampe J, et al. The ascending pathophysiology of cholestatic liver disease. *Hepatol (Baltimore Md)*. (2017) 65:722–38. doi: 10.1002/hep.28965
- Jones DE, Watt FE, Metcalf JV, Bassendine MF, James OF. Familial primary biliary cirrhosis reassessed: a geographically-based population study. *J Hepatol*. (1999) 30:402–7. doi: 10.1016/S0168-8278(99)80097-X
- Hirschfield GM, Liu X, Xu C, Lu Y, Xie G, Lu Y, et al. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *New Engl J Med*. (2009) 360:2544–55. doi: 10.1056/NEJMoa0810440
- Watt FE, James OFW, Jones DEJ. Patterns of autoimmunity in primary biliary cirrhosis patients and their families: a population-based cohort study. *QJM: Monthly J Assoc Physicians*. (2004) 97:397–406. doi: 10.1093/qjmed/hch078
- Alegre E, Rizzo R, Bortolotti D, Fernandez-Landázuri S, Fainardi E, González A. Some basic aspects of HLA-G biology. *J Immunol Res*. (2014) 2014:657625. doi: 10.1155/2014/657625
- Castelli EC, Ramalho J, Porto IOP, Lima THA, Felício LP, Sabbagh A, et al. Insights into HLA-G genetics provided by worldwide haplotype diversity. *Front Immunol*. (2014) 5:476. doi: 10.3389/fimmu.2014.00476
- Contini P, Murdaca G, Puppo F, Negrini S. HLA-G expressing immune cells in immune mediated diseases. *Front Immunol*. (2020) 11:1613. doi: 10.3389/fimmu.2020.01613
- LeMaout J, Yan WH. Editorial: the biological and clinical aspects of HLA-G. *Front Immunol*. (2021) 12:649344. doi: 10.3389/fimmu.2021.649344
- LeMaout J, Le Discorde M, Rouas-Freiss N, Moreau P, Menier C, McCluskey J, et al. Biology and functions of human leukocyte antigen-G in health and sickness. *Tissue Antigens*. (2003) 62:273–84. doi: 10.1034/j.1399-0039.2003.00143.x
- LeMaout J, Krawiec-Radanne I, Dausset J, Carosella ED. HLA-G1-expressing antigen-presenting cells induce immunosuppressive CD4+ T cells. *Proc Natl Acad Sci United States America*. (2004) 101:7064–9. doi: 10.1073/pnas.0401922101
- Le Rond S, Le Maout J, Créput C, Menier C, Deschamps M, Le Friec G, et al. Alloreactive CD4+ and CD8+ T cells express the immunotolerant HLA-G molecule in mixed lymphocyte reactions: *in vivo* implications in transplanted patients. *Eur J Immunol*. (2004) 34:649–60. doi: 10.1002/eji.200324266
- Sabbagh A, Luisi P, Castelli EC, Gineau L, Courtin D, Milet J, et al. Worldwide genetic variation at the 3' untranslated region of the HLA-G gene: balancing selection influencing genetic diversity. *Genes Immun*. (2014) 15:95–106. doi: 10.1038/gene.2013.67
- Rizzo R, Bortolotti D, Bolzani S, Fainardi E. HLA-G molecules in autoimmune diseases and infections. *Front Immunol*. (2014) 5:592. doi: 10.3389/fimmu.2014.00592
- Martelli-Palomino G, Pancotto JA, Muniz YC, Mendes-Junior CT, Castelli EC, Massaro JD, et al. Polymorphic sites at the 3' untranslated region of the HLA-G gene are associated with differential hla-g soluble levels in the Brazilian and French population. *PLoS One*. (2013) 8:e71742. doi: 10.1371/journal.pone.0071742
- Castelli EC, de Almeida BS, Muniz YCN, Silva NSB, Passos MRS, Souza AS, et al. HLA-G genetic diversity and evolutive aspects in worldwide populations. *Sci Rep*. (2021) 11:23070. doi: 10.1038/s41598-021-02106-4
- Xu X, Zhou Y, Wei H. Roles of HLA-G in the maternal-fetal immune microenvironment. *Front Immunol*. (2020) 11:592010. doi: 10.3389/fimmu.2020.592010
- Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, DeMars R. A class I antigen, HLA-G, expressed in human trophoblasts. *Sci (New York NY)*. (1990) 248:220–3. doi: 10.1126/science.2326636
- Melo-Lima BL, Poras I, Passos GA, Carosella ED, Donadi EA, Moreau P, et al. The Autoimmune Regulator (Aire) transactivates HLA-G gene expression in thymic epithelial cells. *Immunology*. (2019) 158:121–35. doi: 10.1111/imm.13099
- Le Discorde M, Moreau P, Sabatier P, Legeais J-M, Carosella ED. Expression of HLA-G in human cornea, an immune-privileged tissue. *Hum Immunol*. (2003) 64:1039–44. doi: 10.1016/j.humimm.2003.08.346
- Amodio G, Gregori S. HLA-G genotype/expression/disease association studies: success, hurdles, and perspectives. *Front Immunol*. (2020) 11:1178. doi: 10.3389/fimmu.2020.01178
- Cirulli V, Zalatan J, McMaster M, Prinsen R, Salomon DR, Ricordi C, et al. The class I HLA repertoire of pancreatic islets comprises the nonclassical class Ib antigen HLA-G. *Diabetes*. (2006) 55:1214–22. doi: 10.2337/db05-0731
- Amiot L, Vu N, Samson M. Biology of the immunomodulatory molecule HLA-G in human liver diseases. *J Hepatol*. (2015) 62:1430–7. doi: 10.1016/j.jhep.2015.03.007
- Souto FJD, Crispim JCO, Ferreira SC, da Silva ASM, Bassi CL, Soares CP, et al. Liver HLA-G expression is associated with multiple clinical and histopathological forms of chronic hepatitis B virus infection. *J Viral Hepatitis*. (2011) 18:102–5. doi: 10.1111/j.1365-2893.2010.01286.x
- Shi W-W, Lin A, Xu D-P, Bao W-G, Zhang J-G, Chen S-Y, et al. Plasma soluble human leukocyte antigen-G expression is a potential clinical biomarker in patients with hepatitis B virus infection. *Hum Immunol*. (2011) 72:1068–73. doi: 10.1016/j.humimm.2011.06.012
- Amiot L, Vu N, Rauch M, L'Helgoualc'h A, Chalmel F, Gascan H, et al. Expression of HLA-G by mast cells is associated with hepatitis C virus-induced liver fibrosis. *J Hepatol*. (2014) 60:245–52. doi: 10.1016/j.jhep.2013.09.006
- Weng P-J, Fu Y-M, Ding S-X, Xu D-P, Lin A, Yan W-H, et al. Elevation of plasma soluble human leukocyte antigen-G in patients with chronic hepatitis C virus infection. *Hum Immunol*. (2011) 72:406–11. doi: 10.1016/j.humimm.2011.02.008
- Littera R, Perra A, Miglianti M, Piras IS, Mocci S, Lai S, et al. The double-sided of human leukocyte antigen-G molecules in type 1 autoimmune hepatitis. *Front Immunol*. (2022) 13:1007647. doi: 10.3389/fimmu.2022.1007647
- Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: A review. *JAMA*. (2018) 320:1360–72. doi: 10.1001/jama.2018.13103
- Mohammadi N, Adib M, Alsahebhosoul F, Kazemi M, Etemadifar M. An investigation into the association between HLA-G 14 bp insertion/deletion polymorphism and multiple sclerosis susceptibility. *J Neuroimmunol*. (2016) 290:115–8. doi: 10.1016/j.jneuroim.2015.11.019
- Consiglio CR, Veit TD, Monticelo OA, Mucenic T, Xavier RM, Brenol JCT, et al. Association of the HLA-G gene +3142C>G polymorphism with systemic lupus erythematosus. *Tissue Antigens*. (2011) 77:540–5. doi: 10.1111/j.1399-0039.2011.01635.x
- Invernizzi P, Selmi C, Mackay IR, Podda M, Gershwin ME. From bases to basis: linking genetics to causation in primary biliary cirrhosis. *Clin Gastroenterol Hepatol: Off Clin Pract J Am Gastroenterol Assoc*. (2005) 3:401–10. doi: 10.1016/S1542-3565(04)00678-0
- Invernizzi P, Selmi C, Poli F, Frison S, Floreani A, Alvaro D, et al. Human leukocyte antigen polymorphisms in Italian primary biliary cirrhosis: a multicenter study of 664 patients and 1992 healthy controls. *Hepatol (Baltimore Md)*. (2008) 48:1906–12. doi: 10.1002/hep.22567
- Invernizzi P, Battezzati PM, Crosignani A, Perego F, Poli F, Morabito A, et al. Peculiar HLA polymorphisms in Italian patients with primary biliary cirrhosis. *J Hepatol*. (2003) 38:401–6. doi: 10.1016/S0168-8278(02)00440-3
- Songini M, Lombardo C. The Sardinian way to type 1 diabetes. *J Diabetes Sci Technol*. (2010) 4:1248–55. doi: 10.1177/193229681000400526
- Pugliatti M, Rosati G, Carton H, Riise T, Drulovic J, Vécsei L, et al. The epidemiology of multiple sclerosis in Europe. *Eur J Neurol*. (2006) 13:700–22. doi: 10.1111/j.1468-1331.2006.01342.x
- Contu L, Arras M, Carcassi C, La Nasa G, Mulargia M. HLA structure of the Sardinian population: a haplotype study of 551 families. *Tissue Antigens*. (1992) 40:165–74. doi: 10.1111/j.1399-0039.1992.tb02041.x
- European Association for the Study of the Liver. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. *J Hepatol*. (2017) 67:145–72. doi: 10.1016/j.jhep.2017.03.022
- Nilsson LL, Funck T, Kjersgaard ND, Hviid TVF. Next-generation sequencing of HLA-G based on long-range polymerase chain reaction. *HLA*. (2018) 92:144–53. doi: 10.1111/tan.13342

50. Kalligeros M, Vassilopoulos A, Vassilopoulos S, Victor DW, Mylonakis E, Noureddin M, et al. Prevalence of steatotic liver disease (MASLD, MetALD, and ALD) in the United States: NHANES 2017–2020. *Clin Gastroenterol Hepatol: Off Clin Pract J Am Gastroenterol Assoc.* (2024) 22:1330–1332.e4. doi: 10.1016/j.cgh.2023.11.003
51. Di Gaetano C, Fiorito G, Ortu MF, Rosa F, Guarrera S, Pardini B, et al. Sardinians genetic background explained by runs of homozygosity and genomic regions under positive selection. *PLoS One.* (2014) 9:e91237. doi: 10.1371/journal.pone.0091237
52. Zimmer V, Lammert F. Looking into the crystal ball: predicting non-response to ursodeoxycholic acid in primary biliary cholangitis. *EBioMedicine.* (2017) 15:10–1. doi: 10.1016/j.ebiom.2016.12.008
53. Arns T, Antunes DA, Abella JR, Rigo MM, Kavraki LE, Giuliani S, et al. Structural modeling and molecular dynamics of the immune checkpoint molecule HLA-G. *Front Immunol.* (2020) 11:575076. doi: 10.3389/fimmu.2020.575076
54. Rebmann V, van der Ven K, Pässler M, Pfeiffer K, Krebs D, Grosse-Wilde H. Association of soluble HLA-G plasma levels with HLA-G alleles. *Tissue Antigens.* (2001) 57:15–21. doi: 10.1034/j.1399-0039.2001.057001015.x
55. Favoino E, Favia IE, Vettori S, Vicenti C, Prete M, Valentini G, et al. Clinical correlates of human leucocyte antigen (HLA)-G in systemic sclerosis. *Clin Exp Immunol.* (2015) 181:100–9. doi: 10.1111/cei.12633
56. Palladini G, Ferrigno A, Richelmi P, Perlini S, Vairetti M. Role of matrix metalloproteinases in cholestasis and hepatic ischemia/reperfusion injury: A review. *World J Gastroenterol.* (2015) 21:12114–24. doi: 10.3748/wjg.v21.i42.12114
57. Jacob-Ferreira ALB, Lacchini R, Gerlach RF, Passos CJS, Barbosa F, Tanus-Santos JEA. A common matrix metalloproteinase (MMP)-2 polymorphism affects plasma MMP-2 levels in subjects environmentally exposed to mercury. *Sci Total Environ.* (2011) 409:4242–6. doi: 10.1016/j.scitotenv.2011.07.013
58. Zhang X, Yu L, Ding Y. Human leukocyte antigen G and miR-148a are associated with the pathogenesis of intrahepatic cholestasis of pregnancy. *Exp Ther Med.* (2014) 8:1701–6. doi: 10.3892/etm.2014.1986
59. Hardie C, Green K, Jopson L, Millar B, Innes B, Pagan S, et al. Early molecular stratification of high-risk primary biliary cholangitis. *EBioMedicine.* (2016) 14:65–73. doi: 10.1016/j.ebiom.2016.11.021
60. Liu L, Wang L, Zhao L, He C, Wang G. The role of HLA-G in tumor escape: manipulating the phenotype and function of immune cells. *Front Oncol.* (2020) 10:597468. doi: 10.3389/fonc.2020.597468
61. Lleo A, Maroni L, Glaser S, Alpini G, Marziani M. Role of cholangiocytes in primary biliary cirrhosis. *Semin Liver Dis.* (2014) 34:273–84. doi: 10.1055/s-0034-1383727
62. Contini P, Ghio M, Poggi A, Filaci G, Indiveri F, Ferrone S, et al. -B₂-C and -G molecules induce apoptosis in T and NK CD8+ cells and inhibit cytotoxic T cell activity through CD8 ligation. *Eur J Immunol.* (2003) 33:125–34. doi: 10.1002/immu.200390015
63. Crabtree E, Song L, Llanga T, Bower JJ, Cullen M, Salmon JH, et al. AAV-mediated expression of HLA-G1/5 reduces severity of experimental autoimmune uveitis. *Sci Rep.* (2019) 9:19864. doi: 10.1038/s41598-019-56462-3
64. Chen S, Li MQ, Duan W-J, Li B-E, Li S-X, Lv T-T, et al. Concomitant extrahepatic autoimmune diseases do not compromise the long-term outcomes of primary biliary cholangitis. *Hepatobiliary Pancreat Dis Int.* (2022) 21:577–82. doi: 10.1016/j.hbpd.2022.05.009
65. Niegowska M, Paccagnini D, Burrai C, Palermo M, Sechi LA. Antibodies against proinsulin and homologous MAP epitopes are detectable in Hashimoto's thyroiditis Sardinian patients, an additional link of association. *PLoS One.* (2015) 10:e0133497. doi: 10.1371/journal.pone.0133497
66. Kemp EH, Metcalfe RA, Watson PF, Weetman AP. HLA-G does not have a pathophysiological role in Graves' disease. *J Clin Pathol.* (2003) 56:475–7. doi: 10.1136/jcp.56.6.475
67. Dardano A, Rizzo R, Polini A, Stignani M, Tognini S, Pasqualetti G, et al. Soluble human leukocyte antigen-g and its insertion/deletion polymorphism in papillary thyroid carcinoma: novel potential biomarkers of disease? *J Clin Endocrinol Metab.* (2012) 97:4080–6. doi: 10.1210/jc.2012-2231