Genetic predictors of the response to the treatment of hepatitis C virus infection

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ABSTRACT

The genome-wide association studies have identified a strong association between ***interleukin 28B (IL28B) gene*** polymorphisms and the response to treatment in patients with hepatitis C virus (HCV) infection. The aim of the study was to evaluate the association between three most widely studied ***IL28B gene*** polymorphisms and the response to antiviral treatment of chronic hepatitis C. We performed the genotyping of the three ***IL28B gene*** polymorphisms: ***rs12979860, rs8099917***, and ***rs12980275*** in 72 Caucasian patients with chronic hepatitis C, previously treated with the combination therapy of pegylated interferon alpha (PEGIFN α) and ribavirin (RBV). The patients included in the study had finished the treatment regimen at least 6 months before enrolling in the study. We used the sustained viral response (SVR) for the evaluation of the effectiveness of the antiviral treatment, and it was tested with an assay with a sensitivity of 20 IU/mL. An SVR was achieved in 59.7% (43/72) of the treated patients. The three ***IL28B gene*** polymorphisms (CC genotype of ***rs12979860***, TT genotype of ***rs8099917***, and AA genotype of ***rs12980275***) were associated with the SVR (p = 0.029, p = 0.016, and p = 0.028, respectively) in the study patients with chronic hepatitis C treated with the combination therapy of PEGIFN α and RBV. The association of ***IL28B gene*** polymorphisms with the treatment response points to the possibility of personalized medicine for the treatment of HCV infection.

KEY WORDS: Single nucleotide polymorphisms; interferon-lambda protein; hepatitis C chronic; treatment
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INTRODUCTION

Hepatitis C virus (HCV) infects an estimated number of 200 million people worldwide and is associated with an increased risk of the development of liver cirrhosis and hepatocellular carcinoma [1,2]. HCV viruses are classified into six genotypes, from 1 to 6. The HCV genotype 1 is the most common genotype worldwide and one of the most difficult to treat [3]. Before 2011, the standard treatment for chronic hepatitis C included pegylated interferon alpha 2a or 2b (PEGIFN) in combination with ribavirin (RBV) [4,5]. In 2011, two first-generation HCV protease inhibitors (boceprevir and telaprevir), given in combination with PEGIFN and RBV, were approved for the treatment of HCV genotype 1 infections in many countries [6,7]. In 2014, a combination therapy of the second-generation protease inhibitor (simeprevir) or polymerase inhibitor (sofosbuvir) with PEGIFN/RBV was approved to improve the treatment response in patients infected with HCV genotype 1 [8,9]. The primary goal of the treatment is HCV eradication, which is synonymous with the sustained viral response (SVR). The SVR is defined as undetectable HCV RNA in serum, 24 weeks after the completion of the antiviral treatment [10].

The PEGIFN/RBV treatment is prolonged and expensive, complicated by side effects leading to treatment discontinuation, and only about one-half of the treated patients achieve the SVR [11]. The addition of the first-generation protease inhibitors to the PEGIFN/RBV therapy has led to higher SVR rates (70-80%) for patients naive

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to the HCV treatment while SVR rates were lower for patients who failed to respond to previous treatment with PEGIFN/RBV [12]. For all these reasons, the prediction of the response rate before starting the treatment of chronic hepatitis C is of great importance. The viral and host factors are involved in predicting the treatment response. The viral factor, HCV genotype, is used for making the decision for the treatment duration and the prediction of the response rate, although the predictive value of HCV genotype is only 55% [13]. The patients with HCV genotype 2 or 3 are treated for 24 weeks and have a relatively high rate of SVR (70-80%). Those infected with HCV genotype 1 or 4 have a much lower rate of SVR (40%) despite the need for 48 weeks of treatment [14]. The host genetic factors may also predict the outcome of the chronic hepatitis C treatment. Four genome-wide association studies (GWAS) had already associated treatment-induced clearance of HCV following PEGIFN/RBV therapy with the single nucleotide polymorphisms (SNPs) near the ***interleukin 28B (IL28B) gene*** on chromosome 19 [15-18]. The ***IL28B gene*** encodes Type III IFN: IFN lambda (IFNλ), which possess antiviral activities against HCV [19,20]. The GWAS showed that SNPs near the ***IL28B gene*** (CC for ***rs12979860***, TT for ***rs8099917***, and AA for ***rs12980275***) were associated significantly with a successful treatment of chronic hepatitis C.

The aim of the study was to evaluate the association between three most widely studied ***IL28B gene*** polymorphisms and the response to the treatment of chronic hepatitis C.

MATERIALS AND METHODS

A group of 72 adult Caucasian patients with chronic hepatitis C, treated routinely with an antiviral therapy, was investigated in the study. The study was approved by the local Ethics Committee and written informed consent was obtained from each study participant. The patients included in the study had finished the treatment regimen at least 6 months before enrolling in the study. The patients were previously treated with a standard of care protocol with PEGIFN α in combination with RBV. A standard dose of e PEGIFN α was administered subcutaneously once a week (PEGIFN α-2a: 180 μg and PEGIFN α-2b: 1.5 μg/kg). RBV was administered daily with a dose of 1000 mg for patients with a body weight of less than 75 kg and 1200 mg for patients with a body weight over 75 kg when combined with PEGIFN α-2a. When combined with PEGIFN α-2b, the RBV dose was 800 mg for patients with a body weight of less than 65 kg, 1000 mg for patients with a body weight between 65-85 kg and 1200 mg for patients with a body weight higher than 85 kg. The treatment duration was 24 weeks for patients infected with HCV genotype 3, and 48 weeks for patients infected with HCV genotype 1. SVR was used for the assessment of the antiviral treatment effectiveness. SVR is defined as an absence of detectable HCV RNA in serum, 6 months after the completion of the antiviral treatment.

Peripheral blood samples in ethylenediamine tetraacetic as an anticoagulant were obtained from each patient enrolled in the study.

HCV quantification

HCV RNA was extracted from plasma using QIAamp Viral RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Reverse transcriptase-polymerase chain reaction (RT-PCR) assay for HCV quantification was done with HCV Real-TM Quant (Sacace Biotechnologies, Como, Italy) on Stratagene MX3005P real-time PCR system (Agilent Technologies, Edinburgh, UK) according to the manufacturer's instructions. The detection limit of the assay was 20 IU/mL.

IL28B polymorphisms genotyping

Peripheral blood mononuclear cells (PBMCs) were isolated with density-gradient centrifugation using Histopaque-1077 (Sigma-Aldrich, Munich, Germany) and homogenized in Tri Reagent Solution (Ambion, Life Technologies, Carlsbad, CA, USA). Genomic DNA was extracted from PBMCs homogenized in Tri Reagent Solution (Ambion, Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions and genotyped for three ***IL28B*** polymorphisms: ***rs8099917***, ***rs12979860***, and ***rs12980275***. The ***rs8099917*** polymorphism was genotyped using the TaqMan predesigned SNP genotyping assay (reference C_11710096_10, Applied Biosystems) according to the manufacturer's recommended protocol in a total volume of 25 μL. The two later SNPs were genotyped using custom designed TaqMan assays with the following primes and probes: TCTACTGAACCAGGGAGCTC, GCGCGGAGTGCAATTCAAC, 6Fam-TGGTTCACGCC TTC, Vic-TGGTTCGCGCCTTC for ***rs12979860***, and GTG CTGAGAGAAGTCAAATTCC, CCGCTACCCGGCAAA TATT, 6Fam-ACACGTCCGTTTCTA, Vic-AGACACGT CTGTTTCTA for ***rs12980275*** [21]. For both assays, 20 ng of DNA was used in a total volume of 25 μL including 12, 5 μL TaqMan Universal PCR master Mix (×2), 1 μM of each primer and 200 nM of each probe. The PCR reaction conditions were as follows: Initial denaturing at 95°C for 10 minutes; 40 cycles of 15 seconds at 92°C and 1 minutes at 64°C to reduce miss priming. Thermal cycling was performed using a Stratagene MX3005P real-time PCR system (Agilent Technologies, Edinburgh, UK). Both positive and negative controls were included in each genotyping assay.

A statistical analysis of data was performed using the SPSS software version 17 (IBM Corporation). The parametric variables were presented as the mean and standard deviations. A logistic regression model was used to determine the genetic predictors of the SVR. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were derived from the logistic regression model. A $p < 0.05$ was considered statistically significant.

RESULTS

The SVR was achieved in 59.7% (43/72) of the treated patients, confirmed by RT-PCR assay with a detection limit of 20 IU/mL. The HCV genotype 1 was present in most of the patients (95.8%, 69/72). The demographic data of the patients, distribution of HCV genotypes, and SVR rate are presented in Table 1.

The distribution of the frequencies of *rs12979860* genotypes in the study group was as follows: 20 (28%) patients with CC genotype, 41 (57%) patients with CT genotype, and 11 (15%) patients with TT genotype (Table 2). The distribution of the frequencies of *rs8099917* genotypes was: 42 (58%) patients with TT genotype, 23 (32%) patients with TG genotype, and 7 (10%) patients with GG genotype (Table 2). The distribution of the frequencies of *rs12980275* genotypes was: 23 (32%) patients with AA genotype, 39 (54%) patients with AG genotype, and 10 (14%) patients with GG genotype (Table 2).

The logistic regression analysis showed that the CC genotype of *rs12979860* was associated with the achievement of SVR as compared with non-CC genotypes (OR = 1.30, 95% CI 1.03-1.62, $p = 0.029$) (Table 3). The SVR was achieved in 80% of patients with the CC genotype of *rs12979860* compared with 51.9% of patients with the non-CC genotypes. The SVR was achieved in significantly more patients with the TT genotype of *rs8099917* than in patients with non-TT genotypes (OR = 1.33, 95% CI 1.05-1.66, $p = 0.016$) (Table 3). Furthermore, the AA genotype of *rs12980275* was associated with the achievement of SVR as compared with non-AA genotypes (OR = 1.29, 95% CI 1.03-1.62, $p = 0.028$) (Table 3).

TABLE 1. The demographic data, distribution of HCV genotypes, and SVR rate in patients

Patients (*n*)	72
Gender, *n* (%)	
Man	46 (63.9)
Women	26 (36.1)
Age, years (mean±SD)	42.0±11.2
HCV genotype, *n* (%)	
G1	69 (95.8)
G1+G3	2 (2.8)
G3	1 (1.4)
SVR rate, n (%)	43 (59.7)

HCV: Hepatitis C virus; SVR: Sustained viral response, SD: Standard deviation

TABLE 2. Distribution of the frequencies of the *IL28B gene* polymorphisms in the study participants

rs12979860	CC	CT	TT
n (%)	20 (28)	41 (57)	11 (15)
rs8099917	TT	TG	GG
n (%)	42 (58)	23 (32)	7 (10)
rs12980275	AA	AG	GG
n (%)	23 (32)	39 (54)	10 (14)

IL28B: Interleukin 28B

TABLE 3. Association between the SVR and *IL28B gene* polymorphisms

IL28B gene polymorphisms	Treatment response
rs12979860 genotypes	SVR[a]
CC (*n*=20)	16 (80.0%)
Non-CC (CT, TT) (*n*=52)	27 (51.9%)
OR[b] (95% CI)[c]	1.30 (1.03-1.62)
p	0.029
rs8099917 genotypes	SVR[a]
TT (*n*=42)	30 (71.4%)
Non-TT (TG, GG) (*n*=30)	13 (43.4%)
OR[b] (95% CI)[c]	1.33 (1.05-1.66)
p	0.016
rs12980275 genotypes	SVR[a]
AA (*n*=23)	18 (78.3%)
Non-AA (AG, GG) (*n*=49)	25 (51.0%)
OR[b] (95% CI)[c]	1.29 (1.03-1.62)
p	0.028

[a]Achieved SVR, [b]Odds ratio, [c]95% confidence interval, *IL28B*: Interleukin 28B; SVR: Sustained viral response

DISCUSSION

The GWAS [15-18] identified that homozygosis for the C allele of *rs12979860*, homozygosis for the T allele of *rs8099917*, and homozygosis for the A allele of *rs12980275* were favorable genotypes of the *IL28B gene* polymorphisms which predicted the SVR in patients with chronic hepatitis C treated with PEGIFN/RBV. The same favorable genotypes of the *IL28B gene* polymorphisms were associated with the SVR in the patients of this study. Most of the studies investigated the effect of the SNPs near the *IL28B gene* on SVR in patients with HCV genotype 1. Almost all of the treated patients in our study were infected with HCV genotype 1, except for two patients infected with dual infection (genotype 1 and 3) and only one patient infected with genotype 3. The studies of Rauch et al. [18] and Mangia et al. [22] confirmed similar effects of the *IL28B gene* polymorphisms on SVR in Caucasian patients infected with HCV genotypes 2, 3, and 4 as in the patients with HCV genotype 1.

The distribution of the frequencies of *rs12979860* genotypes in our study group was: CC in 28%, CT in 57%, and TT in 15% of the participants. In Latvia, the majority of the people are Caucasians, and they reported a similar distribution of the frequencies of *rs12979860*: CC in 33%, CT in 53%, and TT

in 14% of the study participants (Caucasians, ***n*** = 142) [23]. In northern Italy, the distribution of ***rs8099917*** genotypes was: TT in 55%, TG in 40%, and GG in 5% of the study participants (Caucasians, ***n*** = 175) [24]. The distribution of the frequencies of ***rs8099917*** genotypes in our study group was similar, TT in 58%, TG in 32%, and GG in 10% of the participants.

The SVR rate was 59.7% in our study group, and it was associated significantly with favorable genotypes of the IL28B gene polymorphisms. Consistent to our findings were the results of the studies of Shaikh et al. [25] and Domagalski et al. [26]. The study of Shaikh et al. included a cohort of 220 Caucasian (Pakistani) patients with chronic hepatitis C treated with PEGIFN/RBV [25]. The SVR rate was 45.5%, and there was a significant association between the SVR and ***IL28B gene*** polymorphisms. The CC genotype of ***rs12979860***, TT genotype of ***rs8099917***, and AA genotype of ***rs12980275*** were associated with the successful treatment (p = 0.001, p = 0.032, and p = 0.0001, respectively). Domagalski et al. determined the predictability of ***IL28B gene*** polymorphisms on the treatment response in a cohort of 174 Caucasian (Polish) patients infected with HCV genotype 1 and 4 treated with PEGIFN/RBV [26]. The CC genotype of ***rs12979860***, TT genotype of ***rs8099917***, and AA genotype of ***rs12980275*** were associated significantly with the successful treatment (p = 0.001, p = 0.016, and p = 0.002, respectively).

The ***IL28B gene*** encodes cytokine type III IFN, which serves as a natural barrier to a viral infection. The human body is capable of producing cytokines differently depending on the polymorphism of the cytokine production genes [27]. However, the dynamic interactions between genes or between genes and the existing environment affect cytokine production and thus the immune response [28]. About 20% of treated patients with a favorable CC genotype of ***rs12979860*** did not achieve SVR [29]. Other factors, such as ethnicity and HCV genotype should be used together with the ***IL28B*** genotype as pretreatment predictors of response.

CONCLUSION

Genetic analysis of the host may predict the patients that are more likely to respond to the antiviral treatment, since ***IL28B gene*** polymorphisms can influence treatment response, suggesting the possibility of personalized medicine for the treatment of hepatitis C.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

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