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CLINICAL STUDIES

IL28B polymorphisms are markers of therapy response and are influenced by genetic ancestry in chronic hepatitis C patients from an admixed population

Lourianne N. Cavalcante[1,2], Kiyoko Abe-Sandes[3,4], Ana Luisa D. Angelo[3], Taisa M. B. Machado[3], Denise C. Lemaire[3,5], Carlos M. C. Mendes[6], João R. Pinho[7], Fernanda Malta[7], Luiz G. C. Lyra[1,2] and André C. Lyra[1,2]

1 Department of Medicine, Federal University of Bahia (UFBA) Salvador, Bahia, Brazil
2 Gastro-Hepatology Service – Hospital São Rafael – Monte Tabor Salvador, Bahia, Brazil
3 Laboratory of Immunology – Health Sciences Institute (ICS), Federal University of Bahia (UFBA) Salvador, Bahia, Brazil
4 Department of Life Sciences, State University of Bahia (UNEB) Salvador, Bahia, Brazil
5 Bahiana School of Medicine and Public Health (EBMSP) Salvador, Bahia, Brazil
6 Fima Lifshitz Research Center, Federal University of Bahia Bahia, Brazil
7 Institute of Tropical Medicine – Faculty of Medicine, University of São Paulo, São Paulo, SP, Brazil

Keywords
antiviral therapy – genetic ancestry – hepatitis C – *IL28B* – polymorphism – single nucleotide

Abbreviations
AIM, ancestry informative markers; CHC, chronic hepatitis C; GWAS, genome-wide association studies; HCV, hepatitis C virus IL, interleukin; NR/R, non-response/relapse; OR, odds ratio; RVR, rapid virological response; SNP, single nucleotides polymorphisms; SVR, sustained virological response.

Correspondence
André Castro Lyra, R Sócrates Guanaes Gomes 84/401, Salvador, Bahia, CEP: 40 296 720, Brazil
Tel: 55 71 3351 8097
Fax: 55 71 3281 6855
e-mail: aclyra@live.com

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Abstract

Background: IL28B polymorphisms are predictors of therapy response in hepatitis C virus (HCV) patients. We do not know whether they are markers of treatment response in admixed populations or not. *Aims:* To determine whether *IL28B* polymorphisms are predictors of therapy response in patients with HCV from an admixed population and are influenced by genetic ancestry. *Methods:* rs12979860 and rs8099917 were genotyped in 222 HCV patients treated with pegylated interferon and ribavirin. Ancestry was determined using genetic markers. *Results: IL28B* rs12979860 C/C was associated with sustained virological response (SVR), whereas C/T and T/T were associated with failure to therapy ($P = 1.12 \times 10^{-5}$). *IL28B* rs8099917 T/T was associated with SVR, and G/G and G/T were associated with nonresponse/relapse (NR/R) ($P = 8.00 \times 10^{-3}$). Among HCV genotype 1 patients with C/C genotype, genomic ancestry did not interfere with therapy response. Among patients with rs12979860 T/T genotype, African genetic contribution was greater in the NR/R group ($P = 1.51 \times 10^{-3}$), whereas Amerindian and European genetic ancestry contribution were higher in the SVR group ($P = 3.77 \times 10^{-3}$ and $P = 2.16 \times 10^{-2}$ respectively). Among HCV type 1 patients with rs8099917 T/T, African genetic contribution was significantly greater in the NR/R group ($P = 5.0 \times 10^{-3}$); Amerindian and European ancestry genetic contribution were greater in the SVR group. *Conclusion: IL28B* rs12979860 and rs8099917 polymorphisms were predictors of therapy response in HCV genotypes 1, 2 and 3 subjects from an admixed population. Genomic ancestry did not interfere with response to therapy in patients with rs12979860 C/C, whereas it interfered in patients with C/T and T/T genotypes. Among HCV genotype 1 rs8099917 T/T patients, genomic ancestry interfered with response to therapy.

Infection by hepatitis C virus (HCV) is a worldwide endemic disease, an important aetiology of cirrhosis and hepatocellular carcinoma, and is the leading cause of liver transplantation in adults (1, 2). Treatment with pegylated interferon alpha associated with ribavirin for 48 weeks is recommended for patients infected with HCV genotype 1 with a sustained virological response (SVR) rate of approximately 50%, whereas subjects infected with HCV genotypes 2 or 3 may be treated for 24 weeks, and the SVR rate may be as high as 80% (3–5). Host characteristics such as advanced liver fibrosis, male gender, insulin resistance, absence of adherence to therapy, co-infection with hepatitis B or HIV, liver iron overload, are associated with lower SVR rates (6). In addition, several studies have shown that ancestry influences antiviral therapy response, and African descendants have lower SVR rates compared with whites (7, 8). Of note, known factors like therapy adherence,

HCV genotype, kind of pegylated interferon or social status cannot explain these differences (9). Variations in genetic and immunological background possibly explain these differences and they can be useful as therapy response markers.

Several genome-wide association studies (GWAS) have observed that numerous single nucleotides polymorphisms (SNP) affect interferon drug response and also the spontaneous viral clearance to HCV. Recently, the *IL28B* gene polymorphisms have been reported as the strongest predictor of HCV therapy response, early viral kinetics and spontaneous viral clearance, and they might explain part of the association among therapy response and the host ethnicity (10–16). *IL28B* contributes to viral resistance and is known to be unregulated by interferon and by RNA virus infection. In addition, polymorphisms in *MxA, osteopontin* and *KIR* receptor genes have been reported to be associated with interferon response (17–19).

The rs12979860 *IL28B* variant was strongly associated with SVR in HCV genotype 1 infected North Americans. In patients of European ancestry, the C/C genotype was associated with a two-fold greater rate of SVR than the T/T genotype, three-fold in the African American and two-fold in Hispanic population groups, and when C/C genotype was compared with other host variables as baseline viral load, liver fibrosis and ethnicity, the *IL28B* polymorphism was strongly and independently associated with therapy response (10). The C allele is more frequently found in European ancestry people, and then it was hypothesized that part of the differences in SVR rates could be accounted for by the difference in frequency of the C allele between African Americans and individuals of European ancestry. Studies have showed that African American patients with the C/C genotype had a significantly higher rate of SVR than European Americans who were non-C/C (10). Data showed that genotype 1 chronic hepatitis C (CHC) in Asian subjects is associated with higher rates of virological response compared with that in Caucasians and frequency of *IL28B* alleles, and genotypes may explain this differences (20). Other *IL28B* polymorphisms (rs12980275 and rs8099917) have been studied and the non-favourable alleles were strongly associated with virological non-response to HCV therapy in Japanese population (OR 20.3 and OR 30.0 respectively) (11). Allele G from *IL28B* at rs8099917 is more frequent in Asiatic population, and probably this is the reason that the predictive value of these polymorphisms in the Japanese study appears to be stronger than those observed in the studies of African and European Americans.

In HCV genotype 2 or 3 infected patients, the role of *IL28B* gene polymorphisms is uncertain, especially if we consider their different variants. A multicentre trial that characterized *IL28B* at rs1297960 in 268 Caucasian concluded that *IL28B* polymorphism was associated with SVR in patients who did not achieve a rapid virological response (RVR) (21).

Of note, the frequency of *IL28B* alleles in admixed populations from different geographical regions is not known. There is no data showing that *IL28B* polymorphisms can be applied as a predictor of HCV antiviral therapy response in populations highly admixed from Europeans, Africans and Amerindians.

In this study, two *IL28B* SNPs were analysed with regard to therapy response and ancestry in a diverse sample stratified in two groups: HCV genotype 1 and HCV genotypes 2 plus 3. The *IL28B* variant, rs12979860 (T > C), is upstream of the *IL28B* gene. Another variant, rs8099917 (T > G), is located in 80-kilobase region, encoding three cytokines (IL-28B, IL-28A and IL-29). The aim of this study was to analyse association between *IL28B* SNPs and HCV therapy response in an admixed population.

Materials and methods

Study design and patients

This is a case–control concurrent study. Between January 2010 and March 2011, 283 adult patients chronically infected with HCV genotypes 1, 2 and 3 from the outpatient clinic, who had been treated with a combination of pegylated interferon alpha-2a or alpha-2b and ribavirin, were consecutively screened. Among them, 222 were enrolled in the protocol, and 61 were not included because of ongoing antiviral therapy, co-infection with HIV/HBV or incomplete data. This study was approved by the institution's ethics committee, and all patients provided written informed consent. All procedures were conducted in accordance with provisions of the Declaration of Helsinki.

Inclusion criteria

Patients with CHC with viral genotype 1, 2 or 3; older than 18 years who were treated with combination antiviral therapy with pegylated interferon alpha 2a or 2b plus ribavirin.

Exclusion criteria

Hepatitis B or HIV co-infection; alcohol intake $\geq$ 40 g ethanol/day or other concomitant chronic liver diseases.

Hepatitis C virus genotype was determined using the Inno-LiPA HCV assay (Innogenetics, Zwijnaarde, Belgium) and the chronic infection was defined by detectable HCV RNA for at least 6 months (using the COBAS® AMPLICOR HCV Test, v2.0, Roche Molecular Systems, Pleasanton, CA, USA). SVR was defined as undetectable HCV RNA in serum 24 weeks after discontinuation of therapy. Patients infected with HCV genotypes 2 or 3 were treated for 24 weeks and patients with HCV genotype 1 received 48 weeks of treatment. Non-response to therapy was defined as HCV viral load decline less than 2 logs at week 12 during therapy or detectable serum HCV RNA at any other time during

therapy up to 48 weeks. Relapse was defined as undetectable HCV RNA in serum at the end of therapy followed by detectable HCV RNA after discontinuation of therapy. For analysis, we separated patients into two groups: SVR (including individuals who achieved SVR) and non-response/relapse (NR/R) (with individuals who were non-responder or relapse to therapy). Liver biopsies were reviewed only by one pathologist using the Metavir scoring system and variables considered for analysis were: mild and moderate (F0–F2) and severe (F3–F4) liver fibrosis (22). Other data were obtained from patient's interview and charge review.

DNA extraction

Samples of peripheral blood from each subject were collected in 0.5 M EDTA tubes, and genomic DNA was extracted from mononuclear cells using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

IL28B genotyping

The rs12979860 and rs8099917 were genotyped using the ABI TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA) and predesigned commercial genotyping assays (ABI assay C_11710096_10). The PCR reactions were performed in 96-well microplates with ABI 7300 Real-Time PCR (Applied Biosystems). Allele discrimination was achieved by fluorescence detection using SDS software version 1.3.1 (Applied Biosystems).

Ancestry analysis

Ancestry informative markers

Genetic ancestry was determined by analysing seven ancestry informative markers (AIMs), and the allele*1 was defined as the presence of insertion or the lack of restriction enzyme site (23). The AIMs used were selected based on previous studies that analysed a panel of 48 ancestry markers in different populations (23, 24). We chose seven AIMs with the largest ethnic-geographical differential frequencies (higher than 48%). The *Alu* insertion and *indel* polymorphisms were detected using conventional PCR, and SNPs were detected using Real-Time PCR (TaqMan™ System by Applied Biosystems). The AIMs analysed were: African ancestry, *AT3-I/D* (76bp *indel*, *locus* 1q25.1, *rs3138521*) and *LPL* [T > C (SNP), *locus* 8p21.3, *rs285*](25); European ancestry, *Sb19.3* (*Alu insertion, locus* 19p12, *rs3138524*), *APO* (*Alu insertion*, *locus* 11q23.3, *rs3138522*) and *FY-Null* [A > G (SNP), *locus* 1q23.2, *rs2814778*]; and Amerindian ancestry markers, *PV92* (*Alu insertion, locus* 16q23.3, *rs3138523*), *CKMM* [C > T (SNP), *locus* 19q13.32, *rs4884*] (24, 25). In analysis, the allele*1 frequency in each genetic ancestry marker studied was considered.

Ancestral population

A total of 482 participants comprising the ancestral populations were used as reference groups in the genetic ancestry analysis. The African ancestral population consisted of 134 Nigerians; the European ancestral population consisted of 23 Germans and 83 Spanish; the Amerindian ancestral population consisted of 242 Native Americans. These samples were kindly provided by Mark Shriver, MD, PHD. All ancestry markers analysed in this study population were also tested in the ancestral population.

Morphological phenotypic ancestry

Two trained researchers performed the morphological phenotypic classification of ancestry using the Krieger's and Parra's criteria (26, 27). These criteria evaluate skin colour (white, brown or black), nose shape (prominent or upturned tip, depressed tip or flat), lip thickness (thin, medium or thick) and hair texture (straight, wavy or curly hair). People were classified according to their phenotypic characteristics as white, mulatto (a person who has both black and white phenotypic ancestry) or black. If a person had a combination of white skin colour, upturned nose tip, fine lips and straight hair, they were classified phenotypically as white; if they had black skin colour, flat nose, thick lips and curly hair, they were classified as black. If they had intermediate or mixed characteristics, they were classified as mulatto. An atlas of the human race was used to define and classify participants' nose and lip shapes (28).

Self-reported ancestry (self-classification of race/colour)

Patients defined themselves as white, mulatto, black or other (Amerindian or Asian) according to race/colour classification from Brazilian Institute of Geography and Statistics (IBGE) (29).

The patients were divided into two groups based on their phenotypic and self-reported ancestries: whites and African descendants (composed of mulattos and blacks).

Statistical analyses

The sample size was estimated to obtain a statistical power of 80% to detect an association between *IL28B* and response to antiviral therapy (SVR and NR/R). Two-sided tests were utilized with an alpha value of 0.05 and a confidence interval of 95%. To describe the study sample, we used proportions for categorical variables, as well as median, mean and standard deviation for continuous variables. Proportions were compared using the Chi-square test and Fisher's exact test. Continuous variables were compared using Student's *t*-test for parametric variables and the Mann–Whitney test, when the distributions were not parametric. As a result of a

concern that there was collinearity between *IL28B* variants and also among genetic ancestries, they were analysed separately. The R PROJECTS software version 2.11.1 for Windows (31 May 2010, Statistics Department of the University of Auckland, Auckland, New Zealand, http://www.r-project) was used to analyse these data. Allele frequencies and analysis of differentiation between groups were performed using the GENEPOP online version 4.0.10 (Laboratiore de Genetique et Environment, Montpellier, France) (30). Estimation of ancestral contribution was calculated using the ADMIX95 software (Departamento de Genética de la Facultad de Medicina, Universidad de la República, Montevideo, Uruguay, www.genetica.fmed.edu.uy) (31) and the estimation of ancestry contribution of each individual was calculated using the STRUCTURE software version 2.2 (Department of Human Genetics, University of Chicago, Chicago, IL, USA) (32).

Results

The sample comprised 222 subjects; 88 (39.6%) had achieved SVR, 106 (47.7%) were non-responders and 28 (12.7%) were relapsers to antiviral therapy. A total of 139 patients (62.6%) were males and 83 (37.4%) were females; 102 (53.7%) patients had Metavir score F3/F4. There were 166 (74.7%) subjects infected by HCV genotype 1, and among them, 60 (36.1%) were sustained virological responders; 56 (25.3%) were infected by HCV genotypes 2 or 3 and 28 of them (50%) had achieved SVR.

Factors associated with therapy failure at baseline included severe liver fibrosis (OR 2.19, $P = 1.10 \times 10^{-2}$), HCV genotype 1 (OR 1.77, $P = 4.50 \times 10^{-2}$) and HCV viral load ($P = 4.47 \times 10^{-2}$). There were no significant associations between therapy response and gender, age or aminotransferase levels (Table 1).

Our study population was admixed and tri-hybrid, with the genetic contribution of European (0.333), Amerindian (0.217) and especially African genetic contribution (0.450). The AIMs – *APO, PV92* and *LPL* were in Hardy–Weinberg equilibrium ($P = 0.06$, 0.18 and 0.59 respectively). Frequencies of AIMs in the ancestral populations and from our population with CHC are described in Table 2. African genetic ancestry contribution was associated to therapy failure ($P = 1.89 \times 10^{-3}$) and Amerindian genetic ancestry contribution was associated to SVR ($P = 5.86 \times 10^{-6}$) (Table 1). When the same analysis was performed in the subgroup of HCV genotype 1 infected patients, the results were similar

Table 1. Baseline characteristics of patients with chronic hepatitis C according to antiviral therapy response

	SVR, $n = 88$ (39.6%)	NR/R, $n = 134$ (60.4%)	*P*	OR	CI 95%
Age, *y*	52 (46.3; 56.8)	52.5 (47; 59.8)	0.25		
Gender, *n* (%)					
Male	60 (43.2)	79 (56.8)	0.20	1.49	0.85–2.63
Female	28 (33.7)	55 (66.3)			
Ancestry classification					
Genetic ancestry contribution (mean)					
European	0.336	0.329	0.45		
Amerindian	0.324	0.287	5.86×10^{-6}		
African	0.335	0.374	1.89×10^{-3}		
Self-reported ancestry, *n* (%)					
Whites	32 (47.8)	35 (52.2)	0.14	1.57	0.88–2.80
African-descendans	56 (36.8)	96 (63.2)			
Missing	0	03			
Phenotypic ancestry, *n* (%)					
Whites	41 (45.6)	49 (54.4)	0.21	1.46	0.84–2.53
African-descendans	47 (36.4)	82 (63.6)			
Missing	0	03			
HCV genotype *n* (%)					
1	60 (36.1)	106 (63.9)	4.50×10^{-2}	1.77	0.96–3.26
2/3	28 (50.0)	28 (50.0)			
Liver fibrosis (Metavir) *n* (%)					
F0–F2	42 (47.7)	46 (52.3)	1.10×10^{-2}	2.19	1.21–3.98
F3–F4	30 (29.4)	72 (70.6)			
Missing	16	16			
HCV RNA viral load (IU/L)(Log_{10})	5.58 (5.1; 5.9)	5.93 (5.6; 6.1)	4.47×10^{-2}		
ALT (IU/L)*	97 (64; 148)	97 (61; 139.3)	1.00		
AST (IU/L)*	69 (53; 89)	76.5 (50.8; 100.3)	0.89		

Age, AST, ALT, HCV viral load were showed as median (p25; p75).

*Alanine aminotransferasis (ALT) and aspartato aminotransferasis (AST) normal values were 40 UI/L.

CI 95%, confidence interval 95%; NR/R, non-response or relapse; *P*, *P*-value; OR, odds ratio; SVR, sustained virological response.

Table 2. Allele*1 frequencies from ancestry informative markers in the ancestral populations and in the admixed study population with chronic hepatitis C (CHC)

Markers	Ancestral populations*1			Study population CHC*2
	African	European	Amerindian	
Fynull	0.001	0.998	1.000	0.657
APO	0.415	0.903	0.645	0.692
LPL	0.971	0.492	0.442	0.634
AT3-I/D	0.858	0.280	0.061	0.531
SB19.3	0.420	0.925	0.977	0.575
CKMM	0.164	0.313	0.904	0.352
PV92	0.225	0.152	0.792	0.275

*1Frequencies by Shriver (38).

*2Frequencies from an admixed population with CHC.

and the NR/R group had a higher contribution of African genetic ancestry than the SVR group (0.381 vs 0.334, respectively, $P = 2.0 \times 10^{-3}$), whereas in the SVR group, there was a predominance of Amerindian ancestry compared with the NR/R group (0.281 vs 0.323, $P = 1.64 \times 10^{-5}$). There was no difference between the amount of European contribution (0.323 in NR/R and 0.335 in SVR group, $P = 0.23$). Among the HCV genotype 2/3 subgroup of patients, we did not observe differences in the genetic ancestry contribution among NR/R and SVR groups: Amerindian contribution was 0.325 in the NR/R group and 0.351 in the SVR group, $P = 0.23$; European, 0.331 in the NR/R and 0.302 in the SVR group, $P = 0.15$; African 0.342 and 0.357 in the NR/R and SVR groups respectively; $P = 0.59$. There was no significant association between therapy response and self-reported and phenotypic ancestries (Table 1).

IL28B

The *IL28B* rs12979860 was analysed in 221 subjects, and rs8099917 was analysed in 222 subjects. Both *IL28B* SNPs were in Hardy–Weinberg equilibrium (rs8099917, $P = 1.00$; rs12979860, $P = 0.38$), but analysis of linkage disequilibrium showed that they were highly associated ($P < 1.10 \times 10^{-7}$). The Amerindian ancestry *CK/MM* is located close to *IL28B* on chromosome 19 (19q13), but they were not in genotypic linkage disequilibrium (rs8099917, $P = 0.14$; rs12979860, $P = 0.80$).

IL28B rs12979860

In rs12979860 SNP, C allele frequency was 0.457 with a greater frequency in the SVR group (0.585 in the SVR group vs NR/R 0.450, $P = 1.7 \times 10^{-3}$); it was also more frequent among sustained virological responders when we analysed HCV genotype 1 patients (0.623 in SVR vs 0.440 in NR/R group, $P = 3.35 \times 10^{-3}$) and genotype 2/3 (0.614 in SVR vs 0.450 in NR/R group, $P = 0.15$) infected subjects separately. When all patients were analysed, T allele frequency was 0.547, and it was higher in the NR/R groups (NR/R group T frequencies: in the whole sample, 0.550; HCV genotype 1, 0.557 and HCV genotype 2/3, 0.538).

We found similar genotype frequency distribution in the HCV genotype 1 subgroup [C/C 23.5% ($n = 39$), C/T 56% ($n = 93$) and T/T 20.5% ($n = 34$)] and among patients with HCV genotypes 2/3 [C/C 27.3% ($n = 15$), C/T 50.9% ($n = 28$) and T/T 21.8% ($n = 12$)]. The C/C genotype was significantly associated with SVR and C/T and T/T with failure to therapy when all HCV genotypes were analysed together and when they were analysed separately (HCV genotype 1 and HCV genotypes 2/3). The frequency of SVR among patients with C/C genotype in the whole sample, HCV genotype 1 and HCV genotypes 2/3 groups was 67, 64 and 73% respectively (Fig. 1A). We observed a strong association between C/C genotype and SVR when compared with the other two genotypes combined (C/T + T/T) ($P = 4.64 \times 10^{-6}$, OR 4.55). Similar results were observed in the HCV genotype 1 ($P = 5.32 \times 10^{-5}$, OR 4.69) and HCV genotypes 2/3 ($P = 3.66 \times 10^{-2}$, OR 4.13) subgroups (Fig. 1B).

IL28B rs8099917

By analysing rs8099917, a higher frequency of T allele (0.807) and lower of G allele (0.193) were observed in a whole sample. We found similar genotype frequency distribution in the HCV genotype 1 subgroup [T/T 62% ($n = 103$), G/T 34.3% ($n = 57$) and G/G 3.6% ($n = 6$)] and among patients with HCV genotypes 2/3 [T/T 64.3% ($n = 36$), G/T 33.9% ($n = 19$) and G/G 1.7% ($n = 1$)]. The G/G and G/T genotypes were significantly associated with failure to obtain SVR when all HCV genotypes were analysed together and when they were analysed separately (HCV genotype 1 and HCV genotypes 2/3). On the other hand, the frequency of SVR was greater among patients with T/T genotype compared with the other two genotypes (Fig. 2A). The G allele was associated with NR/R to antiviral therapy, and its frequency was 0.130 in SVR group vs 0.240 in NR/R group, $P = 6.5 \times 10^{-3}$. Similar results were found for HCV genotype1 group (G 0.120 in the SVR group vs G 0.240 in the NR/R group, $P = 3.00 \times 10^{-2}$) and for HCV genotypes 2/3 group (G 0.110 in the SVR group vs G 0.250 in the NR/R group, $P = 0.05$).

We observed a strong association between G/G + G/T genotypes and failure to obtain SVR when compared with the T/T genotype ($P = 2.78 \times 10^{-3}$, OR 1.38). Similar results were observed in the HCV genotype 1 ($P = 3.03 \times 10^{-2}$, OR 1.32) and HCV genotypes 2/3 ($P = 4.96 \times 10^{-2}$, OR 1.57) subgroups (Fig. 2B).

Genomic ancestry and *IL28B* polymorphisms

By analysing genetic contribution according to *IL28B* rs12979860 genotypes, we found that among HCV type 1 patients with the C/C genotype, genomic ancestry does

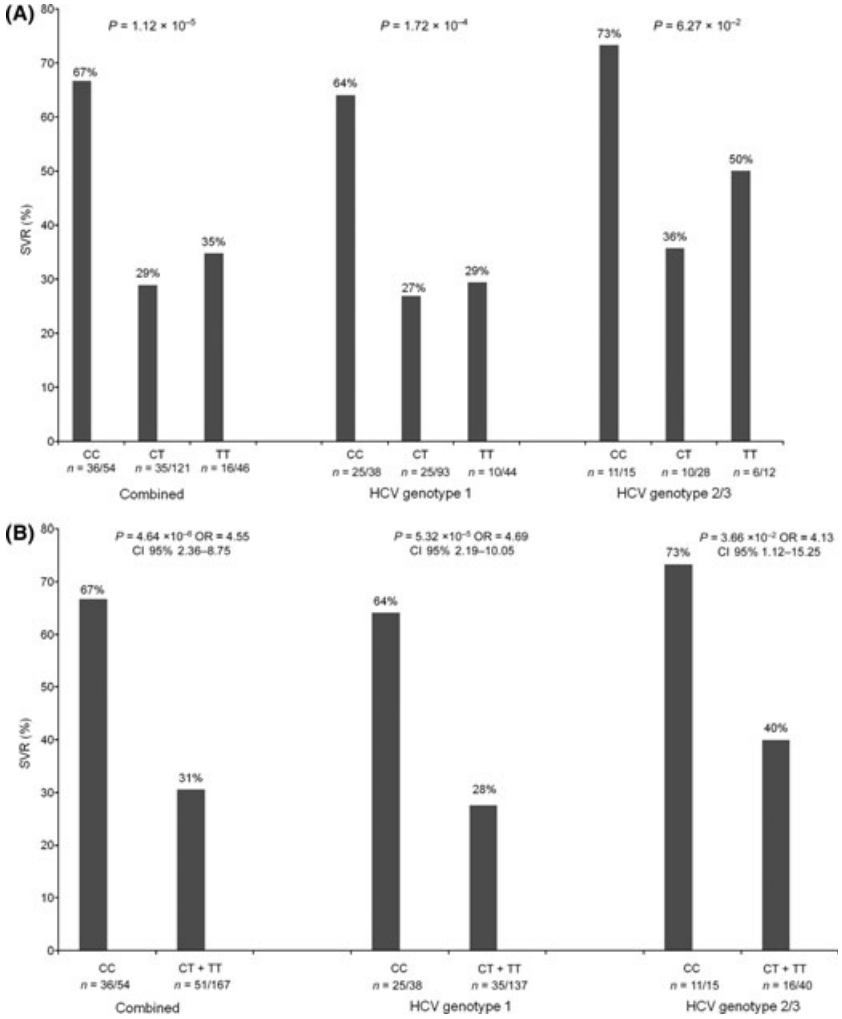

Fig. 1. *IL28B* rs12979860 according to therapy response. (A) Analysis considering *IL28B* genotypes separately. (B) Analysis considering C/T and T/T genotypes together vs C/C.

not appear to interfere with response to antiviral therapy, as European, African and Amerindian genetic contribution were similar between sustained virological responders and non-responders/relapsers. On the other hand, among HCV type 1 patients with the T/T and C/T genotypes, genomic ancestry does appear to interfere with response to antiviral therapy. African genetic contribution was significantly greater among patients in the NR/R group compared with the SVR group; on the other hand, Amerindian ancestry genetic contribution was significantly higher in the SVR group. European ancestry was associated with SVR only among patients with the T/T genotype (Table 3). There was no significant association between therapy response and genetic ancestry contribution among HCV genotypes 2/3 infected patients.

Of note, ancestry classified using self-reported or morphological phenotypic methods did not interfere with HCV therapy response in any of the *IL28B* rs12979860 genotypes (Table 4).

Analysis with rs8099917 showed that among HCV genotype 1 patients with the T/T, genomic ancestry appears to interfere with response to antiviral therapy. African genetic contribution was significantly greater among patients in the NR/R group compared with the SVR group; Amerindian and European ancestry genetic contribution were greater in the SVR group (Table 3). There was no statistically significant association between therapy response and genetic ancestry contribution among HCV genotypes 2/3 infected patients.

Of note, in patients with the T/T genotype, there were a greater frequency of African-descents classified using morphological phenotypic and self-reported ancestry methods among non-responders/relapsers (Table 4).

Discussion

This study showed that in an admixed population, both rs12979860 and rs8099917 *IL28B* polymorphisms are

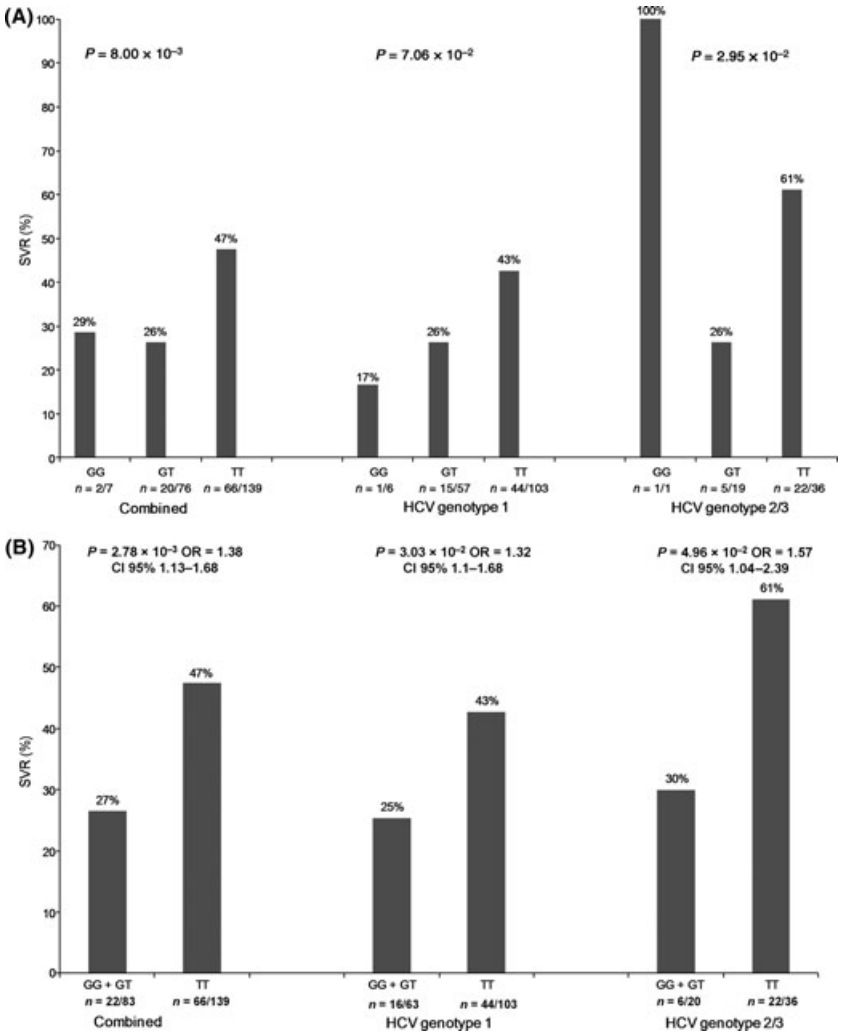

Fig. 2. *IL28B* rs8099917 according to therapy response. (A) Analysis considering *IL28B* genotypes separately. (B) Analysis considering G/G and G/T genotypes together vs T/T.

strong predictors of response to combination of antiviral therapy with pegylated interferon alpha and ribavirin in chronically infected patients with HCV. These results are in agreement with data showed in previous studies from more homogenous populations (10, 11). Of note, *IL28B* polymorphisms were influenced by genetic ancestry in subgroups of patients.

We evaluated a tri-hybrid population, not only mixed with a predominantly African ancestry contribution but also with a European and Amerindian contribution. This has been confirmed by genetic AIMs of ancestry and by the historical context of the Brazilian colonization (33). The AIMs *SB19.3, CKMM*, *AT3* and *Fy-Null* were not in Hardy–Weinberg equilibrium, but in an admixed population as ours, and it might reflect recent admixture or wedding among phenotipically similar people. In admixed populations, it should be necessary to use more objective methods to evaluate the patients' ethnic background when estimating patients' health outcomes. Therefore, we have chosen AIMs with high frequency differential (higher than 48%) that have been previously studied in the Brazilian population with good accuracy to measure ancestry (27). As a result of the high frequency of racial inter-mixing over time, we observed a heterogeneous population, and probably this is the reason that self-reported and phenotypic racial features were poor predictors of genetic ancestry and SVR in our study. On the other hand, even in Europe, North America and Africa where there appears to be a low frequency of racial inter-mixing, self-reported ancestry and morphological ancestry methods can be useful to define race and to predict SVR to antiviral therapy for CHC patients.

Many GWAS have reported associations of different SNPs in *IL28B* with response to antiviral therapy. Particularly in HCV genotype 1 infected patients, these SNPs

Table 3. Genetic ancestry contribution according to *IL28B* genotypes and antiviral therapy response

rs12979860						*rs8099917*					
			Ancestry						Ancestry		
			Mean	SD	*P*				Mean	SD	*P*
HCV genotype 1											
CC	European	SVR	0.339	0.021	0.50	GG	European	SVR	0.372		0.65
		NR/R	0.356	0.093				NR/R	0.314	0.106	
	Amerindian	SVR	0.322	0.023	0.37		Amerindian	SVR	0.330		0.65
		NR/R	0.300	0.084				NR/R	0.296	0.063	
	African	SVR	0.338	0.037	0.86		African	SVR	0.299		0.66
		NR/R	0.343	0.113				NR/R	0.369	0.137	
CT	European	SVR	0.330	0.024	0.50	GT	European	SVR	0.343	0.023	0.99
		NR/R	0.320	0.103				NR/R	0.343	0.103	
	Amerindian	SVR	0.322	0.031	2.28×10^{-3}		Amerindian	SVR	0.332	0.026	4.7×10^{-2}
		NR/R	0.280	0.088				NR/R	0.284	0.079	
	African	SVR	0.331	0.086	6.61×10^{-2}		African	SVR	0.294	0.096	0.12
		NR/R	0.377	0.125				NR/R	0.356	0.126	
TT	European	SVR	0.349	0.026	2.16×10^{-2}	TT	European	SVR	0.334	0.023	0.07
		NR/R	0.309	0.067				NR/R	0.309	0.085	
	Amerindian	SVR	0.326	0.025	3.77×10^{-3}		Amerindian	SVR	0.319	0.027	4.0×10^{-3}
		NR/R	0.274	0.067				NR/R	0.279	0.087	
	African	SVR	0.325	0.020	1.51×10^{-3}		African	SVR	0.345	0.040	5.0×10^{-3}
		NR/R	0.413	0.114				NR/R	0.399	0.116	
HCV genotypes 2/3											
CC	European	SVR	0.324	0.025	0.79	GG	European	SVR	–		
		NR/R	0.328	0.035				NR/R			
	Amerindian	SVR	0.338	0.028	0.40		Amerindian	SVR	–		
		NR/R	0.370	0.111				NR/R			
	African	SVR	0.339	0.039	0.47		African	SVR	–		
		NR/R	0.374	0.139				NR/R			
CT	European	SVR	0.333	0.023	0.46	GT	European	SVR	0.333	0.013	0.73
		NR/R	0.356	0.089				NR/R	0.348	0.070	
	Amerindian	SVR	0.308	0.020	0.47		Amerindian	SVR	0.314	0.020	0.78
		NR/R	0.290	0.071				NR/R	0.301	0.079	
	African	SVR	0.354	0.038	0.94		African	SVR	0.352	0.028	0.99
		NR/R	0.350	0.139				NR/R	0.351	0.132	
TT	European	SVR	0.362	0.025	0.82	TT	European	SVR	0.332	0.028	0.23
		NR/R	0.353	0.073				NR/R	0.355	0.090	
	Amerindian	SVR	0.339	0.033	0.35		Amerindian	SVR	0.331	0.030	0.25
		NR/R	0.302	0.069				NR/R	0.308	0.081	
	African	SVR	0.300	0.048	0.49		African	SVR	0.335	0.045	0.55
		NR/R	0.346	0.116				NR/R	0.355	0.133	

In hepatitis C virus (HCV) genotype 2/3 NR/R subgroup nobody had G/G genotype and only one in sustained virological response (SVR).

(rs12979860 C/C, rs8099917 T/T and rs12980275 A/A) have been demonstrated to be highly associated with SVR to pegylated interferon/ribavirin treatment. Those results also showed an association between self-reported ancestry and SVR. Of note, the rs12979860 is just 4378 bases from rs8099917, and it has been described as a high linkage between them (10, 16). In our study, we also found this high linkage as well.

In *IL28B* rs12979860, we detected a 24% C/C genotype frequency among our study sample. This is lower than the frequency of 35% found in a previous study when all ethnic groups were analysed. However, it appears to be an intermediate prevalence compared with the C/C genotype frequency of 16% among African Americans and 39% among European Americans (10). It is interesting to speculate that this intermediate frequency reflects the ethnic admixture of our population. Of note, the C allele frequency in our study sample was closer to one observed among the African Americans in the North American study. This reflects the higher rate of heterozygotes detected in our sample, which perhaps is related to the greater African genetic contribution in our population. It is important to mention that in our study, the C/C genotype in rs12979860 was a strong predictor of SVR for HCV genotype 1 (OR 4.69) and HCV genotype 2/3 (OR 4.13). A previous US study also showed C/C in rs12979860 to be a strong predictor of SVR for HCV genotype 1 and HCV genotype 2/3 (34). However, the C/C genotype is still controversial as a predictor of SVR among HCV genotypes 2/3 infected

Table 4. Ancestry analysis classified according to morphological phenotypic characteristics and self-reported classification, *IL28B* genotypes and antiviral therapy response

		SVR (%)	NR/R (%)	*P*	CI 95%
rs12979860					
Phenotypic classification					
Whites	CC	18 (75.0)	6 (25.0)	0.38	0.616–6.49
African-descents		18 (60.0)	12 (40.0)		
Whites	CT	16 (32)	34 (68)	0.68	0.57–2.80
African-descents		19 (27.1)	51 (72.9)		
Whites	TT	7 (43.8)	9 (56.3)	0.52	0.46–5.83
African-descents		9 (32.1)	19 (67.9)		
Self-reported classification					
Whites	CC	16 (80.0)	4 (20.0)	0.14	0.77–10.18
African-descents		20 (58.8)	14 (41.2)		
Whites	CT	11 (29.7)	26 (70.3)	1.00	0.45–2.43
African-descents		24 (28.9)	59 (71.1)		
Whites	TT	5 (50)	5 (50)	0.46	0.50–8.76
African-descents		11 (32.4)	23 (67.6)		
rs8099917					
Phenotypic classification					
Whites	GG	2 (50)	2 (50)	0.43	0.19–1.33
African-descents		0	3 (100)		
Whites	GT	10 (25.6)	29 (74.4)	1.00	0.78–1.35
African-descents		10 (27.8)	26 (72.2)		
Whites	TT	29 (61.7)	18 (38.3)	0.03	0.44–0.97
African-descents		37 (41.1)	53 (58.9)		
Self-reported classification					
Whites	GG	1 (50)	1 (50)	1.00	0.12–13.96
African-descents		1 (20)	4 (80)		
Whites	GT	7 (24.1)	22 (75.9)	0.79	0.28–2.34
African-descents		13 (28.3)	33 (71.7)		
Whites	TT	24 (66.7)	12 (33.3)	0.01	1.27–6.24
African-descents		42 (41.6)	59 (58.4)		

CI 95%, confidence interval 95%; NR/R, non-response or relapse; OR, odds ratio; *P*, *P*-value; SVR, sustained virological response.

patients. In a recent published European study, C/C genotype was a strong predictor of SVR; however, in another study, it was a predictor of SVR only in patients who had not achieved RVR (21). Also, we found a slightly higher frequency (28%) of this protective genotype among HCV genotype 2/3 infected patients compared with HCV genotype 1 in contrast to greater frequencies described by other authors (35–37).

We evaluated the relationship between genetic ancestry contribution, *IL28B* rs12979860 and response to HCV therapy. Interestingly, HCV genotype 1 patients who had the C/C genotype did not have their chances of obtaining SVR influenced by genetic ancestry contribution. On the other hand, genetic ancestry contribution interfered with the antiviral HCV genotype 1 response rates of subjects who had the C/T and especially T/T genotype. In these subgroups of patients, a higher African genomic contribution was associated with fewer chances to obtain SVR, and a greater Amerindian and European (the latter for T/T genotype only) was associated with more chances to obtain SVR. Therefore, the C/C genotype appears to be a more important marker of therapy response than ancestry in our admixed population. From another standpoint, patients who have the other two unfavourable genotypes are influenced by other factors such as the amount of genetic contribution they have received. Of note, when we analysed our population using phenotypic and self-reported ancestry, there was no association between these classification methods and response to antiviral therapy. Therefore, our study showed that in admixed populations, they should not be used to determine the chances of obtaining SVR. North American studies have found that in patients of European ancestry, the C/C genotype was associated with a two-fold greater rate of SVR than the T/T genotype, three-fold greater rate in African Americans and two-fold higher rate in Hispanic populations (10). Results from recent spontaneous HCV clearance data have shown that the strength of the protective C/C effect was similar in individuals of African and European ancestry (15).

As previously mentioned, Amerindian genetic ancestry was a strong protective factor associated with SVR in this admixed sample. Although we did not observe a high Amerindian ancestry contribution (0.217), it was more associated to SVR than European ancestry.

One Amerindian AIM (*CKMM*) is close to *IL28B* rs12979860; however, they are not linked. In addition, CKMM is a good marker to differentiate African, European and Amerindian populations with high differential frequency (23).

In *IL28B* rs8099917, we detected a high T allele frequency (0.807), and it was significantly associated with SVR. The G allele frequency was low (0.193), and it was more frequent among non-responders/relapsers. This is agreement with previous studies which found that G allele was associated with failure to antiviral treatment and T allele with SVR. The most significant associations were found in Asiatic populations (11, 12). Also, the T/T genotype frequency in our admixed population was 63% (HCV genotype 1, 62% and HCV genotypes 2/3, 64%), only slightly higher than the 58% frequency found in HCV genotype 1 Swiss population (16).

We also evaluated the relationship between genetic ancestry contribution, *IL28B* rs8099917 and response to HCV therapy. Interestingly, in contrast to *IL28B* rs12979860, HCV genotype 1 patients who had the favourable genotype (T/T) had their chances of obtaining SVR influenced by genetic ancestry contribution. As this genotype was highly frequent in our population, probably other factors such as the amount of genetic contribution they have received are also important to obtain SVR. A higher African genetic contribution was associated with non-response to therapy, and again a higher Amerindian contribution was a predictor of SVR. These findings reinforce the protective role of the Amerindian genomic ancestry for HCV treatment.

In summary, *IL28B* rs12979860 and rs8099917 were predictors of therapy response in our admixed population, for both HCV genotypes 1 and HCV genotypes 2/3 patients. *IL28B* rs12979860 C/C genotype was the most important factor associated with SVR. In patients with the unfavourable *IL28B* rs12979860 genotypes, a higher African genomic contribution was associated with fewer chances to obtain SVR, and a greater Amerindian and European was associated with more chances to obtain SVR. When we analysed our population using phenotypic and self-reported ancestry, there was no association between these classification methods and response to antiviral therapy. Although *IL28B* rs8099917 was a predictor of therapy response among our patients, it was not as strong as *IL28B* rs12979860.

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