Human leukocyte antigen (HLA-F) polymorphism is associated with chronic HBV infection

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Abstract

Human leukocyte antigen (HLA)-F has been involved in immune regulation of infectious diseases. However, the role of HLA-F polymorphisms in hepatitis B infection outcomes remains unclear. Here, we aimed to determine HLA-F polymorphism implication in chronic HBV. Genotype analysis was performed for three single nucleotide polymorphisms (SNPs) of HLA-F and one SNP of HLA-E using PCR-SSP, in 252 Tunisian patients with chronic HBV infection stratified according to their HBV DNA levels (140 patients with low HBV DNA levels < 2000 IU/mL and 112 patients with high HBV DNA levels ≥ 2000 IU/mL) and 240 healthy controls (CTRL). The three HLA-F SNPs (HLA-F*01:02, -F*01:03 and -F*01:04) have the same allelic and genotypic frequencies in patients and in CTRL. We reported a low HLA-F*01:02 and F*01:04 allelic frequencies in the Tunisian population; however, high HLA-F*01:03 allele frequencies were observed (17%). A significant association was found between the HLA-F*01:03 allele and decreased level of HBV DNA ($P = 0.02$ OR 0.56, 95% CI 0.35–0.92). No significant differences were observed in haplotype distribution between patients and CTRL. A significant association of HLA-F*01:03 with the level of HBV DNA suggests an important role of HLA-F in HBV replication control.

Keywords Human leukocyte antigen-F · Hepatitis B virus · Chronic hepatitis · Single nucleotide polymorphism · Fibrosis

Introduction

Hepatitis B virus (HBV) is a significant problem for global health, with 240 million people worldwide chronically infected and about 780,000 deaths each year throughout the

world (World H, Organization 2015). Additionally, HBV infection is at the origin of different clinical liver diseases such as acute and chronic hepatitis, cirrhosis and eventually hepatocellular carcinoma (HCC) (Ganem and Prince 2004). The outcome of HBV infection is influenced not only by viral and environmental factors, but also by the host immune response which plays a critical role in determining the course of the infection (Thursz et al. 2011). Several studies suggest that HBV develops strategies to evade host immune surveillance. The exact mechanism of viral persistence and immune evasion requires further investigations (Tan et al. 2008; Wieland et al. 2004). Indeed, the study of host immune tolerance during HBV infection is important to understand the outcome of HBV infection.

The non-classical human leukocyte antigen (HLA)-class Ib molecules, including HLA-E, -F, and G, are considered as tolerogenic molecules. HLA-G is known for its inhibition of the cytolytic functions of natural killer (NK) cells, cytotoxic T lymphocyte (CTL) and dendritic cells (DCs) (Braud et al. 1998; Carosella et al. 2008; Lepin et al. 2000). Nevertheless, in pathological conditions, HLA-E, -F and

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-G polymorphisms and expression have been associated with several tumors (Leone et al. 2013), inflammatory diseases (Baricordi et al. 2008; Zidi et al. 2015), autoimmune diseases (Rizzo et al. 2013) and viral infection (Zidi et al. 2015).

The HLA-F locus is located at the terminal end of the chromosome 6 and has low levels of allelic polymorphisms (Geraghty et al. 1987). Only 22 HLA-F alleles encoding four distinct proteins (HLA-F*01:01, HLA-F*01:02, HLA-F*01:03 and HLA-F*01:04) have been reported. These alleles differ by a single nucleotide. Indeed, HLA-F*01:02 has a T in the place of C (HLA-F*01:01) at position 38 in exon 1, resulting in a valine in the place of an alanine. The HLA-F*01:03 allele contains a T-to-C mutation at position 814 in exon 4, exchanging a serine for a proline. A C-to-A substitution at position 212 in exon 2 discriminates HLA-F*01:04 from HLA-F*01:01, exchanging a proline for a glutamine (Moscoco et al. 2007; Robinson et al. 2013).

The clinical implications of HLA-F polymorphisms and the linkage disequilibrium (LD) with HLA-E in patients with chronic HBV infection have not been considerably investigated. In a previous study, we reported the association between HLA-E polymorphism and HLA-E soluble in HBV infection (Zidi et al. 2016). In the current study, we extend the genotyping of HLA-E to investigate the impact of haplotypes and the characteristics of LD between HLA-F and HLA-E loci in chronic infection with HBV. To our knowledge, this is the first report studying the prevalence of HLA-F polymorphisms in Tunisian patients infected with HBV and its association with clinical outcome of HBV infection.

Materials and methods

Patients and controls

Two hundred and fifty-two Tunisian patients, chronically infected with HBV, recruited from the infectious diseases service of CHU Farhat Hached Sousse were enrolled in this study. All patients were seropositive for the hepatitis B surface antigen (HBsAg) for more than 6 months.

HBV markers including HBsAg, HBe antigen (HBeAg), HBe antibodies (anti-HBe) and anti-core antibodies (anti-HBc) were detected by microparticle enzyme immunoassay technology (AxSYM, Abbott Laboratories, Abbott Park, USA). Patients with other liver diseases (including autoimmune liver disease and alcoholic liver disease) or other viral diseases (including hepatitis C virus (HCV), hepatitis delta virus and HIV) were excluded from the study. No patient has received antiviral treatment.

The patients were followed regularly with the measurements of liver function tests including aspartate

aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBil), γ -glutamyltransferase (GGT) and alkaline phosphatase (ALP).

Patients were stratified into two subgroups according to their HBV DNA levels: 140 patients with low serum HBV DNA levels (< 2000 IU/mL) (61 man and 79 women; Mean age 35.98 ± 1.04 years) and 112 patients with high serum HBV DNA levels (≥ 2000 IU/mL) (65 man and 47 women; Mean age 31.25 ± 1.24 years). The value of HBV DNA levels was expressed as log of measured HBV copy numbers.

A percutaneous liver biopsy was performed for 72 chronically HBV-infected patients during the study period. Hepatic fibrosis stage was assessed using the METAVIR scoring system. Based on fibrosis score, HBV patients were divided in two subgroups: the first includes 61 patients with mild to moderate fibrosis (METAVIR F0, F1 or F2) and the second includes 11 patients with severe fibrosis (METAVIR F3 or F4).

Two hundred and forty ethnically and geographically matched healthy blood donors served as controls group that were seronegative for any known serological marker of HBV, HCV and HIV infection. They were recruited from the regional center of blood transfusion of Farhat Hached University Hospital. All patients and healthy controls gave their consent for the performed study.

Genotyping assay of HLA SNPs

Genomic DNA was extracted from 200 μ l of peripheral blood using the QIAamp DNA blood kit according to the manufacturer's instructions (Qiagen, Chatsworth, CA, USA). Genotyping of three SNPs of HLA-F: HLA-F*01:02 C>T (rs17875379), HLA-F*01:03 T>C (rs1736924), HLA-F*01:04 C>A (rs17875380), and HLA-E*01:01:01:03 A>G (rs1264457) was performed by polymerase chain reaction sequence-specific priming (PCR-SSP); three sets of amplification primers, as previously described, for HLA-F SNPs (Pan et al. 2013). The final volume of 10 μ l of PCR contained 100 ng genomic DNA, 1X taq polymerase buffer, 1.5 mM $MgCl_2$, 0.2 mM concentrations of each dNTP, 1U of taq DNA polymerase, 0.4 μ M of each specific primer and 0.2 μ M of internal control primers, which amplified a human growth hormone sequence for the validation of the PCR amplification system (forward: 5'-CAGTGCCTTCCCAACCATTCCCTT A-3'; reverse 5'-ATCCACTCACGGATTCTGTGTGTTTC-3'). Genotype analysis of HLA-E*01:01:01:03 A>G (rs1264457) was performed with a polymerase chain reaction sequence-specific primer typing method as described previously (Zidi et al. 2016). The PCR products were visualized by 2% agarose gel electrophoresis.

Statistical analysis

Statistical analyses were performed using SPSS version 17.0. Allele and genotype frequencies were compared among different groups using the χ^2 test, and the Fisher exact test was used when appropriate. The odds ratio (OR) [95% confidence interval (CI)] was calculated to estimate the relative risk. P values < 0.05 were considered significant. Correction of Bonferroni was applied for genotype comparisons. The Haploview software (version 4.2) and SNP Analyzer (version 2.0) were used for analysis of the Hardy–Weinberg equilibrium (HWE), haplotype inference and LD parameters.

Results

Characteristics of the study population

Our study included 252 patients (126 man and 126 women) and 240 healthy controls (117 man and 123 women), whose mean ages were [$\pm$ standard error of the mean (SEM)] 35.59 ± 0.68 and 34.4 ± 0.67 years, respectively. There were no significant differences in gender, age and ethnicity between the two groups. The clinical profiles of HBV-infected patients with high or low HBV DNA levels are shown in Table 1. We observed significant differences in the HBV DNA levels between the two groups ($P = 0.001$).

Genotypes and allele distribution of HLA-F and HLA-E SNPs among patients and healthy controls

All polymorphisms of HLA-F and -E SNPs were in HWE ($P > 0.05$). The genotype frequencies of the four SNPs among patients infected with HBV and controls are as shown

in Table 2. No significant differences in genotype and allele frequencies among these two groups were reported for HLA-F*01:03 T>C and HLA-F*01:04 C>A polymorphisms ($P = 0.99$; $P = 0.64$, respectively; Table 2). Interestingly, HLA-F*01:02 C>T is significantly associated with HBV infection ($P_{\text{corrected}} = 0.01$ for alleles and $P_{\text{corrected}} = 0.03$ for genotypes, Fisher test). The HLA-F*01:02 T/T genotype was not found in both groups. For the HLA-E*01:01/01:03 A>G polymorphism, there was no significant difference in the allele frequencies among patients and healthy controls ($P = 0.85$). The frequencies of the AA, AG and GG genotypes of HLA-E*01:01/01:03 A>G were 34.2, 45 and 20.8% in healthy controls, and 29, 54.4 and 16.7% in patients with chronic HBV infection ($P = 0.83$ and $P = 0.08$). Increasing sample size of HLA-E*01:01/01:03 A>G genotyping in this study is consistent with our previously published result (Zidi et al. 2016).

Genotype and allele distribution of HLA-F and HLA-E SNPs according to the levels of HBV DNA

To investigate the possible implication of the HLA-F and HLA-E polymorphisms in the progression of HBV infection, we stratified chronic HBV carriers into two groups according to the HBV DNA levels (high: ≥ 2000 IU/mL; low: < 2000 IU/mL). As indicated in Table 3, our results showed no significant differences in genotype and allele distribution, HLA-F*01:02 C > T, HLA-F 01:04 C/A and HLA-E*01:01/01:03 A > G SNPs (data not shown), in patients with high HBV DNA levels and those with low HBV DNA levels. On the contrary, the frequency of HLA-F*01:03 C allele among patients with low HBV DNA levels (21%) was higher than those with high HBV DNA levels (13%) ($P = 0.02$). The combined CC + TC (dominant model) genotypes of HLA-F* 01:03 were also associated

Table 1 Demographic characteristics and clinical profiles of HBV-infected patients' subgroups with high or low HBV DNA levels

	Patients with low HBV DNA levels (N = 140)	Patients with high HBV DNA levels (N = 112)	P value
Liver function tests (LFT)			
ALT (IU/L)*	24.28 $\pm$ 0.98	35.55 $\pm$ 3.17	<0.0001
AST (IU/L)*	22.87 $\pm$ 0.90	30.24 $\pm$ 2.33	<0.0001
GGT (IU/L)*	24.61 $\pm$ 1.95	27.03 $\pm$ 3.45	0.2
ALP (IU/L)*	86.78 $\pm$ 5.03	84.46 $\pm$ 6.88	0.8
T Bil (μ mol/L)*	9.48 $\pm$ 0.54	10.97 $\pm$ 0.72	0.37
HBV DNA Loads (IU/mL, log)*	2.12 $\pm$ 0.06	4.38 $\pm$ 0.11	0.001
Liver biopsy specimen available during the study period	6	66	
Stage of fibrosis			
F0–F2	6	55	
F3–F4	0	11	

*Mean $\pm$ SEM

Table 2 Genotype distribution and allele frequencies of HLA-F and HLA-E polymorphisms between patients with chronic HBV infection and healthy controls

Allele/Genotype distribution		Patients <i>n</i> (%)	Controls <i>n</i> (%)	<i>P</i> value*	<i>P</i> _{corrected} value	OR [95% CI]
HLA-F*01:02 C/T						
Allele	T	0 (0)	6 (1.25)	0.01		0.07 [0.004–1.28]
	C	504 (100)	474 (98.75)			
Genotype	TT	0 (0)	0 (0)	–		–
	TC	0 (0)	6 (2.5)	0.01	0.03	0.07 [0.004–1.28]
	CC	252 (100)	234 (97.5)	**		
	TT + TC vs CC	0	6	0.01	0.03	0.07 [0.004–1.28]
HLA-F*01:03 T/C						
Allele	C	87 (17)	83 (17)	0.99		0.99 [0.71–1.38]
	T	417 (83)	397 (83)			
Genotype	CC	11 (4)	10 (4)	0.92	2.76	1.04 [0.43–2.52]
	CT	65 (26)	63 (26)	0.91	2.73	0.97 [0.65–1.46]
	TT	176 (70)	167 (70)	**		
	CC + CT vs TT	76 (30)	73 (30)	0.95	2.85	0.98 [0.67–1.45]
HLA-F*01:04 C/A						
Allele	A	1 (1)	2 (1)	0.64		0.47 [0.04–5.25]
	C	503 (99)	478 (99)			
Genotype	AA	0 (0)	(0)	–		–
	AC	1 (0.5)	2 (1)	0.61	1.83	0.47 [0.04–5.26]
	CC	251 (99.5)	238 (99)	**		
	AA + AC vs CC	1 (0.5)	2 (1)	0.61	1.83	0.47 [0.04–5.26]
HLA-E*01:01/01:03 A/G						
Allele	G	316 (47.7)	256 (47.1)	0.85		1.02 [0.81–1.2]
	A	347 (52.4)	287 (52.9)			
Genotype	GG	42 (16.7)	48 (20.8)	0.83		0.9 [0.56–1.59]
	AG	137 (54.4)	104 (45)	0.08		1.4 [0.94–2.14]
	AA	73 (29)	79 (34.2)	**		
	GG + AG vs AA	179	152	0.21		1.2 [0.86–1.87]

Risk alleles marked in bold letters

**Reference genotype. *Fisher test exact

with a low risk of high HBV DNA levels (OR 0.54; 95% CI 0.31–1.03; $P = 0.03$). However, we lost this association after correction for multiple comparisons, possibly due to the low number of samples present in the different cohorts. These results suggest that the HLA-F*01:03 “C” allele may have an important role in the regulation of serum HBV viral level.

Genotype and allele distribution of HLA-F and HLA-E SNPs according to the stage of fibrosis

After stratification according to the stage of liver fibrosis, no significant differences were observed in the distribution of the HLA-F*01:02 C>T alleles, HLA-F*01:04 C>A and HLA-E*01:01/01:03 A>G alleles (data not shown) between patient groups with mild to moderate fibrosis and those

with severe fibrosis. Interestingly, the frequencies of HLA-F*01:03 “C” allele is significantly associated with liver fibrosis stages (OR 2.88; 95% CI 1.02–8.09; $P = 0.038$), suggesting that HLA-F*01:03 T>C polymorphism may be involved in the progression of liver fibrosis (Table 4).

Genotypes and alleles distribution of HLA-F and HLA-E SNPs according to the gender of patients and healthy controls

When allele and genotype frequencies of HLA-F and HLA-E polymorphisms were stratified by gender (Table 5), no significant difference in the distributions of HLA-F*01:02 C>T, HLA-F*01:03 T>C and HLA-F*01:04 C>A among patients with chronic HBV infection and healthy controls were found.

Table 3 Genotype distribution and allele frequencies of HLA-F polymorphisms between patients with low or high HBV DNA levels

Allele/Genotype distribution		Patients with low HBV DNA levels (N = 140) N (%)	Patients with high HBV DNA levels (N = 112) N (%)	P value*	P _{corrected} value	OR [95% CI]
HLA-F*01:02 C/T						
Allele	T	0 (0)	0 (0)	—		—
	C	280 (100)	224 (100)			
Genotype	TT	0 (0)	0 (0)	—		—
	TC	0 (0)	0 (0)	—		—
	CC	140 (100)	(112) (100)	**		—
HLA-F*01:03 T/C						
Allele	C	58 (21)	29 (13)	0.02		0.56 [0.35–0.92]
	T	222 (79)	195 (87)			
Genotype	CC	8 (6)	3 (3)	0.16	0.48	0.39 [0.1–1.5]
	CT	42 (30)	23 (20)	0.06	0.18	0.57 [0.31–1.03]
	TT	90 (64)	86 (77)	**		—
	CC + CT vs TT	50 (36)	26 (23)	0.03	0.09	0.54 [0.31–0.95]
HLA-F*01:04 C/A						
Allele	A	1 (0.5)	0 (0)	1.54		0.41 [0.01–10.23]
	C	279 (99.5)	224 (100)			
Genotype	AA	0 (0)	0 (0)	—		—
	AC	1 (1)	0 (0)	0.37	1.11	0.41 [0.01–10.24]
	CC	139 (99)	112 (100)	**		—
	AA + AC vs CC	1 (1)	0 (0)	0.37	1.11	0.41 [0.01–10.24]

Risk alleles marked in bold letters

**Reference genotype. *Fisher test exact

Furthermore, comparison of the frequency distribution between male and female patients with chronic HBV infection revealed the presence of the “G” allele (OR 1.41; 95% CI 1.0–1.99; $P = 0.046$) and GG genotype (OR 2.15; 95% CI 1.03–4.48; $P = 0.038$) of the HLA-E*01:01/01:03 A>G to be higher in males with chronic HBV infection compared to females chronically infected with HBV. Conversely, no significant differences were observed in the distribution of the HLA-E*01:01/01:03 A>G alleles and genotypes between patients and healthy controls with respect to gender.

Haplotype association analysis

Haplotype analysis was performed for patients with chronic HBV infection and healthy controls with an emphasis on the characteristics LD between HLA-F and HLA-E and to evaluate the haplotype frequencies for each group. All polymorphisms with MAF (minor allele frequency) < 0.01 are excluded. A total of four haplotypes were derived from the observed genotypes. The HLA-F*01:03 T>C and HLA-E*01:01/01:03 A>G haplotype frequencies are represented in Table 6. The highest frequency of haplotype in cases and controls was TA (H1) (45.6 and 50.9%, respectively) and the lower haplotype was “CA” (H4) (8.6 and 6.8%, respectively).

No statistically significant differences were observed in the haplotype distribution between patients with chronic HBV infection and healthy controls.

Discussion

Diverse and complex clinical manifestations are seen following HBV exposure in adulthood. Indeed, acute infection is usually asymptomatic and the majority of subjects are able to eradicate completely the virus through a strong immune response directed against viral antigens. By contrast, in chronic carriers of HBV, the immune response is weak and less vigorous than patients with self-limited HBV infection. Patients with persistent infection are at increased risk of chronic liver disease complications (liver fibrosis, cirrhosis and hepatocellular carcinoma) (Chisari and Ferrari 1995). Several studies suggest that a complex combination of virological, immunological and host genetic factors may influence the natural history of disease (Chapman and Hill 2012). In this respect, the human leukocyte antigen (HLA) class I and II are suggested to be associated with the outcome of HBV infection (Chapman and Hill 2012).

Table 4 Genotype distribution and allele frequencies of HLA-F polymorphisms in patients according to the stage of liver fibrosis

Allele/genotype distribution		Fibrosis METAVIR score		<i>P</i> value	<i>P</i> _{corrected} value	OR [95% CI]
		F0–F1 (<i>N</i> = 61) <i>N</i> (%)	F2–F4 (<i>N</i> = 11) <i>N</i> (%)			
HLA-F*01:02 C/T						
Allele	T	0 (0)	0 (0)	–	–	–
	C	122 (100)	22 (100)			
Genotype	TT	0 (0)	0 (0)	–	–	–
	TC	0 (0)	0 (0)	–	–	–
	CC	61 (100)	11 (100)	**		
	TT + TC vs CC	0 (0)	0 (0)			–
HLA-F*01:03 T/C						
Allele	C	17 (14)	7 (32)	0.038		2.88 [1.02–8.09]
	T	105 (86)	15 (68)			
Genotype	CC	2 (4)	2 (18)	0.093	0.27	7.66 [0.9–64.94]
	TC	13 (21)	3 (27)	0.42	1.26	1.76 [0.38–8.06]
	TT	46 (75)	6 (55)	**		
	CC + TC vs TT	25 (15)	5 (45)	0.15	0.45	2.55 [0.68–9.58]
HLA-F*01:04 C/A						
Allele	A	0 (0)	0 (0)	–	–	–
	C	122 (100)	22 (100)			
Genotype	AA	0 (0)	0 (0)	–	–	–
	CA	0 (0)	0 (0)	–	–	–
	CC	61 (100)	11 (100)	**		
	AA + CA vs CC	0 (0)	0 (0)	–	–	–

Risk alleles marked in bold letters

**Reference genotype.*Fisher test exact

Here, we aim to investigate the association between four SNPs in HLA-F and HLA-E genes (HLA-F*01:02 C>T, F*01:03 T>C, HLA-F*01:04 C>A and HLA-E*01:01/01:03 A>G) and the susceptibility to different clinical status of chronic infection with HBV in a Tunisian population. Our results revealed a statistically significant protective association of HLA-F*01:02 “T” allele with chronic HBV infection (OR 0.07; 95% CI 0.004–1.28; *P* = 0.01; Table 2). The frequency of HLA-F*01:02 C>T heterozygote genotype is increased in healthy control group versus patients with chronic HBV infection (2.5 vs 0%, respectively). Furthermore, our data showed the absence of the homozygous HLA-F*01:02 T/T genotype in our samples. This finding is consistent with the observations made on different Chinese populations (Pan et al. 2013).

No significant differences were found in the distribution of allele and genotype frequencies between patients and healthy controls concerning HLA-F*01:03 T>C polymorphism (Table 2). This finding is in concordance with previous published data reporting no association between HLA-F*01:03 T>C polymorphism and chronic HBV infection in a Chinese population (Zhang et al. 2012). However, the HLA-F*01:03 “C” allele was found to be the minor allele

in the Tunisian population, but it was detected with high prevalence in our cohort compared to the Chinese population (17 vs 1.9%, respectively) (Zhang et al. 2012). These discrepancies regarding the distribution of alleles could be due to differences in study cohorts and ethnicity. In addition, no significant association was observed for HLA-F*01:04 C>A polymorphism in relation with the chronic infection with HBV (*P* = 0.64; Table 2). In agreement with the observations of Zhang et al., no significant association was observed with chronic HBV infection (Zhang et al. 2012). In our cohort, the HLA-F*01:04 “A” allele was found to be the minor allele (1%) compared to the “C” allele (99%) in healthy controls (Table 2). Similar allelic frequencies were detected in the controls group of the Chinese population (*A* = 0.8%, and *C* = 99.2%) (Zhang et al. 2012).

The comparison of the genotype and allele distribution of HLA-F*01:03 T>C polymorphism with regard to viral load revealed an interesting and a significant difference between patients with high DNA levels and those with low HBV DNA levels (*P* = 0.02), which we lost after correction for multiple comparisons, possibly due to the low number of samples present in the different cohorts. As shown in Table 3, the frequency of the HLA-F*01:03 “C” allele

Table 5 HLA-F and HLA-E allele and genotype frequencies in patient with chronic HBV infection and healthy controls

Allele/genotype distribution		CHB Patients		Healthy controls		P value ^a	OR ^a [95% CI]	P value ^b	OR ^b [95% CI]	P value ^c	OR ^c [95% CI]
		Male	Female	Male	Female						
HLA-F*01:02 C/T											
Allele	T	1	0	6	0	1	-	-	-	-	-
	C	273	260	224	244						
Genotype	TT	0	0	0	0	1	-	-	-	-	-
	TC	1	0	6	0	1	-	-	-	-	-
	CC	136	130	109	122	1	-	-	-	-	-
	TT + TC vs CC	1	0	6	0	1	-	-	-	-	-
HLA-F*01:03 T/C											
Allele	C	47	40	30	37	0.64	1.11 [0.7–1.77]	0.18	1.4 [0.85–2.3]	0.86	1.04 [0.64–1.7]
	T	219	208	196	201						
Genotype	CC	9	2	2	4	0.06	4.07 [0.85–19.38]	0.06	4.02[0.84–19.15]	0.68	0.5 [0.08–2.8]
	TC	29	36	26	29	0.27	0.72 [0.41–1.28]	0.99	0.99[0.54–1.82]	0.45	1.24[0.69–2.2]
	TT	95	86	85	86	-	-	-	-	-	-
	CC + TC vs TT	38	38	28	33	0.71	0.9 [0.52–1.54]	0.5	1.2 [0.68–2.1]	0.61	1.15 [0.66–2]
HLA-F*01:04 A/C											
Allele	A	0	1	0	2	-	-	-	-	-	-
	C	272	259	230	246						
Genotype	AA	0	0	0	0	-	-	-	-	-	-
	CA	0	1	0	2	-	-	-	-	-	-
	CC	136	129	115	122						
	AA + CA vs CC	0	1	0	2	-	-	-	-	-	-
HLA-E*01:01/01:03 A/G											
Allele	G	139	106	97	103	0.046	1.41 [1–1.99]	0.18	1.2 [0.88–1.8]	0.79	0.95 [0.66–1.36]
	A	139	150	123	139						
Genotype	GG	32	19	23	25	0.038	2.15 [1.03–4.48]	0.21	1.5 [0.76–3.2]	0.54	0.79 [0.38–1.66]
	AG	75	68	51	53	0.23	1.41 [0.8–2.49]	0.09	1.6 [0.91–2.99]	0.29	1.34 [0.76 –2.35]
	AA	32	41	36	43	-	-	-	-	-	-
	GG + AG vs AA	107	87	74	78	0.09	1.5 [0.94–1.67]	0.08	1.6 [0.92–2.85]	0.55	1.16 [0.69–1.97]

^aMale patients vs female patients^bMale patients vs male healthy controls^cFemale patients vs female healthy controls

Table 6 Haplotype frequencies of HLA-F and HLA-E genes between patients and healthy controls

Haplotypes	Sequences	Cases, controls frequencies	Frequency	Chi square	P value	OR
H1	TA	0.456; 0.509	0.482	1.137	0.286	0.87 [0.677–1.12]
H2	TG	0.367; 0.342	0.354	0.052	0.819	1.03 [0.788–1.35]
H3	CG	0.091; 0.081	0.086	1.245	0.264	1.26 [0.838–1.9]
H4	CA	0.086; 0.068	0.078	0.116	0.733	1.1 [0.63–1.92]

was much higher in patients with low HBV DNA levels as compared to patients with low HBV DNA levels (21 vs 13%, respectively). This result suggests that the HLA-F*01:03 “C” allele may favor the inhibition of HBV replication (OR 0.56; 95% CI 0.35–0.92; $P = 0.02$). In addition, haplotype analysis including HLA-F SNPs and HLA-E revealed no significant differences between patients and healthy controls.

Elsewhere, we present evidence of significant association of the HLA-F*01:03 T>C polymorphism with liver fibrosis severity in patients with chronic HBV infection ($P = 0.038$). The HLA-F 01:03 “C” allele is more prevalent among patients with severe fibrosis (F3–F4) than with mild–moderate fibrosis (F0–F2) (32% vs 14%, respectively; Table 4). This finding suggests that HLA-F*01:03 “C” allele may influence the progression of liver fibrosis in HBV carriers. In accordance with this hypothesis, Xu et al. reported that HLA-F expression was associated with poor survival in HCC patients and correlated with tumor cell invasion and metastasis (Xu et al. 2015).

After stratification according to gender, the statistical analysis revealed that the HLA-E*01:03 alleles were associated with males chronically infected with HBV ($P = 0.046$). In the literature, infected males with HBV are also six times more likely to be susceptible than women to develop chronic HBV infection (Lee et al. 1999). The HLA-E*01:03 genotype has been correlated with susceptibility to various diseases including bacterial infection and early transplant-related mortality of bone marrow, and the outcome of hematopoietic stem cell (Ludajic et al. 2009; Tamouza et al. 2005). The HLA-E*01:03 genotype has been associated with high level of HLA-E molecule on the cell surface (Strong et al. 2003). The HLA-E*01:03 allele may increase the susceptibility to males to develop a chronic infection with HBV.

Studies concerning the function of HLA-F allelic variation have been rarely reported. The HLA-F*01:03 “C” allele is characterized by a synonym substitution (serine to proline) that may influence the alpha 3 ($\alpha 3$) domains binding to the light chain $\beta 2$ -microglobulin ($\beta 2 m$). This mutation is known to influence the affinity of peptides binding and T cell recognition (Howcroft and Singer 2003). In chronic hepatitis B infection, the HBV-specific CD8+ T cells play an important role in the inhibition of virus replication (Penna et al. 1991). Inappropriate viral antigen recognition by cytotoxic T lymphocytes (CTLs) and substitutions of T cell receptor (TCR)

contact sites could influence the CTL response, which may induce persistent HBV infection (Chisari and Ferrari 1995). Here, we propose that HLA-F*01:03 T>C polymorphism may decrease the affinity of the HLA-F peptide-binding groove to some HBV viral peptides (such as HBsAg) that may cause the downregulation of the HBV-specific CD8+ T cells. These mechanisms may, therefore, be involved in the escape from HBV antiviral immune responses. In accordance with our previous study showing that the 14-bp del/del genotype of HLA-G is associated with low HBV DNA levels (Zidi et al. 2015), we suggest a possible gene–gene interaction involvement in the HBV infection progression.

In conclusion, HLA-F*01:03 “C” allele may be associated with low HBV DNA levels and the severity of liver fibrosis in HBV carriers, suggesting that the HLA-F*01:03 “C” allele may affect the regulation of serum HBV DNA levels and the progression of HBV-related liver disease. Different studies will reveal if HLA-F and HLA-E polymorphisms may play a critical role in the immune response of hepatitis B infection and be considered as prognostic markers for HBV infection outcome.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interests.

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