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Title: *HLA-E* variants are associated with sustained virological response in HIV/HCV coinfected patients on HCV therapy

Running head: *HLA-E* variants and HCV treatment

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ABSTRACT

Objectives: To analyze whether human leukocyte antigen (HLA)-E allelic variants are associated with and may predict response to peg-interferon (IFN) alpha plus ribavirin treatment in HIV/hepatitis C virus (HCV) coinfected patients.

Design: Retrospective follow-up study

Methods: We studied 321 naïve patients who started HCV-treatment. *HLA-E* genotyping was performed by restriction fragment length polymorphism. A sustained virological response (SVR) was defined as undetectable plasma HCV-RNA up through 24 weeks after the end of HCV-treatment.

Results: The *HLA-E*0101* allele increased the odds of achieving SVR for all patients (adjusted odds ratio (aOR)=2.03 (95% confidence interval (95%CI)=1.35–3.06); p=0.001), for HCV genotype (GT) 1/4 patients (aOR=1.62 (95%CI=1.03–2.54), p=0.035), and for GT2/3 patients (aOR=9.87 (95%CI=2.47–31.89), p=0.001). For decision tree analysis, the SVR rate increased from 0% to 82.6% and then to 92.5% in GT2/3 patients when the count of *HLA-E*0101* alleles increased. In GT1/4 patients with rs8099917 TT genotype, the SVR rate increased from 33.3% to 54.8% and then to 61.8% when the count of *HLA-E*0101* alleles increased. In GT1/4 patients with rs8099917 GT/GG genotype, the SVR rate increased from 15.4% to 22% and then to 44% when the count of *HLA-E*0101* alleles increased. The overall percentage of patients correctly classified was 73.2% and the area under the receiver operating characteristic curves (AUROC) was 0.803±0.024.

Conclusions: The *HLA-E*0101* allele was associated with increased odds of HCV clearance and could help to predict SVR among HIV/HCV coinfected patients on HCV therapy. This would helpful to avoid treatment in those less likely to respond to pegIFNα/RVB treatment.

Keywords: *HLA-E*; AIDS; HCV therapy; *IL28B*; HCV clearance; single nucleotide polymorphism

INTRODUCTION

Through the use of highly active antiretroviral therapy (HAART), human immunodeficiency virus (HIV) infection has become a chronic disease, and thus its mortality has declined [1]. As a result, chronic hepatitis C (CHC) has turned into an important comorbidity and a major cause of death among HIV/hepatitis C virus (HCV) coinfected patients [2].

HCV therapy with pegylated-interferon-alpha plus ribavirin (pegIFNα/RVB) is still in use among HIV/HCV coinfected patients [3], even in combination with new therapies such as Telaprevir or Boceprevir [4]. However, not all HIV/HCV coinfected patients achieve the desired sustained virological response (SVR) [4]. Due to this fact, the identification of predictors for HCV therapy success is particularly desirable to ensure an adequate selection of the best candidates and to minimize any undesirable toxicity. To date, the best baseline predictors of response to current HCV-therapy are HCV genotype, baseline serum HCV RNA level, liver fibrosis, and single-nucleotide polymorphisms around *interleukin 28B* (*IL28B*) gene [5]. However, an unexplained variability in treatment outcome still remains, suggesting that other host genetic factors may play an important role in pegIFNα/RBV treatment success [6].

The human leukocyte antigen (HLA)-E molecule is a ligand for CD94/NKG2 receptors (CD94/NKG2A (inhibitory) and CD94/NKG2C (activator)) on natural killer (NK) cells and NK cytotoxic T lymphocytes (NK-CTL), as well as a ligand for the αβ T-cell receptor (TCR) on CD8+ T lymphocytes (CTL) [7, 8]. The *HLA-E* gene is minimally polymorphic. One single nucleotide substitution (A to G at position 382, exon 3) results in an amino acid change from arginine (R) to a glycine (G) at position 107 of the α2 heavy-chain domain [9, 10]. This non-synonymous change generates two variants (*HLA-E*0101* and *HLA-E*0103*), which differ at only one amino acid position.

HLA-E plays an important role in regulating antiviral immunity [7]. Both HIV and HCV infections are associated with enhanced HLA-E expression, which may contribute to viral persistence as an additional viral evasion strategy targeting the antiviral activities of NK cells [11, 12]. Furthermore, the presence of a *HLA-E*0101/HLA-E*0101* genotype might confer protection from HIV infection [13] and HCV infection [14]. Finally, the NK receptor has recently been associated with pegIFNα/RBV therapy-induced clearance, in combination with *IL28B* genotype [15, 16].

The aim of the present study was to analyze whether *HLA-E* allelic variants are associated with and may predict the response to pegIFNα/RBV treatment in HIV/HCV coinfected patients.

PATIENTS AND METHODS

Patients

We carried out a retrospective follow-up study on 321 HIV/HCV coinfected patients, who started treatment with pegIFNα/RBV on regular follow-up for the first time, at two reference HIV hospitals located in Madrid, Spain. The study population was comprised of HIV/HCV coinfected individuals who had completed a course of pegIFNα/RBV therapy and were genotyped for *HLA-E* variants. This study was conducted in accordance with the Declaration of Helsinki. Patients gave their written consent for the study and it was approved by the Institutional Ethics Committee.

Information was obtained from medical records as previously described [17]: age, gender, risk category, weight, height, nadir $CD4^+$ T cell count, antiretroviral therapy, HCV genotype, and liver fibrosis. In addition during HCV therapy, a blood sample was taken from each patient to analyze complete blood counts, $CD4^+$ T-cells, plasma HIV viral load (HIV-RNA) and plasma HCV viral load (HCV-RNA).

DNA Genotyping

Genomic DNA was extracted from peripheral blood by using Qiagen columns (QIAamp DNA Blood Midi/Maxi; Qiagen, Hilden, Germany). The *IL28B* polymorphism rs8099917 was genotyped by the Spanish National Genotyping Centre (CeGen; http://www.cegen.org/). Genotyping was performed by using the GoldenGate® assay with VeraCode® Technology (Illumina Inc. San Diego, CA, USA). The *HLA-E* polymorphism rs1264457 was genotyped by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) in order to differentiate between *HLA-E*0101* and *HLA-E*0103* variants. After PCR, fragments were digested by the restriction enzyme HpaII. The PCR amplicon of the *HLA-E*0103* allele presents a restriction site for HpaII, producing two fragments of 260 and 20 bp, while the *HLA-E*0101* allele has no restriction site for the enzyme. Primer sequences have been previously reported [18].

Hepatitis C therapy

Treatment regimens included pegIFNα 2a or 2b at standard doses (180 µg/week or 1.5 µg/kg/week, respectively) plus weight-adjusted RBV dosing (1000 mg/day for patients weighing <75 kg and 1200 mg/day for patients weighing ≥75 kg). Following international guidelines [19], patients with HCV genotypes 1 or 4 received either 48 or 72 weeks of treatment, and patients with HCV genotype 2 or 3 were treated for 24 or 48 weeks, according to virological response at week 4. Early stopping rules were applied for subjects with suboptimal virological response at week 12 [19]. A SVR was defined as an undetectable serum HCV-RNA level (<10 IU/mL) up through 24 weeks after the end of treatment. Patients not fulfilling SVR criteria were considered as non-responders.

Statistics

For this study, we excluded patients from the analysis that had their HCV treatment prematurely interrupted due to adverse events, abandonment or loss of follow-up. The statistical analysis was carried out by on-treatment analysis of observed data.

The statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) 15.0 (SPSS INC, Chicago, IL, USA). All p-values were two-tailed. Statistical significance was defined as $p<0.05$. Categorical data and proportions were analyzed using the chi-squared test or Fisher's exact test. Kruskal-Wallis test was used to compare data among independent groups. P-values were adjusted by Bonferroni correction.

Statistically significant deviations from the expected genotypic frequencies were determined using the Hardy-Weinberg principle with a p-value cutoff of <0.01 [20]. The trend data was tested by Gamma correlation coefficient (γ; values between -1 and +1), a non-parametric test for measuring the correlation between ordinal variables. Next, we performed univariate and multivariate logistic regression analyses to investigate the association between *HLA-E* alleles and HCV treatment response. In each multiple logistic regression analysis, we included the number of *HLA-E*0101* alleles and the most significant covariables, which were selected by a stepwise algorithm (at each step, factors are considered for removal or entry: a p-value for entry of 0.20 and exit of 0.15). The covariables included in stepwise analysis were gender, age, body mass index (≥25 kg/m^2), nadir CD4$^+$ T-cells, undetectable HIV-RNA (<50 copies/mL), CD4+ T-cells, HAART, HCV-RNA ≥500,000 IU/ml, HCV genotype (GT1/4 vs. GT3), significant fibrosis (F≥2), and rs8099917 TT genotype.

Classification and regression tree (CART) was used to classify SVR according to *HLA-E* and *IL28B* genotypes, and HCV-genotype. CART is a prognostic system with a hierarchical structure, based on recursive portioning that builds a decision tree to identify subgroups at higher odds of SVR. A number of different configurations were evaluated using 25-fold cross-validation to determine the optimal split. The accuracy was evaluated by calculating area under the receiver operating characteristic curves (AUROC). Criteria to qualify for accuracy were as follows: 0.90–1 =excellent, 0.80–0.90 =good, 0.70–0.80 =fair, and 0.60–0.70 =poor.

RESULTS

Patients

Table 1 shows the clinical and epidemiological characteristics of 321 HIV/HCV coinfected patients on HCV treatment. Of note, patients with *HLA-E*0101/*0101* genotype had higher percentages of GT2/3 and the favorable rs8099917 TT genotype, which are both relevant predictive factors of virological response.

Table 1. Main baseline epidemiological and clinical characteristics of HIV/HCV coinfected patients on HCV therapy.

		HLA-E			
	All patients	**E0103/E0103**	**E0101/E0103**	**E0101/E0101**	**(*) p-value**
No.	321	46	129	146	
Male	244 (76%)	36 (78.3%)	95 (73.6%)	113 (77.4%)	0.976
Age (years)	41.9 (38.6;45.1)	41 (38;45)	42 (38.6;45)	42 (39;46)	0.896
IVDU (**)	281 (89.2%)	42 (93.3%)	110 (86.6%)	129 (90.2%)	0.784
HAART	270 (84.1%)	39 (84.8%)	108 (83.7%)	123 (84.2%)	1.000
HIV markers					
Nadir CD4+ T-cells/µL	226 (131;341)	206 (114;316)	247 (144;343)	200 (118;354)	0.600
Nadir CD4+ <200 cells/µL	136 (42.4%)	19 (41.3%)	63 (48.8%)	54 (37%)	0.359
CD4+ T-cells/µL	460 (362;669)	413 (317;536)	486.5 (400;664)	460 (347;703)	0.124
CD4+ ≥500 cells/µL (**)	142 (44.7%)	14 (30.4%)	56 (44.1%)	72 (49.7%)	0.203
HIV-RNA <50 copies/mL	208 (65.6%)	31 (67.4%)	84 (66.7%)	93 (64.1%)	0.998
HCV markers (**)					
HCV-genotype 1/4	216 (67.5%)	41 (89.1%)	83 (64.3%)	92 (63.4%)	**0.009**
HCV-RNA ≥500.000 IU/mL	232 (73.5%)	40 (88.9%)	92 (72.4%)	100 (69.9%)	0.112
Liver fibrosis (**)		n=44	n=111	n=129	
Significant fibrosis (F≥2)	182 (62.8%)	30 (68.2%)	74 (65.5%)	78 (58.6%)	0.793
Advanced fibrosis (F≥3)	100 (34.5%)	21 (47.7%)	36 (31.9%)	43 (34.5%)	0.346
***IL28B* genotype (**)**					
rs8099917 TT	200 (62.9%)	17 (37%)	77 (59.7%)	106 (74.1%)	**<0.001**

Values expressed as absolute number (percentage) and median (percentiles 25 and 75). P-values were adjusted by Bonferroni correction. In bold, p-values with statistical significance.
Abbreviations: IVDU, intravenous drug users; HAART, highly active antiretroviral therapy; HCV, Hepatitis C virus; HCV-RNA, HCV plasma viral load; HIV, Human immunodeficiency virus; HIV-RNA, HIV plasma viral load; IL28B, interleukin 28B; HLA-E, human leukocyte antigen E. (*), The test used to analyze the data was Kruskal-Wallis, a non-parametric test to compare data among independent groups. (**), Sometimes, the percentages were not calculated from all patients because some data were missing.

HLA-E alleles are associated with sustained virological response

The Hardy-Weinberg equilibrium was evaluated for rs8099917 at *IL28B* (p=0.220) and rs1264457 at *HLA-E* (p=0.049), considering equilibrium when p-values were higher than the cut-off of 0.01. We analyzed the data according to genetic models of additive, dominant, and recessive inheritance. The additive genetic model showed the strongest association with SVR. In the additive model, the risk conferred by an allele is increased r-fold for heterozygotes and 2r-fold for homozygotes with two copies of a specific allele. In this case, homozygotes for the minor allele, heterozygotes, and homozygotes for the major allele were coded as 0, 1, and 2, respectively.

We found a significant trend of achieving SVR when the number of *HLA-E*0101* alleles increased irrespective of the HCV genotype or *IL28B* genotype (**Figure 1**). Moreover, there was also a significant trend of achieving successful virological response for each *HLA-E*0101* allele at different endpoints during HCV treatment (see **supplementary data**). However, rs8099917 TT was associated with virological response only in GT1/4 patients, but not in GT2/3 patients (see **supplementary data**).

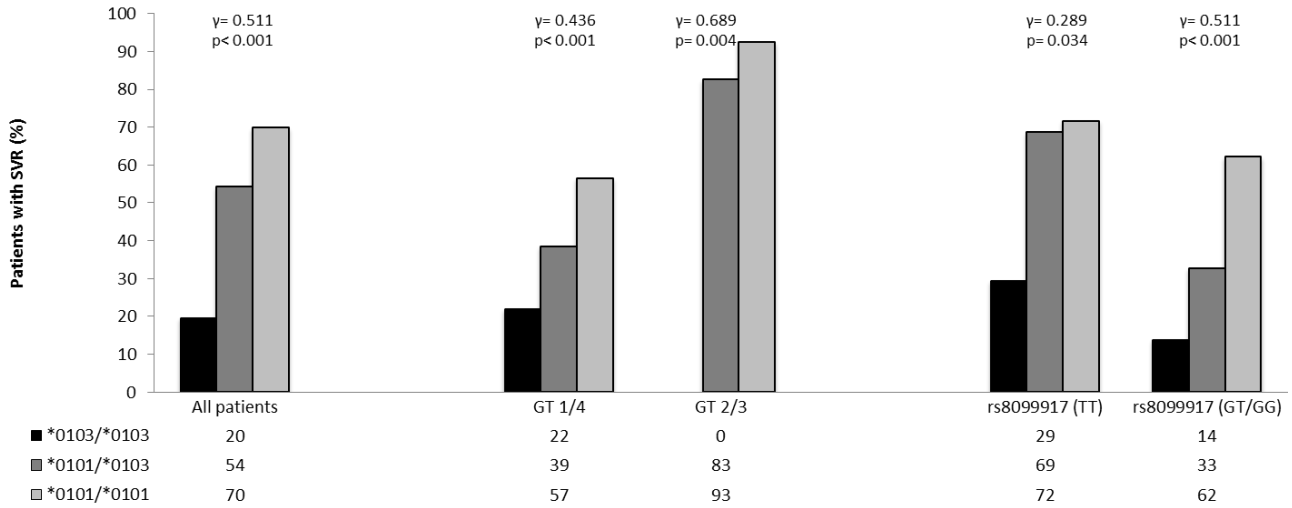

Figure 1. Rates of sustained virological response (SVR) in 321 HIV/HCV coinfected patients on HCV treatment according to *IL28B, HLA-E* and HCV genotypes. Abbreviations: SVR, sustained virological response; HCV, hepatitis C virus; IL28B, interleukin 28B; HLA-E, human leukocyte antigen E; p, significance value; γ, gamma correlation coefficient. The test used to analyze the trend of data was the gamma correlation coefficient (γ; values between -1 and +1), a non-parametric test for measuring the association between two ordinal variables.

Next, we analyzed the influence of the *HLA-E*0101* allele on SVR using a logistic regression analysis adjusted by the most relevant characteristics of patients (**Table 2**). The *HLA-E*0101* allele increased odds of achieving SVR (adjusted odds ratio (aOR)=2.04). Moreover, patients had increased odds of achieving SVR per *HLA-E*0101* allele in patients infected with GT1/4 (aOR=1.59) or GT2/3 (aOR=6.51), and patients with rs8099917 TT (aOR=2.14) or rs8099917 GT/GG (aOR=2.65). In addition, we did not find any statistically significant interaction between *HLA-E* and *IL28B* genotypes regarding HCV therapy response.

Table 2. Summary of logistic regression analysis for achieving sustained virological response (SVR) per *HLA-E*0101* allele in all patients on HCV treatment and stratified by *IL28B* and HCV genotypes.

	Non adjusted		**(*) Adjusted**	
	OR (95%CI)	**p-value**	**OR (95%CI)**	**p-value**
All patients	2.66 (1.89 – 3.76)	<0.001	2.04 (1.35 – 3.08)	0.001
HCV genotypes				
Genotype 1/4	2.13 (1.43 – 3.15)	<0.001	1.59 (1.02 – 2.48)	0.040
Genotype 2/3	6.71 (2.26 – 19.87)	0.001	6.51 (2.17 – 19.47)	0.001
***IL28B* genotype**				
rs8099917 TT	1.83 (1.16 – 2.89)	0.009	2.14 (1.16 – 3.97)	0.015
rs8099917 GT/GG	3.24 (1.79 – 5.88)	<0.001	2.65 (1.34 – 5.22)	0.005

Abbreviations: HCV, hepatitis C virus; *IL28B*, gene of interleukin 28B; *HLA-E*, gene of human leukocyte antigen E; OR, odds ratio; 95% CI, 95% of confidence interval. (*), Logistic regression analyses were adjusted by the most significant covariables, such as gender, age, HCV genotype (GT1/4 vs. GT3), HCV-RNA ≥500,000 IU/ml, significant fibrosis (F≥2), and rs8099917 TT genotype at *IL28B,* HAART.

Prediction of sustained virological response

Figure 2 shows a decision tree analysis with the three variables that had the highest significant associations with SVR (HCV genotype, *IL28B* genotype, and *HLA-E* genotype).

In patients infected with GT2/3, SVR rate increased from 0% to 82.6% and then to 92.5% when the count of *HLA-E*0101* alleles increased. In patients infected with GT1/4 and with the favorable rs8099917 TT genotype, SVR rate increased from 33.3% to 54.8% and then to 61.8% when the count of *HLA-E*0101* alleles increased. In patients infected with GT1/4 and with the unfavorable rs8099917 GT/GG genotype, SVR rate increased from 15.4% to 22% and then to 44% when the count of *HLA-E*0101* alleles increased.

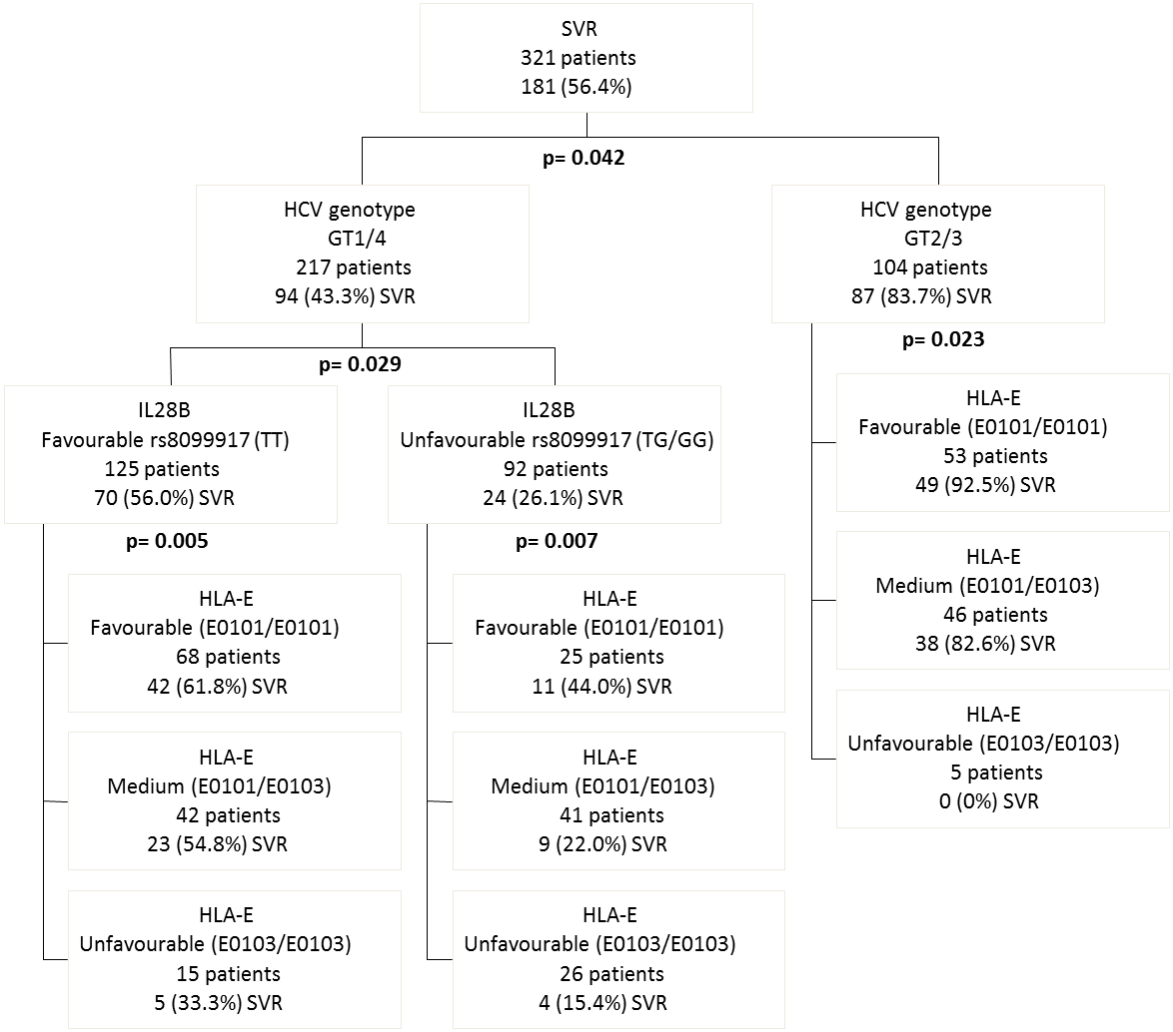

Figure 2. Distribution of patients with sustained virological response (SVR) according to IL28B, HLA-E and HCV genotypes. Abbreviations: SVR, sustained virological response; HCV, hepatitis C virus; IL28B, interleukin 28B; HLA-E, human leukocyte antigen E. The test used to analyze the data was a classification and regression tree (CART), which built a decision tree to identify subgroups at higher odds of SVR.

The overall percentage of patients correctly classified (accuracy) was 73.2% and the AUROC of this decision tree was 0.803±0.024. For those patients with a non-SVR response, non-SVR was predicted for only 59.3% of them, which means that 40.7% of the non-SVR patients were inaccurately classified as SVR patients. For those patients with a SVR response, SVR was predicted for 84% of them, which means that 16% of the SVR patients were inaccurately classified as non-SVR patients. After 25-fold cross-validation, the overall percentage of patients correctly classified was 71.7%.

DISCUSSION

This study shows that patients carrying the *HLA-E*0101* allele had a higher probability of successful HCV treatment. Moreover, a predictive model with HCV genotype, rs8099917 genotype, and *HLA-E* genotype had an accuracy value close to 75%.

The mechanism behind the association between *HLA-E* and treatment-induced HCV clearance is unknown. On the one hand, a failure in the immune response related to HLA-E may be a reason. As a novel strategy adopted by HCV to evade NK cell-mediated responses an up-regulation of CD94/NKG2A inhibitory receptors is known to occur during HCV infection on both NK cells and NK-CTL, which are more susceptible to inhibition via HLA-E/NKG2A interactions [11]. In addition, the *HLA-E*0101/*0101* genotype has been associated with low HLA-E surface expression [21]. Thus, the HLA-E–mediated inhibition of NK cell function could be less effective in carriers of the *HLA-E*0101/*0101* genotype, which would help in producing a more effective immune response against HCV. Therefore, since the interaction of HLA-E is NKG2-specific, our data might support the role of NK function in HCV clearance during pegIFNα/RVB therapy via killing virally infected cells [22]. In addition, HIV infection has been associated with deregulated expression of CD94/NKG2A and CD94/NKG2C on both NK cells and CD8+ T cells [23-25]. Thus, the effect of HLA-E polymorphisms might be different in HIV/HCV co-infected and HCV mono-infected patients. On the other hand, it might be argued that a more potent T-cell mediated immune response can explain the better treatment outcomes for patients with the *HLA-E*0101/*0101* genotype. These patients have a high frequency of antiviral HLA-E–restricted CD8+ T cells with a high degree of HLA-E–restricted IFN-γ secretion, which is associated with low HCV viral load [14]. Moreover, the natural ligands for HLA-E are nonamer peptides derived from the leader sequence of classical MHC I molecules, which show higher binding affinity to the *HLA-E*0103* allele. This increased affinity is due to a higher stability of complexes formed between the leader peptides and HLA-E*0103 compared to those formed by HLA-E*0101 [21]. Under this assumption, it is possible that HLA-E*0101 could more readily be available than HLA-E*0103 to bind to HCV peptides, which would facilitate HLA-E–restricted CD8+ T cell responses [14].

IL28B polymorphisms are already used as a predictive marker of treatment response to pegIFNα/RVB in clinical practice [5]. To date, many articles have assessed the influence of *IL28B* polymorphisms on the SVR in CHC patients, where rs8099917 and rs12979860 have been the most studied [26]. Although rs12979860 is more likely to be correlated with SVR in the Caucasian population, we recently have shown a strong association of rs8099917 with SVR in HCV/HIV-coinfected patients [17]. In the current study, we did not analyze rs12979860 but only rs8099917, which is in high linkage disequilibrium with rs12979860 in the European population [27]. In addition, rs8099917 has been less studied than rs12979860 in Caucasian populations, and therefore additional results involving rs8099917 would be of interest.

Curiously, in our study we have shown that *HLA-E* variants had a significant role similar to that of rs8099917 variants in predicting HCV treatment response among HCV/HIV-coinfected patients. Furthermore, the inclusion of both genetic markers had an additive effect on the ability to predict SVR. This genetic evidence supports an underlying physiological mechanism for HCV viral control involving a relationship between *IL28B* and *HLA-E*. In agreement with this, Golden-Mason et al. recently reported that higher expression levels of inhibitory NKG2A receptors were present in patients who failed to achieve SVR and in patients carrying the unfavorable *IL28B* allele [16]. Moreover, it should be noted that there may be a different distribution of favorable and unfavorable genotypes of rs12979860 and rs8099917 in our cohort, and this could affect the relationship found with *HLA-E* alleles differently.

The combination of *IL28B* genotype and plasma levels of Interferon-γ Inducible Protein-10 (IP-10) has also been found to be useful to predict SVR among CHC patients [28-31]. *IL28B* encodes IFN-λ3, which induces HCV antiviral activity through interferon-stimulated genes (ISGs) [32]. The so-called favorable *IL28B* genotypes (associated with better HCV treatment response) had low hepatic expression levels of *IL28B* and ISGs, but are induced more strongly after administration of IFN-α treatment [33]. IP-10 is one of those ISGs induced during the immune response against viral infections [34], but it is unclear why high IP-10 levels are associated with a poor response to HCV therapy. In our study, we found that the accuracy of our algorithm was similar to other models that use a combination of *IL28B* genotype and IP-10 levels [28-31]. However,

we did not have data for IP-10, and it might be possible that the diagnostic accuracy of our algorithm (HCV, *IL28B*, and *HLA-E* genotypes) would be enhanced with this biomarker.

This study has other limitations that must be taken into account for the correct interpretation of the data. Our study design is retrospective and contains a low number of patients. All selected patients met a set of criteria for starting HCV treatment (e.g., no alcohol abuse, high CD4 cell counts, controlled HIV replication, and good treatment adherence), and this may have introduced a selection bias. Besides, this study was carried out entirely in Caucasians, therefore as the frequency of these alleles differs among different ethnicities, it would be necessary to perform an independent replication of this study for different ethnic groups. We did not have a second independent cohort of HCV-infected patients in which to confirm the proposed association of HLA-E variants with HCV therapy outcome. Finally, interferon therapy regimens were not identical since they varied in some characteristics such as pegIFNα 2a or 2b and likely ribavirin dose. Our study is not a clinical trial, and this lack of uniformity may have a significant unaccounted for effect on the interpretation of our results. Instead, each physician administered the appropriate HCV therapy regimen according to his/her criteria and by following local and/or international guidelines. However, the data presented here are entirely derived from routine clinical practice.

In conclusion, the *HLA-E*0101* allele was associated with increased odds of HCV clearance and could help to predict SVR among HIV/HCV coinfected patients on HCV therapy. It would be helpful to categorize patients before HCV therapy so as to avoid prescribing treatment in those less likely to respond to pegIFNα/RVB treatment.

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AUTHORS CONTRIBUTIONS

MGF and SR performed all statistical analysis, interpretation of the data and wrote the manuscript. JB, VS, and SR participated in the study concept and design. JB, VS, JC, JCL, and PM participated in patient selection, collection of samples and acquisition of data. AFR, MAJS, MGA, JMB, JM, and NR participated in sample preparation, DNA isolation and genotyping pre-procedure, and contributed with critical revision of the manuscript. SR supervised the study.

All authors revised the manuscript from a draft by SR.

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SUPPLEMENTARY DATA

Outcome variables

Virological response to HCV treatment was estimated by detection of plasma HCV viral load at different endpoints. The following on-treatment virological responses were assessed: a) rapid virological response (RVR): no detectable viral load (<10 IU/mL) after 4 weeks of treatment; b) early virological response (EVR): viral load dropped by 99% (2 $\log_{10}$); c) end-of-treatment virological response (EOTVR): no detectable viral load at completion of treatment; d) sustained virological response (SVR): no detectable viral load six months after treatment cessation. Once treatment has been initiated, an undetectable plasma HCV RNA level at week 4 is the best predictor of cure. Thus, we also looked at the SVR among HIV/HCV coinfected patients without RVR (SVR/no-RVR).

We also defined several patterns of virological failure to HCV therapy related to plasma HCV kinetics: a) partial responder (partial-R) is someone who has had EVR but does not achieve SVR; b) relapser is a patient who has had EOTVR but whose HCV viral load rebounded after they completed HCV treatment. Null-responder (non-EVR) and non-responder (non-SVR) were not analyzed because these failure patterns are complementary of EVR and SVR, respectively.

HLA-E and IL28B alleles are associated with response rates to HCV treatment

The rates of virological response at different endpoints according to *HLA-E* and *IL28B* genotypes are shown in **Supplementary data** (**SD)1**. We found a significant positive trend of achieving the four efficacy endpoints of HCV treatment (RVR, EVR, EOTVR, and SVR) and SVR/no-RVR as the number of *HLA-E*0101* alleles increased. We also found that the rates of virological response at different end-points were higher in patients with favorable *IL28B* genotype (rs8099917 TT) compared to patients with unfavorable *IL28B* genotype (rs8099917 GG/GT) (**SD1**). In addition, these rates of virological response were similar for *HLA-E*0101/*0101* and rs8099917 TT patients. In contrast, we found a significant negative association of being a partial-R or relapser with the presence of *HLA-E*0101/*0101* but not with rs8099917 TT genotype (**SD1**).

As expected, we found that patients infected with GT1/4 had lower rates of virological response than patients infected with HCV genotypes 2 or 3 (GT2/3) (*data not shown*). We also found that rs8099917 TT was associated with virological response only in GT1/4 patients, but not in GT2/3 patients (**SD2**). However, when we analyzed the influence of *HLA-E* genotypes, we observed that *HLA-E*0101* allele was associated with virological response in both groups of patients according to HCV-genotype (GT1/4 versus GT2/3 patients; **SD3**) and *IL28B* genotype (rs8099917 TG/GG versus rs8099917 TT; **SD4**).

Supplementary data 1. Virological response rates during HCV treatment among HIV/HCV coinfected patients according to *HLA-E* alleles and *IL28B* genotypes.

	HLA-E					**rs8099917 *IL28B***				
rs8099917 *IL28B*	***0103/*0103**	***0101/*0103**	***0101/*0101**	**γ**	**(*) p-value**	**GG**	**GT**	**TT**	**γ**	**(*) p-value**
Virological response										
RVR (n=225)	4 (12.5%)	23 (26.4%)	49 (46.2%)	0.483	**<0.001**	2 (28.6%)	18 (22.8%)	56 (39.7%)	0.350	**0.009**
EVR (n=288)	22 (53.7%)	78 (70.9%)	108 (78.8%)	0.320	**0.004**	6 (66.7%)	55 (55.6%)	150 (82%)	0.520	**<0.001**
EOTVR (n=316)	19 (41.3%)	78 (61.9%)	110 (76.4%)	0.422	**<0.001**	4 (44.4%)	51 (46.8%)	152 (75.2%)	0.535	**<0.001**
SVR (n=321)	9 (19.6%)	70 (54.3%)	102 (69.9%)	0.511	**<0.001**	4 (44.4%)	40 (35.7%)	136 (66.3%)	0.525	**<0.001**
SVR/no-RVR (n=149)	3 (10.7%)	28 (43.8%)	30 (52.6%)	0.456	**<0.001**	1 (20%)	15 (24.6%)	43 (50.6%)	0.510	**0.001**
HCV treatment failure										
Partial-R (n=208)	13 (59.1%)	17 (21.8%)	13 (12%)	-0.548	**<0.001**	2 (33.3%)	17 (30.9%)	29 (19.3%)	-0.297	0.082
Relapser (n=207)	10 (52.6%)	11 (14.1%)	9 (8.2%)	-0.562	**0.001**	0 (0%)	11 (21.6%)	20 (13.2%)	-0.222	0.290

In bold, p-values with statistical significance.

Abbreviations: RVR, rapid virological response; EVR, early virological response; EOTVR, end-of-treatment virological response; SVR, sustained virological response; partial-R, partial responder; HCV, Hepatitis C virus; HCV-RNA, HCV plasma viral load; HIV, Human immunodeficiency virus; p: significance value; γ, gamma correlation coefficient.

(*), The test used to analyze the trend of data was the gamma correlation coefficient (γ; values between -1 and +1), a non-parametric test for measuring the correlation between two ordinal variables.

Supplementary data 2. Virological response rates during HCV treatment in HIV/HCV coinfected patients on HCV treatment according to rs8099917 *IL28B* and HCV genotypes.

	HCV genotype 1/4					HCV genotype 2/3				
rs8099917 *IL28B*	**GG**	**GT**	**TT**	**γ**	**(*) p-value**	**GG**	**GT**	**TT**	**γ**	**(*) p-value**
Virological response										
RVR (n=225)	0 (0%)	8 (13.6%)	24 (27.2%)	0.460	**0.012**	2 (100%)	10 (52.6%)	32 (60.3%)	0.032	0.898
EVR (n=288)	3 (50)%	35 (45.5%)	84 (75%)	0.531	<0.001	3 (100%)	19 (90.5%)	66 (93%)	0.065	0.880
EOTVR (n=316)	1 (16.7%)	32 (37.6%)	83 (65.9%)	0.543	**0.001**	3 (100%)	18 (78.3%)	69 (90.8%)	0.361	0.262
SVR (n=321)	1 (16.7%)	22 (25%)	70 (55.1%)	0.573	**<0.001**	3 (100%))	17 (73.9%)	66 (84.6%)	0.211	0.452
SVR/no-RVR (n=149)	1 (20%)	9 (17.6%)	26 (40.6%)	0.490	**0.005**	0 (0%)	5 (55.6%)	17 (81%)	0.545	0.183
HCV treatment failure										
Partial-R (n=208)	2 (66.7%)	14 (40%)	23 (27.4%)	-0.324	0.103	0 (0%)	3 (15.8%)	6 (9.1%)	-0.188	0.619
Relapser (n=207)	0 (0%)	10 (31.3%)	15 (18.1%)	-0.314	0.190	0 (0%)	1 (5.6%)	5 (7.2%)	0.234	0.622

In bold, values with statistical significance.

Abbreviations: RVR, rapid virological response; EVR, early virological response; EOTVR, end-of-treatment virological response; SVR, sustained virological response; partial-R, partial responder; HCV, Hepatitis C virus; HCV-RNA, HCV plasma viral load; HIV, Human immunodeficiency virus; p: significance value; γ, gamma correlation coefficient.

(*), The test used to analyze the trend of data was the gamma correlation coefficient (γ; values between -1 and +1), a non-parametric test for measuring the correlation between two ordinal variables.

Supplementary data 3. Virological response rates during HCV treatment in HIV/HCV coinfected patients on HCV treatment according to *HLA-E* and HCV genotypes.

	HCV genotype 1/4					HCV genotype 2/3				
***HLA-E* genotypes**	***0103/*0103**	***0101/*0103**	***0101/*0101**	**γ**	**(*) p-value**	***0103/*0103**	***0101/*0103**	***0101/*0101**	**γ**	**(*) p-value**
Virological response										
RVR (n=225)	4 (14.3%)	7 (13%)	22 (32.8%)	0.434	**0.009**	0 (0%)	16 (48.5%)	27 (71.1%)	0.564	**0.003**
EVR (n=288)	19 (51.4%)	41 (59.4%)	59 (68.6%)	0.232	0.059	3 (75%)	37 (90.2%)	48 (96%)	0.501	0.173
EOTVR (n=316)	18 (43.9%)	39 (47.6%)	59 (65.6%)	0.307	**0.006**	1 (20%)	39 (86.6%)	50 (94.3%)	0.658	**0.021**
SVR (n=321)	9 (22%)	32 (38.6%)	52 (56.5%)	0.436	**<0.001**	0 (0%)	38 (82.6%)	49 (92.5%)	0.689	**0.004**
SVR/no-RVR (n=149)	3 (12.5%)	14 (29.8%)	19 (42.2%)	0.419	**0.007**	0 (0%)	14 (82.4%)	10 (90.9%)	0.800	**0.009**
HCV treatment failure										
Partial-R (n=208)	10 (52.6%)	14 (34.1%)	11 (18.6%)	-0.463	**0.004**	3 (100%)	3 (8.1%)	2 (4.1%)	-0.695	0.051
Relapser (n=207)	9 (50%)	89 (20.5%)	8 (13.6%)	-0.490	**0.010**	1 (100%)	3 (7.7%)	1 (2%)	-0.731	0.107

In bold, values with statistical significance.

Abbreviations: RVR, rapid virological response; EVR, early virological response; EOTVR, end-of-treatment virological response; SVR, sustained virological response; Full-R, full responder; partial-R, partial responder; OR, odds ratio; 95% CI, 95% of confidence interval; HCV, Hepatitis C virus; HCV-RNA, HCV plasma viral load; HIV, Human immunodeficiency virus; p: significance value; γ, gamma correlation coefficient.

(*), The test used to analyze the trend of data was the gamma correlation coefficient (γ; values between -1 and +1), a non-parametric test for measuring the correlation between two ordinal variables.

Supplementary data 4. Virological response rates during HCV treatment in HIV/HCV coinfected patients on HCV treatment according to *HLA-E* and *IL28B* genotypes.

	rs8099917 (GT/GG)					rs8099917 (TT)				
***HLA-E* genotypes**	***0103/*0103**	***0101/*0103**	***0101/*0101**	**γ**	**(*) p-value**	***0103/*0103**	***0101/*0103**	***0101/*0101**	**γ**	**(*) p-value**
Virological response										
RVR (n=225)	2 (10%)	6 (17.1%)	12 (41.4%)	0.552	**0.006**	2 (16.7%)	17 (32.7%)	36 (48.6%)	0.387	**0.010**
EVR (n=288)	10 (38.5%)	24 (53.3%)	25 (71.4%)	0.408	**0.006**	12 (80%)	54 (83.1%)	80 (80.8%)	-0.039	0.827
EOTVR (n=316)	9 (31%)	21 (41.2%)	25 (69.4%)	0.471	**0.001**	10 (58.8%)	57 (76%)	82 (78.1%)	0.175	0.252
SVR (n=321)	4 (13.8%)	17 (32.7%)	23 (62.2%)	0.612	**<0.001**	5 (29.4%)	53 (68.8%)	76 (71.7%)	0.289	**0.034**
SVR/no-RVR (n=149)	2 (11.1%)	6 (20.7%)	8 (41.7%)	0.551	**0.014**	1 (10%)	22 (62.9%)	20 (52.6%)	0.198	0.294
HCV treatment failure										
Partial-R (n=208)	6 (60%)	8 (33.3%)	3 (12%)	-0.629	**0.003**	7 (58.3%)	9 (16.7%)	10 (12.5%)	-0.449	**0.023**
Relapser (n=207)	5 (55.6%)	4 (19%)	2 (8%)	-0.669	**0.011**	5 (50%)	7 (12.3%)	7 (8.5%)	-0.465	**0.044**

In bold, values with statistical significance.

Abbreviations: RVR, rapid virological response; EVR, early virological response; EOTVR, end-of-treatment virological response; SVR, sustained virological response; Full-R, full responder; partial-R, partial responder; OR, odds ratio; 95% CI, 95% of confidence interval; HCV, Hepatitis C virus; HCV-RNA, HCV plasma viral load; HIV, Human immunodeficiency virus; p: significance value; γ, gamma correlation coefficient.

(*), The test used to analyze the trend of data was the gamma correlation coefficient (γ; values between -1 and +1), a non-parametric test for measuring the correlation between two ordinal variables.