Interleukin 28B Polymorphism as a Predictive Factor to Treatment Response in Chronic Hepatitis C Genotype 4 Egyptian Patients

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Single nucleotide polymorphisms (SNPs) in the IL-28B gene are considered one of the most important baseline predictors of sustained virological response (SVR) to peg-interferon (PegIFN) and ribavirin (Rbv) in patients with hepatitis C virus (HCV) genotype 1 infection, and much less so in HCV-2 and -3. Whether this holds true for HCV-4 patients too is unknown. The purpose of this study was to determine the relevance of SNP in the IL28B gene region rs12979860 to treatment response in a well characterized cohort of Egyptian patients with genotype 4 HCV infection. This study included 43 patients with chronic genotype 4 HCV infection previously treated with standard doses of PegIFN-α/Rbv. IL28B SNP genotyping at rs12979860 was detected using the LightMix® Kit. Each of CC and CT genotype were present in 34.8% of patients, while 30% had the TT genotype. Seventeen (39.5%) of patients achieved a SVR, while 26 (60.5%) did not. Of the 17 responders, 13 had genotype CC, 4 had genotype CT, while none with genotype TT responded. Patients having CC genotype achieved significantly higher SVR rates (86.6%) compared with CT/TT patients (14%). By univariate analysis, fibrosis stage F0-2 (OR 10, 95%CI 1.78-56.15, P=0) and IL-28B genotype CC (OR 17.87, 95%CI 2.73-116.87, P=0.003) were significantly associated with SVR. In conclusion, IL-28B polymorphism is significantly associated with response to PegIFN and Rbv in Egyptian patients with chronic genotype 4 HCV infection. The advance knowledge of patient’s genotype could be in the future an important component of the clinical decision of initiating treatment.

Keywords: Hepatitis C virus, IL28B, Single nucleotide polymorphism, Sustained virological response.

INTRODUCTION

Hepatitis C virus is a major public health problem, as 150 million people are chronically infected worldwide and are at risk of developing liver cirrhosis and/or liver cancer while more than 350 000 people die every year from hepatitis C-related liver diseases. Egypt is considered one of the countries with the highest rate of chronic hepatitis C infection accounting for 15% (WHO, 2012).

The current recommended therapy of chronic HCV infection is the combination of a pegylated interferon (PegIFN) α and ribavirin (Rbv) for 24 or 48 weeks,
according to patient’s HCV genotype (Ridruejo et al., 2011). The rates for sustained virological response (SVR) are heterogeneous and vary significantly according to HCV genotype and baseline viral load, with a poor outcome in patients infected with genotype 1 or 4 and with a high viral load at treatment initiation (Manns et al., 2001; Fried et al., 2002). Besides these viral factors, host factors such as age, body weight, insulin resistance, stage of fibrosis, and compliance influence the outcome of antiviral treatment in patients with chronic hepatitis C. It is also known that chronic hepatitis C patients of European or Asian (Yan et al., 2008; Liu et al., 2008) ancestry have a higher rate of SVR than African American or Hispanic patients (Muñoz et al., 2004), reinforcing the influence of genetic host factors (Stättermayer et al., 2011).

Lately, three genome-wide association studies (GWAS) identified genetic variations in the IL28B gene on chromosome 19, that influence the results of the treatment in genotype 1 infected HCV individuals. This genetic polymorphism is also associated with the spontaneous viral clearance in acute infection with HCV (Ge et al., 2009; Tanaka et al., 2009; Suppiah et al., 2009).

IL28B (IFN-λ3) belongs to the family of type III IFNs, which share functional characteristics with type I IFNs (IFN-α and IFN-β). All are produced in response to viral infections and mediate their effects throughout the JAK-STAT signal transduction pathway (Kotenko et al., 2003; Sheppard et al., 2003). As IFN-λ3 is active against HCV in vitro following interaction with its receptor (Robek et al., 2005; Marcello et al., 2006) IL28B gene polymorphisms might influence viral control modulating the expression and/or functionality of IFN-λ3 (Rallón et al., 2012).

Two single nucleotide polymorphisms (SNPs) in the IL28B gene region, rs12979860 and rs8099917, were found to predict treatment outcome in the more difficult to cure genotype 1 patients and spontaneous clearance in acutely infected individuals (Ge et al., 2009; Thomas et al., 2009). The strongest association signal to date has been for SNP rs12979860 (Ge et al., 2009). Patients with the CC genotype of rs12979860 had a greater SVR rate to the treatment with PegIFN/Rbv in comparison to the non-CC genotypes (C/T or T/T) (Sheppard et al., 2003; Thomas et al., 2009).

The predictive power of IL28B SNPs in genotype 1 patients has been validated in many studies conducted in different geographical areas, to the point that this test has become part of the therapeutic algorithm (Suppiah et al., 2009; Tanaka et al., 2009; Rauch et al., 2010; McCarthy et al., 2010; Afshal et al., 2011). However, the role of IL28B as a moderator of treatment outcome in other HCV genotypes is less clear. Indeed, in HCV-2 and -3 patients the impact of IL28B polymorphisms on SVR rates are less pronounced, because acceptable rates of viral clearance have been documented even in the unfavorable IL28B genotypes, CT and TT (Mangia et al., 2010). Relatively unknown is the role of rs12979860 in patients with HCV-4, the most prevalent agent of chronic hepatitis C in the Middle East and North Africa (Nicola et al., 2012).

The purpose of this study was to underline the relevance of SNP in the IL28B gene region rs12979860 to treatment response in a well characterized cohort of Egyptian patients with genotype 4 HCV infection who were treated with PegIFNα and Rbv.

**Patients and methods**

The present study included 43 patients with chronic hepatitis C (HCV) genotype 4 who were recruited from Tropical Medicine Department, Ain Shams University Hospital and Yassin Abdel Gaffar Center for Hepatology and Gastroenterology. Patients were previously treated with standard doses of PegIFN-α/Rbv therapy and had well documented treatment response data records.

Treatment regimens included pegylated IFN-α 2a at standard doses (180 mg/week) plus weight-adjusted ribavirin (1000 mg/day for patients weighing 75 kg and 1200 mg/day for patients weighing ≥75 kg). Following international guidelines patients received either 48 or 72 weeks of treatment, according to the virological response at week 4.

According to the previously established response criteria (Ghany et al., 2009), responses were defined as Sustained Virological Response (SVR): HCV RNA negative 24 weeks after cessation of treatment, Relapse: reappearance of HCV RNA in serum after therapy is discontinued and Non-response: failure to clear HCV RNA from serum after 24 weeks of therapy. Baseline, weeks 12, 24, and 48 serum viral load results were clearly documented in clinical records.

Clinical and other laboratory data obtained from their medical records included: manifestations of hepatitis and liver cell failure such as jaundice, hepatomegaly, tenderness in the right hypochondrium, ascites, splenomegaly, lower limb edema, liver fibrosis stage, Child classification, complete blood picture, α-fetoprotein, and liver function profile (serum bilirubin, aspartate transaminase [AST], alanine transaminase [ALT], alkaline phosphatase).

Patients were excluded if they had hepatitis B virus (HBV) or human immunodeficiency virus (HIV) co-infection, Schistosomiasis, decompensated liver disease, drug dependence, previously transplanted, or on hemodialysis. Patients with poorly controlled diabetes, severe depression, autoimmune diseases, or concomitant malignant neoplastic diseases were also excluded.

An informed consent was obtained from the patients to make their medical records available for this study and to perform rs12979860 genotype testing. The study protocol was approved by the Ethics and Research Committee of Ain Shams University.
IL28B rs12979860 single nucleotide polymorphism genotyping

After 5 ml of peripheral blood was drawn from all the patients, total genomic DNA was extracted from peripheral blood mononuclear cells using MagNA Pure Compact Nucleic Acid Isolation Kit I (Cat. No.03730964001-Roche-Germany) according to the manufacturer’s instructions. IL28B polymorphism at rs12979860 was detected using the LightMix® Kit IL28B Cat.-No. 40-0588-32 Version 11/2010. The DNA isolated from whole blood samples was genotyped according to the manufacturer’s instructions by a LightCycler® FastStart DNA Master HybProbe kit Cat.-No. 03 003 248 001 using (LightCycler® 1.x / 2.0 Instruments) Cat.-No. 04 929 292 001. A 139 bp long fragment was amplified with specific primers and analyzed in a subsequent melting curve analysis, using a SimpleProbe® oligomer which is specific for the -3176 C allele (rs12979860). The supplied control DNA allowed for the accurate comparison with unknown samples. Negative control: the template DNA was replaced with water, Positive control: the template DNA was replaced with the provided control DNA.

The reaction mix (15 μl) consisted of 9.4 μl PCR-grade water, 1.6 μl (Mg2+ solution 25 mM), 2 μl reagent mix from LightCycler® FastStart DNA Master HybProbe kit and 2 μl Roche master (premixed lyophilized primers and probes). After mixing gently and spinning down, the reaction mix was transferred to a LightCycler® capillary (LightCycler® 1.x / 2.0 Instrument) Cat.-No. 04 929 292 001 and a 5 μl of sample or control DNA (control DNA; IL28B allele C, allele T, allele C/T) was then added for a final reaction volume of 20 μl.

The protocol consisted of 4 program steps; Denaturation at 95°C for 10 minutes; Cycling (45 cycles each at 95°C for 5 seconds, 60°C for 10 seconds and 72°C for 15 seconds); Melting (95°C for 20 seconds, 40°C for 20 seconds and 85°C 0 second); and finally Cooling at 40°C for 30 seconds.

The resulting PCR fragments were analyzed with a SimpleProbe® probe. The genotypes were identified by running a melting curve with specific melting points (Tm). Comparing the number of melting peaks and the Tm of the test genotypes to the positive controls, homozygote genotypes (TT and CC) showed single peaks at 53.25°C and 63.6°C, respectively. Heterozygote genotype (CT showed 2 peaks at 52.65°C and 63.4°C (figure 1).

Statistical analysis

Data analyses were performed by IBM computer using SPSS windows (V 6.2) package as follows: Quantitative variables were presented as medians or mean±standard deviation (mean±SD), whereas qualitative variables were described as number and percentages. The Chi-square test was employed to compare categorical variables. Logistic regression test was used for univariate analysis to identify the variables predicting a SVR to treatment. A probability P value <0.05 was considered statistically significant.
Table 1. Demographic, clinical and laboratory data of the 43 study patients

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>32 (74.4%)</td>
<td>11 (25.6%)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>Range (median)</td>
<td>31-54 (42)</td>
</tr>
<tr>
<td></td>
<td>mean±SD</td>
<td>42.86667±7.44049</td>
</tr>
<tr>
<td></td>
<td>&lt;40</td>
<td>13 (30%)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>F1</td>
<td>8 (18.6%)</td>
</tr>
<tr>
<td>stage</td>
<td>F2</td>
<td>16 (37.2%)</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>10 (23.2%)</td>
</tr>
<tr>
<td></td>
<td>F4</td>
<td>9 (21%)</td>
</tr>
<tr>
<td>Basal viral load</td>
<td>Range (median)</td>
<td>900-456 870 00 (876556)</td>
</tr>
<tr>
<td>low viral load</td>
<td>mean±SD</td>
<td>3713424.53333±9661764.56662</td>
</tr>
<tr>
<td></td>
<td>&lt;400 000 IU/ml</td>
<td>16 (37%)</td>
</tr>
<tr>
<td>ALT*</td>
<td>Range (median)</td>
<td>21-204 (50.5)</td>
</tr>
<tr>
<td></td>
<td>mean±SD</td>
<td>64.46667±40.87216</td>
</tr>
<tr>
<td></td>
<td>elevated ALT*</td>
<td>10 (33%)</td>
</tr>
</tbody>
</table>

*ALT alanine transaminase * >2 fold increase

Table 2. Response to treatment among the three IL28B genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Response to treatment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Response</td>
<td>No response</td>
</tr>
<tr>
<td>CC</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>TT</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>TC</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>18</td>
</tr>
</tbody>
</table>

RESULTS

The demographic, clinical and laboratory data of the 43 patients enrolled in the study are presented in Table 1. Seventy-four percent were males, the median age was 42 (70%>40 yrs), 63% had a basal viral load >400 000 IU/ml and 44% with fibrosis stage F3-4. Among the studied population, each of rs12979860 CC and CT genotype were equally present in 15 (34.8%) patients, while 13 (30%) were with genotype TT (figure 2).

As regards response to treatment, 17 (39.5%) of the study population achieved a SVR, while 26 (60.5%) did not. In the latter group 18 were non responders and 8 were relapers. Of the 17 patients with a SVR, 13 had genotype CC and 4 were with genotype CT. None of the patients with genotype TT attained a SVR (figure 2).

By stratifying patients on the basis of the IL28B genotype (CC versus CT/TT), the CC genotype patients achieved significantly higher SVR rates (13/15, 86.6%) compared with CT/TT patients (4/28, 14%) (P < 0.001). A lower rate of relapse was also observed among patients with CC genotype in relation to those the non CC patients (1/15; 6.7% versus 7/28; 25%) (figure 3).

When evaluating predictors of response, we found that the fibrosis stage F0-2 (OR 10, 95%CI 1.78-56.15, P = 0) and IL28B genotype CC (OR 17.87, 95%CI 2.73-116.87, P = 0.003) were significantly associated with SVR. Sex, age, low viral load and ALT values (more than 2 time upper limit of normal, normal value <35 IU/ml) did not influence SVR (table 3).

DISCUSSION

Despite all efforts made to eradicate the HCV infection, after the standard therapy (PegIFN and Rbv for 48 weeks) only 40–50% of the HCV infected individuals with genotype 1 achieve SVR (Ionită-Radu, 2011). In addition to limited efficacy, treatment is often poorly tolerated because of side effects that prevent some patients from completing therapy. Since our goal today is the eradication of HCV
Table 3. Predictors of SVR, univariate analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Sex</td>
<td>0.26</td>
<td>0.62</td>
<td>1.094</td>
</tr>
<tr>
<td>Age &lt;40 yrs</td>
<td>0.250</td>
<td>0.042</td>
<td>1.483</td>
</tr>
<tr>
<td>Stage</td>
<td>10</td>
<td>1.781</td>
<td>56.150</td>
</tr>
<tr>
<td>Low viral load</td>
<td>0.429</td>
<td>0.90</td>
<td>2.051</td>
</tr>
<tr>
<td>Elevated ALT *</td>
<td>0.333</td>
<td>0.067</td>
<td>1.652</td>
</tr>
<tr>
<td>Genotype CC</td>
<td>17.875</td>
<td>2.734</td>
<td>116.877</td>
</tr>
</tbody>
</table>

*Statistically significant
* ALT alanine transaminase >2 fold increase
infection, our prior concern has become the identification of predictor factors concerning treatment response (Ge et al., 2009).

Single nucleotide polymorphisms (SNPs) in the IL28B region are considered the strongest baseline predictors of a SVR to PegIFN and Rbv in patients with HCV genotype 1 infection, and much less so in HCV-2 and -3 (Mangia et al. 2010). Whether this holds true for HCV-4 patients too is unknown. The amount of available data regarding the SNPs prevalence and response to treatment in HCV-4 patients is, however, limited and often gathered on ethnically heterogeneous populations (Antaki et al., 2012).

To our knowledge, no data was published regarding the IL28B SNPs prevalence in our Egyptian population. The current study however showed that among the studied Egyptian chronic HCV-4 patients, the rs12979860 CC and CT genotypes were equally present in 34.8% of patients, while 30% patients had genotype TT. By comparing these results with the study of Ridruejo et al. (2011) including Argentine patients, the CC genotype was presented by 18%, the TT genotype by 19% while the CT genotype presented 63%. De Nicola et al. (2012) in their study in Italy detected the genotype CC in 23% of the population studied and 63% had the CT genotype, while 14% presented the TT genotype. In the first study on IL28B gene polymorphism in Latvia, the most common genotype of IL28B was CT with an incidence of 53%, followed by the CC genotype (33%), while 14% of patients showed the TT genotype (Tolmane et al., 2012).

Although HCV-4 was initially branded as a difficult-to-treat genotype, reported data, especially from Egypt, where HCV-4 represents >90% of HCV infections, have suggested SVR rates between 43 and 70%, that is, intermediate between those reported for HCV-1 and HCV-2 or HCV-3 (Antaki et al., 2010; De Nicola et al, 2012).

Of the 43 Egyptian patients included in this study, 39.5% achieved a SVR. These results are a bit lower than those recorded previously. The low sample size could be a reason for that. Patients with the CC genotype achieved significantly higher SVR rates compared with CT/TT patients (86.6% versus 14%). Moreover, a lower rate of relapse was also observed among them in relation to non CC genotypes (6.7% versus 25%).

In earlier studies conducted on HCV-1 patients in different geographical areas, patients with CC genotype achieved SVR more often than CT or TT subgroups. Ge et al. (2009) found in rs12979860 CC patients a SVR of 77% in Caucasians and 53% in African Americans. In patients of European ancestry, as well as in African-American and Hispanic patients, the CC genotype was associated with a two-fold greater SVR rate than the TT genotype. CT being closer to TT than to CC. The relationship between this polymorphism and the SVR explained much of the difference in the response between European-American and African-American patients, as the T allele is significantly more frequent in the latter than in the former. This finding was also confirmed in subsequent studies where Thompson et al. (2010) showed that the rate of SVR observed in Caucasians with the CC IL28B type was 69% and in Hispanics was 56%, while in African Americans was 48%. Ridruejo et al (2011) also reported that the SVR rates in Argentine patients with a European ancestry were 64% in rs12979860 CC genotype. In a study in Latvia on patients with genotype 1, SVR was achieved in 84% of CC subgroup versus 47.6% in non-CC subgroups (Tolmane et al 2012).

While referring to chronic HCV-4 patients, in accordance with our findings, De Nicola et al. (2012) reported significantly higher rates of SVR in CC patients compared with CT/TT patients (88% versus 38%), while also showing lower relapse rates (0% versus 36%). Moreover, in a study by Antaki et al. (2012) among chronic hepatitis C patients, all from Syria, a SVR was achieved in 62% of CC compared with 35% of rs12979860 CT/TT carriers. They concluded that SNP near IL28B strongly predicts virological response to therapy among chronic hepatitis C with HCV-4.

IL28B polymorphisms have been shown to influence on-treatment viral kinetics; they are linked to a genetic predisposition to respond to IFN administration by mounting an efficient antiviral response. The exact mechanisms behind this association are still only partially understood, with the most accepted theory supporting a correlation between IL28B genotypes and IFN-stimulated gene (ISG) expression (Sarasin-Filipowicz et al., 2008). One product of the IL28B gene, IFN-λ3, triggers an antiviral cascade via the Jak-Stat signaling pathway that is similar to, and probably synergistic with that of type I IFNs, although it uses a distinct receptor (Chevaliez et al., 2007). Like type I IFNs, LIFNs have antiviral activity against HCV both in vitro and in vivo. Exogenous administration of LIFNs is associated with a slower but more sustained induction of ISGs than α IFN (Marcello et al., 2006). These findings and further study of the functional mechanism underlying the IL28B response association, may help identify patients for whom therapy is likely to be successful, and highlight the IFN-λ signaling axis as a potential target for novel antiviral drug development (Ge et al., 2009).

Parameters for the prediction of SVR in patients with chronic hepatitis C before initiation of antiviral therapy are important in order to be able to estimate the potential for treatment success. They can help clinicians in the decision on whether or not to start antiviral therapy and this information can also motivate patients who might have a high chance for virological response. Different studies have shown that HCV genotype, HCV RNA concentration, age, gender, body mass index (BMI), fibrosis stage, ALT, and gamma glutamyltranspeptidase (GGT) levels, insulin resistance as well as host genetic polymorphisms of several genes (HLA, chemokines, interleukins and ISG) are associated with SVR (Kau et al., 2008).
IL28B SNP has been validated as the strongest pretreatment predictor of a SVR to PegIFN plus Rbv in Caucasian, Afro-American, and Asian HCV-1 patients (Suppiah et al., 2009; Tanaka et al., 2009; McCarthy et al., 2010; Rauch et al., 2010). Ridrujeuo et al. (2011) also in their univariate and multivariate analysis showed that rs12979860 CC genotype as well as viral load < 400,000 IU/ml, and F0-2 were associated with SVR in HCV-1. They concluded that IL28B genotypes should be added to baseline predictors of SVR to PegIFN/Rbv therapy in Latin American patients with European ancestry. In addition, Tolmane et al. (2012) in their study in Latvia showed that IL28B gene polymorphism in HCV-1 patients was considered a strong predictor of treatment result. They also observed that fibrosis stage was higher in non-responders group, in comparison to responders group.

Concerning HCV-2 and -3 patients, Mangia et al. (2010) analysed pretreatment clinical variables that were associated with SVR. IL-28B type, BMI of 27 or greater, and fibrosis stage F0–F2, were the only baseline variables that were associated with SVR on univariable analysis. In a multivariable logistic regression model IL-28B genotype predicted SVR.

Although the predictive role of rs12979860 in HCV-4 patients is relatively unidentified, several studies lately started to address this topic. The current study showed that the fibrosis stage F0-2 and IL-28B genotype CC were significantly associated with SVR. These findings came in line with the preceding studies targeting the impact of IL28B polymorphisms on SVR rates. Two subgroup analyses of HIV HCV coinfected and Egyptian patients were first carried out and found the IL28B CC genotype to be an independent predictor of an SVR to PegIFN plus Rbv (Rallón et al., 2011, Stättermayer et al., 2011).

Another study that evaluated factors playing a role in the outcome of therapy in HCV-4 patients was De Nicola et al. (2012) who showed that baseline variables associated with a SVR were female gender, fibrosis stage, Egyptian ethnicity and the IL28 genotype CC. By logistic regression, the IL28B rs12979860 CC genotype was an independent predictor of SVR. The present study also agreed with Antaki et al. (2012) in which predictors of SVR were similar to ours with the exception of SNP at marker rs12979860 being replaced by rs8099917. In their study both the absence of advanced fibrosis and polymorphisms near IL28B were independent predictors of SVR, confirming that algorithms of treatment for HCV-4 should include at least these two variables.

In conclusion, IL-28B polymorphism is significantly associated with response to PegIFN and Rbv for Egyptian patients with chronic genotype 4 HCV infection. However, larger scale studies are required to verify the predictive role IL28B SNPs in HCV-4 patients. The advance knowledge of host genotype of patients infected with HCV-4 could in the future become an important component of the clinical decision to initiate treatment. Besides increasing the chances to achieve SVR, determining IL28B SNPs prior to initiating treatment will be cost-effective and reduces adverse effects.

REFERENCES


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