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ORIGINAL ARTICLE

ASSOCIATION BETWEEN THE IL28B RS12979860 POLYMORPHISM AND THERAPY RESPONSE IN PATIENTS INFECTED WITH GENOTYPE 1 OF HEPATITIS C VIRUS IN CENTRAL BRAZIL

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ABSTRACT

Single nucleotide polymorphisms (SNPs) upstream of the IL28B gene have been associated with the spontaneous clearance of hepatitis C virus (HCV) and with a sustained virological response (SVR) to HCV infection treatment. This study aimed to investigate the association between IL28B SNP rs12979860 and SVR in patients with hepatitis C in Central Brazil. A total of 101 HCV genotype 1 mono-infected chronic patients treated with pegylated interferon and ribavirin (PEG-IFN/RBV) were studied in the City of Goiânia, Central Brazil. Analysis of rs12979860 showed that the most prevalent genotype was CT (57.4%), followed by CC (23.8%) and TT (18.8%). An overall SVR rate of 28.7% (95% CI: 20.4-38.7) was found in the study population. In a multivariate analysis, only IL28B rs12979860 CC genotype (OR: 3.77; 95% CI: 1.13-12.60; p = 0.031) was associated with SVR. These findings show that IL28B SNP rs12979860 is a strong predictor of SVR in the PEG-IFN/RBV treatment in patients infected with genotype 1 of HCV in Central Brazil.

KEY WORDS: IL28B; rs12979860; hepatitis C treatment; sustained virological response.

RESUMO

Associação entre o polimorfismo IL28B rs12979860 e a resposta terapêutica em pacientes infectados com o genótipo 1 do vírus da hepatite C no Brasil Central

Polimorfismos de nucleotídeo único (SNPs) relacionados ao gene IL28B têm sido associados à eliminação espontânea do vírus da hepatite C (VHC) e à resposta virológica sustentada (RVS) no tratamento da infecção pelo VHC. Este estudo teve como objetivo investigar a associação entre o SNP IL28B rs12979860 e a RVS em pacientes com hepatite C no Brasil Central. Um total de 101 pacientes crônicos monoinfectados com o genótipo 1 do VHC, tratados com interferon peguilado e ribavirina (PEG-IFN/RBV), foi estudado na cidade de Goiânia, estado de Goiás, Brasil Central. A análise de rs12979860 mostrou que o genótipo mais prevalente foi CT (57,4%), seguido por CC (23,8%) e TT (18,8%). A taxa global de

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28,7% (IC 95%: 20,4-38,7) para a RVS foi encontrada na população estudada. Na análise multivariada, somente o genótipo CC para o SNP rs12979860 (OR: 3,77; IC 95%: 1,13-12,60; p = 0,031) mostrou-se associado à RVS. Estes achados mostram que o SNP IL28B rs12979860 é um forte preditor de RVS no tratamento com PEG-IFN/RBV nos pacientes infectados com o genótipo 1 do VHC no Brasil Central.

DESCRITORES: IL28B; rs12979860; tratamento da hepatite C; resposta virológica sustentada.

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic hepatic diseases including cirrhosis and hepatocellular carcinoma (WHO, 2015). Currently, there is no hepatitis C vaccine, although HCV infection is potentially curable. In developed countries, several direct acting antiviral (DAAs) treatments for hepatitis C are now available (Webster et al., 2015). Nevertheless, the limitations of DAAs include high cost and potential low genetic barriers to resistance. Therefore, in most developing countries, therapy with pegylated interferon-alfa (PEG-IFN-α) in combination with ribavirin (RBV) will still be used to treat HCV infection in the immediate future (Liu et al., 2015). In Brazil, new DAAs in interferon-free combinations are now available (Brasil, 2015).

Both viral and host factors may influence the outcome of PEG-IFN/RBV therapy. Regarding the viral factors, HCV genotypes are a well-known and widely observed factor (Liu et al., 2015; Matsuura et al., 2014). Among host genetic factors, polymorphisms near the interleukin-28B (IL-28B) locus on chromosome 19 have been associated with response to hepatitis C treatment. Concerning the single nucleotide polymorphism (SNP) rs12979860, the homozygous CC genotype is associated with a better response to therapy than heterozygous CT or the homozygous TT genotype (Ge et al., 2009; Olmedo et al., 2015). In addition, IL28B genotyping may be useful to identify patients who would benefit from shorter treatment with a first-generation DAA (Muir et al., 2013; Matsuura et al., 2014). Also, the IL28B rs12979860 CC genotype may affect the response to IFN-free combination therapy in HCV genotype 1 infected patients (Chu et al., 2012; Zeuzem et al., 2013). It was suggested that the importance of innate immunity and IL28B SNP genotype may affect HCV therapy efficacy in certain IFN-free regimens (Matsuura et al., 2014).

In Brazil, HCV genotype 1, the most prevalent, is known as a difficult to treat genotype (Lampe et al., 2013; Webster et al., 2015). Concerning host factors, the Brazilian population has a wide range of genetic backgrounds due to the high degree of miscegenation, which may also influence the response to the HCV therapy (Cavalcante et al., 2012). However, there are few studies determining the IL28B polymorphisms in Brazilian HCV genotype 1 mono-infected patients treated with PEG-IFN and RBV (Cavalcante et al., 2012; Ramos et al., 2012; Garcia et al., 2013; Vasconcelos et al., 2014), and there is available data on these patients in Central Brazil. Therefore, the aim of the study was to estimate the

frequency of the genotypic variants of IL28B SNP rs12979860 (C/T) and to evaluate the association of this polymorphism with sustained virological response (SVR) to PEG-IFN/RBV treatment among patients infected with genotype 1 of HCV in Central Brazil.

MATERIAL AND METHODS

From January 2012 to February 2014, adult patients (older than 18 years of age) chronically infected with HCV genotype 1, who had been treated with a combination of PEG-IFN and RBV [48-wk combination of PEG-IFN-α 2a/2b and RBV (15 mg/kg)], were consecutively subjected to the genotyping of IL28B SNP rs12979860 at the Institute of Tropical Pathology and Public Health in the City of Goiania, Central Brazil. Patients who were co-infected with hepatitis B virus or human immunodeficiency virus or had other concomitant chronic liver diseases were excluded from the study.

Data on treatment outcome and a blood sample were collected for each enrolled patient. The treatment outcome of SVR was defined by a negative result for HCV RNA detection up to 6 months after treatment. Patients who did not achieve SVR (including those who relapsed) were categorized as non-responders (NR). Written informed consent was obtained from all participants prior to the start of the study. The protocol used in this study was approved by the Ethics Committee of the Federal University of Goiás.

Whole-blood samples of all patients were subjected to DNA extraction using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Polymerase chain reaction (PCR) targeting the IL28B SNP rs12979860 was performed, and PCR products were genotyped by restriction fragment length polymorphism (RFLP) analysis using BstUI restriction endonuclease (New England Biolabs, UK), essentially as described previously (Fabris et al., 2011).

Allele frequencies were estimated by direct allele counting. Both deviation from Hardy-Weinberg equilibrium and comparisons between groups were assessed using the chi-square test. To determine the baseline factors predicting SVR, these variables were initially assessed by univariate, a p value ≤ 0.20 was used to select variables for inclusion in a multivariate logistic regression model to estimate adjusted odds ratios (ORs) and 95% confidence intervals (CIs). P values < 0.05 were considered statistically significant. The data were analyzed using SPSS version 17.0 (SPSS for Windows, Chicago, IL, US).

RESULTS

A total of 101 HCV genotype 1 infected patients treated with PEG-IFN/RBV were included in the present study. Most of the study participants (73.3%) were previously treatment-naïve. The mean age was 48.2 years, 54.5% were

males, 69.3% were non-White (brown/*pardo* 62.4% and black 6.9%), with a mean body mass index (BMI) of 25.6 Kg/cm^2 and 77.2% with basal HCV RNA ≥ 600.000 IU/mL. Histopathological analysis of liver biopsy tissues was only available for 77 patients; of these, 64.9% had Metavir F0-F2 scores (Table 1). An overall SVR of 28.7% (95% CI: 20.4-38.7) was found in the study population.

As shown in Table 2, the SNP rs12979860 genotypic frequencies found in the whole sample (n = 101) and in the SVR group (n = 29) were in Hardy-Weinberg equilibrium (p = 0.128 and 0.925, respectively), but not that observed in the non-responders (NR) group (n = 72) (p = 0.029). Overall, the most prevalent genotype was CT (n = 58, 57.4%), followed by CC (n = 24, 23.8%) and TT (n = 19, 18.8%). The frequency of the CC genotype was higher in the SVR group. On the other hand, the frequencies of CT and TT genotypes were higher in the NR group (p = 0.006).

Table 3 shows the association between the baseline characteristics and SVR. Univariate analysis revealed that the mean BMI (p = 0.048), liver fibrosis stage F0-F2 (p = 0.019), and rs12979860 CC genotype (p = 0.002) were significantly associated with SVR. In a multivariate analysis, only rs12979860 CC genotype (OR: 3.77; 95% CI: 1.13-12.60; p = 0.031) was independent factor associated with SVR in HCV genotype 1 infected patients.

Table 1. Baseline characteristics of patients chronically infected with hepatitis C virus (HCV) genotype 1 in Central Brazil (n = 101)

Variables	
Age (years) (mean ± sd)	48.2 ± 10.6
Gender (male) [n (%)]	55 (54.5)
Race/ethnicity [n (%)]	
White	31 (30.7)
Black/Brown	70 (69.3)
BMI (Kg/cm^2) (mean ± sd)	25.6 ± 4.5
HCV RNA viral load (IU/mL) [n (%)]	
< 600.000	23 (22.8)
≥ 600.000	78 (77.2)
Liver fibrosis (Metavir)* [n (%)]	
F0-F2	50 (64.9)
F3-F4	27 (35.1)

Sd: standard deviation; BMI: body mass index *Histopathological analysis of liver biopsy tissues was only available for 77 patients: F0-F2 and F3-F4: Metavir score (mild-moderate and severe-cirrhosis, respectively)

Table 2. Genotypic and allelic frequencies of the rs12979860 interleukin-28B gene polymorphism among patients chronically infected with hepatitis C virus genotype 1 treated with pegylated interferon and ribavirin.

	All patients n (%)			SVR n (%)			NR n (%)			p-value**
	Observed	Expected		Observed	Expected		Observed	Expected		
Genotype										
CC	24 (23.8)	27.8 (27.5)		13 (44.8)	13.1 (45.2)		11 (15.3)	15.6 (21.6)		
CT	58 (57.4)	50.4 (49.9)		13 (44.8)	12.8 (44.1)		45 (62,5)	35.8 (49.8)		
TT	19 (18.8)	22.8 (22.6)		3 (10.3)	3.1 (10.7)		16 (22.2)	20.6 (28.6)		0.006
Allele										
C	106 (52.5)	p-value*	0.128	39 (67.2)	p-value*	0.925	67 (46.5)	p-value*	0.029	
T	96 (47.5)	χ^2	2.313	19 (32.8)	χ^2	0.009	77 (53.5)	χ^2	4.721	

*Hardy-Weinberg equilibrium;**Chi-square test for sustained virological responders (SVR) *vs* non-responders (NR) genotype frequencies.

DISCUSSION

Table 3. Predictors of sustained virological response, univariate and multivariate logistic regression analyses

Variables	Unadjusted OR	95% CI	P value	Ajusted OR*	95% CI	P value
Age (years)	0.97	0.93-1.01	0.151	0.97	0.92-1.02	0.293
Gender (male)	0.58	0.24-1.38	0.218			
Race/ethnicity						
White	1.00					
Black/Brown	1.57	0.59-4.19	0.365			
Body mass index (Kg/cm^2)	0.89	0.80-1.00	0.048	0.95	0.83-1.10	0.522
HCV RNA < 600.000 IU/mL	0.69	0.26-1.86	0.464			
Liver fibrosis (Metavir) (n = 77)						
F0-F2	1.00			1.00		
F3-F4	4.50	1.19-17.05	0.019	2.94	0.71-12.12	0.136
IL28B rs12979860 CC genotype	4.51	1.70-11.93	0.002	3.77	1.13-12.60	0.031

OR = Odds ratio; CI = Confidence interval; *Adjusted by age, body mass index, liver fibrosis and IL28B rs12979860 CC genotype.

Hepatitis C involves a quite complex interaction between the host and HCV. Treatment decisions in patients with chronic hepatitis C are based on demographic, virological, and clinical characteristics of infected patients. Accumulating evidence supports that the analysis of the IL28B SNP rs12979860 may be a useful tool to determine which chronic HCV genotype 1 infected patient would be most likely to respond to PEG-IFN/RBV treatment (Olmedo et al., 2015).

Analysis of rs12979860 showed that the most prevalent genotype was CT (57.4%) in the study population, followed by CC (23.8%) and TT (18.8%). These frequencies were comparable with those reported in HCV infected patients in Brazil (Cavalcante et al., 2012; Ramos et al., 2012; Garcia et al., 2013; Grandi et al., 2013; da Silva Conde et al., 2014; Vasconcelos et al., 2014; Bertol et al., 2015).

In this study, a significant association was observed between SVR and the rs12979860 CC genotype. This result was consistent with previous reports among HCV genotype 1 mono-infected patients treated with PEG-IFN and RBV in Brazil (Cavalcante et al., 2012; Ramos et al., 2012; Garcia et al., 2013; Vasconcelos et al., 2014). It is important to emphasize that the CC genotype was a strong predictor of SVR to PEG-IFN and RBV therapy in the study population (OR: 3.77; 95% CI: 1.13-12.60; $p = 0.031$). Similar results were previously reported in another Brazilian cohort of chronically infected patients with HCV genotype 1 (Cavalcante et al., 2012). Additionally, in a recent systematic review, Olmedo et al. (2015) confirmed the association of the rs12979860 with SVR in treatment-naive patients infected with HCV genotype 1 (CC versus CT/TT-genotype; OR: 4.18; 95% CI: 3.37-5.17).

An overall SVR of 28.7% was found in these chronically HCV genotype 1 infected patients, and 32.4% among those who were previously treatment-naïve, percentages which were similar to those reported in two other Brazilian cohorts of chronically infected patients with HCV genotype 1 (29.4-48%) (Brandão et al., 2006; Azevedo et al., 2012). However, when these rates were compared to those observed in studies with American and European populations (40-50%) (Webster et al., 2015), our SVR rates were slightly lower. As suggested elsewhere (Ramos et al., 2012), this may be explained by the higher frequency of the rs12979860 CT genotype in Brazilian patients chronically infected with HCV.

This study suffered a few limitations. Firstly, the sample size was relatively small. Secondly, the race/ethnicity categories (white, brown, and black) were self-reported by each participant. Of note, it is difficult to classify race/ethnicity of the study participants since the Brazilian population has a wide range of genetic backgrounds due to the high degree of miscegenation (Cavalcante et al., 2012). Additionally, IL28 polymorphisms are influenced by race/ethnicity (Ge et al., 2009, Cavalcante et al., 2012), which may be a

confounding factor in this study. Therefore, IL28B polymorphisms did not account for all of the ethnic differences in response to PEG-IFN/RBV therapy (Matsuura et al., 2014).

In conclusion, this study shows that the IL28B SNP rs12979860 CC genotype is present in 23.8% of HCV genotype 1 infected patients in Central Brazil and this polymorphism is associated with SVR to PEG-IFN and RBV therapy, reinforcing its role as a predictor of patient treatment outcome.

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REFERENCES

1. Azevedo FK, Azevedo CC, Souto FJ. Assessment of the treatment of chronic hepatitis C in the state of Mato Grosso, Central Brazil. *Mem Inst Oswaldo Cruz 107*: 217-223, 2012.
2. Bertol BC, Moreira S, Garcia RFL, Ferreira LE, Debortoli G, Pinho Mde S, Amendola-Pires M, Maciel AM, Brandão-Mello CE, de França PHC. IL28B gene polymorphisms in mono- and HIV-coinfected chronic hepatitis C patients. *Front Microbiol 6*: 1-8, 2015.
3. Brandão C, Barone A, Carrilho F, Silva A, Patelli M, Caramori C, Focaccia R, Pereira L, Pedroso M, Tatsch F, Pessoa M, Pegasys Brazilian Study Group. The results of a randomized trial looking at 24 weeks vs. 48 weeks of treatment with peginterferon alpha-2a (40 kDa) and ribavirin combination therapy in patients with chronic hepatitis C genotype 1. *J Viral Hepat 13*: 552-559, 2006.
4. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de DST, Aids e Hepatites Virais. *Protocolo clínico e diretrizes terapêuticas para hepatite C e coinfecções/ Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de DST, Aids e Hepatites Virais. – Brasília: Ministério da Saúde,2015*.Available:http://www.aids.gov.br/sites/default/files/anexos/publicacao/2015/58192/pcdt_capa_miolo_09_2015_baixa_pdf_31917.pdf . Accessed in: 29/09/2015.
5. Cavalcante LN, Abe-Sandes K, Angelo AL, Machado TM, Lemaire DC, Mendes CM, Pinho JR, Malta F, Lyra LG, Lyra AC. IL28B polymorphisms are markers of therapy response and are influenced by genetic ancestry in chronic hepatitis C patients from an admixed population. *Liver Int 32*: 476-486, 2012.
6. Chu TW, Kulkarni R, Gane EJ, Roberts SK, Stedman C, Angus PW, Ritchie B, Lu XY, Ipe D, Lopatin U, Germer S, Iglesias VA, Elston R, Smith PF, Shulman NS. Effect of IL28B genotype on early viral kinetics during interferon-free treatment of patients with chronic hepatitis C. *Gastroenterology 142*: 790-795, 2012.
7. da Silva Conde SR, Soares Monteiro JC, Silva Dos Santos BT, Fonseca Filgueiras NK, de Almeida Lins PA, Bonfim Freitas F, da Silva Graça E, Demachki S, Ferreira de Araújo MT, Ishak R, Rosário Vallinoto AC. SNP rs8099917 in gene IL28B might be associated with risk of chronic infection by HCV but not with response to treatment. *Biomed Res Int 2014*: 1-6, 2014.
8. Fabris C, Falleti E, Cussigh A, Bitetto D, Fontanini E, Bignulin S, Cmet S, Fornasiere E,

Fumolo E, Fangazio S, Cerutti A, Minisini R, Pirisi M, Toniutto P. IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. *J Hepatol 54*: 716-722, 2011.

9. Garcia RF, Moreira S, de Araújo Ramos AL, Ferreira LE, de Mattos AA, Tovo CV, Nader LA, Ramos JA, Rondinelli E, de Jesus Dominici A, Garcia CE, de Souza Leite Pinho M, Brandão-Mello CE, Villela-Nogueira CA, de França PH. Interleukin 28B-related polymorphisms: a pathway for understanding hepatitis C virus infection? *World J Gastroenterol 19*: 7399-7404, 2013.
10. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature 461*: 399-401, 2009.
11. Grandi T, da Silva CM, Amaral KM, Picon PD, Costi C, da Fré NN, Fiegenbaum M, Niel C, Rossetti ML. Response to treatment in Brazilian patients with chronic hepatitis C is associated with a single-nucleotide polymorphism near the interleukin-28B gene. *Mem Inst Oswaldo Cruz 108*: 48-53, 2013.
12. Lampe E, Lewis-Ximenez L, Espírito-Santo MP, Delvaux NM, Pereira SA, Peres-da-Silva A, Martins RM, Soares MA, Santos AF, Vidal LL, Germano FN, de Martinez AM, Basso R, Pinho JR, Malta FM, Gomes-Gouvêa M, Moliterno RA, Bertolini DA, Fujishima MA, Bello G. Genetic diversity of HCV in Brazil. *Antivir Ther 18*: 435-444, 2013.
13. Liu B, McGilvray I, Chen L. IFN-λ: A new class of interferon with distinct functions-implications for hepatitis C virus research. *Gastroenterol Res Pract 2015*: 1-9, 2015.
14. Matsuura K, Watanabe T, Tanaka Y. Role of IL28B for chronic hepatitis C treatment toward personalized medicine. *J Gastroenterol Hepatol 29*: 241-249, 2014.
15. Muir AJ. IL28B in the era of direct-acting antivirals for hepatitis C. *J Clin Gastroenterol 47:* 222-227, 2013.
16. Olmedo DB, Cader SA, Porto LC. IFN-λ gene polymorphisms as predictive factors in chronic hepatitis C treatment-naive patients without access to protease inhibitors. *J Med Virol 87*: 1702-1715, 2015.
17. Ramos JA, Ramos AL, Hoffmann L, Perez RM, Coelho HS, Urményi TP, Silva R, Rondinelli E, Villela-Nogueira CA. A single nucleotide polymorphism, rs129679860, in the IL28B locus is associated with the viral kinetics and a sustained virological response in a chronic, monoinfected hepatitis C virus genotype-1 Brazilian population treated with pegylated interferon-ribavirin. *Mem Inst Oswaldo Cruz 107*: 888-892, 2012.
18. Vasconcelos LR, Moura P, Carmo RF, Pereira LB, do Carmo RF, Pereira LB, Cavalcanti Mdo S, Aroucha DC, Dutra RA, Pereira LM. Low IL10 serum levels as key factor for predicting the sustained virological response to IFNα/ribavirin in Brazilian patients with HCV carrying IL28B CT/TT genotype. *Hum Immunol 75*: 895-900, 2014.
19. Webster DP, Klenerman P, Dusheiko GM. Hepatitis C. *Lancet 385*: 1124-1135, 2015.
20. WHO. *World Health Organization. Hepatitis C. 2015.* Available: http://www.who.int/mediacentre/factsheets/fs164/en/. Accessed in: 16/09/2015.
21. Zeuzem S, Soriano V, Asselah T, Bronowicki JP, Lohse AW, Müllhaupt B, Schuchmann M, Bourlière M, Buti M, Roberts SK, Gane EJ, Stern JO, Vinisko R, Kukolj G, Gallivan JP, Böcher WO, Mensa FJ. Faldaprevir and deleobuvir for HCV genotype 1 infection. *N Engl J Med 369*: 630-639, 2013.