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Research paper

IFNL4 ss469415590 polymorphism contributes to treatment decisions in patients with chronic hepatitis C virus genotype 1b, but not 2a, infection

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ABSTRACT

Recently, the dinucleotide variant ss469415590 (TT/ΔG) in a novel gene, interferon lambda 4 (*IFNL4*), was identified as a stronger predictor of hepatitis C virus (HCV) clearance in individuals of African ancestry compared with rs12979860. We aimed to determine whether this variant contributes to treatment decisions in a Chinese population. A total of 447 chronic hepatitis C (CHC) patients (including 328 treated with interferon alpha-2b and ribavirin), 129 individuals who had spontaneously cleared HCV (SHC), and 169 healthy controls were retrospectively investigated. ss469415590 genotyping was performed using a mass spectrometry method (SEQUENOM). A higher proportion of SHC individuals carried the TT/TT genotype compared with CHC patients (95.3% vs. 88.8%, $P = 0.027$). In patients with HCV genotype 1b, the ss469415590 variant was independently associated with sustained virologic response (SVR) (odds ratio [OR] = 3.247, 95% confidence interval [CI] = 1.038–10.159, $P = 0.043$) and on-treatment virological responses, including rapid (RVR), complete early (cEVR), early (EVR), and end-of-treatment (ETVR), with a minimal OR of 3.73. Especially for patients with high viral load ($\geq 4 \times 10^5$ IU/ml), ΔG allele carriers had a lower chance of achieving SVR compared with those carrying the TT/TT genotype (7.1% vs. 36.0%, $P = 0.034$, OR [95% CI] = 7.24 [1.02–318.45], negative predictive value = 92.9%). In patients with HCV genotype 2a, no significant association between the ss154949590 variant and the virological response was identified ($P > 0.05$). Additionally, we found that ss154949590 was in complete linkage disequilibrium with rs12979860. In conclusion, the *IFNL4* ss154949590 TT/TT genotype favors spontaneous clearance of HCV. This same variant is associated with treatment-induced clearance in patients with genotype 1b, but not 2a. ss469415590 (or rs12979860) genotyping should be considered for patients with HCV genotype 1b and high viral load when making a choice between standard dual therapy and an IFN-free direct-acting antiviral regimen.

1. Introduction

Hepatitis C virus (HCV) infects 130 to 170 million people worldwide (Lavanchy, 2009) and up to 4 million individuals are newly infected with HCV annually (Westbrook and Dusheiko, 2014). Up to 64–82% of acutely infected individuals fail to clear the virus and thus develop chronic hepatitis C (CHC), with as many as 10–20% progressing to cirrhosis over 20–30 years (Westbrook and Dusheiko, 2014).

CHC patients experience significant differences in response to antiviral therapy depending on the HCV genotype. Treatment with pegylated interferon and ribavirin (IFN/RBV) results in a sustained virologic response (SVR) in approximately 50% of CHC patients infected with HCV genotype 1 and 75% of those infected with genotype 2 (Hadziyannis et al., 2004; Manns et al., 2001). In addition to limited efficacy, IFN/RBV combination treatment is often poorly tolerated because of side effects that prevent some patients from completing therapy.

A milestone in hepatitis C research was the discovery of genetic markers—such as the rs12979860 and rs8099917 single nucleotide polymorphisms—located on chromosome 19q13.13 near *IFNL3* (also known as *IL28B*, encoding IFNL3) that have been associated with responses to HCV treatment and spontaneous clearance of the virus in several genome-wide association studies (Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009). However, molecular investigations

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; cEVR, complete early virological response; CHC, chronic hepatitis C; CI, confidence interval; ETVR, end-of-treatment virological response; EVR, early virological response; GGT, gamma-glutamyl transferase; HCV, hepatitis C virus; HGB, hemoglobin; *IFNL4*, interferon lambda 4; IL28B, interleukin-28B; OR, odds ratio; RBC, red blood cell; RBV, ribavirin; RVR, rapid virological response; SNP, single nucleotide polymorphism; SVR, sustained virologic response; TC, serum total cholesterol; TG, triglyceride; ULN, upper limit of normal; URVR, ultra-rapid virological response; WBC, white blood cell.

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into the function of *IFNL3* could not explain these findings (Ray, 2013). For instance, these markers are not consistently associated with hepatic *IFNL3* mRNA expression (Dill et al., 2011; Honda et al., 2010; Marukian et al., 2011). Furthermore, Urban et al. found that the only coding polymorphism in *IFNL3*, rs8103142 (encoding a p.Arg70Lys alteration in exon 2), which is in strong linkage disequilibrium with rs12979860 ($r^2 = 0.8–1.0$), does not seem to affect the function of the IFNL3 protein (Urban et al., 2010). More recently, a new interferon family gene, *IFNL4*, which harbors a dinucleotide variant (ss469415590) with two alternative alleles, TT or ΔG, was identified (Prokunina-Olsson et al., 2013). The one-base deletion in the ΔG variant results in a frameshift, which in turn produces the full-length protein designated as IFNL4, while the TT variant does not produce IFNL4 (Prokunina-Olsson et al., 2013). IFNL4 impairs spontaneous clearance and IFNα-based treatment-induced clearance of HCV (Prokunina-Olsson et al., 2013). A correlation between *IFNL4* polymorphisms and the activity of IFNL4 protein was confirmed and a causal link between reduced antiviral activity of the IFNL4 protein and reduced hepatic interferon-stimulated gene (ISG) expression as well as improved HCV clearance was established (Terczynska-Dyla et al., 2014).

Direct-acting antiviral agents (DAA) with or without IFN/RBV yield highly promising SVR rates (Jacobson et al., 2014; Pawlotsky, 2014). However, DAAs are expensive and not affordable for many patients (Drenth, 2013). Presently, these agents have not been approved in many countries, including China. In addition, IFN-free treatment may be associated with slightly higher relapse rates and/or drug resistance, as reported recently with some regimens (Schinazi et al., 2014). Thus, if a DAA treatment fails, there is an increased risk of resistant HCV strains being selected that may compromise future treatment options (Ciesek and Manns, 2011; Sarrazin et al., 2012). Consequently, there is still an urgent need to distinguish the patients who might benefit from the standard regimen with IFN/RBV only based on pre-treatment predictors of response.

In this context, our study aimed to (1) assess the association of the novel *IFNL4* polymorphism ss469415590 with spontaneous HCV clearance, (2) evaluate the predictive value of this SNP for treatment responses, (3) clarify the distribution of *IFNL4* variants in non-SVR patients, and (4) identify patients with a high probability of treatment failure according to genetic variations in *IFNL4*, pre-treatment viral loads, and standard biochemical variables in a Chinese population predominantly infected with HCV genotypes 1b or 2a.

2. Materials and methods

2.1. Study population

A total of 745 Chinese Han adults were evaluated retrospectively. All subjects attended the "Epidemiological investigation of hepatitis C virus infection in FuYu country of Jilin Province" at the First Hospital of Jilin University from 2009 to 2010. The prevalence of HCV in this area is high as a consequence of illicit drug (caffeine sodium benzoate) use involving shared syringes in the 1980s (data not published). Enrolled individuals were classified into three groups: 447 patients with CHC, 129 who had spontaneously cleared HCV, and 169 healthy controls. All subjects met the following inclusion criteria: Chinese, Han, treatment-naïve, hepatitis B surface antigen (HBsAg)-negative, human immune deficiency virus (HIV)-negative, and with sufficient stored DNA to perform *IFNL4* genotyping. The following exclusion criteria were employed: the presence of decompensated liver disease, hepatocellular carcinoma, or a previous history of antiviral therapy for CHC. Patients were considered to have CHC if they were anti-HCV positive and HCV RNA positive in serum. Those with spontaneous viral clearance were anti-HCV positive and HCV RNA negative. Healthy controls were anti-HCV negative and had no evidence of liver disease on physical examination, biochemistry testing, and abdominal ultrasound.

Written informed consent for genetic testing was obtained from all participants, and the study protocol was approved by the Ethics Committee of the First Hospital of Jilin University.

2.2. Treatment and follow-up

Of the 447 CHC patients, 328 had received IFN/RBV treatment in the form of subcutaneous injections of recombinant interferon-α2b (5 MU, three times/week; Beijing Kawin Technology Share-holding Co, Ltd., Beijing, China) and oral ribavirin (900 mg/day) for at least 12 weeks and treatment compliance rates were greater than 80% before 12 weeks. HCV RNA was quantified at weeks 0, 2, 4, 12, 24, 36, and 48 during treatment, and weeks 24, 48, and 96 after treatment cessation.

2.3. Outcomes

On-treatment responses were defined as follows: ultra-rapid virological response (URVR), HCV RNA load <15 IU/ml after 2 weeks of therapy; rapid virological response (RVR), <15 IU/ml after 4 weeks of therapy; complete early virological response (cEVR), <15 IU/ml after 12 weeks of therapy; early virological response (EVR), a more than 2 $\log_{10}$ decrease in HCV RNA load from baseline after 12 weeks of therapy, and end-of-treatment virological response (ETVR), <15 IU/ml at the end of treatment. SVR was defined as HCV RNA load <15 IU/ml at least 24 weeks after the completion of therapy. Non-SVR outcomes included null response, on-treatment viral breakthrough, and relapse. Null response was defined as viral load >15 IU/ml throughout the duration of therapy. On-treatment viral breakthrough was defined as HCV RNA load >15 IU/ml after an initial response (HCV RNA load <15 IU/ml) during treatment. Virologic relapse was defined as HCV RNA load <15 IU/ml at the end of treatment but >15 IU/ml during follow-up.

2.4. Laboratory tests

HCV genotyping in patients with CHC was performed by multicolor fluorescence PCR with an HCV RNA genotype kit (BioAssay Science & Technology Co. Ltd., China). HCV genotyping in the SHC group was performed by HCV serotyping assay using a fully automatic HISCL-5000 immunoanalyzer (Sysmex Co., Hyogo, Japan). A genotype of 1 or 2 can be determined. In China, the overwhelming majority of HCV strains are 1b or 2a. Thus, this assay was suitable. HCV RNA levels were determined by a real-time polymerase chain reaction assay (COBAS AmpliPrep HCV Monitor/COBAS TaqMan HCV test, Roche, Grenzach, Germany) with a lower limit of quantification of 15 IU/ml. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total cholesterol (TC) and triglyceride (TG) levels, and hemoglobin (HGB) levels, as well as white blood cell (WBC), red blood cell (RBC), and platelet counts, were determined by routine laboratory techniques.

2.5. IFNL4 ss469415590 genotyping

Genotyping of ss469415590 was performed using a mass spectrometry method (SEQUENOM, BioMiao Biological Technology, Beijing, China). The PCR primers were: forward 5′-ACGTTGGATGGCTCCAGCGA GCGGTAGTG-3′; reverse 3′-ACGTTGGATGTGGGTCCTGTGCACGGTGA-5′; and UEP ggcttTGCACGGTGATCGCAG.

2.6. Statistical analysis

Normally distributed variables are presented as mean ± standard deviation. Differences were evaluated by the Student's t-test. Skewed distributed variables are presented as median (range) and differences were tested by the Wilcoxon rank-sum test. Categorical variables are presented as numbers (percentage) and differences were assessed using the Pearson Chi-square test, linear-by-linear association, or Fisher's