Tumour necrosis factor -308 and -238 promoter polymorphisms are predictors of a null virological response in the treatment of Brazilian hepatitis C patients

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Certain host single nucleotide polymorphisms (SNPs) affect the likelihood of a sustained virological response (SVR) to treatment in subjects infected with hepatitis C virus (HCV). SNPs in the promoters of interleukin (IL)-10 (-1082 A/G, rs1800896), myxovirus resistance protein 1 (-123 C/A, rs17000900 and -88 G/T, rs2071430) and tumour necrosis factor (TNF) (-308 G/A, rs1800629 and -238 G/A, rs361525) genes and the outcome of PEGylated α-interferon plus ribavirin therapy were investigated. This analysis was performed in 114 Brazilian, HCV genotype 1-infected patients who had a SVR and in 85 non-responders and 64 relapsers. A significantly increased risk of having a null virological response was observed in patients carrying at least one A allele at positions -308 [odds ratios (OR) = 2.58, 95% confidence intervals (CI) = 1.44-4.63, p = 0.001] or -238 (OR = 7.33, 95% CI = 3.59-14.93, p < 0.001) in the TNF promoter. The risk of relapsing was also elevated (-308: OR = 2.87, 95% CI = 1.51-5.44, p = 0.001; -238: OR = 4.20, 95% CI = 1.93-9.10, p < 0.001). Multiple logistic regression of TNF diplotypes showed that patients with at least two copies of the A allele had an even higher risk of having a null virological response (OR = 16.43, 95% CI = 5.70-47.34, p < 0.001) or relapsing (OR = 6.71, 95% CI = 2.18-20.66, p = 0.001). No statistically significant association was found between the other SNPs under study and anti-HCV therapy response.

Key words: TNF - polymorphisms - HCV - virological response - Brazil

Infection with hepatitis C virus (HCV) may result in different clinical outcomes, ranging from viral elimination to the development of end-stage liver disease. Hepatitis C treatment generally consists of a combination of PEGylated interferon (PEG-IFN) alpha and the antiviral drug ribavirin (RBV) (Fried et al. 2002). Analysis of HCV isolates has shown substantial heterogeneity of nucleotide sequences, leading to the classification of HCV into six main genotypes with numerous subtypes. Because HCV genotype 1, the most prevalent genotype in Brazil, is less sensitive to therapy, patients have to be treated for 48 weeks vs. 24 weeks for the other genotypes (Hadziyannis et al. 2004). Sustained virological response (SVR), which is defined as having no detectable HCV RNA six months after stopping treatment, occurs in less than 50% of patients infected with genotype 1 isolates. Moreover, interindividual variations in therapeutic response have been observed.

Single nucleotide polymorphisms (SNPs) in genes encoding for cytokines involved in pro and anti-inflammatory effects may modulate, directly or indirectly, the benefits of antiviral therapy (Ge et al. 2009, Dogra et al. 2011). Genome-wide association studies have suggested that common SNPs located on a linkage disequilibrium (D) block in the vicinity of three genes on chromosome 19, namely interferons λ1 [*interleukin (IL)-29*], λ2 (*IL-28A*) and λ3 (*IL-28B*), are strongly associated with the therapeutic response of HCV genotype 1-infected patients treated with PEG-IFN/RBV (Suppiah et al. 2009, Tanaka et al. 2009, Romero-Gomez et al. 2011).

Tumour necrosis factor (TNF) and IL-10 participate in the regulation of the cellular immune response to HCV infection (Larrea et al. 1996, Napoli et al. 1996). Heterogeneity in the promoter region of the *IL-10* gene has been reported to play a role in determining the initial and sustained IFN-α treatment response in chronic hepatitis C (Yee et al. 2001, Persico et al. 2006). TNF is a potent pro-inflammatory cytokine and an antagonist of IL-10. Well-characterised *TNF* polymorphisms at posi-

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tions -308 and -238 have been shown to influence TNF expression (Cheong et al. 2006). These polymorphisms have also been reported to be associated with the pathogenesis of acute and chronic HCV infection, viral persistence and response to IFN-α therapy (Dai et al. 2006, Thio 2008).

Human myxovirus resistance protein 1 (MxA) is a key mediator of the IFN-induced response against a wide range of single-stranded RNA viruses. Polymorphisms in the *Mx1* gene promoter have been associated with both spontaneous resolution of HCV infection and favourable responses in hepatitis C treatment (Hijikata et al. 2000, 2001, Knapp et al. 2003b).

A recent study from our laboratory has shown that the response to treatment in Brazilian patients with hepatitis C was associated with a SNP near the *IL-28B* gene (Grandi et al. 2013). The aim of the present study, which was performed with the same group of patients (n = 263), was to determine whether *IL-10*, *TNF* and *Mx1* gene promoter polymorphisms are relevant for the response to PEG-IFN/RBV therapy.

SUBJECTS, MATERIALS AND METHODS

Patients and follow-up - The 263 patients enrolled in this study were the same individuals who participated in a previous study (Grandi et al. 2013). Briefly, the study population consisted of 154 males (48.0 ± 10.4 years old) and 109 females (55.5 ± 10.3 years old) living in the southernmost state of Brazil (Rio Grande do Sul). All of the patients were treatment-naïve and chronically infected with an HCV genotype 1 isolate. Therapy with PEG-IFN 2a or 2b plus RBV was planned for a 48-week duration, but was interrupted after 12 weeks for non-responders (see below). Written informed consent was obtained from each patient. The study protocol was conducted in accordance with the provisions of the ethical guidelines of the Declaration of Helsinki and was approved by the Research Ethical Committee of the Public Health School of Rio Grande do Sul, Brazil. One hundred and seventy eight (67.7%) of the 263 patients reached end of treatment response (ETR). However, during follow-up evaluations, 64/178 patients who had achieved ETR were classified as relapsers. Therefore, 114/263 (43.3%) patients showed SVR and 149 (56.7%) did not show SVR. The patients with SVR had a significantly lower viral load than the others (Grandi et al. 2013). No correlation was observed between the type of PEG-IFN (2a or 2b) administered and the proportion of patients with SVR.

HCV load was measured to evaluate the response to PEG-IFN/RBV treatment. Early virological response was defined as an at least 2-log reduction in viral load after 12 weeks of therapy. An absence of detectable virus by conventional polymerase chain reaction (PCR) at 48 weeks of treatment is referred to as an ETR. SVR was assessed 24 weeks after the conclusion of treatment (week 72). During follow-up evaluations, relapse was defined as having detectable HCV RNA levels in patients who had achieved ETR. All other patterns of viral load were classified as a virological non-response (Strader et al. 2004).

Serum HCV RNA levels were classified as low (< 600,000 IU/mL) or high (≥ 600,000 IU/mL) viral load for analysis.

Analysis of polymorphisms - Polymorphisms in the *IL-10* (-1082 A/G, rs1800896), *TNF* (-308 G/A, rs1800629 and -238 G/A, rs361525) and *Mx1* (-123 C/A, rs17000900 and -88 G/T, rs2071430) gene promoters were assessed. Among the different *IL-10* gene polymorphisms, the SNP at position -1082 was prioritised as the most cited in the literature (Persico et al. 2006). For this purpose, genomic DNA was extracted from dried blood samples preserved in Whatman FTA elute cards (GE Healthcare, Uppsala, Sweden), following the manufacturer's instructions and amplified by PCR. Table I shows the sequences of the oligonucleotide primers and PCR conditions used in this study. PCR mixes contained 10-100 ng of genomic DNA, 2.5 mM $MgCl_2$, 500 mM dNTPs, 12.5 pmol of each primer and 1 U of Taq polymerase in a final volume of 25 μL. Direct sequencing of the PCR products was performed in both directions using a BigDye Terminator v.1.1 cycle sequencing kit (Life Technologies, Carlsbad, CA, USA). These procedures resulted in a success rate of genotyping calls ranging from 97.5-99% in the different files. For quality control purposes, 5% of the samples were selected randomly to be genotyped twice independently, which resulted in a concordance rate of 99.9%.

Statistical analysis - Allele frequencies were calculated using the gene counting method. Both deviation from Hardy-Weinberg equilibrium and allelic distributions between groups were assessed by χ^2 tests or, when appropriate, by Fisher's exact test using GraphPad InStat software v.2.04a (GraphPad Software, La Jolla, CA, USA). Haplotypes and linkage D were estimated using ARLEQUIN software, v.3.1 (Excoffier et al. 2007). D theoretical maximum (D_{max}) and D/D_{max} (D') values were calculated as described previously (Lewontin 1998). Univariate logistic regression analyses were used to determine the predictors of treatment success. Age, gender, baseline viral load and the presence of liver cirrhosis as well as the previously genotyped IL-28B SNP rs12979870 (Grandi et al. 2013) were included as covariates in a multivariate logistic regression model to estimate adjusted odds ratios (OR) and 95% confidence intervals (CI). Mean adjusted variables were compared among *TNF* genotypes and haplotypes using ANOVA or Student's *t* test. The general linear model was used to test the association of the *TNF* polymorphisms and the virological response. A multiple logistic regression analysis was conducted to estimate the OR with 95% CI. The statistical analysis was performed using SPSS v.16.0 (SPSS Inc, Chicago, IL, USA) statistical package.

RESULTS

Gene polymorphisms and the response to PEG-IFN/RBV therapy - The distribution of genotypes was consistent with the proportions expected under Hardy-Weinberg equilibrium. The associations between polymorphisms and treatment response are shown in Table II. No significant differences were detected in the distribution of the *IL-10* -1082 genotypes between patients with SVR,

TABLE I

Oligonucleotide primers and polymerase chain reaction (PCR) conditions used in this study

Gene	Polymorphisms	Oligonucleotide primers		PCR conditions	References
		Direction	Sequence 5'→3'		
IL-10	-1082 A/G	Sense	ATCCAAGACAACACTACTAA	95ºC 5 min; 95ºC 40 s, 66ºC min, 72ºC 30 s (35x); 72ºC 7 min	Wu et al. (2002)
		Antisense	TAAATATCCTCAAAGTTCC	-	-
TNF	-308 G/A and -238 G/A	Sense	CAAACACAGGCCTCAGGACTC	94ºC 5 min; 94ºC 30 s, 54ºC 45 s, 72ºC 30 s (35x); 72ºC 7 min	Spriewald et al. (2005)
		Antisense	AGGGAGCGTCTGCTGGCTG	-	-
Mx1	-123 C/A and -88 G/T	Sense	TGAAGACCCCCAATTACCAA	94ºC 5 min; 94ºC 30 s, 60ºC 30 s, 72ºC 1 min (40x); 72ºC 7 min	Knapp et al. (2003b)
		Antisense	CTCTCGTTCGCCTCTTTCAC	-	-

IL: interleukin; Mx1: myxovirus resistance protein 1; TNF: tumour necrosis factor.

relapsers and non-responders. Similarly, the analyses of two biallelic polymorphisms in the *Mx1* gene promoter (-123 C/A and -88 G/T) did not show any significant association between the genotype and the response to PEG-IFN/RBV treatment. The genotypes GG, GA and AA at position -308 in the *TNF* promoter were found in 183 (69.6%), 73 (27.6%) and seven (2.7%) patients, respectively. At position -238, the corresponding distribution was 205 (77.9%), 54 (20.5%) and four (1.5%), respectively. Both *TNF* gene polymorphisms -308 G/A and -238 G/A were significantly associated with a null virological response to therapy with PEG-IFN/RBV ($p < 0.001$), with higher SVR frequencies among patients with the GG genotype. A cumulative effect was observed since 80.6% of the individuals showing two-four A alleles were non-responders, compared to 35.4% and 20.9% of the subjects with one and no A allele, respectively (Table II).

TNF polymorphisms -308 and -238 are predictors of a null virological response - A logistic regression analysis was performed to determine whether *TNF* genotypes and diplotypes are predictors of a null virological response or relapsing. A multivariate model was designed with age, sex, baseline viral load, presence of liver cirrhosis and *IL-28B* SNP rs12979870 as covariates because they might constitute confounding variables. Comparisons were performed between patients with ETR and non-responders and comparisons were performed between patients with SVR and relapsers. The results are shown in Table III. After adjusting for eventual confounding effects, it was observed that carriers of one or two A allele(s) at position -308 exhibited a significantly increased risk of having a null virological response (OR = 2.58, CI = 1.44-4.63, $p = 0.001$) and relapsing (OR = 2.87, CI = 1.51-5.44, $p = 0.001$). Similar increasing risks were observed for patients with A allele(s) at position -238 (OR = 7.33, CI = 3.59-14.93, $p < 0.001$ for null virological response and OR = 4.20, CI = 1.93-9.10, $p < 0.001$ for relapsing). An analysis performed with a non-adjusted logistic model also revealed increased risks (see footnote of Table III).

Because each *TNF* polymorphism (-308 and -238) was associated with a treatment outcome, a possible combined effect when both polymorphisms were present was investigated. The haplotype frequencies for two *TNF* gene polymorphisms were estimated using a maximum likelihood method. The polymorphisms were in low linkage D (D' = 0.31 and $r^2 = 0.06$) and the haplotype frequencies were 76.8%, 7.6%, 11.8% and 4.9% for G/G, G/A, A/G and A/A, respectively (data not shown). The haplotype combinations (diplotypes) were assessed in terms of the association with (i) ETR (after 48 weeks of treatment) and (ii) SVR within the group of patients who had achieved ETR (72 weeks). A multivariate model showed a cumulative effect of -308/-238 polymorphisms on the treatment outcome. Individuals with two-four copies of the A allele at positions -308 and -238 exhibited a significantly increased risk of having a null virological response (OR = 16.43, CI = 5.70-47.34, $p < 0.001$) and relapsing (OR = 6.71, CI = 2.18-20.66, $p = 0.001$) (Table III).

TABLE II

Genotypes and alleles frequencies of *IL-10*, *Mx1* and *TNF* polymorphisms in patients with sustained virological response (SVR), relapsers and non-responders

Genotypes and alleles	Number of patients n (%)				p	
	All (n = 263)	With SVR (n = 114)	Relapsers (n = 64)	Non-responders (n = 85)	SVR vs. relapsers	SVR vs. non-responders
IL-10 -1082 A/G						
AA	116 (44.1)	45 (39.5)	36 (56.2)	35 (41.2)	NS	NS
AG	107 (40.7)	49 (43)	20 (31.3)	38 (44.7)	-	-
GG	40 (15.2)	20 (17.5)	8 (12.5)	12 (14.1)	-	-
A	339 (64.4)	139 (61)	92 (71.9)	108 (63.5)	NS	NS
G	187 (35.6)	89 (39)	36 (28.1)	62 (36.5)	-	-
Mx1 -123 C/A						
CC	211 (80.5)	94 (82.5)	51 (79.7)	66 (78.6)	NS	NS
CA	47 (17.9)	18 (15.8)	11 (17.2)	18 (21.4)	-	-
AA	4 (1.5)	2 (1.7)	2 (3.1)	0 (0)	-	-
C	469 (89.5)	206 (90.3)	113 (88)	150 (89)	NS	NS
A	55 (10.5)	22 (9.6)	15 (12)	18 (11)	-	-
Mx1 -88 G/T						
GG	202 (76.8)	91 (79.8)	47 (73.4)	64 (75.3)	NS	NS
GT	55 (20.9)	21 (18.4)	15 (23.4)	19 (22.3)	-	-
TT	6 (2.3)	2 (1.8)	2 (3.1)	2 (2.4)	-	-
G	459 (87.3)	203 (89)	109 (85.2)	147 (86.5)	NS	NS
T	67 (12.7)	25 (11)	19 (14.8)	23 (13.5)	-	-
TNF -308 G/A						
GG	183 (69.6)	91 (79.8)	48 (75)	44 (51.8)	NS	< 0.001
GA	73 (27.6)	21 (18.4)	16 (25)	36 (42.3)	-	-
AA	7 (2.7)	2 (1.8)	0 (0)	5 (5.9)	-	-
G	439 (83.5)	203 (89)	112 (87.5)	124 (72.9)	NS	< 0.001
A	87 (16.5)	25 (11)	16 (12.5)	46 (27.1)	-	-
TNF -238 G/A						
GG	205 (77.9)	101 (88.6)	55 (85.9)	49 (57.6)	NS	< 0.001
GA	54 (20.5)	13 (11.4)	9 (14.1)	32 (37.6)	-	-
AA	4 (1.5)	0 (0)	0 (0)	4 (4.7)	-	-
G	464 (88.2)	215 (94.3)	119 (93)	130 (76.5)	NS	< 0.001
A	62 (11.8)	13 (5.7)	9 (7)	40 (23.5)	-	-
TNF -308/-238 diplotypes[a]						
No A allele	153	81 (52.9)	40 (26.1)	32 (20.9)	NS	< 0.001
1 A allele	79	28 (35.4)	23 (29.1)	28 (35.4)	-	-
2-4 A alleles	31	5 (16.1)	1 (3.2)	25 (80.6)	-	-

a: combinations are as follows. No A allele: G/G + G/G; one A allele: G/G + G/A and G/G + A/G; two A alleles: G/G + A/A, G/A + G/A, G/A + A/G and A/G + A/G; three A alleles: G/A + A/A and A/G + A/A; four A alleles: A/A + A/A. Assuming that the larger the number of copies of allele A, the greater the risk, the samples were divided into categories, according to the number of A copies. However, none of the samples showed three A alleles and only four showed four A alleles. For this reason, all the samples with two-four A alleles were grouped in a unique category. In the genotypic model, dominant model was used because having one allele increases the chance of not responding to therapy. The statistical power of the sample to detect an association for the non-significant single nucleotide polymorphisms (SNPs) [interleukin (*IL*)-*10* and myxovirus resistance protein 1 (*Mx1*)] with an odds ratio of 3 ranged from 61-91%. NS: not significant; TNF: tumour necrosis factor.

TABLE III

Logistic regression model adjusted by age, sex, baseline viral load, presence of liver cirrhosis and interleukin (*IL*)-*28B* polymorphism for association between tumour necrosis factor (*TNF*) genotypes and diplotypes and virological response

	Patients with ETR vs. non-responders		Patients with SVR vs. non-responders + relapsers	
Features	Adjusted OR (95% CI)	p	Adjusted OR (95% CI)	p
TNF -308				
GA+AA	2.58 (1.44-4.63)[a]	0.001	2.87 (1.51-5.44)[b]	0.001
Age	0.99 (0.97-1.02)	0.929	1.01 (0.99-1.04)	0.208
Sex	0.74 (0.41-1.34)	0.323	0.55 (0.30-0.99)	0.047
Viral load ≥ 600,000 IU/mL	2.59 (1.20-5.60)	0.015	3.71 (1.86-7.39)	< 0.001
Liver cirrhosis	2.32 (1.11-4.84)	0.024	2.12 (0.94-4.76)	0.069
IL-28B	6.94 (2.01-23.91)	0.002	6.29 (2.67-14.82)	< 0.001
TNF -238				
GA+AA	7.33 (3.59-14.93)[c]	< 0.001	4.20 (1.93-9.10)[d]	< 0.001
Age	0.99 (0.96-1.02)	0.636	1.01 (0.98-1.04)	0.259
Sex	0.91 (0.49-1.71)	0.788	0.64 (0.35-1.16)	0.146
Viral load ≥ 600,000 IU/mL	2.85 (1.26-6.44)	0.012	3.55 (1.78-7.10)	< 0.001
Liver cirrhosis	2.84 (1.30-6.17)	0.008	2.32 (1.02-5.26)	0.043
IL28B	9.22 (2.47-34.43)	0.001	6.75 (2.77-16.42)	< 0.001
TNF -308/-238 diplotypes				
1 A allele	1.88 (0.99-3.56)	0.052	2.68 (1.41-5.11)	0.003
2-4 A alleles	16.43 (5.70-47.34)	< 0.001	6.71 (2.18-20.66)	0.001
Age	0.99 (0.97-1.02)	0.948	1.01 (0.98-1.04)	0.235
Sex	0.75 (0.40-1.41)	0.385	0.58 (0.31-1.05)	0.075
Viral load ≥ 600,000 IU/mL	2.79 (1.22-6.36)	0.014	3.88 (1.92-7.86)	< 0.001
Liver cirrhosis	2.60 (1.20-5.61)	0.015	2.24 (0.97-5.15)	0.056
IL-28B	7.10 (1.98-25.34)	0.003	6.53 (2.72-15.68)	< 0.001

a: non-adjusted odds ratios (OR) = 3.36, p < 0.001; *b*: non-adjusted OR = 2.45, p = 0.002; *c*: non-adjusted OR = 5.71, p < 0.001; *d*: non-adjusted OR = 2.69, p < 0.001. OR were calculated by logistic regression, taking GG genotype as a reference. To calculate unadjusted OR, genotype was the only parameter considered. For adjusted OR, all other variables were included. CI: confidence interval; ETR: expected treatment response; SVR: sustained virological response.

DISCUSSION

Host immune response as a consequence of genetic background has been shown to play a crucial role in HCV infection pathogenesis and interindividual heterogeneity of disease outcomes (Amini & Poustchi 2012). Cytokine production varies among individuals and these variations are associated with SNPs located in the coding and promoter regions of cytokine genes (Ollier 2004). TNF, in particular, has been reported to play a critical role in the host immune response to HCV infection (Hohler et al. 1998, Dai et al. 2006).

The *TNF* gene promoter has been shown to contain numerous binding sites for transcription factors. Therefore, the presence of SNPs in the *TNF* gene promoter might influence the transcriptional regulation of the gene. However, whereas some studies have reported a significant association between *TNF* polymorphisms and response to hepatitis C therapy, others have not (Rosen et al. 2002, Barrett et al. 2003, Dai et al. 2006, Kusumoto et al. 2006). Currently, the G/A genotypes associated with polymorphisms at positions -308 and -238 are the best characterised. Hohler et al. (1998) reported an association between the A allele of the polymorphism G/A at position -238 and chronic hepatitis C, suggesting that this polymorphism may contribute to viral persistence. Dai et al. (2006) suggested that the *TNF* polymorphism at position -308 may be a predictor of treatment failure in patients treated with a combination of IFN-α and RBV. However, studies conducted in the United States of America, Ireland and Japan have been unable to identify any association between TNF genetic polymorphisms and histological severity or response to antiviral therapy (Hohler et al. 1998, Ollier 2004, Amini & Poustchi 2012).

In the present study, a significant association was found between each of the two *TNF* promoter polymorphisms and SVR rates after combination therapy with PEG-IFN and RBV. Serum HCV RNA levels and the presence of A alleles at positions -308 and -238 were predictors of SVR in patients infected with HCV genotype 1 (Table III). The disadvantage for a patient of having A alleles at those positions was even more evident when all diplotype combinations were considered, indicating a cumulative effect. Therefore, carrying two or more copies of the A allele may be a valuable indicator for predicting treatment difficulties. Furthermore, this study also confirmed that the *IL-28B* polymorphism was an independent predictor of SVR in the study participants (Table III). These results, corroborating those of a recently published paper (Pasha et al. 2013), suggested that polymorphisms could be used as tools of clinical utility to categorise patients before starting hepatitis C treatment.

SNPs in the *IL-10* promoter region have been associated with a beneficial treatment response and to a lesser extent with the spontaneous resolution of HCV infection (Yee et al. 2001, Vidigal et al. 2002, Knapp et al. 2003a, Mangia et al. 2004). However, other investigations have reported that the GG genotype does not influence HCV infection outcomes or the response to PEG-IFN/RBV therapy (Barrett et al. 2003, Chuang et al. 2009). Here, no significant association was found between the *IL-10* -1082 A/G polymorphism and the response to PEG-IFN/RBV therapy.

Infection with HCV leads to a rapid type I IFN response within the liver. Antiviral proteins involved in the type I IFN pathway, such as the MxA protein, together with pro-inflammatory cytokines, have been associated with treatment responses in patients with chronic hepatitis due to HCV genotype 1 infection (Cheong et al. 2006, Persico et al. 2006). Previous studies have reported that *Mx1* polymorphisms are important in predicting the IFN therapy response among patients with chronic hepatitis C (Hijikata et al. 2001, Knapp et al. 2003b, Suzuki et al. 2004), although another study did not confirm these results (Vidal et al. 2012). However, in the present study, no correlation was established between the polymorphisms -123 C/A and -88 G/T in the *Mx1* gene promoter and the response to PEG-IFN/RBV therapy.

In a general manner, the discrepancies between studies may be due not only to the type of therapy (monotherapy with IFN alone vs. combination therapy with RBV), but also to differences between ethnic groups (Layden-Almer et al. 2003, Conjeevaram et al. 2006). In this respect, it is noteworthy that the genetic structure of the Brazilian population is one of the most heterogeneous in the world, due to the ethnic mix of the population resulting from five centuries of massive interethnic crosses between people from different continents (Alves-Silva et al. 2000, Pena et al. 2011). Different from what occurs in other countries, the majority of Brazilians cannot be classified in a determined ethnic group based only on skin colour. However, in an attempt to allow comparisons with other studies, it may be interesting to mention that the genomic proportions of European, African and Amerindian ancestry in the South Brazil, where this study was conducted, have been reported to be 79.5%, 10.3% and 9.4%, respectively (Pena et al. 2011). Therefore, the genetic admixture of the Brazilian population constitutes a limitation of this study, as it was not possible to determine whether the three different genetic backgrounds were equally distributed among the patients with SVR, ETR, relapse or non-responders.

In conclusion, the results from this study corroborate that the PEG-IFN/RBV therapy response to chronic hepatitis C may be associated, at least in part, with host genetic factors, particularly *TNF* promoter polymorphisms. A replication study with a larger, well-characterised independent cohort will be necessary to confirm these associations.

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