

Original article

# Impact of rs12979860 polymorphism on liver morphology in chronic HCV infection

Tadeusz Wojciech Łapiński<sup>1</sup>, Magdalena Rogalska-Płonska<sup>1</sup>, Anatol Panasiuk<sup>1</sup>, Oksana Kowalczyk<sup>2</sup>, Jacek Nikliński<sup>2</sup>, Robert Flisiak<sup>1</sup>

<sup>1</sup>Department of Infectious Diseases and Hepatology, Medical University of Białystok, Białystok, Poland

<sup>2</sup>Department of Clinical Molecular Biology, Medical University of Białystok, Białystok, Poland

## Abstract

**Aim of the study:** To determine distribution of rs12979860 genotypes, their correlations with viral load as well as inflammatory activity and stage of liver fibrosis in patients infected with HCV genotype 1.

**Material and methods:** The study included 132 patients infected with HCV genotype 1b. Serum viral loads were obtained with the PCR method. Rs12979860 polymorphisms were determined by sequencing of PCR products. Liver biopsy was performed in all patients.

**Results:** CT, TT and CC alleles of rs12979860 polymorphism were detected in 58%, 20% and 22% of patients respectively. The highest viral load was observed in the TT and the lowest in the CC group ( $72.0 \times 10^6$  IU/ml vs.  $2.1 \times 10^6$  IU/ml,  $p < 0.005$ ). A significant correlation was demonstrated between patient's age and inflammatory activity as well as degree of liver fibrosis. No association was found between liver histopathology and HCV viral load or rs12979860 genotypes.

**Conclusions:** There is an association between HCV viral load and rs12979860 polymorphism. Inflammatory activity and stage of liver fibrosis depend on age, but there is no relationship with rs12979860 genotypes and HCV viral load.

**Key words:** HCV, polymorphism rs12979860, liver histology.

## Address for correspondence

Prof. Tadeusz Wojciech Łapiński, Department of Infectious Diseases and Hepatology, Medical University of Białystok, 14 Żurawia St., 15-540 Białystok, Poland, e-mail: twlapinski@gmail.com

## Introduction

Efficacy of the standard therapy for chronic HCV genotype 1 infection, consisting of pegylated interferon alpha (PegIFN $\alpha$ ) and ribavirin (RBV), is 40-60%. Addition of the first generation direct acting antivirals (DAA) – telaprevir or boceprevir – increases efficacy in treatment naive patients up to 75%. Further new DAA drugs, in the late stage of development or even registration procedures, combined with PegIFN $\alpha$  or RBV, as well as within interferon-free regimens, allow a sustained virological response (SVR) to be achieved in more than 90% of patients, regardless of their prior treatment response or severity of liver disease [1,2].

However, due to high costs, in the near future access to these drugs will probably be limited to patients with the highest risk of progression of liver disease.

High potency of new antivirals is not necessarily equivocal to their ability to improve liver function and structure. Adverse effects of these drugs may occasionally have a deteriorating effect on liver morphology. Currently, the inhibitory or even reversing activity of liver fibrosis is in fact documented only for  $\alpha$  interferons, used in the treatment of chronic hepatitis C (CHC) [3,4].

A single nucleotide polymorphism (SNP) in the rs12979860 region of human chromosome 19 is responsible for encoding specific amino acid sequences of the protein molecules called exons. Exons are separated in

the gene by introns, which are non-coding sequences. Just before transferring the RNA particle from the nucleus to the cytoplasm, the introns are cleaved and exons are combined to form a complete particle of messenger RNA (mRNA) encoding polypeptides. Newly formed nucleic acid encodes numerous polypeptides playing an important role in the immune activity, metabolic processes and apoptosis of the cell [5]. The impact of rs12979860 polymorphism has been documented mainly in relation to the efficacy of antiviral therapy in patients infected with genotype 1 HCV. Numerous studies have revealed that CC configuration of alleles in the rs1297986 region, in contrast to CT and TT, is a positive prognostic factor of spontaneous clearance of infection and response to the treatment [6,7].

Rs12979860 polymorphism and the treatment response after 4 and 12 weeks of therapy were considered the most important predictors of treatment efficacy [8]. Introduction of DAA to the antiviral therapy compensated for unfavorable genetic conditions associated with the T allele. However, the influence of rs12979860 polymorphism on the immune status as well as cell metabolic pathways including self-repairing abilities remains to be clarified.

The aim of the study was to determine the influence of rs12979860 genotypes on HCV viral load before treatment as well as to reveal possible correlations between rs12979860 polymorphism and inflammatory activity or stage of fibrosis.

## Material and methods

The study included 132 patients: 62 women and 70 men, aged 19 to 66 (mean age 41). All patients were chronically infected with genotype 1b HCV and were treatment naïve. Patients with HBV or HIV coinfections and with noninfectious liver diseases which could influence the morphology or functioning of the liver were excluded.

HCV-RNA in serum was measured by the RT-PCR method with starters of reaction specific to the 5' end of the noncoding region in the viral genome (5'-UTR). The viral genotype was determined by direct sequencing of the PCR product.

Rs12979860 polymorphism was evaluated using sequencing of the PCR product, including the SNP specified earlier. Sequencing was performed by chain termination synthesis using Big Dye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Isolation of DNA from whole blood and RNA from serum was performed in an automated system with a magnetic technique for the extraction of nucleic acids EasyMag (BioMerieux, France) according to the manufacturer's

protocol, using ready-made reagents of this manufacturer. Blood proteins were denatured before isolation by incubation with proteinase K (Sigma-Aldrich). PCR reaction was performed using *Taq* DNA polymerase and a dNTP mixture (EURx, Gdańsk). Reverse transcription was performed using *Tth* polymerase (Epicentre, USA). Products of PCR intended for sequencing and those obtained in chain termination synthesis were purified by sets of reagents "Gel-Out" and "Ex-Terminator" (DNA II Gdańsk). DNA sequencing was performed in the automated capillary sequencer Genetic Analyzer 3500 (Applied Biosystems, USA). The obtained sequences were analyzed by the BLAST program (NCBI, USA).

Percutaneous liver biopsy was performed during qualification for the treatment. The procedure was executed in local anesthesia (lidocaine). The sample was obtained from the right liver lobe by the Menghini method using disposable sets – Hepafix Luer Lock (Braun) and the 16G needle. Immediately after the liver tissue was taken, it was fixed in 4% buffered formalin solution and embedded in paraffin. The sections were stained with hematoxylin and eosin to determine the inflammatory cell infiltrations and with Sirius red, visualizing the connective tissue. Morphology of the liver (inflammatory activity and stage of fibrosis) was evaluated according to the Scheuer score ranging from 0 to 4 [9].

The study was performed with the approval of the local Bioethical Committee in patients who provided informed consent.

Statistical analysis was performed using the Mann-Whitney *U*, Spearman's correlation and logistic regression tests. A *p* value < 0.05 was considered statistically significant.

## Results

CT genotype of rs12979860 polymorphism was detected in 58% of patients, CC and TT in 22% and 20% respectively. Prevalence of CC, CT and TT genotypes among women and men was comparable (Fig. 1).

The highest HCV viral load was detected in the group of patients with genotype TT and the lowest was associated with CC genotype. As shown in Figure 2, statistically significant differences were demonstrated between particular genotypes.

A statistically significant correlation was demonstrated between severity of inflammation or stage of liver fibrosis and patients' age (Fig. 3).

Inflammatory activity exceeding a Scheuer score of 2 was found in 8 out of the 28 patients with CC genotype (29%). We did not find patients with stage of fibrosis exceeding a score of 2 in this group. Among patients with CT and TT genotypes, inflammatory ac-

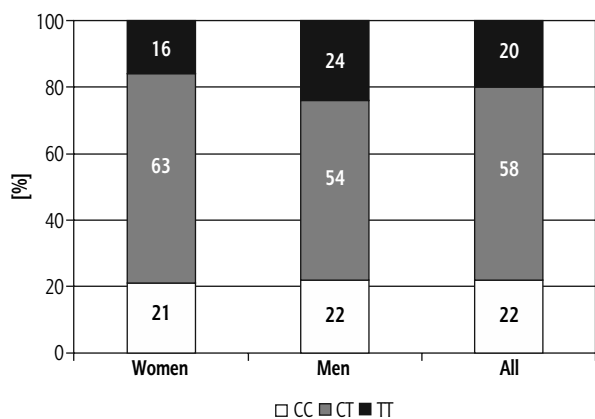


Fig. 1. Prevalence of genotypes of the rs12979860 polymorphism in the study group

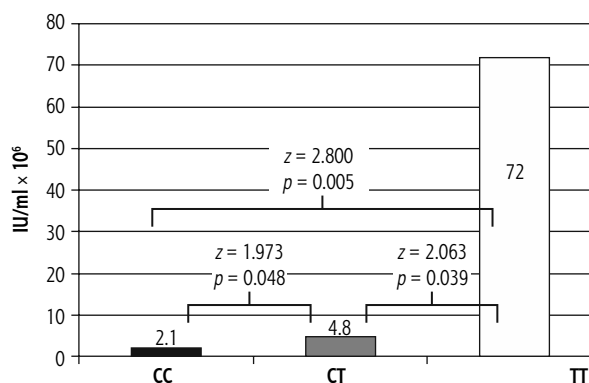


Fig. 2. Viral loads of genotype 1b HCV depending on rs12979860 polymorphism

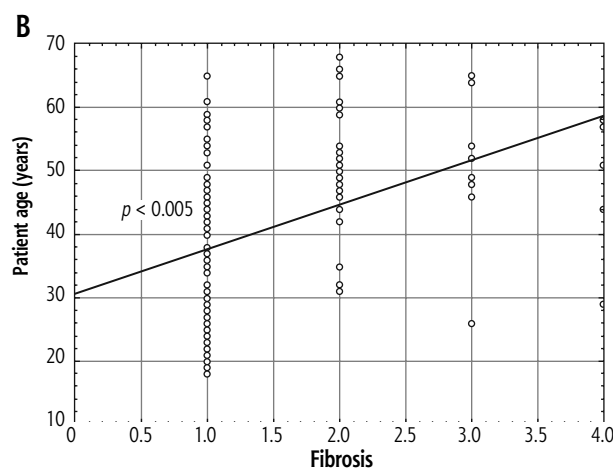
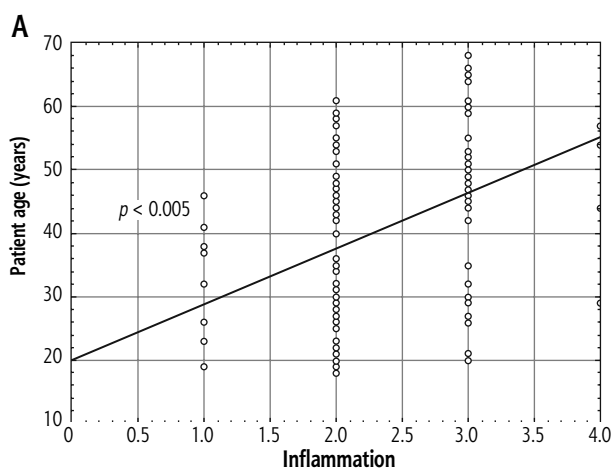


Fig. 3. Relationship between the patients' age and severity of inflammation (A) and stage of fibrosis (B) in the liver (logistic regression test)

tivity exceeding a score of 2 was more frequent compared to CC genotype (38% and 37% respectively), but it was not statistically significant. Prevalence of liver fibrosis exceeding a score of 2 in these two groups was also comparable (12% and 11% respectively) (Table 1).

The lowest stages of fibrosis and inflammatory activity were observed among patients with CC genotype. Yet, there were no statistically significant differences as compared to the patients with CT/TT genotypes (Table 2).

No correlations between HCV viral load and inflammatory activity or stage of fibrosis were found (Table 3).

## Discussion

The prevalence of CT and TT genotypes of rs12979860 polymorphism in a homogeneous group of genotype 1 HCV infected patients was 78%, regardless of gender. Such a high percentage of the T allele in

the studied population is unfavorable in comparison to other European countries, where the frequency of CC genotype exceeds 30% [6,10]. The obtained results suggest that genotype 1 HCV infected patients in Poland mostly belong to the group of patients poorly responding to the treatment with interferon and ribavirin and will require more efficient, DAA-based therapy.

In previous studies an association between low viral load and CC genotype of rs12979860 polymorphism was also observed. In the study published by Honda *et al.* [11], the reason for this may be intense synthesis of proinflammatory cytokines, which is genetically conditioned by CC genotype and may induce inhibition of replication and clearance of the virus. Further proof supporting the hypothesis of more frequent spontaneous HCV elimination in patients with CC genotype of rs12979860 is the fact that patients with this genotype represent a smaller proportion of genotype 1 HCV infected patients (33.9%) compared to the healthy population (49%). This association is

**Table 1.** Inflammatory activity and stage of fibrosis in relation to the rs12979860 polymorphism

Assessed parameter	Points	n (%)		
		CC (n = 28)	CT (n = 77)	TT (n = 27)
Inflammatory activity	0-2	20 (71%)	48 (62%)	17 (63%)
	3-4	8 (29%)	29 (38%)	10 (37%)
Stage of fibrosis	0-2	28 (100%)	68 (88%)	24 (89%)
	3-4	0	9 (12%)	3 (11%)

**Table 2.** Mean score values for degree of fibrosis and inflammatory activity depending of the rs12979860 polymorphism

Assessed parameter		rs12979860		
		CC (n = 28)	CT (n = 77)	TT (n = 27)
Inflammatory activity	x	2.36	2.38	2.39
	± SE	0.09	0.08	0.15
Stage of fibrosis	x	1.25	1.49	1.49
	± SE	0.09	0.09	0.15

**Table 3.** Serum HCV viral load in relation to the stage of fibrosis and inflammatory activity

		Inflammation		Fibrosis	
		0-2	3-4	0-2	3-4
HCV RNA (IU/ml)	x	$2.1 \times 10^7$	$1.5 \times 10^7$	$2.1 \times 10^7$	$9.4 \times 10^6$
	± SE	$1.1 \times 10^7$	$1.0 \times 10^7$	$9.2 \times 10^7$	$5.8 \times 10^7$

not observed in patients infected with genotypes 2 and 3 HCV (42.7%) [12].

Di Marco *et al.* [13] reported a correlation between increasing age of patients infected with genotypes 1 and 4 HCV and the stage of fibrosis. In our study, this finding was confirmed in relation not only to fibrosis but also to inflammatory activity.

Polymorphic changes in particular regions of the genome may influence the inflammatory activity and fibrosis in the liver. The studies conducted by Trépo *et al.* [14] in patients infected with genotypes 1, 2 and 4 HCV revealed an association between existence of the G nucleotide in the rs738409 region and fibrosis as well as steatosis of the liver. Rizk *et al.* [15] found a 13-fold greater degree of fibrosis in patients infected with genotype 4 HCV who also had TT genotype of rs281437 compared to those with CC genotype.

The opinions regarding the effect of rs12979860 polymorphism on histological changes in the liver are contrary. Sato *et al.* [16] analyzed data of 28 clinical studies which included patients infected with different HCV genotypes and suggested that existence of CC genotype of the rs12979860 polymorphism and TT genotype of the rs8099917 polymorphism promotes more intensive inflammation and fibrosis in the liver. The influence of genotypes of studied polymorphisms

on liver steatosis has not been demonstrated. The authors concluded that patients with mentioned genotypes of polymorphisms are characterized by more intensive proinflammatory cytokine synthesis resulting in substantial progression of inflammation and fibrosis if the virus is not cleared. However, these conclusions are questionable because each viral genotype has a different influence on the morphology of the liver and may also have a different effect, depending on the polymorphisms.

According to Honda *et al.* [11], among genotype 1b HCV infected patients there are no associations between degree of liver fibrosis and rs8105790, rs11881222, rs12979860, rs8099917 or rs7248668 polymorphisms. This study confirmed the relationship between the polymorphisms and stimulation of the type 1 signal transducers and activators of transcription (STAT1). The effect of STAT1 stimulation is increased expression of interferon stimulated genes (ISG), which may lead to stimulation of proinflammatory cytokine synthesis. Increased activity of proinflammatory cytokines may indeed be responsible for the severity of inflammation and necrosis but probably not for fibrosis. An opposite opinion is presented by Falleti *et al.* [10], who reported a relationship between TT genotype and stage of fibrosis in the liver of HCV infected patients. The authors

suggested the possible encoding of pro-fibrotic factors by CT and TT genotypes of rs12979860. The results of the Eurich *et al.* [17] study also indicated the unfavorable properties of TT genotype, as it was demonstrated that HCV infected patients having the T allele of rs12979860 polymorphism had a high probability of cancer recurrence and significant progression of fibrosis after liver transplantation due to hepatocellular carcinoma.

Lin *et al.* [18] also evaluated the fibrotic process in the liver of genotype 1 HCV infected patients, and their results did not confirm any association between histologic changes in the liver and rs12979860 genotypes. Also, the study by Sarrazin *et al.* [12] carried out in patients infected with genotypes 2 and 3 HCV did not confirm the relationship between rs12979860 polymorphism and histological changes in the liver.

## Conclusions

In the studied population the T allele of rs12979860 polymorphism was found in 78% of patients infected with genotype 1b HCV, and its presence especially as TT genotype was associated with higher HCV viral load. Hepatic inflammation and fibrosis correlated with the age of patients but not with the rs12979860 genotypes.

## Disclosure

Authors report no conflict of interest.

## References

- Jaroszewicz J, Flisiak R, Dusheiko G. A pill for HCV – myth or foreseeable future? *Liver Int* 2014; 34: 6-11.
- Flisiak R, Jaroszewicz J, Parfieniuk-Kowierda A. Emerging treatments for hepatitis C. *Expert Opin Emerg Drugs* 2013; 18: 461-475.
- Poynard T, McHutchison J, Manns M, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002; 122: 1303-1313.
- Poynard T. Treatment of cirrhotic patients in the pegylated interferon era. *Dig Liver Dis* 2004; 36 Suppl 3: S344-348.
- Sheppard P, Kindsvogel W, Xu W, et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat Immunol* 2003; 4: 63-68.
- Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; 461: 798-801.
- Łapiński TW, Pogorzelska J, Kowalczyk O, et al. SNP RS12979860 related spontaneous clearance of hepatitis C virus infection in HCV/HIV-1 coinfecting patients. *Przegl Epidemiol* 2013; 67: 407-409, 517-519.
- Neumann AU, Pianko S, Zeuzem S, et al. Positive and negative prediction of sustained virologic response at weeks 2 and 4 of treatment with albinterferon alfa-2b or peginterferon alfa-2a in treatment-naive patients with genotype 1, chronic hepatitis C. *J Hepatology* 2009; 51: 21-28.
- Scheuer PJ. Classification of chronic viral hepatitis: A need for reassessment. *J Hepatol* 1999; 13: 372-374.
- Falletti E, Bitetto D, Fabris C, et al. Role of interleukin 28B rs12979860 C/T polymorphism on the histological outcome of chronic hepatitis C: relationship with gender and viral genotype. *J Clin Immunol* 2011; 31: 891-899.
- Honda M, Sakai A, Yamashita T, et al. Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C. *Gastroenterology* 2010; 139: 499-509.
- Sarrazin C, Susser S, Doehring A, et al. Importance of IL28B gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients. *J Hepatol* 2011; 54: 415-421.
- Di Marco V, Bronte F, Calvaruso V, et al. IL28B polymorphisms influence stage of fibrosis and spontaneous or interferon-induced viral clearance in thalassemia patients with hepatitis C virus infection. *Haematologica* 2012; 97: 679-686.
- Trépo E, Pradat P, Potthoff A, et al. Impact of PNPLA3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. *Hepatology* 2011; 54: 60-69.
- Rizk NM, Derbala MF. Genetic polymorphisms of ICAM 1 and IL28 as predictors of liver fibrosis severity and viral clearance in hepatitis C genotype 4. *Clin Res Hepatol Gastroenterol* 2013; 37: 262-268.
- Sato M, Kondo M, Tateishi R, et al. Impact of IL28B genetic variation on HCV-induced liver fibrosis, inflammation, and steatosis: a meta-analysis. *PLoS One* 2014; 9: e91822.
- Eurich D, Boas-Knoop S, Bahra M, et al. Role of IL28B polymorphism in the development of hepatitis C virus-induced hepatocellular carcinoma, graft fibrosis, and posttransplant antiviral therapy. *Transplantation* 2012; 93: 644-649.
- Lin CY, Chen JY, Lin TN, et al. IL28B SNP rs12979860 is a critical predictor for on-treatment and sustained virologic response in patients with hepatitis C virus genotype-1 infection. *PLoS One* 2011; 6: e18322.