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► To cite this version:

Magda Rybicka, Anna Woziwodzka, Alicja Sznarkowska, Tomasz Romanowski, Piotr Stalke, et al.. Genetic variation in IL-10 influences the progression of hepatitis B infection.. International Journal of Infectious Diseases, 2020, 96, pp.260 - 265. 10.1016/j.ijid.2020.04.079 . hal-02917279

HAL Id: hal-02917279

<https://hal.science/hal-02917279>

Submitted on 2 Feb 2021

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Genetic variation in *IL-10* influences the progression of hepatitis B infection



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ARTICLE INFO

Article history:

Received 17 February 2020

Received in revised form 27 April 2020

Accepted 28 April 2020

Keywords:

HBV

Cirrhosis

Fibrosis

IL10

Single-nucleotide polymorphism

ABSTRACT

Objectives: The outcomes of hepatitis B virus (HBV) infection vary substantially among affected individuals, providing evidence of the role of host genetic background in the susceptibility to HBV persistence and the dynamics of liver injury progression to cirrhosis and hepatocellular carcinoma (HCC). **Methods:** Six single-nucleotide polymorphisms within the interleukin 10 gene (*IL10*) were genotyped by MALDI-TOF mass spectrometry in 857 patients with chronic HBV infection (CHB), 48 patients with resolved HBV infection, and 100 healthy volunteers. Associations of the selected polymorphisms with susceptibility to chronic HBV infection, liver injury progression, and outcomes were investigated.

Results: *IL10* −819T (rs1800871), −592A (rs1800872), and +504T (rs3024490) alleles were associated with treatment-induced hepatitis B surface antigen (HBsAg) seroclearance. Additionally, *IL10* ATAC haplotype increased the chance of HBsAg loss and was significantly more frequent in patients with less liver injury. Moreover rs1800871TT, rs1518110TT, rs1800872AA, and rs3024490TT genotypes were identified as predictors of a lower FIB-4 score (<0.5).

Conclusions: This study indicates that polymorphisms within the promoter region and intronic sequences of *IL10* are associated with chronicity of hepatitis B and with HBV-induced liver damage.

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Introduction

Despite progress in implementing vaccination programmes and in the development of new treatment perspectives, hepatitis B virus (HBV) infections remain a major health problem worldwide, contributing considerably to cirrhosis- and hepatocellular carcinoma (HCC)-related mortality of 0.5–1 million per year. As much as one-third of the general population carries serological markers of HBV infection (Hu and Ren, 2017).

Outcomes of HBV infection vary substantially among affected individuals, providing evidence of the role of host genetic background in the susceptibility to HBV persistence and the

dynamics of liver injury progression to cirrhosis and HCC. The response of the immune system to HBV infection is complex, and a broad range of cytokines such as interferon (IFN)- α/β , IFN- γ , and tumour necrosis factor (TNF)- α are involved in the early phase of infection. An effective antiviral response, mainly mediated by CD4+ and CD8+ T-cells, natural killer cells, and monocytes, may result in immune controlled HBV replication (functional cure). By contrast, in children and adults with a compromised immune system, active viral replication can become persistent. In chronic HBV infection, both the number of regulatory T-cells and levels of inhibitory interleukin 10 (IL-10) and transforming growth factor beta (TGF- β) increase, leading to HBV-specific CD8+ T-cell exhaustion and rendering viral eradication from the liver impossible (Peeridogaheh et al., 2018).

IL-10 is recognized as a key cytokine regulating the immune response to HBV infection. Recently, a subset of IL-10-producing B-cells, known as regulatory B-cells (Bregs), have been shown to

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regulate HBV-specific CD8+ T-cell immunity (Das et al., 2012; Liu et al., 2016). Moreover, down-regulation of IL-10 restores the function of exhausted HBV-specific CD8+ T-cells (Das et al., 2012). In patients with chronic hepatitis B (CHB), both the levels of IL-10 and number of regulatory B-cells increase (Das et al., 2012). Serum IL-10 levels reflect the dynamics of viral load and liver inflammation, and are correlated with spontaneous flares of liver disease (Das et al., 2012). Moreover, IL-10 might substantially affect the antiviral immune response, as it inhibits the production of proinflammatory cytokines such as IFN- γ , TNF- α , IL-1 β , and IL-6 (Walter, 2014).

The capacity for IL-10 production is regulated on the transcriptional level and is affected by single-nucleotide polymorphisms (SNPs) located in the 5'-flanking promoter region of the *IL10* gene: -1082A/G (rs1800896), -819C/T (rs1800871), and -592C/A (rs1800872) (Edwards-Smith et al., 1999; Reuss et al., 2002; Höhler et al., 2005). Numerous studies have aimed to elucidate the role of these SNPs in the anti-HBV response, and a recent meta-analysis conducted by Shu et al. confirmed their clinical significance in the course of HBV infection (Shu et al., 2015). Additionally, the -1353G/A (rs1800893) promoter and +954G/T (rs1518110) intronic *IL10* SNPs have been shown to influence the outcome of HBV infection in African Americans (Truelove et al., 2008). Another *IL10* intronic variant +504G/T (rs3024490) has been related to susceptibility to CHB in the Chinese Han population (Zhang et al., 2014).

In CHB, activation of the immune response, required for virus eradication from hepatocytes, is also associated with inflammation that leads to liver damage. The host *IL10* genetic background is a risk factor for liver injury and the development of cirrhosis in CHB (Ghaleh Baghi et al., 2015; Guo et al., 2015; Yao et al., 2015). Interestingly, the role of IL-10 in liver disease is not limited to HBV-infected patients, and the interactions of the *IL10* promoter genotype and liver outcome have also been observed in hepatitis C virus (HCV) infection (Guo et al., 2015) and alcohol-related cirrhosis (Yang et al., 2014). Indeed, there is evidence suggesting that IL-10, apart from its well-documented role in regulating the

immune response to HBV infection, also exerts a modulatory effect in liver fibrogenesis (Louis et al., 1998; Thompson et al., 1998).

This study was performed to investigate the impact of four SNPs located in the promoter region of *IL10* (rs1800896, rs1800871, rs1800872, rs1800893) and two *IL10* intronic variants (rs1518110, rs3024490) on the course of CHB in a European population.

Materials and methods

Patients

The study group consisted of 857 patients with CHB and 48 untreated patients in the hepatitis B surface antigen (HBsAg)-negative phase of disease (HBsAg-negative, hepatitis B core antibody (anti-HBc) reactive). The blood samples of 648 patients were collected from the ANRS CO22 HEPATHER cohort (ClinicalTrials.gov registry number NCT01953458). The remaining 257 CHB patients were recruited from the Department of Infectious Diseases, Medical University of Gdansk, and the Hepatology Outpatients Clinic of the Pomeranian Centre for Infectious Diseases and Tuberculosis in Gdansk in 2014–2016. The control group comprised 100 blood donors with confirmed absence of HIV, HBV, and HCV infections from the Gdansk Regional Centre of Blood Donations and Haemotherapy (Figure 1). Patients with a history of schistosomiasis infection, heavy alcohol abuse, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or inherited liver diseases were excluded to prevent interference with the association with HBV infection.

Blood tests, including hepatitis B serology (HBsAg, hepatitis B e antigen (HBeAg), hepatitis B e antibody (anti-HBe)) and the quantification of HBV DNA, were performed on all recruited patients. Liver biopsies were collected from 132 patients. The specimens were assessed for inflammation activity and stage of fibrosis according to Scheuer scores. For 725 patients, liver fibrosis or cirrhosis was assessed according to the Metavir scoring system, as described by Carrat et al. (2019). Significant fibrosis was defined as $F \geq 2$ for the Metavir and Scheuer staging systems and cirrhosis

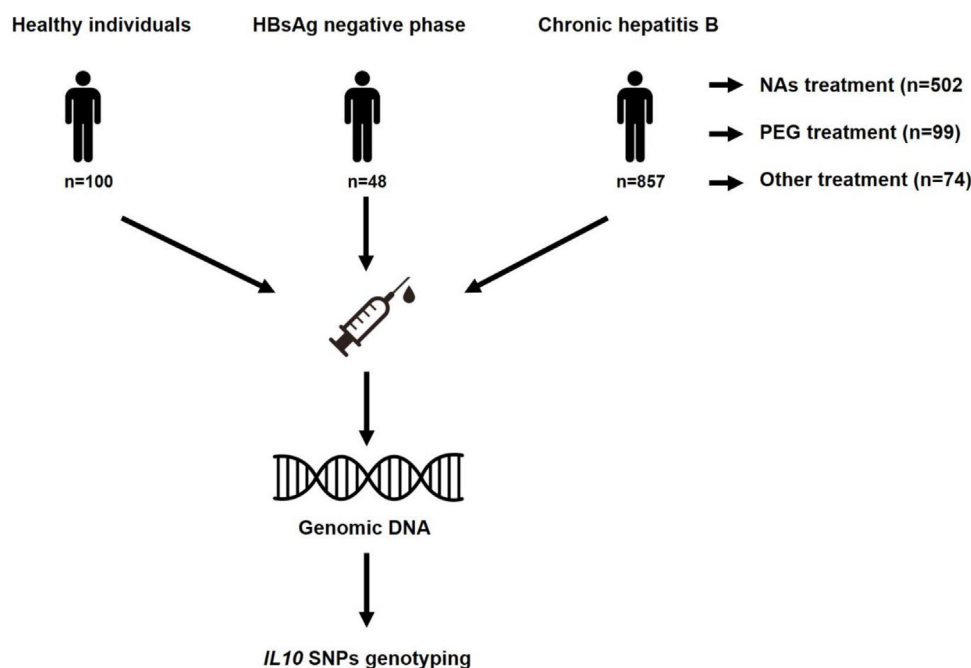


Figure 1. Diagram presenting study group.

Table 1
Primers used for MALDI-TOF MassARRAY single-nucleotide polymorphism genotyping.

NCBI ID	PCR forward primer ^a	PCR reverse primer ^a	Extension primer
rs1800871	ATGCTAGTCAGGTAGTGCTC	GGTGTACCCCTGTACAGGTG	<u>CCCCCTGTACAGGTGATGTAA</u>
rs1800872	AAAGGAGCCTGGAACACATC	TCCTCAAAGTCCCAAGCAG	<u>CAAGAGACTGGCTTCTACAG</u>
rs1800893	CTGACTATAGAGTGGCAGG	CCTGCCATTCCAGTTTACAG	TGTAAGTGGGAGGAACA
rs1800896	GACAACACTACTAAGGCTTC	ATTCCATGGAGGCTGGATAG	TTACCTATCCCTACTTCCCC
rs1518110	ATGCGGTCTTTTGTATGCC	TGATTTTTTTGGCCAGAGC	GGCCAGAGCCAATTT
rs3024490	GGGAGCATCTTCACCTCGAA	CTTATAAGATCCTGCTGGCC	GGCTAGGAGAAGTAAAGAAA

MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight.

^a Each PCR primer also contained a 5'-ACGTTGGATG tag. 5' tags on extension primers are marked in bold underline.

was defined as $F \geq 4$. The Fibrosis-4 score (FIB-4) (Sterling et al., 2006) and aspartate aminotransferase (AST)-to-platelet ratio index (APRI) (Wai 2003) were also used to estimate the amount of scarring in the liver.

Of the 675 patients who received treatment according to Polish or French National Health Service recommendations, 99 (14.5%) were treated with pegylated interferon alpha (PEG-IFN- α), 502 (74.5%) with nucleoside/nucleotide analogues (NAs), and 74 (11%) with combination NA therapy. The treatment response was monitored by the measurement of HBV DNA and HBsAg loss at week 24 after treatment discontinuation for PEG-IFN- α -treated individuals, and at week 72 of treatment for NA-treated patients. At the 48-week follow-up, an analysis was conducted to further monitor HBsAg loss. Sustained virological response (SVR) was defined as an undetectable HBV DNA level at 24 weeks after treatment discontinuation.

The study was performed with the approval of the local independent bioethics committee at the Medical University of Gdansk in compliance with the Declaration of Helsinki, and written consent was obtained from each patient.

SNP genotyping

Genomic DNA was isolated from the whole blood samples using the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche, Germany) according to the manufacturer's protocol. The polymorphisms of the *IL10* gene (-592C/A, rs1800872; -819C/T, rs1800871; -1082G/A, rs1800896; -1353C/T, rs1800893; +504G/T, rs3024490; +954G/T, rs1518110) were analysed by MassARRAY platform with iPLEX Pro chemistry (Agena Bioscience, USA) according to the standard protocol. Amplification and extension primers (Table 1) were designed with Agena Assay Design Suite v2. During the initial amplification PCR, six different products of ~100 bp containing the SNPs of interest were amplified. Next, unincorporated dNTPs were removed with shrimp alkaline phosphatase. Finally, after single-nucleotide extension reaction, allele-specific products of distinct masses were obtained. The purified extension reaction products with an anion-exchange resin, were spotted onto SpectroCHIP using a MassARRAY RS1000 Nanodispenser. Mass spectra were acquired with a MassARRAY Analyzer 4 mass spectrometer and were analysed with MassARRAY Typer 4.0 software.

Statistical analysis

The statistical analysis was conducted using Statistica 13.3 (StatSoft, USA). MIDAS software was used to assess the linkage disequilibrium (LD) and deviations from Hardy-Weinberg equilibrium (HWE) of the analysed SNPs. The Chi-square test and Fisher's exact test were applied to analyse the distribution of nominal variables. Haplotype blocks were constructed using HaploView 4.2 program. Quantitative variables were expressed as the median values (unless stated otherwise) and compared with the Mann-Whitney *U*-test or Kruskal-Wallis test. Logistic

regression was conducted to determine the associations between analysed variables adjusted for possible confounders. All statistical tests were two-tailed. *p*-Values of less than 0.008 were considered significant after the Bonferroni correction was applied to account for multiple testing.

Results

Study group characteristics

All enrolled individuals were unrelated Caucasian adults; 857 were patients with CHB, 48 were individuals who had recovered spontaneously from HBV infection (HBsAg-negative phase), constituting the functional cure control group, and 100 were volunteers, constituting the healthy control group. Their mean age was 52.0 years, 56.9 years, and 27.3 years, respectively. The baseline characteristics of the study and control groups are shown in Table 2.

The genotypes of six SNPs within *IL10* were obtained for all 1005 subjects included in the study, with a success rate of 100%. All SNPs were variable in the study group. Genotypic and allelic frequencies of the analysed SNPs for the CHB and control groups are shown in **Supplementary Material Table S1 and Table S2. For the control groups**, the distribution of genotypes followed HWE ($p > 0.05$), except for rs1800871, rs1800872, and rs1518110 in individuals with functional cure. For CHB patients, the distributions were not consistent with HWE ($p < 0.05$). Significant differences in genotypic distribution of rs1800871, rs1800872, and rs1518110 were found between healthy blood donors and individuals with HBsAg loss. Moreover, the genotypic distribution of rs1800893 differed between the CHB patients and the healthy control group (**Supplementary Material Table S1**). No statistically significant differences in allelic distribution of *IL10* polymorphisms were observed between the CHB and the control groups (**Supplementary Material Table S2**).

The haplotype analysis showed linkage disequilibrium between rs1800871 and rs1800872 ($r^2 = 0.88$), rs1518110 ($r^2 = 0.74$), and rs3024490 ($r^2 = 0.78$); between rs1800893 and rs1800896 ($r^2 = 0.89$); between rs1518110 and rs1800872 ($r^2 = 0.68$); and between rs1518110 and rs3024490 ($r^2 = 0.72$). All remaining pairs of SNPs were independent ($r^2 < 0.2$).

In this study, rs1800896CC and rs1800893CC genotypes were more common in patients with lower baseline HBV DNA levels (<2000 IU/ml) (sex-adjusted GG, GA vs. AA: odds ratio (OR) 1.72, 95% confidence interval (CI) 1.16–2.57, $p = 0.007$; sex-adjusted TT, CT vs. CC: OR 1.82, 95% CI 1.22–2.70, $p = 0.003$).

IL10 polymorphisms and treatment response

For the CHB patients who underwent antiviral treatment, an analysis was performed to determine whether the *IL10* polymorphisms affected the treatment response expressed as HBsAg clearance. Three of the analysed polymorphisms within *IL10* were significantly associated with treatment-induced HBsAg loss at 24

Table 2
Baseline demographic and clinical characteristics of the study and control groups.

	CHB patients (n = 857)	Individuals with functional cure (n = 48)	Healthy controls (n = 100)
Age, years	51 ± 1	55.7 ± 1.4	27.3 ± 0.9
Sex, % female	37%	30%	22%
Origin, % Caucasian	100%	100%	100%
ALT, IU/l	97 ± 53	29.50 ± 1.91	–
AST, IU/l	219 ± 91	30.25 ± 2.83	–
HBV DNA, kIU/ml	6957 ± 1728	–	–
HBsAg, % positive	100%	0%	–
HBeAg, % positive	13%	–	–
Anti-HBsAg, % positive	0%	100%	–
Anti-HBcAg, % positive	100%	100%	–
Anti-HBe, % positive	82%	–	–
Liver inflammation grade ^a	1.5 (0–2)	–	–
Liver fibrosis stage ^a	1 (0–2)	0.62 (0–4)	–

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus. Data are reported as the mean ± standard error of the mean.

^a Median value (25th–75th percentile).

weeks after discontinuation of PEG-IFN- α . Significantly higher minor allele frequencies at –819T (rs1800871), –592A (rs1800872), and +504T (rs3024490) were observed in patients with HBsAg loss (Table 3). In the group of patients previously treated with NAs, rs1800896 was associated with a higher predisposition to treatment-induced loss of HBsAg (age and sex-adjusted: OR 0.037, 95% CI 0.002–0.66, $p = 0.01$); however significance was lost after Bonferroni correction. Additionally, the *IL10* ATAC haplotype (–1082A/–819T/–592A/–1353C) increased the chance of achieving the HBsAg-negative phase in the PEG-IFN- α -treated (sex and age-adjusted: OR 9.85, 95% CI 1.13–85, $p = 0.0034$) and NA-treated (sex and age-adjusted: OR 20.33, 95% CI 3.25–127, $p = 0.0012$) groups.

The incidence of HBsAg loss was assessed in 344 patients from the original cohort of 675 CHB patients who underwent antiviral treatment, and the clearance rate was found to be 6.98%. Comparison of the genotype and allele frequencies between individuals with and without treatment-induced loss of HBsAg revealed significant differences in all six polymorphisms within *IL10* (Table 4).

Table 3
IL10 polymorphisms and chance of PEG-IFN- α -induced HBsAg loss.^a

<i>IL10</i> genotype	MAF	Odds ratio	95% CI	p -Value
rs1800896 [A/G]				
AA vs. GA	G = 0.27	0.43	0.15–1.27	0.12
AA vs. GG		0.19	0.02–1.61	0.10
rs1800871 [C/T]				
CC vs. CT	T = 0.43	4.43	1.33–14.76	0.016 ^a
CC vs. TT		19.59	1.76–217	0.0068 ^b *
rs1800872 [C/A]				
CC vs. CA	A = 0.39	3.60	1.31–9.89	0.013 ^a
CC vs. AA		12.97	1.72–97	0.007 ^b *
rs1800893 [G/A]				
CC vs. CT	T = 0.28	0.42	0.15–1.26	0.12
CC vs. TT		0.19	0.022–1.62	0.10
rs3024490 [G/T]				
GG vs. GT	T = 0.43	3.67	1.35–10	0.012 ^a
GG vs. TT		13.46	1.81–100	0.009 ^b *
rs1518110 [G/T]				
GG vs. GT	T = 0.42	1.54	0.66–3.63	0.31
GG vs. TT		2.39	0.43–13.18	0.31

CI, confidence interval; HBsAg, hepatitis B surface antigen; *IL10*, interleukin 10 gene; PEG-IFN- α , pegylated interferon alpha; MAF, minor allele frequency.

^a Multivariate logistic regression analysis adjusted for age; HBsAg loss defined as HBsAg negativity at week 24 after treatment discontinuation.

^b Significant p -values ($p < 0.05$); those marked with an asterisk (*) remained significant after Bonferroni correction.

IL10 polymorphisms and liver injury

To examine possible risk factors for liver injury assessed by FIB-4 in the study population, logistic regression analyses were conducted on variables that were *IL10* genotypes, sex, and age. In the multivariate analysis rs1800871TT, rs1518110TT, rs1800872AA, and rs3024490TT genotypes were identified as predictors of a lower FIB-4 score ($p < 0.5$) (Table 5). Similar observations were found for the APRI index results, which were significantly lower in patients with a homozygosity of the minor allele within these four SNPs in the *IL10* gene ($p = 0.003$). Additionally, the *IL10* gene ATAC haplotype (–1082A/–819T/–592A/–1353C) was significantly more frequent in patients with less liver injury (OR 8.94, 95% CI 1.78–44, $p = 0.007$). Moreover, the *IL10* GCCT haplotype (–1082G/–819C/–592C/–1353T) increased the risk of developing cirrhosis (OR 2.61, 95% CI 1.58–4.30, $p = 0.0003$).

Discussion

In its key role, IL-10 acts as an immunosuppressive cytokine by suppressing T-cell proliferation and antigen-presenting cell (APC) functions, and by modulating cytokine and chemokine synthesis (Saraiva and O'Garra, 2010). *IL10* expression is elevated during several chronic viral infections, which serves as a viral strategy to downregulate the host immune response and allow viral persistence in the host (Hyodo et al., 2004; Ohga et al., 2004; Brooks et al., 2006; Kaplan et al., 2008; Brockman et al., 2009). Over recent years it has been established that polymorphism within the *IL10* promoter region influences expression and serum levels of IL-10. Three functional SNPs in the *IL10* promoter have been investigated intensively: 1082A/G, –819T/C, and –592A/C. These SNPs present three major haplotypes: ATA, ACC, and GCC, which are associated with low, medium, and high levels of *IL10* expression, respectively (Turner et al., 1997; Eskdale et al., 1998). These three polymorphisms have been demonstrated to be essential modulators of the immune response against hepatitis viral antigens, suggesting their role in the aetiology of HBV infection (Höhler et al., 2005).

In this study, six common polymorphisms in the *IL10* gene (four from the promoter region and two intronic variants) were genotyped in a cohort of HBV chronically infected patients, a group of individuals in the HBsAg-negative phase, and in healthy blood donors. Differences in genotype distribution in one SNP (rs1800893) between CHB patients and healthy blood donors, and in three SNPs (rs1800871, rs1800872, rs1518110) between healthy blood donors and individuals in the HBsAg-negative phase of HBV infection, were observed. Strikingly, all of the analysed SNPs

Table 4
Genotypic and allelic distribution of analysed *IL10* polymorphisms between CHB patients (HBsAg-positive) and individuals who achieved HBsAg loss induced by antiviral treatment (HBsAg-negative).

SNP ID	Genotypic distribution (%)				Allelic distribution (%)			
	Genotype	HBsAg (+) (n = 320)	HBsAg (-) (n = 24)	p-Value	Allele	HBsAg (+) (n = 640)	HBsAg (-) (n = 48)	p-Value
-592C/A (rs1800872)	CC	185 (58)	6 (25)	0.0007 ^a	C	471 (74)	22 (46)	0.000039 ^a
	CA	101 (31)	10 (42)		A	169 (26)	26 (54)	
	AA	34 (11)	8 (33)					
-819C/T (rs1800871)	CC	182 (57)	6 (25)	0.0005 ^a	C	470 (73.5)	22 (46)	0.000044 ^a
	CT	106 (33)	10 (42)		T	170 (26.5)	26 (54)	
	TT	32 (10)	8 (33)					
-1082G/A (rs1800896)	GG	61 (19)	1 (4)	0.03 ^a	G	263 (41)	10 (21)	0.005653 ^a
	GA	141 (44)	8 (33)		A	377 (59)	38 (79)	
	AA	118 (37)	15 (63)					
-1353C/T (rs1800893)	CC	122 (38)	14 (58)	0.041 ^a	C	363 (57)	37 (77)	0.005809 ^a
	CT	119 (37)	9 (38)		T	277 (43)	11 (23)	
	TT	79 (25)	1 (4)					
+504G/T (rs3024490)	GG	173 (54)	7 (29)	0.005 ^a	G	462 (72)	24 (50)	0.001132 ^a
	GT	116 (36.5)	10 (42)		T	178 (28)	24 (50)	
	TT	31 (9.5)	7 (29)					
+954G/T (rs1518110)	GG	188 (59)	12 (50)	0.0021 ^a	G	476 (74.5)	28 (58)	0.015446 ^a
	GT	100 (31)	4 (17)		T	164 (25.5)	20 (42)	
	TT	32 (10)	8 (33)					

CHB, chronic hepatitis B; HBsAg, hepatitis B surface antigen; HBsAg (+), HBsAg-positive patients; HBsAg (-), HBsAg-negative patients; *IL10*, interleukin 10 gene; SNP, single nucleotide polymorphism.

^a Significant p-values ($p < 0.05$).

Table 5
Relationship between *IL10* polymorphisms and severity of liver disease in HBV-infected patients.^a

<i>IL10</i> genotype	MAF	Odds ratio	95% CI	p-Value
rs1800871 [C/T] CC,CT vs. TT	T = 0.43	8.94	1.78–44.80	0.007 ^b *
rs1800872 [C/A] CC,CA vs. AA	A = 0.39	9.15	1.82–45.89	0.006 ^b *
rs3024490 [G/T] GG,GT vs. TT	T = 0.43	9.02	1.79–45	0.007 ^b *
rs1518110 [G/T] GG,GT vs. TT	T = 0.42	8.84	1.76–44.29	0.008 ^b *

CI, confidence interval; HBV, hepatitis B virus; *IL10*, interleukin 10 gene; MAF, minor allele frequency.

^a Liver fibrosis was assessed by the FIB-4 index.

^b Significant p-values ($p < 0.05$); those marked with an asterisk (*) remained significant after Bonferroni correction.

showed significantly different genotypic and allelic distributions between chronically infected patients and the spontaneously recovered group. Hyodo et al. (2004) showed that IL-10 production is elevated in response to the HBV core antigen (HBCAg) in patients with chronic infection, suggesting that its excessive production may contribute to HBV persistency in these patients (Hyodo et al., 2004). Knowing that all of the analysed SNPs are associated with the *IL10* expression level, it is justified to conclude that *IL10* polymorphisms play an essential role in the immune response against HBV infection.

As well as determining the possible association of *IL10* polymorphisms with the chronicity of HBV infection, this study also investigated whether these could have a predictive value in assessing the response of CHB patients to antiviral therapy (IFN- α and analogues) measured by serum HBsAg loss. In this study, three of the *IL10* treatment response to PEG-IFN- α , and it was determined that the ATAC haplotype increased the chance of treatment-induced HBsAg loss. The ATA haplotype has already been correlated with a low level of *IL10* expression. ATA individuals secrete on average two or three times less IL-10 than GCC individuals (Turner et al., 1997). Heterogeneity in the promoter region of the *IL10* gene affects the initial response to IFN- α therapy

in patients with hepatitis C. Patients who are genetically predisposed to high IL-10 production have shown a poor response to IFN- α . A better response to treatment has also been associated with lower liver fibrosis. Similarly in the present study, alleles that were related to treatment-induced HBV loss were associated with a decreased liver fibrosis scored by FIB-4 and APRI indexes. This indicates that *IL10* polymorphisms might have a prognostic value in assessing the liver condition in chronically infected patients. Moreover, the ATAC haplotype, which was associated with a decreased *IL10* expression and an increased chance of eliminating viral infection, was more often found in patients with less intense liver injury. In contrast, the GCCT haplotype, associated with high *IL10* expression, has been shown to increase the risk of progression to cirrhosis (Shin, 2003). This proves that *IL10* polymorphisms may not only impact the antiviral immune response, but also HBV infection-induced liver fibrosis. In studies on alcohol-induced liver cirrhosis, it was the low expression of the ATA haplotype that was associated with increased fibrosis and a higher risk of alcoholic cirrhosis in Taiwanese patients (Yang et al., 2014). IL-10 has been shown to protect against inflammation-induced liver damage, fibrosis, and cirrhosis due to its immunosuppressive action (Thompson et al., 1998). Interestingly, in the present study the ATAC haplotype was found to be associated with a better liver condition and less fibrosis in chronically infected patients. This leads to the conclusion that lower levels of IL-10 allow the virus to be eradicated more quickly and more effectively, and in this way protect the liver from the virus-induced fibrosis.

What emerges from this study is that polymorphisms within the promoter region and intronic sequences of *IL10* are strongly associated with chronicity of hepatitis B and with the virus-induced liver damage.

This study has some limitations. The influence of the particular polymorphisms on *IL10* expression was not analysed. However, in terms of the SNPs localized in the promoter region, there are many studies pointing to their impact on *IL10* expression (through the modulation of the transcription factor binding sites), although we do not know how the intronic variants affect the IL-10 level and/or activity. It can only be hypothesized that intronic SNPs affect splicing and therefore modulate *IL10* mRNA levels or stability, but this hypothesis awaits verification.

Funding

This work was supported by the National Centre for Research and Development (NCBR, grant number INFECT-ERA/01/2014) and by the French National Research Agency (ANR, grant number ANR-13-IFEC-0006-02) in the frame of the European consortium Infect-ERA hepBccc and ST79 from the Medical University of Gdansk, and the MOBI4Health project, which is funded by the European Union's Seventh Framework Programme under grant agreement number 316094.

Conflict of interest

None to declare.

Acknowledgements

We thank the participants and participating clinicians of the ANRS CO22 HEPATHER cohort at each study site. We thank F. Carrat, S. Pol, and H. Fontaine, the coordinators of the cohort, C. Dorival, project manager, and the ANRS CO22 HEPATHER scientific committee. The ANRS CO22 HEPATHER cohort is sponsored and funded by Inserm-ANRS (France Recherche Nord & Sud Sida-vih Hepatites), ANR (Agence Nationale de la Recherche), DGS (Direction Ge'ne'rale de la Sante'), and MSD, Janssen, Gilead, AbbVie, BMS and Roche.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ijid.2020.04.079>.

References

- Brockman MA, Kwon DS, Tighe DP, Pavlik DF, Rosato PC, Sela J, et al. IL-10 is up-regulated in multiple cell types during viremic HIV infection and reversibly inhibits virus-specific T cells. *Blood* 2009;114(2):346–56. [Cited 18 August 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19365081>.
- Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MBA. Interleukin-10 determines viral clearance or persistence in vivo. *Nat Med* 2006;12(11):1301–9. [Cited 18 August 2019]. Available from: <http://www.nature.com/articles/nm1492>.
- Carrat F, Fontaine H, Dorival C, Simony M, Diallo A, Hezode C, et al. Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. *Lancet* 2019;393(10179):1453–64. [Cited 29 September 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/30765123>.
- Das A, Ellis G, Pallant C, Lopes AR, Khanna P, Peppia D, et al. IL-10-producing regulatory B cells in the pathogenesis of chronic hepatitis B virus infection. *J Immunol* 2012;189(8):3925–35. [Cited 15 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22972930>.
- Edwards-Smith CJ, Jonsson JR, Purdie DM, Bansal A, Shorthouse C, Powell EE. Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis C to interferon alpha. *Hepatology* 1999;30(2):526–30. [Cited 15 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10421663>.
- Eskdale J, Gallagher G, Verweij CL, Keijsers V, Westendorp RGJ, Huizinga TWJ. Interleukin-10 secretion in relation to human IL-10 locus haplotypes. *Proc Natl Acad Sci* 1998;95(16):9465–70. [Cited 18 August 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9689103>.
- Ghaleh Baghi S, Alavian SM, Mehrnosh L, Salimi S. Impact of the IL-10 promoter gene polymorphisms in the severity of chronic hepatitis B infection. *Hepat Mon* 2015;15(7). [Cited 17 April 2019]. Available from: <http://hepatmon.com/en/articles/15501.html>.
- Guo P-F, Jin J, Sun X. Influence of IL10 gene polymorphisms on the severity of liver fibrosis and susceptibility to liver cirrhosis in HBV/HCV-infected patients. *Infect Genet Evol* 2015;30:89–95. [Cited 17 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25514046>.
- Höhler T, Reuss E, Freitag CM, Schneider PM. A functional polymorphism in the IL-10 promoter influences the response after vaccination with HBsAg and hepatitis A. *Hepatology* 2005;42(1):72–6. [Cited 15 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15918171>.
- Hu P, Ren H. Interpretations of EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. *Zhonghua Gan Zang Bing Za Zhi* 2017;25(6):415–8. [Cited 11 October 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28763857>.
- Hyodo N, Nakamura I, Imawari M. Hepatitis B core antigen stimulates interleukin-10 secretion by both T cells and monocytes from peripheral blood of patients with chronic hepatitis B virus infection. *Clin Exp Immunol* 2004;135(3):462–6. [Cited 18 August 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15008979>.
- Kaplan DE, Ikeda F, Li Y, Nakamoto N, Ganesan S, Valiga ME, et al. Peripheral virus-specific T-cell interleukin-10 responses develop early in acute hepatitis C infection and become dominant in chronic hepatitis. *J Hepatol* 2008;48(6):903–13. [Cited 18 August 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18384906>.
- Liu Y, Cheng L-S, Wu S-D, Wang S-Q, Li L, She W-M, et al. IL-10-producing regulatory B-cells suppressed effector T-cells but enhanced regulatory T-cells in chronic HBV infection. *Clin Sci* 2016;130(11):907–19. [Cited 15 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26980345>.
- Louis H, Van Laethem J-L, Wu W, Quertinmont E, Degraef C, Van den Berg K, et al. Interleukin-10 controls neutrophilic infiltration, hepatocyte proliferation, and liver fibrosis induced by carbon tetrachloride in mice. *Hepatology* 1998;28(6):1607–15. [Cited 17 April 2019]. Available from: <http://doi.wiley.com/10.1002/hep.510280621>.
- Ogha S, Nomura A, Takada H, Tanaka T, Furuno K, Takahata Y, et al. Dominant expression of interleukin-10 and transforming growth factor- β genes in activated T-cells of chronic active Epstein-Barr virus infection. *J Med Virol* 2004;74(3):449–58. [Cited 18 August 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15368517>.
- Peeridogaheh H, Meshkat Z, Habibzadeh S, Arzanlou M, Shahi JM, Rostami S, et al. Current concepts on immunopathogenesis of hepatitis B virus infection. *Virus Res* 2018;245:29–43. [Cited 10 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29273341>.
- Reuss E, Fimmers R, Kruger A, Becker C, Rittner C, Höhler T. Differential regulation of interleukin-10 production by genetic and environmental factors – a twin study. *Genes Immun* 2002;3(7):407–13. [Cited 15 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12424622>.
- Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol* 2010;10(3):170–81. [Cited 18 August 2019]. Available from: <http://www.nature.com/articles/nri2711>.
- Shin HD. Interleukin 10 haplotype associated with increased risk of hepatocellular carcinoma. *Hum Mol Genet* 2003;12(8):901–6. [Cited 6 September 2019]. Available from: <https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/ddg104>.
- Shu C, Wang J, He Y, Song T, Chen Z, Tang S, et al. Effects of interleukin 10 polymorphisms on the development of hepatitis B virus infection: a systemic review and meta-analysis. *Int J Clin Exp Med* 2015;8(8):12028–40. [Cited 10 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26550115>.
- Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006;43(6):1317–25. [Cited 7 May 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16729309>.
- Thompson K, Maltby J, Fallowfield J, McAulay M, Millward-Sadler H, Sheron N. Interleukin-10 expression and function in experimental murine liver inflammation and fibrosis. *Hepatology* 1998;28(6):1597–606. [Cited 17 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9828224>.
- Truelove AL, Oleksyk TK, Shrestha S, Thio CL, Goedert JJ, Donfield SM, et al. Evaluation of IL10, IL19, and IL20 gene polymorphisms and chronic hepatitis B infection outcome. *Int J Immunogenet* 2008;35(3):255. [Cited 15 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18479293>.
- Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* 1997;24(1):1–8. [Cited 18 August 2019]. Available from: <http://doi.wiley.com/10.1111/j.1365-2370.1997.tb00001.x>.
- Wai C. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003;38(2):518–26. [Cited 7 May 2019]. Available from: <http://doi.wiley.com/10.1053/jhep.2003.50346>.
- Walter MR. The molecular basis of IL-10 function: from receptor structure to the onset of signaling. *Curr Top Microbiol Immunol* 2014;380:191–212. [Cited 15 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25004819>.
- Yang A-M, Wen L-L, Yang C-S, Wang S-C, Chen C-S, Bair M-J. Interleukin 10 promoter haplotype is associated with alcoholic liver cirrhosis in Taiwanese patients. *Kaohsiung J Med Sci* 2014;30(6):291–8. [Cited 17 April 2019]. Available from: <https://www.sciencedirect.com/science/article/pii/S1607551X14000692>.
- Yao L, Xing S, Fu X, Song H, Wang Z, Tang J, et al. Association between interleukin-10 gene promoter polymorphisms and susceptibility to liver cirrhosis. *Int J Clin Exp Pathol* 2015;8(9):11680. [Cited 10 April 2019]. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4637726/>.
- Zhang T-C, Zhang W-F, Zhao Y-Q, Pan F-M, Gao Y-F, Yuan H, et al. Gene variation in IL10 and susceptibility to chronic hepatitis B. *J Infect* 2014;69(1):75–80. [Cited 15 April 2019]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24631780>.