

## Observational Study

## Impact of hepatitis C virus core mutations on the response to interferon-based treatment in chronic hepatitis C

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### Abstract

#### AIM

To determine whether hepatitis C virus (HCV) core substitutions play a role in the response to interferon-

based treatment in Caucasian patients.

## METHODS

One hundred eight HCV chronically infected patients initiating treatment with pegylated IFN plus ribavirin for 48 wk were tested for baseline substitutions at codons 70 and 91 of the viral core protein (BigDye Terminator vers.3.1, Applied Biosystems,) and for genetic polymorphisms in host *IL28B* gene rs12979860 (Custom TaqMan 5' allelic discrimination assay; Applied Biosystems).

## RESULTS

Of the patients, all were infected with HCV genotype 1b, 44.4% had low baseline HCV viral load, and 37.9% had mild/moderate fibrosis. Only 38.9% achieved therapeutic success, defined as sustained virological response (SVR). Eighty-eight percent of the patients presented at least one substitution at core position 70 (R70Q/H) or/and position 91 (L91M). The favorable *IL28B* CC polymorphism was detected in only 17.6% of the patients. In the univariate analysis, young age ( $P < 0.001$ ), urban residence ( $P = 0.004$ ), *IL28B* CC genotype ( $P < 0.001$ ), absence of core mutations ( $P = 0.005$ ), achievement of rapid virologic response ( $P < 0.001$ ) and early virological response ( $P < 0.001$ ) were significantly correlated with SVR. A multivariate analysis revealed three independent predictors of therapeutic success: young age ( $P < 0.001$ ), absence of core substitutions ( $P = 0.04$ ) and *IL28B* CC genotype ( $P < 0.001$ ); the model correctly classified 75.9% of SVR cases with a positive predictive value of 80.7%.

## CONCLUSION

HCV core mutations can help distinguish between patients who can still benefit from the affordable IFN-based therapy from those who must be treated with DAAs to prevent the evolution towards end-stage liver disease.

**Key words:** Chronic hepatitis C; Caucasian patients; Core substitutions; *IL28B* polymorphism; Treatment

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**Core tip:** The high cost of the newly introduced direct acting antivirals precludes universal replacement of the suboptimal interferon-based therapy for chronic hepatitis C. Therefore, a series of host- and virus-related factors are used as prognostic markers of treatment response. In Asian patients, a newly described viral factor is represented by amino acid substitutions in the hepatitis C virus core protein at positions 70 and 91. The present study confirms that core substitutions are also found in Caucasian patients and, together with age and *IL28B* genotype, can be used as predictors of the outcome of interferon-based therapy.

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## INTRODUCTION

Hepatitis C might become the first curable chronic disease due the remarkable efficacy of the newly introduced direct acting antiviral drugs (DAAs). Interferon-free regimens, based on combinations of DAAs with pan-genotypic activity, allow for shorter courses of treatment without severe side effects<sup>[1]</sup>. Nevertheless, the high cost of DAAs continues to preclude universal replacement of the classic treatment consisting of PEGylated-interferon and ribavirin (PEGIFN/RBV). This therapeutic combination is effective in approximately 50% of hepatitis C virus (HCV) chronically infected patients, with the response rate strongly dependent on the infecting genotype<sup>[2]</sup> and correlated with a series of other viral and host factors, e.g., baseline/on-treatment viral load, liver fibrosis, host *IL28B* polymorphisms<sup>[3-6]</sup>.

Across the 9.6 kb genome of HCV, several regions (specifically HVR1, IFN sensitivity-determining region and an IFN/ribavirin resistance-determining region in NS5A<sup>[7,8]</sup>) have been extensively analyzed in relation to treatment outcome, whereas the more conserved core gene has been mostly used for HCV genotyping and classification. Nevertheless, the core region has been reported to antagonize the antiviral response induced by IFN by interacting with the IFN-activating and signaling pathways<sup>[9,10]</sup>. Substitutions in certain less conserved sites of the core region can give rise to viral quasispecies resistant to interferon treatment<sup>[11]</sup>. Several reports, mainly from Japan, have indicated that amino acid substitutions in positions 70 and 91 of the core protein are associated with the outcome of interferon-based therapy<sup>[12,13]</sup>.

Because most of the studies related to these core mutations have been conducted in Asian populations, the aim of the present study is to determine whether HCV core substitutions are present and play a significant role in the outcome of interferon-based treatment in Caucasian patients, as well to inform better selection and prioritization of those patients who can still benefit from this affordable therapy.

## MATERIALS AND METHODS

### Study population

An observational study was conducted on 108 HCV chronically infected Caucasian patients treated for the

**Table 1** Demographic, clinical and virological characteristics of study patients *n* (%)

Characteristics	Total <i>n</i> = 108	IL28B CC <i>n</i> = 19	IL28B non-CC <i>n</i> = 89	<i>P</i> value
Age (yr), median	53.5 (22.0-68.3)	48.2 (22.1-66.4)	53.8 (22.1-68.3)	0.137
Female	64 (59.3)	8 (42.1)	56 (62.9)	0.094
Residence urban	78 (72.2)	17 (87.5)	61 (68.5)	0.090
Baseline ALT (mg/dL), median	76.5 (16-890)	103 (16-890)	74 (17-256)	0.031
Baseline HCV-RNA (log <sub>10</sub> IU/mL), median	6.1 (4.15-7.3)	6.2 (4.15-7.2)	6.1 (4.15-7.3)	0.333
Mild/moderate fibrosis	38 (58.5)	9 (69.23)	29 (55.8)	0.470
Absence of core mutations - DW	13 (12.0)	6 (31.6)	7 (7.9)	0.011
RVR (%)	14 (13.0)	7 (36.8)	7 (7.9)	0.003
EVR (%)	43 (39.8)	16 (84.2)	27 (30.3)	< 0.001
SVR (%)	42 (38.9)	17 (89.5)	25 (28.1)	< 0.001

HCV: Hepatitis C virus; DW: Double wild-type; SVR: Sustained virological response; EVR: Early virological response; RVR: Rapid virological response.

first time with a combination of PEGylated IFN- $\alpha$ 2a (180  $\mu$ g per week) or PEGylated IFN- $\alpha$ 2b (1.5  $\mu$ g/kg per week) plus ribavirin (1000 or 1200 mg, dependent on body weight) in two tertiary care facilities in Bucharest, Romania. All patients met the following inclusion criteria: 18-65 years of age, detectable HCV viremia, and previously untreated. The exclusion criteria were: HBV or HIV co-infections' malignancies; coexistent liver disease of other etiology; organ recipients, clinically significant pulmonary, renal, cardiovascular or hematological diseases; current pregnancy and lactation. Each patient provided informed consent and the Bioethics Committee of the Institute of Virology approved the study.

#### Measurement of HCV-RNA

HCV-RNA was performed at baseline, weeks 4 and 12 of treatment and 24 wk after treatment completion using COBAS AmpliPrep/COBAS TaqMan Quantitative Test, version 2.0 (Roche Diagnostics GmbH, Germany) with a linear range of HCV-RNA quantification between 15 and 100000000 IU/mL. Rapid virologic response (RVR) was defined as undetectable HCV-RNA at week 4 of treatment. Early virological response (EVR) was defined as undetectable HCV-RNA at week 12 of treatment, and sustained virological response (SVR) was defined as undetectable HCV-RNA 6 mo after treatment completion.

#### Viral genotyping and detection of substitutions at codons 70 and 91 in core protein

Viral RNA was extracted from 140  $\mu$ L serum using a commercial kit (QIAamp Mini Viral Kit, Qiagen). Reverse transcription was performed as described previously<sup>[14]</sup> and the cDNA was used in a semi-nested PCR yielding a 422 bp amplicon spanning the HCV core region<sup>[14,15]</sup>. The amplicons were sequenced (BigDye Terminator v3.1 and 3130 Genetic Analyzer, Applied Biosystems) and the resulting sequences were edited with BioEdit version 7.0.5.3<sup>[16]</sup> and used for genotyping (NCBI BLAST) and to assess the presence of substitutions at positions 70 and 91 in the core protein.

#### Genetic polymorphism in the IL28B gene (rs12979860)

Genetic polymorphism in the *IL28B* gene (rs12979860) was investigated using Custom TaqMan 5' allelic discrimination assay (Assays-by-DesignSM Service for SNP Genotyping Assays, Applied Biosystems, United States) and running a real time PCR on an ABI 7300 instrument with primers and fluorescent probes predesigned by the manufacturer and interpreted using SDS software from Applied Biosystems Inc., United States.

#### Liver fibrosis

Liver fibrosis was assessed using a noninvasive method - transient elastography (FibroScan™) - that discriminated between mild/moderate fibrosis (F1 + F2) and advanced fibrosis (F3 + F4) by assigning a value of liver stiffness lower or higher than 9.5 kPa<sup>[17]</sup>.

#### Statistical analysis

Statistical analysis performed with IBM SPSS Statistics version 20. Univariate analysis was performed for both categorical and continuous variables; *P* values were calculated using the independent samples Mann-Whitney *U* test for continuous variables and Pearson  $\chi^2$  or Fisher's exact test for categorical variables. Variables with statistical significance (*P* < 0.05) in the univariate analysis were introduced into a multivariate logistic regression model.

## RESULTS

#### Characteristics of patients and response to treatment

Patients' characteristics are summarized in Table 1. The median baseline HCV-RNA was 6.1 log<sub>10</sub>IU/mL. Of the 108 patients, 44.4% had a baseline HCV viral load lower than 600000 IU/mL (5.8 log<sub>10</sub>IU/mL) and 37.9% had mild or moderate fibrosis. All patients were infected with HCV genotype 1b. Only 38.9% (42 patients) achieved SVR, and modest percentages had prompt responses during therapy: 13% had RVR and 39.8% had EVR.

The favorable *IL28B* CC genotype was detected in only 17.6% of the patients, and had no significant

**Table 2** Patients characteristics related to core mutation type, univariate analysis

Characteristics	Patients with DW-type infection <i>n</i> = 13	Patients with R70Q/H and/or L91M substitutions <i>n</i> = 95	Patients with L91M substitution <i>n</i> = 40	Patients with R70Q/H substitution <i>n</i> = 8	<i>P</i> value <sup>1</sup>
Age (yr) median	51.5 (29.1-66.54)	53.6 (22.1-68.3)	53.3 (24.4-67.3)	50 (33.8-58.8)	a = 0.607 b = 0.694 c = 0.860
Gender Female (%)	7 (53.8)	57 (60)	22 (55)	5 (62.5)	a = 0.767 b = 1.000 c = 1.000
Residence Urban (%)	11 (84.6)	67 (70.5)	33 (82.5)	5 (62.5)	a = 0.509 b = 1.000 c = 0.325
Baseline ALT (mg/dL) median	94 (3-235)	76 (16-890)	78 (17-890)	67 (54-197)	a = 0.592 b = 0.849 c = 1.000
Baseline HCV-RNA (log <sub>10</sub> IU/mL), median	6.25 (4.15-6.9)	6.05 (4.15-7.3)	6.05 (4.1-7.3)	6.3 (5.2-7.1)	a = 0.966 b = 1.000 c = 0.414
Mild/moderate fibrosis (F1 + F2, %)	4 (57.1)	34 (58.6)	14 (58.3)	4 (57.14)	a = 0.881 b = 0.925 c = 0.283
IL28B CC (%)	6 (46.2)	13 (13.7)	8 (20.0)	1 (12.5)	a = 0.011 b = 0.080 c = 0.174
RVR (%)	6 (46.2)	8 (8.4)	4 (10.0)	0 (0.0)	a = 0.002 b = 0.009 c = 0.005
EVR (%)	10 (76.9)	33 (34.7)	15 (37.5)	3 (37.5)	a = 0.005 b = 0.024 c = 0.164
SVR (%)	10 (76.9)	32 (33.7)	15 (37.5)	2 (25.0)	a = 0.005 b = 0.024 c = 0.032

<sup>1</sup>*P* value symbols a, b, c: cases from each category type of viral substitutions (columns 3, 4, 5) vs cases without viral mutation [double wild-type (DW)-type - column 2]. HCV: Hepatitis C virus; SVR: Sustained virological response; EVR: Early virological response; RVR: Rapid virologic response.

correlation with patients' demographic characteristics (age, gender, urban residence; Table 1).

Patients with *IL28B* genotype CC had the highest therapeutic success rates than those with TT or CT genotypes for the following outcomes: RVR (36.8% vs 7.9%, OR = 6.8, *P* = 0.003); EVR (84.2% vs 30.3%, OR = 12.2, *P* < 0.001), and SVR (89.5% vs 28.1%, OR = 21.8, *P* < 0.001).

#### Impact of core mutations on the treatment response

According to the sequencing results, only 12% of the patients were infected with double wild-type (DW) strains - defined as presence of arginine (R) and leucine (L) at core position 70 and 91, respectively - while the rest had glutamine/histidine at position 70 (R70Q/H), or/and methionine at position 91 (L91M). Of the patients, R70Q/H substitution was present in viral isolates infecting 7.4%, and L91M was observed in 37%, while patients displaying both mutations represented 43.5% of the study population.

The presence of any substitutions at positions 70 and 91 of the core protein was associated with lower rates of RVR, EVR, and SVR (Table 2). Patients infected with DW-type strains obtained SVR more frequently than patients with R70Q/H substitution (76.9% vs 37.5%, OR = 5.6, *P* = 0.032), or L91M substitution

(76.9% vs 25.0%; OR = 12, *P* = 0.032). Furthermore, looking simultaneously at both mutation sites, patients infected with HCV DW-type strains obtained SVR significantly more frequently than patients with substitution at any 70 or 91 positions (76.9% vs 33.7%, *P* = 0.032; OR = 6.6, *P* = 0.005).

No significant differences related to demographic characteristics or virological parameters (HCV viremia, ALT, liver fibrosis) were detected between the patients infected with DW-type strains and those with R70Q/H and L91M substitutions (Table 2).

#### Predictive factors for treatment success

In the univariate analysis, young age, urban residence, *IL28B* CC genotype, absence of core mutations and achievement of RVR and EVR were significantly correlated with the rate of therapeutic success, defined as SVR (Table 3).

Direct logistic regression was performed to assess the impact of different viral and host factors on the likelihood of achieving SVR, and the overall model contained all predictors with statistically significance in the univariate analysis. In the multivariate analysis, young age (less than 50 years old), absence of any type of core mutations, and presence of *IL28B* CC genotype were independently associated with

**Table 3 Predictive factors for sustained virological response, univariate analysis *n* (%)**

Variable	SVR <i>n</i> = 42	No SVR <i>n</i> = 66	<i>P</i> value
Age (yr), median	48.1 (22.1-68.3)	54.7 (22.1-67.3)	< 0.001
Female gender	22 (52.4)	42 (63.6)	0.246
Urban residence (%)	37 (88.1)	41 (62.1)	0.004
Baseline ALT (mg/dL), median	79 (16-890)	75 (17-231)	0.688
Baseline HCV-RNA, median	6.2 (4.15-7.3)	6.2 (4.8; 7.3)	0.167
Mild/moderate fibrosis	18 (75.0)	20 (48.8)	0.107
IL28B type CC (%)	17 (40.5)	2 (3.0)	< 0.001
Absence of core mutations - DW	10 (23.8)	3 (4.5)	0.005
Presence of any core mutation, (%)	32 (76.2)	63 (95.5)	0.005
Viral mutation in core 91 only (%)	15 (60.0)	25 (84.3)	0.024
Viral mutation in core 70 only (%)	2 (16.7)	6 (66.7)	0.032
RVR (%)	13 (31.0)	1 (1.5)	< 0.001
EVR (%)	37 (88.1)	6 (9.1)	< 0.001

HCV: Hepatitis C virus; DW: Double wild-type; SVR: Sustained virological response; EVR: Early virological response; RVR: Rapid virologic response.

**Table 4 Multivariate analysis on independent predictive factors for sustained virological response**

Variable	Adjusted OR	95%CI	<i>P</i> value
Age (< 50 yr)	0.976	0.967-0.986	< 0.001
Absence of core mutations	4.710	1.10-21.30	0.04
IL28B polymorphism	19.200	4.05-91.40	< 0.001

achieving SVR (Table 4).

This multivariate model correctly classified 75.9% of cases with a positive predictive value of 80.7%.

## DISCUSSION

We report that the absence of substitutions in core positions 70 and 91 is a good predictor for achieving SVR after PEG-IFN/RBV treatment in Caucasian patients; together with *IL28B* polymorphisms and age, this absence can be used to stratify HCV-infected patients according to the likelihood of response to a currently suboptimal, but affordable, interferon-based therapy.

The success rate of IFN-based therapy was rather low in the present cohort, despite the fact that several baseline predictors suggested a promising patient profile (relatively young age (median: 53.5 years), low baseline HCV viral load in half of the cases, and minimal fibrosis in more than one-third of the cases). Nevertheless, the on-treatment viral kinetic response was modest, with a minority of patients achieving undetectable viral replication after 4 wk of therapy. An analysis of *IL28B* polymorphism rs12979860, the most important predictor of SVR in patients without RVR<sup>[18]</sup>, revealed that non-CC *IL28B* genotypes were predominant (82.4%). These variants are associated with endogenous activation of the innate immune responses, higher baseline expression levels of IFN-stimulated genes, and a constant activation of the IFN signaling pathway that renders patients unresponsive to IFN treatment<sup>[18,19]</sup>. The exclusive presence of subtype 1b and the low prevalence of the favorable

CC genotype can explain, at least in part, the low rate of virological response, but a series of others factors must be taken into consideration: questionable or inconsistent patient adherence to treatment, presence of adverse events that could determine temporary treatment interruptions (none were acknowledged by the study patients), and preexisting mutations in other genomic regions that could render the virus less susceptible to the prescribed drugs. In this study, core protein substitutions at positions 70 and 91 were present in viral isolates infecting 88% of the patients and linked with a significantly decreased probability of achieving SVR.

There has been increased evidence that substitutions at position 70 in the core protein are found regularly in Asian HCV 1b infected patients, with the mutant clone R70Q detectable even in newly infected people and no distinguishing characteristics for the mutant strain in terms of viral fitness or demographic distribution<sup>[20]</sup>. Studies investigating HCV core mutations in Caucasian patients are scarce, but two studies of very limited numbers of patients have suggested an association between R70 and an increased response to therapy<sup>[21,22]</sup>.

In our study, we detected high rates of core protein substitutions at positions 70 and 91 in 108 Caucasian patients infected with HCV subtype 1b; these substitutions had no significant association with viral load, but were significantly associated with a low therapeutic success rate. Our results are in accordance with previous reports that indicated an absence of substitutions at positions 70 and 91 in core protein as a significant predictor for the success of IFN-based therapy<sup>[12,21,22]</sup>. These results have also been confirmed *in vitro*; Funaoka *et al.*<sup>[23]</sup> evaluated the effect of interferon-alpha on HCV core mutants (R70Q/H and L91M) in terms of viral replication and response to treatment and found a significantly higher degree of IFN resistance compared to the wild-type virus, associated with decreased expression of the IFN-stimulated genes. The proposed hypothesis for the interferon resistance of core mutants was related to the inhibition of the interferon signaling pathway,

potentially involving SOCS3 (suppressor of cytokine signaling). These proteins are stimulated by various cytokines including IL6, which was upregulated in cells transfected with a core mutant. This mechanism can be observed *in vivo* as well, as chronically HCV infected patients have increased levels of inflammatory cytokines, including IL-6 and TNF-alpha<sup>[24]</sup>.

Another interesting mechanism that might explain the role of core substitutions in interferon resistance is their potential influence on the expression of minicore proteins- isotypes of the normal core protein, that lack an N-terminal segment<sup>[25]</sup>. Two important minicore proteins terminate in the vicinity of amino acids 70 and 91; consequently, any structural changes in these amino acids can alter the expression of minicore proteins and implicitly the HCV functioning and IFN sensitivity<sup>[11]</sup>.

To our knowledge, this is one of the first studies conducted on Caucasian patients that extend and confirm the results obtained in Asian populations related to the impact of amino acid substitution in the HCV core region on treatment response. Although our study does not involve a very large number of patients, further sampling is unlikely, as the clinical facilities investigated in this study treat subjects from all over the country. Performing mathematical modeling of the cost-effectiveness of sequencing for HCV core mutations would be beneficial. Although the cost of this test may be rather high, it is significantly lower than the prohibitive cost of DAAs. Several recent studies have attempted to provide an estimate on the cost-effectiveness of interferon-free regimens (assuming a price of \$100000 and a success rate of 90%). Their results support a delay in treatment for patients with mild degrees of fibrosis<sup>[26-28]</sup>. As such, the potentially beneficial role of core sequencing in selecting patients from this subclass who are responsive to interferon-based therapy may outweigh the potential price limitation. This is particularly true for at this time, when there is a constant need for ethical, evidence-based criteria for the prioritization of interferon-free treatment in countries that cannot yet afford the universal introduction of the new highly active antivirals. In addition, there have been reports that HCV core substitutions can also predict the primary outcomes of therapy using first generation protease inhibitors<sup>[29]</sup> and that the *IL28B* genotype is furthermore predictive of the response to triple therapy in patients infected with HCV genotype 1<sup>[30]</sup>. Consequently, future studies will be needed to extend our results to patients treated with the novel categories of antivirals recently introduced for the treatment of HCV chronic infection.

Moreover, recent studies have also indicated that HCV core substitutions are involved in the progression of chronic hepatitis C to hepatocellular carcinoma (HCC)<sup>[31]</sup>. The R70Q variant has been associated with an increased malignancy risk<sup>[32-34]</sup>, while the implication of core 91 substitution was dubitable<sup>[11]</sup>. A

very recent study using deep-sequencing reported that the presence of baseline HCV strains harboring more than 42% non-R70 quasispecies or more than 98.5% non-L91 mutants was associated with an increased HCC risk<sup>[35]</sup>. As long as the residual risk for HCC development in compensated cirrhotic patients with SVR after IFN treatment remains quite high (3.4% at 5 years and 23.7% at 20 years<sup>[36]</sup>), the impact of core mutations on the transforming capacity of HCV core protein is worth studying.

In conclusion, this study reports absence of core genomic mutations associated with IL28B CC polymorphism as prognostic markers for a favorable outcome in HCV chronically infected Caucasian patients treated with interferon-based regimens. Core genomic mutations can be used to tailor treatment and distinguish between those patients who can respond to the affordable bi-therapy and those who must be urgently treated with DAAs to prevent evolution towards end-stage liver disease or HCC.

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## COMMENTS

### Background

Interferon-free regimens, based on combinations of direct acting antivirals (DAAs) with pan-genotypic activity, have a remarkable efficacy and might transform hepatitis C into a curable chronic disease. Nevertheless, the high cost of DAAs still precludes the universal replacement of the classic PEGylated-interferon and ribavirin therapy (PEG-IFN/RBV) that is dependent on a series of host and viral factors. In Asian patients, a newly described viral factor influencing the outcome of bi-therapy treatment is represented by amino acid substitutions in hepatitis C virus (HCV) core protein positions 70 and 91.

### Research frontiers

There is very scarce information on the presence and significance of HCV core substitutions in Caucasian populations. Nevertheless, a number of studies have indicated that amino acid substitutions in the core protein play an important role in the very early dynamics of viral replication during bi-therapy and triple therapy of chronic hepatitis C as well as in the evolution toward hepatocellular carcinoma.

### Innovations and breakthroughs

This is one of the first studies confirming that HCV core substitutions are not specific to the Asian population, being also found in Caucasian patients. Moreover, it demonstrates that absence of core genomic mutations, together with young age and *IL28B* CC genotype, is a prognostic marker for favorable outcome in chronic HCV-infected Caucasian patients treated with PEG-IFN/RBV.

### Applications

The research hotspot is the identification of a new viral factor that can help distinguish between patients who can still benefit from the affordable IFN-based therapy from those who must be urgently treated with DAAs to prevent the evolution towards end-stage liver disease. This is an important practical instrument in countries with developing economies that cannot afford universal introduction of DAA because it can facilitate the prioritization of patients who will benefit from less expensive therapeutic regimens. Further application of these results can be derived from the recently reported role of core substitution in the progression of HCV infection to hepatocellular carcinoma. As long as a residual risk for HCC development persists even in patients successfully treated with interferon or with IFN-free regimens, the impact of core mutations on the transforming capacity of the HCV core protein is worth studying.

### Terminology

HCV core gene is a conserved part of the viral genome that is mostly used for HCV genotyping and classification. Nevertheless, the core region can antagonize the antiviral response induced by IFN, interacting with the IFN-activating and signaling pathways. Substitutions in less conserved sites (positions 70 and 91 in the core region) can contribute to resistance to interferon treatment.

### Peer-review

The manuscript is very well written and clearly states its aims and conclusions. It looks overall good with some limitations of low number of patients and low SVR. Questions were raised concerning the cost of core mutations testing. A paragraph responding to the study limitations and the necessity of core sequencing cost effectiveness evaluation was added by the authors in the revised version.

## REFERENCES

- Peter J, Nelson DR. Optimal interferon-free therapy in treatment-experienced chronic hepatitis C patients. *Liver Int* 2015; **35** Suppl 1: 65-70 [PMID: 25529089 DOI: 10.1111/liv.12718]
- Navaneethan U, Kemmer N, Neff GW. Predicting the probable outcome of treatment in HCV patients. *Therap Adv Gastroenterol* 2009; **2**: 287-302 [PMID: 21180557 DOI: 10.1177/1756283X09339079]
- Enomoto H, Nishiguchi S. Factors associated with the response to interferon-based antiviral therapies for chronic hepatitis C. *World J Hepatol* 2015; **7**: 2681-2687 [PMID: 26609345 DOI: 10.4254/wjh.v7.i26.2681]
- Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 12324553 DOI: 10.1056/NEJMoa020047]
- Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749 DOI: 10.1016/S0140-6736(01)06102-5]
- Fabris C, Falletti 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* 2011; **54**: 716-722 [PMID: 21146242 DOI: 10.1016/j.jhep.2010.07.019]
- Polyak SJ, McArdle S, Liu SL, Sullivan DG, Chung M, Hofgärtner WT, Carithers RL, McMahon BJ, Mullins JI, Corey L, Gretch DR. Evolution of hepatitis C virus quasispecies in hypervariable region 1 and the putative interferon sensitivity-determining region during interferon therapy and natural infection. *J Virol* 1998; **72**: 4288-4296 [PMID: 9557719]
- El-Shamy A, Shoji I, Saito T, Watanabe H, Ide YH, Deng L, Kawata S, Hotta H. Sequence heterogeneity of NS5A and core proteins of hepatitis C virus and virological responses to pegylated-interferon/ribavirin combination therapy. *Microbiol Immunol* 2011; **55**: 418-426 [PMID: 21371092 DOI: 10.1111/j.1348-0421.2011.00331.x]
- Blindbacher A, Duong FH, Hunziker L, Stutvoet ST, Wang X, Terracciano L, Moradpour D, Blum HE, Alonzi T, Tripodi M, La Monica N, Heim MH. Expression of hepatitis c virus proteins inhibits interferon alpha signaling in the liver of transgenic mice. *Gastroenterology* 2003; **124**: 1465-1475 [PMID: 12730885]
- Heim MH, Moradpour D, Blum HE. Expression of hepatitis C virus proteins inhibits signal transduction through the Jak-STAT pathway. *J Virol* 1999; **73**: 8469-8475 [PMID: 10482599]
- El-Shamy A, Hotta H. Impact of hepatitis C virus heterogeneity on interferon sensitivity: an overview. *World J Gastroenterol* 2014; **20**: 7555-7569 [PMID: 24976696 DOI: 10.3748/wjg.v20.i24.7555]
- Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 2007; **46**: 403-410 [PMID: 17126448 DOI: 10.1016/j.jhep.2006.09.019]
- Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, Tanaka E, Onji M, Toyota J, Chayama K, Yoshioka K, Izumi N, Akuta N, Kumada H. Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study. *J Gastroenterol* 2009; **44**: 952-963 [PMID: 19517057 DOI: 10.1007/s00535-009-0087-x]
- Dinu S, Calistru PI, Ceaușu E, Târdeil G, Oprisan G. Screening of protease inhibitors resistance mutations in hepatitis c virus isolates infecting romanian patients unexposed to triple therapy. *Roum Arch Microbiol Immunol* 2015; **74**: 7-17 [PMID: 26727849]
- Sultana C, Oprisan G, Szmal C, Vagu C, Temereanca A, Dinu S, Teleman MD, Ruta S. Molecular epidemiology of hepatitis C virus strains from Romania. *J Gastrointest Liver Dis* 2011; **20**: 261-266 [PMID: 21961093]
- Hall T. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 1999; **41**: 95-98. Available from: URL: <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>
- Castéra L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Ledinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350 [PMID: 15685546 DOI: 10.1053/j.gastro.2004.11.018]
- Hayes CN, Imamura M, Aikata H, Chayama K. Genetics of IL28B and HCV--response to infection and treatment. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 406-417 [PMID: 22641049 DOI: 10.1038/nrgastro.2012.101]
- Feld JJ, Hoofnagle JH. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. *Nature* 2005; **436**: 967-972 [PMID: 16107837 DOI: 10.1038/nature04082]
- Furui Y, Hoshi Y, Murata K, Ito K, Suzuki K, Uchida S, Satake M, Mizokami M, Tadokoro K. Prevalence of amino acid mutation in hepatitis C virus core region among Japanese volunteer blood donors. *J Med Virol* 2011; **83**: 1924-1929 [PMID: 21915867 DOI: 10.1002/jmv.22216]
- Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, Belle SH, Di Bisceglie AM, Aurora R, Tavis JE. Pretreatment sequence diversity differences in the full-length hepatitis C virus

- open reading frame correlate with early response to therapy. *J Virol* 2007; **81**: 8211-8224 [PMID: 17522222 DOI: 10.1128/JVI.00487-07]
- 22 **Alestig E**, Arnholm B, Eilard A, Lagging M, Nilsson S, Norkrans G, Wahlberg T, Wejstål R, Westin J, Lindh M. Core mutations, IL28B polymorphisms and response to peginterferon/ribavirin treatment in Swedish patients with hepatitis C virus genotype 1 infection. *BMC Infect Dis* 2011; **11**: 124 [PMID: 21569441 DOI: 10.1186/1471-2334-11-124]
  - 23 **Funaoka Y**, Sakamoto N, Suda G, Itsui Y, Nakagawa M, Kakinuma S, Watanabe T, Mishima K, Ueyama M, Onozuka I, Nitta S, Kitazume A, Kiyohashi K, Murakawa M, Azuma S, Tsuchiya K, Watanabe M. Analysis of interferon signaling by infectious hepatitis C virus clones with substitutions of core amino acids 70 and 91. *J Virol* 2011; **85**: 5986-5994 [PMID: 21490101 DOI: 10.1128/JVI.02583-10]
  - 24 **Sekiguchi S**, Kimura K, Chiyo T, Ohtsuki T, Tobita Y, Tokunaga Y, Yasui F, Tsukiyama-Kohara K, Wakita T, Tanaka T, Miyasaka M, Mizuno K, Hayashi Y, Hishima T, Matsushima K, Kohara M. Immunization with a recombinant vaccinia virus that encodes nonstructural proteins of the hepatitis C virus suppresses viral protein levels in mouse liver. *PLoS One* 2012; **7**: e51656 [PMID: 23284733 DOI: 10.1371/journal.pone.0051656]
  - 25 **Eng FJ**, Walewski JL, Klepper AL, Fishman SL, Desai SM, McMullan LK, Evans MJ, Rice CM, Branch AD. Internal initiation stimulates production of p8 minicore, a member of a newly discovered family of hepatitis C virus core protein isoforms. *J Virol* 2009; **83**: 3104-3114 [PMID: 19129450 DOI: 10.1128/JVI.01679-08]
  - 26 **Chan K**, Lai MN, Groessl EJ, Hanchate AD, Wong JB, Clark JA, Asch SM, Gifford AL, Ho SB. Cost effectiveness of direct-acting antiviral therapy for treatment-naive patients with chronic HCV genotype 1 infection in the veterans health administration. *Clin Gastroenterol Hepatol* 2013; **11**: 1503-1510 [PMID: 23707354 DOI: 10.7326/M14-1152]
  - 27 **Deuffic-Burban S**, Schwarzwinger M, Obach D, Mallet V, Pol S, Pageaux GP, Canva V, Deltenre P, Roudot-Thoraval F, Larrey D, Dhumeaux D, Mathurin P, Yazdanpanah Y. Should we await IFN-free regimens to treat HCV genotype 1 treatment-naive patients? A cost-effectiveness analysis (ANRS 95141). *J Hepatol* 2014; **61**: 7-14 [PMID: 24650691 DOI: 10.1016/S0168-8278(14)60087-8]
  - 28 **Leidner AJ**, Chesson HW, Xu F, Ward JW, Spradling PR, Holmberg SD. Cost-effectiveness of hepatitis C treatment for patients in early stages of liver disease. *Hepatology* 2015; **61**: 1860-1869 [PMID: 25677072 DOI: 10.1002/hep.27736]
  - 29 **Akuta N**, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Chayama K, Nakamura Y, Kumada H. Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. *Hepatology* 2010; **52**: 421-429 [PMID: 20648473 DOI: 10.1002/hep.23690]
  - 30 **Chayama K**, Hayes CN, Abe H, Miki D, Ochi H, Karino Y, Toyota J, Nakamura Y, Kamatani N, Sezaki H, Kobayashi M, Akuta N, Suzuki F, Kumada H. IL28B but not ITPA polymorphism is predictive of response to pegylated interferon, ribavirin, and telaprevir triple therapy in patients with genotype 1 hepatitis C. *J Infect Dis* 2011; **204**: 84-93 [PMID: 21628662 DOI: 10.1093/infdis/jir210]
  - 31 **Akuta N**, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. *Hepatology* 2007; **46**: 1357-1364 [PMID: 17657816 DOI: 10.1002/hep.21836]
  - 32 **Kobayashi M**, Akuta N, Suzuki F, Hosaka T, Sezaki H, Kobayashi M, Suzuki Y, Arase Y, Ikeda K, Watahiki S, Mineta R, Iwasaki S, Miyakawa Y, Kumada H. Influence of amino-acid polymorphism in the core protein on progression of liver disease in patients infected with hepatitis C virus genotype 1b. *J Med Virol* 2010; **82**: 41-48 [PMID: 19950230 DOI: 10.1002/jmv.21629]
  - 33 **Miura M**, Maekawa S, Kadokura M, Sueki R, Komase K, Shindo H, Ohmori T, Kanayama A, Shindo K, Amemiya F, Nakayama Y, Kitamura T, Uetake T, Inoue T, Sakamoto M, Okada S, Enomoto N. Analysis of viral amino acids sequences and the IL28B SNP influencing the development of hepatocellular carcinoma in chronic hepatitis C. *Hepatol Int* 2012; **6**: 386-396 [PMID: 22020823 DOI: 10.1007/s12072-011-9307-6]
  - 34 **Araujo OC**, Barros JJ, do Ó KM, Nabuco LC, Luz CA, Perez RM, Niel C, Villela-Nogueira CA, Araujo NM. Genetic variability of hepatitis B and C viruses in Brazilian patients with and without hepatocellular carcinoma. *J Med Virol* 2014; **86**: 217-223 [PMID: 24338810 DOI: 10.1002/jmv.23837]
  - 35 **El-Shamy A**, Pendleton M, Eng FJ, Doyle EH, Bashir A, Branch AD. Impact of HCV core gene quasispecies on hepatocellular carcinoma risk among HALT-C trial patients. *Sci Rep* 2016; **6**: 27025 [PMID: 27246310 DOI: 10.1038/srep27025]
  - 36 **Bruno S**, Di Marco V, Iavarone M, Roffi L, Crosignani A, Calvaruso V, Aghemo A, Cabibbo G, Viganò M, Boccaccio V, Craxi A, Colombo M, Maisonneuve P. Survival of patients with HCV cirrhosis and sustained virologic response is similar to the general population. *J Hepatol* 2016; **64**: 1217-1223 [PMID: 27059129 DOI: 10.1016/j.jhep.2016.01.034]

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