ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Clinical importance of pharmacogenetics in the treatment of hepatitis C virus infection

ADINA MARIA KAMAL[1)], PAUL MITRUŢ[1)], KAMAL CONSTANTIN KAMAL[2)], OANA-SORINA TICA[3)], MIHAELA NICULESCU[4)], DRAGOŞ OVIDIU ALEXANDRU[5)], ANDREI-ADRIAN TICA[6)]

[1)]*Department of Internal Medicine, University of Medicine and Pharmacy of Craiova, Romania*
[2)]*Department of Family Medicine, University of Medicine and Pharmacy of Craiova, Romania*
[3)]*Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania*
[4)]*Department of Anatomy, University of Medicine and Pharmacy of Craiova, Romania*
[5)]*Department of Medical Informatics, University of Medicine and Pharmacy of Craiova, Romania*
[6)]*Department of Pharmacology, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

Globally, over 4% of the world population is affected by hepatitis C virus (HCV) infection. The current standard of care for hepatitis C infection is combination therapy with pegylated interferon and ribavirin for 48 weeks, which yield a sustained virological response in only a little over half of the patients with genotype 1 HCV. We investigated the clinical importance of pharmacogenetics in treatment efficacy and prediction of hematotoxicity. A total of 148 patients infected with HCV were enrolled. All patients were treated for a period of 48 weeks or less with pegylated interferon and ribavirin. Four genotypes were investigated: inosine triphosphatase (ITPA) rs1127354, C20orf194 rs6051702, interferon lambda (IFNL)3 rs8099917, IFNL3/4 rs12979860 in the population from southwestern Romania. Genetic variants for rs129798660 and rs6051702 proved once more to represent an indisputable clinical tool for predicting sustained virological response (SVR) (69.23%, *chi*-square p=0.007846, p<0.05 and 63.29%, *chi*-square p=0.007846, p<0.05, respectively). ITPA genetic variants protect against ribavirin-induced hemolytic anemia and C20orf194 also proved to be protective against thrombocytopenia. These clinical findings strengthen the belief that pharmacogenetics should play a constant role in treatment decisions for patients infected with hepatitis C virus.

Keywords: hepatitis C virus, inosine triphosphatase, single nucleotide polymorphism, ribavirin, interleukin 28B.

Introduction

Hepatitis C virus infection represents a major health problem worldwide, affecting over 180 million people [1]. Since the first discovery of hepatitis C virus, over 20 years ago, two scientific breakthroughs have taken the medical society closer to eradication of HCV infection. The first breakthrough was the discovery of the single nucleotide polymorphism (SNP) near the interleukin 28B (IL28B) gene, strongly associated with HCV clearance, spontaneous or on treatment [2]. The second breakthrough resides in the discovery of interferon (IFN)-free direct antiviral agents, which yield a sustained virological response in up to 90–95% of the cases, depending on HCV genotype [3].

In most countries, the standard of care remains the combined therapy with pegylated interferon (PegIFN) and ribavirin (RBV) for 24–48 weeks [4, 5]. The most important drawback of combination therapy is the development of adverse events, amongst which hematological side effects influence the treatment outcome the most. Ribavirin-induced hemolytic anemia, forces the reduction of start doses, thus diminishing the possibility to obtain a sustained virological response, defined as undetectable viral load 24 weeks after completion of treatment. Using genome-wide association studies, there have been identified a number of single nucleotide polymorphisms located on chromosome 20p13 on the inosine triphosphatase gene, encoding the inosine triphosphate pyrophosphohydrolase, a protein that hydrolyses inosine triphosphate to the monophosphate derivate [6]. Reports show that SNP of ITPA influence ribavirin-induced hemolytic anemia [7]. Some of ITPA gene variants have been reported to protect against ribavirin-induced hemolytic anemia in patients treated for chronic hepatitis C.

The purpose of this paper is to evaluate, in the population in southwest of Romania, the factors related to the host associated with response to treatment with IFN and RBV for HCV infection. Genetic factors have a huge impact in producing great individual variation in terms of drug efficacy. Thus, arose the concept of pharmacogenetics, defined as the study of individual variations (polymorphisms) of specific genes on the action and effects of drugs. We aim through this paper, to bring more into attention the concept of pharmacogenetics and pharmacogenomics.

Development of pharmacogenomics could allow in the future – using appropriate tests, predictive therapeutic response – drug selection, determining the optimal dose and avoiding adverse reactions or, in short, personalize medication.

Patients, Materials and Methods

Patients

Between January 2012 and August 2015, blood samples

were collected from 148 patients with HCV infection admitted in the II[nd] Department of Internal Medicine, Emergency County Hospital, Craiova, or from "Renaşterea" Medical Centre, Craiova, Romania. All patients signed the informed consent prior to study initiation. Study protocol was designed in according to the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Review Committee of the University of Medicine and Pharmacy of Craiova.

The patients included, tested positive for HCV infection, had compensated liver disease and were willing to participate in the study. Patients with HIV/HBV (human immunodeficiency virus/hepatitis B virus) co-infection were excluded, as well as patients with alcoholic liver disease, decompensated liver cirrhosis, pulmonary tuberculosis, or patients that were/are in treatment for any malignant tumor. All patients were treated with pegylated interferon alpha-2a or -2b and ribavirin 800/1000/1200 mg, over a period of 48 weeks or less.

We monitored the patients for a period of 18 months, medical examinations being performed at 4, 12, 24 and 48 weeks from the start of the treatment and at 24 weeks after completion of therapy. At each visit, a complete blood count was performed and the patient was handed a questionnaire to record subjective adverse effects that occurred during treatment.

The collected blood samples were stored in an ultra-freezer, at -86^{0}C, until the time of analysis.

Genotyping

Four genotypes were analyzed in order to investigate the clinical importance of pharmacogenetics in the treatment for HCV infection.

Genotyping was performed with TaqMan Pre-designed SNP Genotyping Assay, small scale, human, using the real-time polymerase chain reaction (RT-PCR) technique on the Corbett Rotor-Gene 6200 RPM platform with interchangeable blocks ViiA™ 7 Applied Biosystems and automatic pipetting Thermocycler Eppendorf epMotion 5070 Mastercycler. Sequencing was performed using CEQ™ 8000 Genetic Beckman Coulter sequence analysis system.

Statistical analysis

All data collected was stored in Microsoft Excel files (Microsoft Co., Redmond, WA, USA) and data processing was performed using ChiTest, Pivot Tables, functions chart analysis; fundamental statistical parameters, mean and standard deviation and coefficient of variation were calculated.

Results

We included, in this study, 148 patients diagnosed with chronic HCV infection. Baseline features of the study population are presented in Table 1.

Table 1 – ***Baseline characteristics of study group***

Characteristics	Total	ITPA gene		C20orf194 gene	
		CC	*AC+CT*	*AA*	*AC+CC*
No. of patients (%)	148 (100%)	134 (90.54%)	14 (9.46%)	102 (68.92%)	46 (31.08%)
Gender – female / male ratio (%)	103 / 45 (69.59% / 30.41%)	89 / 45 (66.42% / 33.58%)	14 / 0 (100% / 0%)	71 / 31 (69.61% / 30.39%)	32 / 14 (69.57% / 30.43%)
Age mean±SD [years]	53.12±10.85	52.95±10.62	54.79±13.17	52.35±10.50	54.83±11.50
No. of patients (%) with anemia	87 (58.78%)	83 (61.94%)	4 (28.57%)	67 (65.69%)	20 (43.48%)
Baseline Hb concentration [g/dL]	13.44±0.88	13.45±0.89	13.32±0.86	13.46±0.85	13.39±0.95
No. of patients (%) with baseline Hb concentration <13 g/dL	50 (33.78%)	46 (34.33%)	4 (28.57%)	34 (33.33%)	16 (34.78%)
Baseline platelet count [10^4/mm^3]	158 557.43± 60 280.17	155 332.09± 59 208.18	189 428.57± 63 953.97	165 318.63± 57 906.92	143 565.22± 63 329.35
Baseline WBC count [10^3/mm^3]	3934.32± 1571.89	3994.18± 1584.81	3361.43± 1361.36	4038.43± 1542.44	3703.48± 1628.68
HCV RNA load [log_{10} IU/mL]	6.35±6.84	6.39±6.86	5.72±5.59	6.41±6.91	6.18±6.38
No. of patients (%) with indicated genotypes of gene: IL28B CC CT TT					
CC	52 (35.14%)	48 (35.82%)	4 (28.57%)	41 (40.20%)	11 (23.91%)
CT	75 (50.68%)	65 (48.51%)	10 (71.43%)	50 (49.02%)	25 (54.35%)
TT	21 (14.19%)	21 (15.67%)	0 (0.00%)	11 (10.78%)	10 (21.74%)
No. of patients (%) with indicated genotypes of gene: IFNL3 rs8099917					
GG	12 (8.11%)	12 (8.96%)	0 (0.00%)	6 (5.88%)	6 (13.04%)
GT	57 (38.51%)	50 (37.31%)	7 (50.00%)	38 (37.25%)	19 (41.30%)
TT	79 (53.38%)	72 (53.73%)	7 (50.00%)	58 (56.86%)	21 (45.65%)
Fibrosis F1/F2/F3/F4	5/53/58/32	3/48/52/31	2/5/6/1	2/38/39/23	3/15/19/9
Obtained SVR	81 (54.73%)	71 (52.99%)	10 (71.43%)	62 (60.78%)	19 (41.30%)
No. of cases (%) with ribavirin dose reduction	43 (29.05%)	41 (30.60%)	2 (14.29%)	29 (28.43%)	14 (30.43%)

ITPA: Inosine triphosphatase; C20orf194: Chromosome 20 open reading frame 194; SD: Standard deviation; Hb: Hemoglobin; WBC: White blood cell; HCV: Hepatitis C virus; RNA: Ribonucleic acid; IL28B: Interleukin 28B; IFNL: Interferon lambda; F1: Minimal fibrosis; F2: Fibrosis occurred and spread inside the areas of the liver including blood vessels; F3: Fibrosis spread and connected to other liver areas that contain fibrosis; F4: Cirrhosis or advance liver fibrosis; SVR: Sustained virological response.

Out of the total 148 patients included in the study, 81 (54.27%) obtained SVR, while 67 (45.27%) patients did not. Of the total 67 patients that did not obtain SVR, nine (13.43%) patients were relapsers, one (1.49%) patient was interrupted for tuberculosis occurrence, two (2.98%) patients were interrupted due to decompensated liver cirrhosis and 55 (82.08%) patients did not have undetectable viral load 24 weeks after completion of therapy.

First, we genotyped interferon lambda 3 rs8099917, IFNL3/4 rs12979860, inosine triphosphatase rs1127354 and chromosome 20 open reading frame 194 (C20orf194) rs6051702 in all 148 patients included. Fifty-two and 78 patients presented the major and minor allele, respectively for, rs12979860. Seventy-nine and 69 patients presented the major and minor allele, respectively, for rs8099917.

Achieving the sustained virological response according to IFNL3 rs8099917, IFNL3/4 rs12979860

We investigated whether any of the genotypes could predict SVR in the given population as it has previously been reported. For IFNL3/4 rs12979860 interleukin 28B (IL28B), CC genotype proved to have the highest rate for predicting SVR as 36 (69.23%, *chi*-square p=0.007846, p<0.05) of the patients had undetectable viremia at the end of treatment. Response rates according to IL28B genotype are presented in the graphic below (Figure 1).

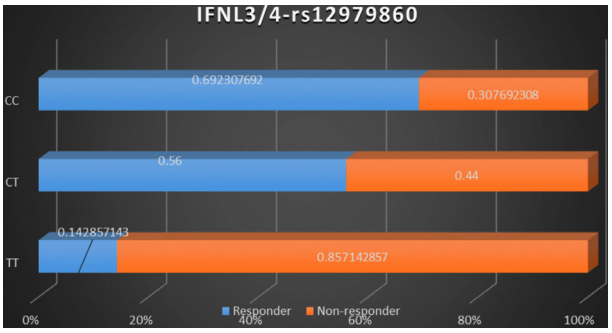

Figure 1 – ***Response rates according to IL28B genotype.***

Next, we investigated the correlations of rs8099917 genotypes to the rates of response to treatment for chronic HCV infection. The major allele proved to impact de rates of response the most, as patients with TT genotype achieved sustained virological response in 63.29% of the cases (*chi*-square p=0.007846, p<0.05) (Figure 2).

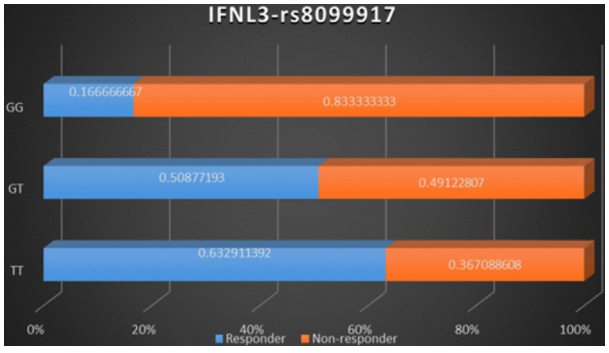

Figure 2 – ***Response rates according to rs8099917 polymorphisms.***

As previously studied, we confirmed the findings in our study cohort, thus strengthened the belief that CC and TT genotypes are able to predict acquirement of SVR especially when used together rather than separate. The rs8099917 SNP was associated with higher rates of thrombocytes drop, although in our study, no such correspondence could be established. There was evidence that major allele could be associated with higher rates of neutrophil reduction under treatment (data not shown).

Hemoglobin drop >2.5 g/dL during treatment with pegylated interferon and ribavirin

Next, we investigated all factors connected to a drop in hemoglobin (Hb) levels of over 2.5 g/dL from baseline, at week 4 of treatment and the occurrence of anemia in the studied population.

In our cohort, 48 (32.43%) patients experienced a drop in Hb levels of over 2.5 g/dL at week 4. There was a strong statistical correlation between ribavirin doses and the drop in Hb (p=0.003). Moreover, the reduction of ribavirin doses during treatment, correlated more strongly with the drop in Hb. No further connections could be established in relation to the investigated parameter.

Anemia occurred in 81 (58.78%) of the patients with no evident connection to age, gender, body mass index (BMI) or baseline Hb levels. However, anemia was strongly connected to ribavirin doses as expected, and the connection between anemia and ribavirin dose reduction was statistically significant (p=0.0001). There was no statistical correlation between ribavirin dose reduction and SVR in our study as ribavirin doses did not differ considerably from patients who achieved SVR and patients who did not.

ITPA rs1127354 variants protect against Hb drop over 2.5 g/dL at week 4 and against anemia throughout the course of treatment for HCV

We investigated the association between ITPA genetic variants and Hb drop over 2.5 g/dL from baseline at week 4. Minor allele proved to be protective against ribavirin-induced hemolytic anemia. CC genotype correlated significantly to anemia throughout the course of treatment – 83 (64.94%) patients (Figure 3).

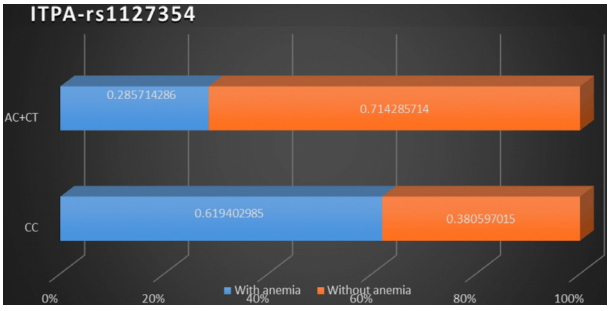

Figure 3 – ***ITPA correlation with ribavirin-induced anemia over the course of treatment.***

We also tested whether SVR could be correlated to any of ITPA genetic variants (*chi*-square p=0.392233) but we could not prove there is a statistically significant difference between CC genotype and the other genotypes in regards to responder status (p>0.05).

No other correspondence could be established between ITPA variants and leucopenia, thrombocytopenia or neutropenia.

C20orf194 rs6051702 variants – complex findings

The association between rs6051702 variants and hematotoxicity was further investigated. The homozygous individuals with AA major allele (67 patients, 65.69%, *chi*-square p=0.011074, p>0.05) were more prone to develop anemia throughout the course of treatment with pegylated interferon and ribavirin, than AC/CC genotypes (20 patients, 43.48%).

No association was found between C20orf194 variants and Hb drop >2.5 g/dL from baseline at week 4, nor with neutropenia and reduction of white blood cell (WBC) count. Unlike ITPA variants, rs6051702 variant AA proved to be protective against thrombocytopenia, statistical analysis bringing evidence that AC/CC genotypes are more prone to develop thrombocytopenia (28 patients, 60.87%, *chi*-square p=0.014425, p<0.05).

Unlike previous reports our data sustain the hypothesis that there is a difference between AA genotype and the other genotypes in regards to responder status (62 patients, 60.78%, *chi*-square p=0.027558, p<0.05). Detailed data regarding responder status is listed in Table 2.

Table 2 – ***Correlations between all four SNP and responder status***

	Genotype	Responder	Non-responder	*Chi*-square *p*
IFNL3 rs8099917	GG	2 (16.67%)	10 (83.33%)	0.007846 (significant)
	GT	29 (50.88%)	28 (49.12%)	
	TT	50 (63.29%)	29 (36.71%)	
IFNL3/4 rs12979860	CC	36 (69.23%)	16 (30.77%)	0.000105 (highly significant)
	CT	42 (56.00%)	33 (44.00%)	
	TT	3 (14.29%)	18 (85.71%)	
ITPA rs1127354	AC+CT	10 (71.43%)	4 (28.57%)	0.392233 (non-significant)
	CC	71 (52.99%)	63 (47.01%)	
C20orf194 rs6051702	AA	62 (60.78%)	40 (39.22%)	0.027558 (significant)
	AC+CC	19 (41.30%)	27 (58.70%)	

SNP: Single nucleotide polymorphism; IFNL: Interferon lambda; ITPA: Inosine triphosphatase; C20orf194: Chromosome 20 open reading frame 194.

Discussion

Combined therapy with PegIFN and RBV is known to be associated with a number of treatment-limiting adverse events. As previously reported in several studies, anemia is one of the most important adverse events known to lead to dose reductions therefore failure to achieve SVR and withdrawal of therapy [8, 9].

Hemolytic anemia induced by ribavirin has been explained by the oxidative stress induced to erythrocytes by triphosphate [10, 11]. The ITPA gene encodes inosine triphosphatase. Mutations in the ITPA gene lead to inosine triphosphatase deficiency, a benign enzymopathy resulting in accumulation of inosine triphosphatase in the erythrocytes thus increasing hematotoxicity [12, 13]. Variations in the gene (rs1127354) lead to ITPase deficiency and thus protects against hemolytic anemia that occurs in the early stages of treatment, in patients infected with HCV [7].

In the present study, we also observed that rs1127354 minor allele protect against hemolytic anemia, and found that CC major allele can predict anemia in the first four weeks of treatment and also throughout the course of therapy. Although ribavirin dose reduction was necessary in our study, we could not correlate the lack of SVR with necessity of dose modification, and also ITPA single nucleotide polymorphisms could not correlate in any way with treatment outcome. Previous reports show that the effect of ribavirin dose reduction is not as important on treatment outcome as long as RBV treatment is not discontinued, which is reconfirmed in our study [14, 15].

The rs6051702 SNP showed considerable linkage with hemoglobin reduction in the first weeks of treatment [7]. We could not find similar correlations in our cohort, but major allele AA individuals were more prone to developing anemia over the course of treatment but not but not necessarily in the first weeks of therapy. We again confirmed the utility of these genetic markers in predicting anemia, in patients treated for HCV infection with combination therapy. Moreover, we observed in our population a high number of patients who were protected for thrombocytopenia being predominantly homozygous for the major allele AA. For the rs1127354 SNP, no such connection could be demonstrated as it has previously been shown [16]. Further studies are needed to investigate whether this information can be used in the future as a predictor for thrombocytopenia in patients undergoing HCV treatment. To our knowledge, it is the first time C20orf194 SNP variants were ever correlated with sustained virological response. We cannot find an explanation for why this occurred. A hypothesis was that patients who developed anemia are more likely to obtain SVR, although, as a separate hypothesis, this could not be demonstrated. Intensive statistical analysis is needed further, in order to determine a link between these two variables.

Since PegIFN and RBV treatment is so burdened by adverse effects, the need to involve some predictors of response, has increased considerably. This has motivated many studies to identify factors related to virus/host involved in a higher response rate to treatment. Patients with genotype 1 HCV infection, can be included in a "difficult to treat" category of patients, having a low rate of SVR. The issue is of high interest in Romania, given that a national multicenter study showed that genotype 1b is almost exclusively present in patients with chronic HCV infection [17].

In 2009, a correlated set of polymorphisms near the gene IL28B were associated with clearance of HCV genotype 1, in patients undergoing treatment with IFN and RBV. The same genotypes were later associated with a spontaneous HCV clearance in untreated patients [2, 18–20]. The single nucleotide polymorphism was located on chromosome 19, 3 kb upstream of the IL28B gene that encodes IFNL3 [21–23]. Interferon lambda has antiviral activity, inhibiting HCV replication, through stimulation of expression of the ISGs (interferon-stimulated genes) and increasing immunity of natural killer (NK) cells and cytotoxicity of antigen-specific CD8+ cell [24–26].

The way IL28B SNPs exerts its antiviral activity, remains questionable. Studies on IFNL expression in peripheral blood mononuclear cells are inconclusive. Most research, points out that the liver tissue mRNA expression

of IL28B is different depending on the genotype IL28B, but there is a different expression of ISG [27].

The second polymorphism, rs8099917, associated with SVR was described in three studies [2, 18, 19]. The polymorphism rs8099917 is located 7.5 kb upstream of the start codon of IL28B, in the intergenomic region of IL28A and IL28B. In these studies, the T-allele was favorable and allele G was unfavorable, yielding three possibilities: homozygosity T/T and G/G and heterozygosity G/T.

In our study, the same correspondence was maintained, as 69.23% of the patients that successfully completed treatment were CC genotype carriers for IL28B gene, and 63.29% of the responders were TT genotype carriers for rs8099917 SNP.

The link between IL28B gene and HCV clearance may influence the decision to initiate antiviral therapy, design and interpretation of clinical trials, treatment economy and the regulatory approval process for new anti-HCV therapeutic agents.

So far, in our country there is no similar study on all four-gene polymorphisms, in terms of response to treatment with IFN and RBV, and impact on hematotoxicity. Our paper tried to draw attention to the importance of determining genetic variations, in order to pinpoint genetic therapy in the future.

Conclusions

We confirmed through our study, that both SNP variants for rs12979860 and rs8099917 are important genetic predictors for sustained virological response. Although they do not provide strong, categorical evidence that certain genotypes are associated with attainment of SVR, they can successfully be utilized as clinical tools in choosing the appropriate treatment. Genetic polymorphisms of ITPA gene were associated with higher rates of Hb level drop at week 4 and throughout the course of therapy. Some variants proved to be protective against ribavirin-induced hemolytic anemia. Similar findings were available for C20orf194 gene polymorphisms. In addition, rs6051702 genotyping showed that AA allele carriers achieved SVR in higher proportions and protected against thrombocytopenia. Since our study was conducted on a small number of cases and all patients included in the study were Romanian, we cannot consider these findings as representative, but extended studies on different ethnicities and on larger cohorts are needed in order to confirm whether this correlation is reliable. There is a strong clinical utility of rs1127354 and rs6051702 polymorphisms. The genetic variants can predict hemolytic anemia in HCV infected patients treated with pegylated interferon and ribavirin. These genetic variants can modulate treatment response as well as treatment methods. Using a combination of these genotypes could lead to a safe and effective treatment for patients with chronic hepatitis C.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007–2013, Project No. POSDRU/159/1.5/S/136893.

References

[1] Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis, 2005, 5(9):558–567.

[2] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet, 2009, 41(10):1105–1109.

[3] Schinazi R, Halfon P, Marcellin P, Asselah T. HCV direct-acting antiviral agents: the best interferon-free combinations. Liver Int, 2014, 34(Suppl 1):69–78.

[4] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, 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(13):975–982.

[5] ***. Correspondence: Peginterferon alfa-2b or alfa-2a with ribavirin for hepatitis C. N Engl J Med, 2009, 361(18):1808–1809.

[6] Fellay J, Thompson AJ, Ge D, Gumbs CE, Urban TJ, Shianna KV, Little LD, Qiu P, Bertelsen AH, Watson M, Warner A, Muir AJ, Brass C, Albrecht J, Sulkowski M, McHutchison JG, Goldstein DB. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. Nature, 2010, 464(7287):405–408.

[7] Thompson AJ, Fellay J, Patel K, Tillmann HL, Naggie S, Ge D, Urban TJ, Shianna KV, Muir AJ, Fried MW, Afdhal NH, Goldstein DB, McHutchison JG. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. Gastroenterology, 2010, 139(4):1181–1189.

[8] Takaki S, Tsubota A, Hosaka T, Akuta N, Someya T, Kobayashi M, Suzuki F, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kumada H. Factors contributing to ribavirin dose reduction due to anemia during interferon alfa2b and ribavirin combination therapy for chronic hepatitis C. J Gastroenterol, 2004, 39(7):668–673.

[9] Gatselis NK, Zachou K, Saitis A, Samara M, Dalekos GN. Individualization of chronic hepatitis C treatment according to the host characteristics. World J Gastroenterol, 2014, 20(11):2839–2853.

[10] De Franceschi L, Fattovich G, Turrini F, Ayi K, Brugnara C, Manzato F, Noventa F, Stanzial AM, Solero P, Corrocher R. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. Hepatology, 2000, 31(4):997–1004.

[11] Russmann S, Grattagliano I, Portincasa P, Palmieri VO, Palasciano G. Ribavirin-induced anemia: mechanisms, risk factors and related targets for future research. Curr Med Chem, 2006, 13(27):3351–3357.

[12] Bierau J, Lindhout M, Bakker JA. Pharmacogenetic significance of inosine triphosphatase. Pharmacogenomics, 2007, 8(9):1221–1228.

[13] Stocco G, Cheok MH, Crews KR, Dervieux T, French D, Pei D, Yang W, Cheng C, Pui CH, Relling MV, Evans WE. Genetic polymorphism of inosine triphosphate pyrophosphatase is a determinant of mercaptopurine metabolism and toxicity during treatment for acute lymphoblastic leukemia. Clin Pharmacol Ther, 2009, 85(2):164–172.

[14] Reddy KR, Shiffman ML, Morgan TR, Zeuzem S, Hadziyannis S, Hamzeh FM, Wright TL, Fried M. Impact of ribavirin dose reductions in hepatitis C virus genotype 1 patients completing peginterferon alfa-2a/ribavirin treatment. Clin Gastroenterol Hepatol, 2007, 5(1):124–129.

[15] Shiffman ML, Ghany MG, Morgan TR, Wright EC, Everson GT, Lindsay KL, Lok AS, Bonkovsky HL, Di Bisceglie AM, Lee WM, Dienstag JL, Gretch DR. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. Gastroenterology, 2007, 132(1):103–112.

[16] Hwang JJ, Lo CC, Lin CH, Cheng HS, Hung IW, Tsai WJ, Hung CH. Association between IPTA gene polymorphisms and hematological abnormalities in hepatitis C virus-infected patients receiving combination therapy. Gut Liver, 2014, 9(2): 214–223.
[17] Grigorescu M; Romanian Society of Gastroenterology and Hepatology. HCV genotype 1 is almost exclusively present in Romanian patients with chronic hepatitis C. J Gastrointestin Liver Dis, 2009, 18(1):45–50.
[18] Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature, 2009, 461(7265):798–801.
[19] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet, 2009, 41(10):1100–1104.
[20] Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY; Swiss Hepatitis C Cohort Study; Swiss HIV Cohort Study. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology, 2010, 138(4):1338–1345.
[21] Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F, Lawitz EJ, McCone J, Shiffman ML, Galler GW, Lee WM, Reindollar R, King JW, Kwo PY, Ghalib RH, Freilich B, Nyberg LM, Zeuzem S, Poynard T, Vock DM, Pieper KS, Patel K, Tillmann HL, Noviello S, Koury K, Pedicone LD, Brass CA, Albrecht JK, Goldstein DB, McHutchison JG. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology, 2010, 139(1):120–129.e18.
[22] McCarthy JJ, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, Tillmann HL, Muir AJ, McHutchison JG. Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. Gastroenterology, 2010, 138(7):2307–2314.
[23] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature, 2009, 461(7262):399–401.
[24] Ionescu AG, Cazacu SM, Streba CT, Forţofoiu MC, Ciurea ME, Ionescu M, Rogoveanu O, Comănescu V, Firu ŞG, Vere CC. Interrelations between hepatic stellate cells and immune system cells in patients with hepatocellular carcinoma. Rom J Morphol Embryol, 2015, 56(2):481–490.
[25] Clark PJ, Thompson AJ. Host genomics and HCV treatment response. J Gastroenterol Hepatol, 2012, 27(2):212–222.
[26] Ionescu AG, Streba LA, Vere CC, Ciurea ME, Streba CT, Ionescu M, Comănescu M, Irimia E, Rogoveanu O. Histopathological and immunohistochemical study of hepatic stellate cells in patients with viral C chronic liver disease. Rom J Morphol Embryol, 2013, 54(4):983–991.
[27] Honda M, Sakai A, Yamashita T, Nakamoto Y, Mizukoshi E, Sakai Y, Yamashita T, Nakamura M, Shirasaki T, Horimoto K, Tanaka Y, Tokunaga K, Mizokami M, Kaneko S; Hokuriku Liver Study Group. Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C. Gastroenterology, 2010, 139(2):499–509.

Corresponding author
Kamal Constantin Kamal, MD, Department of Family Medicine, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Romania; Phone +40748–117 735, e-mail: kamalconstantin@gmail.com

Received: February 8, 2016

Accepted: August 9, 2016