Original paper

The impact of the recipient and donor interferon lambda-3 polymorphism on the course of HCV infection following liver transplantation

Renata Wieczorek-Godlewska[1], Rafał Płoski[2], Agnieszka Perkowska-Ptasińska[1], Olga Tronina[1], Anna Sadowska[1], Marek Pacholczyk[3], Wojciech Lisik[3], Magdalena Durlik[1]

[1]Department of Transplantation Medicine and Nephrology, Medical University of Warsaw, Poland
[2]Medical Genetics Unit, Medical University of Warsaw, Poland
[3]Department of General and Transplant Surgery, Medical University of Warsaw, Poland

Abstract

Aim of the study was to assess the impact of the recipient and donor interferon lambda-3 (IFNL3) single-nucleotide polymorphisms (SNPs) rs12979860 and rs8099917 on the course of hepatitis C virus (HCV) reinfection following liver transplantation.

Material and methods: The study involved 141 subjects after liver transplantation for HCV-induced cirrhosis, performed between 2000 and 2015. It assessed the impact of both SNPs on the outcomes of interferon/ribavirin (IFN/RBV) treatment following transplantation, HCV viral load, laboratory test results, histological lesions in the liver graft, the risk of acute rejection, and the development of hepatocellular carcinoma (HCC) in patient's own liver.

Results: In the case of rs12979860, SVR was achieved in 58.8% of recipients with the CC genotype, and only 12% of recipients with the TT genotype (p = 0.016). Recipients with the rs12979860 CC variant had lower viral load and lower alanine transaminase (ALT) activity than recipients with a non-CC variant. Opposite effects were demonstrated in the analysis of the donors' genotype. Recipients with the unfavorable variants (rs12979860 TT and rs8099917 GG) had a lower risk of graft rejection and tended to have a higher risk of developing HCC in their own liver.

Conclusions: The IFNL3 rs12979860 polymorphism may be considered a predictor for IFN/RBV effectiveness following liver transplantation. The course of HCV reinfection following liver transplantation may be more aggressive if an unfavorable variant in the recipient coexists with a promising variant in the donor. Particularly careful monitoring for HCC in recipients with unfavorable IFNL3 variants is warranted.

Key words: interferon lambda-3 (IFNL3), hepatitis C virus (HCV), liver transplantation.

Address for correspondence

Renata Wieczorek-Godlewska, Department of Transplantation Medicine and Nephrology, Medical University of Warsaw, 59 Nowogrodzka St., 02-006 Warszawa, Poland, phone: +48 22 502 12 32, fax: +48 22 502 21 26, e-mail: godlewska.r@wp.pl

Introduction

In present-day, cirrhosis of the liver induced by chronic infection with hepatitis C virus (HCV) is the most common indication for liver transplantation in adults living in the developed countries [1]. The prognosis for this patient population is, however, much worse as compared with other indications, which is related primarily to frequent HCV reinfection in the donor's liver, occurring in nearly 95% of patients with detectable HCV prior to transplantation [2, 3]. The course of HCV reinfection is more aggressive than infection in the patient's own liver, with more rapid progression of fibrosis and development of cirrhosis

of the graft [4]. Also outcomes of treatment of HCV reinfection in the liver graft using standard pegylated interferon (IFN) and ribavirin are clearly worse than in the case of the patient's own liver: permanent eradication of the virus (sustained virological response – SVR) is achieved only in 24-40% of the treated patients [5-8].

In genome-wide association studies (GWASs) in HCV-infected individuals, single nucleotide polymorphisms (SNPs) were identified and located in the region of IFNL3 (IL28B), which encodes antiviral cytokine interferon lambda 3 (IFNL3, IL28B, interleukin 28B), and is associated with the effectiveness of the interferon/ribavirin treatment. The highest prognostic value was seen in the case of two *loci:* rs12979860 C > T and rs8099917 G > T, and the genotypes correlating with cure were: CC for rs12979860 and TT for rs8099917 [9-12]. Since the source of IFNL3 is composed of both immune cells (especially plasmacytoid dendritic cells [pDCs] and macrophages) and hepatocytes, chimerism occurs after liver transplantation. The patient has two sources of IFNL3: their own immune cells and the donor's hepatocytes, which may affect the immune response to HCV infection following transplantation [13].

The aim of this study is to assess the impact of the recipient and donor IFNL3 single-nucleotide polymorphisms (SNPs) rs12979860 and rs8099917 on the course of HCV reinfection following liver transplantation.

Material and methods

The study involved 184 subjects after liver transplantation for HCV-induced cirrhosis performed between November 2000 and February 2015 at the Department of General and Transplant Surgery, Medical University of Warsaw.

Patients who died within 90 days of transplantation (n = 19), patients with undetectable blood HCV RNA prior to transplantation (n = 9), and patients infected with HCVG3 (n = 15) were excluded from the final analysis. The proper group comprised 141 individuals with HCV reinfection in the liver graft, confirmed by detectable blood HCV RNA following transplantation, infected with HCV genotype 1 (n = 137) or 4 (n = 4). Mean follow-up time was 6.6 ± 3.9 years, ranging between 15.2 years and 1 year.

The study was approved by the Ethics Committee of the Medical University of Warsaw.

IFNL3 genotyping

The recipients' genetic material for IFNL3 polymorphism testing was obtained from fragments of the patients' own liver removed during transplantation and stored as paraffin-embedded blocks. The donors' genetic material was obtained through time-zero biopsies preceding reperfusion and was stored as paraffin-embedded blocks.

DNA was isolated using the Maxwell®16 (Promega GmbH, Germany) instrument for nucleic acid and protein isolation. The Maxwell® 16 FFPE Tissue LEV DNA Purification Kit (Promega GmbH, Germany) was used. Nucleotide sequences within rs12979860 were determined using allele-specific probes and the Custom TaqMan® SNP Genotyping Assay (Applied Biosystems, A Thermo Fisher Scientific Brand). Possible single-nucleotide polymorphisms (SNPs) C > T within rs12979860 included CC, CT, and TT. IFNL3 rs8099917 G > T genotyping was performed using the TaqMan® Pre-Designed SNP Genotyping Assay (Applied Biosystems, A Thermo Fisher Scientific Brand). Possible SNP variants included TT, GT, and GG.

IFNL3 rs12979860 genotyping was performed for 88% of the recipients and 96% of the donors as well as for 120 recipient–donor pairs. In the case of the rs8099917 polymorphism, successful genotyping was performed for 84% of the recipients and 96% of the donors as well as 115 recipient–donor pairs.

Histological examinations

The total of 368 biopsies performed in the study group between November 2000 and December 2015 were included in the analysis. The biopsies were performed 12, 24, and 36 months after transplantation, both for protocol-related reasons and because of clinical indications. Necroinflammatory lesions related to HCV reinfection (grading) and fibrosis severity (staging) were assessed using the Ishak scale [14]. Every biopsy was also assessed for acute liver graft rejection using the Banff 1997 classification [15]. The removed fragments of the patients' own livers were also assessed for hepatocellular carcinoma (HCC).

Antiviral treatment following liver transplantation

In 76 of 141 (54%) patients diagnosed with HCV reinfection of the liver graft, double antiviral therapy with pegylated interferon alpha (PegIFN-α2a or PegIFN-α2b) and ribavirin (RBV) was used. Indications for treatment initiation as well as doses and treatment duration met the then required standards of the American Association for the Study of Liver Diseases (AASLD) and European Association for the Study of the Liver (EASL), and were consistent with the guidelines set out by the Polish Group of Experts on HCV (PGE HCV).

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Treatment was considered successful if no viral load was detectable 24 weeks after treatment completion, i.e. when sustained virologic response (SVR 24) was achieved.

Statistical methods

Continuous variables were presented descriptively as the mean and standard deviation for normal distribution or median and quartiles [1Q-3Q], if the distribution was inconsistent with normal distribution. For normality testing, the Shapiro-Wilk test was used. In the case of categorical variables, the non-parametric Mann-Whitney U test was used. To verify hypotheses on the rates of categorical variables in two subgroups, the χ^2 test was used. To compare the means in more than two subgroups, non-parametric analysis of variance using the Kruskal-Wallis test was carried out. All hypotheses were tested using the significance level of 0.05. The statistical analysis was carried out using R under the GNU GPL license (version 3.2.0).

Results

The distribution of IFNL3 genotypes in the donors and recipients was significantly different for both SNPs of interest. Favorable (from the point of view of cure) HCV genotypes were more common in the donors than in the recipients: for rs12979860, the CC variant was found in 23.4% of the recipients and 38.5% of the donors; $p = 0.013$; for rs8099917, the TT variant was found in 49.6% of the recipients and 64.6% of the donors; $p = 0.023$.

An analysis concerning individual genotypes (CC, CT, and TT for rs12979860 and TT, GT, and GG for rs8099917) did not reveal differences in the recipients'

or donors' demographics, cold ischemic time, immunosuppression, pre-transplantation viral load, incidence of HBV infection, pre-transplantation diabetes mellitus, or incidence of biliary and vascular complications following transplantation.

Impact of the IFNL3 polymorphism on antiviral treatment outcomes (SVR)

Cure, defined as sustained virologic response (SVR24), was achieved in 29 of 76 (38.1%) of the treated subjects.

In the case of the rs127979860 genotype, recipients with the CC variant achieved SVR five times more often than recipients with the TT variant. If the donor had the same CC variant, the chance of achieving SVR was 100%.

In the case of the rs12979860 genotype in the donor, no statistically significant differences were found. There were no statistically significant relationships between the rs8099917 polymorphism in the recipient and the donor, or between the combined recipient and donor polymorphism analysis and antiviral treatment outcomes. The above relationships are presented in Figures 1 and 2.

Impact of the IFNL3 polymorphism on the clinical course of HCV reinfection following liver transplantation

An opposite impact of the same IFNL3 genotype on post-transplantation viral load was seen; it depended on whether it had been found in the recipient or the donor. Recipients with favorable (from the point of view of HCV eradication) CC variant of rs12979860 had the lowest levels of serum HCV RNA following
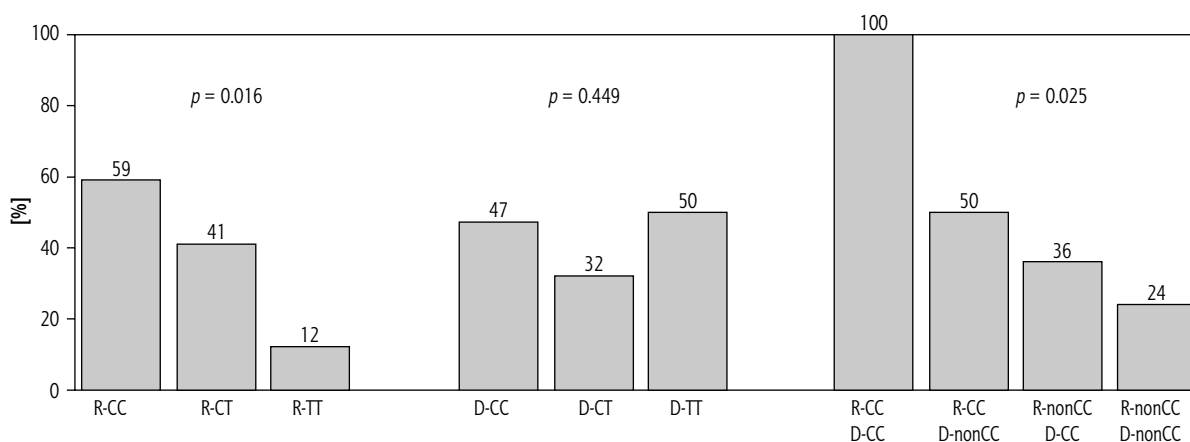

Fig. 1. Sustained virological response (SVR) rates by polymorphism rs12979860 (R-CC, R-CT, R-TT − variants of recipient's genotype, D-CC, D-CT, D-TT − variants of donor's genotype)
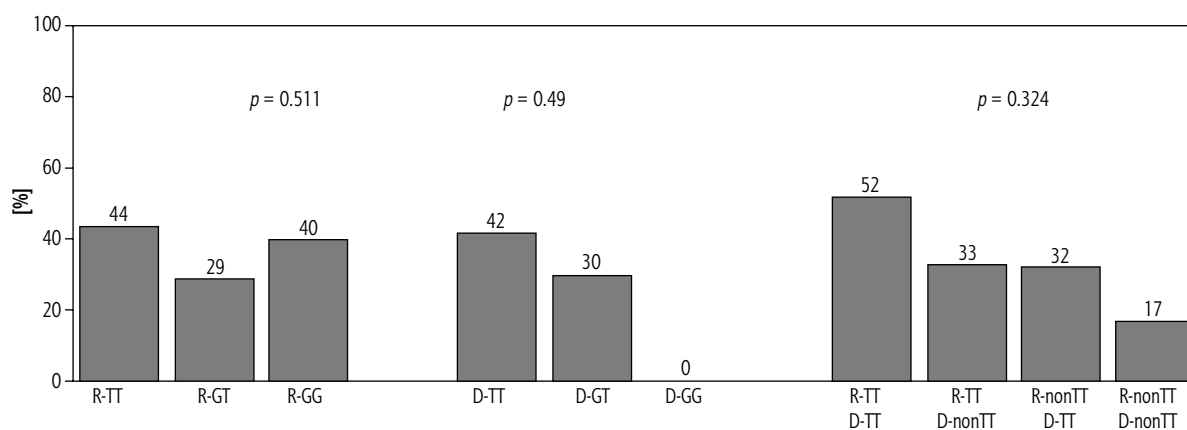
Fig. 2. Sustained virological response (SVR) rates by polymorphism rs8099917 (R-TT, R-GT, R-GG – variants of recipient's genotype, D-TT, D-GT, D-GG – variants of donor's genotype)

transplantation, as compared with other SNPs, and if the same variant had been found in the donor, viral load was the lowest. Similar relationships were reported for rs8099917, although statistically significant differences were reported only for the donor's genotype (Table 1). In a pooled analysis of both the recipient's and the donor's polymorphism, viral load was the highest for the combination of unfavorable (from the point of view of cure) HCV genotype of the recipient and favorable HCV genotype of the donor (for rs12979860, the combination: recipient – nonCC + donor-CC, $p = 0.016$; for rs8099917, the combination: recipient – nonTT + donor – TT, $p = 0.041$).

An analysis of mean serum activity of alanine aminotransferase (ALT) in post-transplantation year one, revealed an opposite impact of the recipient's and donor's genotype – as in the case of viral load: ALT levels were lower with the favorable genotype of the recipient and higher if the same genotype was found in the donor, although statistically significant differences were seen only for the donor's genotype for rs8099917 ($p = 0.034$), and – on the verge of statistical significance – for rs12979860 ($p = 0.051$). The highest post-transplantation activity of ALT was seen with the combina-

tion of an unfavorable recipient's genotype and favorable donor's genotype; at the same time, it is a group of patients with the highest post-transplantation viral load.

No statistically significant differences in the severity of inflammation and fibrosis stage by IFNL3 polymorphism were found, although similar tendencies as for viral load and ALT were seen: median inflammation and median fibrosis were higher in recipients with unfavorable IFNL3 polymorphism, especially if the donor had a favorable variant of the gene.

Impact of the IFNL3 polymorphism on acute rejection rates following liver transplantation

Acute rejection (AR) as evidenced by histological examination was diagnosed in 30 of 141 (21.3%) study subjects. Lower AR rates were reported for carriers of unfavorable homozygotic variants of the gene (TT for rs12979860 and GG for rs8099917), although the differences were statistically significant only for the first SNP. The donor's genotype did not affect the rates of acute rejection. The relationship between acute rejection rates and IFNL3 polymorphism are presented in Figure 3.

Table 1. Hepatits C virus (HCV) viral load following transplantation by interferon lambda-3 (IFNL3) polymorphism in the recipient and the donor

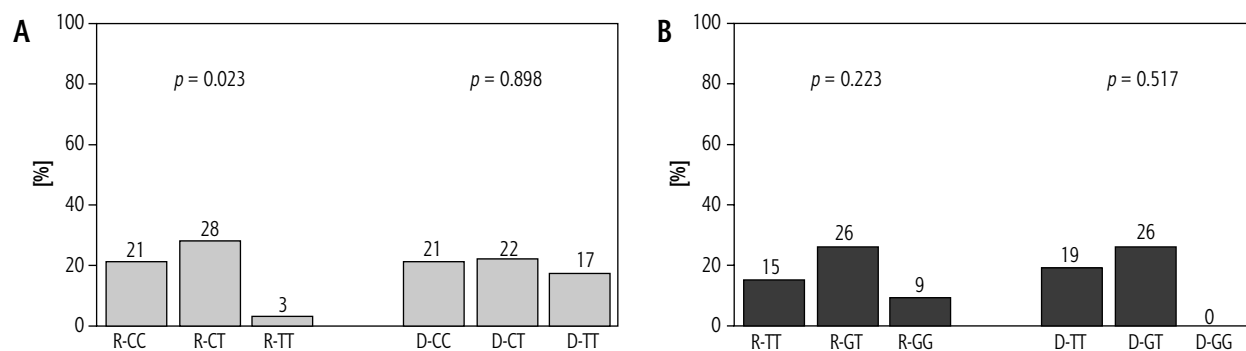| SNP	HCV RNA following transplantation					
	Recipient	Median [1Q-3Q], $\log_{10}$, IU/ml	*p*-value	Donor	Median [1Q-3Q], $\log_{10}$, IU/ml	*p*-value
rs12979860	CC	6.1 [5.4-6.6]	0.011	CC	6.7 [6.2-7.0]	0.001
	CT	6.4 [5.8-6.7]		CT	6.4 [5.9-6.8]	
	TT	6.6 [6.2-7.1]		TT	5.6 [5.1-6.1]	
rs8099917	TT	6.2 [5.7-6.6]	0.368	TT	6.6 [6.2-6.9]	0.002
	GT	6.5 [5.9-6.8]		GT	6.1 [5.8-6.6]	
	GG	6.5 [6.1-6.9]		GG	5.3 [5.2-5.3]	

Fig. 3. Relationship between acute rejection rates and rs12979860 **(A)** and rs8099917 **(B)** polymorphism (R − recipient, D − donor)
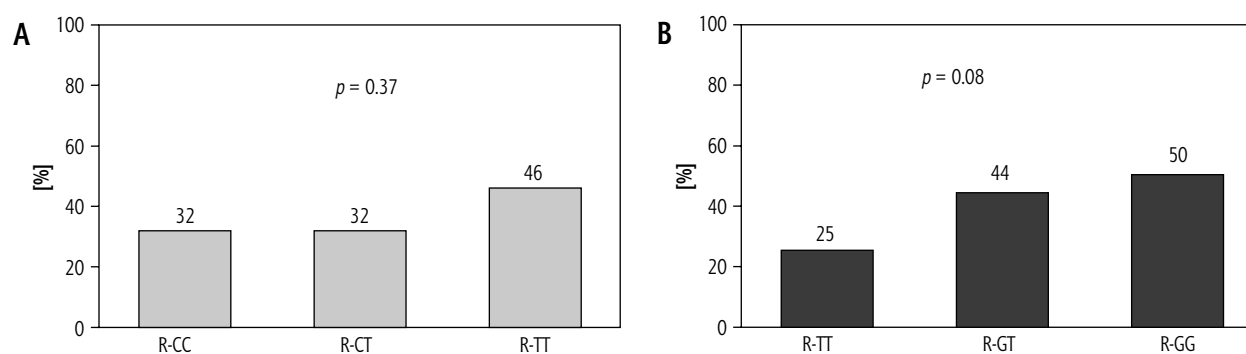

Fig. 4. The incidence of hepatocellular carcinoma (HCC) by the recipient's IFNL3 polymorphism: rs12979860 **(A)** and rs8099917 **(B)**

The risk of hepatocellular carcinoma by IFNL3 genotype in the recipient

Hepatocellular carcinoma in the patient's own liver was found in 46 of 141 (34.6%) of the study subjects. It was demonstrated that the incidence of CC in the patient's own liver tended to be higher among subjects with unfavorable (from the point of view of HCV eradication) variants of the gene (Fig. 4).

Discussion

In the era of direct-acting antivirals (DAAs), IFNL3 polymorphism as the prognostic factor for assessing outcomes of HCV infection treatment is becoming less and less important, but it should not be forgotten altogether. IFNL3 genotyping enables the selection of patients with favorable IFNL3 polymorphism, which is predictive of high SVR rates for standard PegIFN-α/ribavirin-based therapy, comparable to SVR rates for new therapies. One may assume that in future, the problem will lie in the selection of mutated virus variants, which develop resistance to individual DAAs. Such mutations have already been reported in the con-

text of clinical trials. Occurrence of new mutations is likely due to high genetic variability of HCV. Possibly, future treatment regimens may again include agents acting on the immune system of the host, such as interferon.

This paper demonstrates a surprising, opposite impact of the recipient's and donor's IFNL3 genotype on the clinical course of HCV reinfection following liver transplantation. A group of post-transplantation patients (unfavorable SNP of the recipient and favorable SNP of the donor) has been identified, in whom one may suspect a particularly aggressive HCV-reinfection of the liver graft associated with high viral load, high ALT activity, and an increased risk of progression of post-transplantation fibrosis. This group of patients requires urgent antiviral treatment following transplantation, preferably with DAAs. The cause of the opposite impact of the same SNP in both the recipient and the donor remains unclear. Similar relationships have been reported by other authors [16-18], however, no paper discusses these interesting though controversial results.

An explanation of these relationships may possibly be found in the paper by Chen *et al.* who detect-

ed different expression of interferon-stimulated genes (ISG) in hepatocytes and hepatic macrophages in response to HCV infections. In the group of IFN/RBV responders, expression of basic interferon-stimulated genes (ISG15 and MxA) was relatively higher in macrophages and lower in hepatocytes. Unlike in non-responders, in whom high ISG induction in the liver and low in macrophages predominated [19]. Regrettably, the study by Chen *et al.* did not examine the IFNL3 polymorphism or correlation between the genotype, and ISG expression in individual cell types. However, the model described appears to perfectly apply to liver transplantation: the source of interferon lambda is composed of both the donor's hepatocytes and the recipient's immune cells, which populate the graft, and the acquired response of the recipient's macrophages is considerably impaired as a result of immunosuppression, while inborn response of the donor's hepatocytes is compromised only to a low degree. According to this theory, the highest viral load and ALT activity were found in the case of this combination: unfavorable recipient's genotype (low ISG expression in macrophages) and favorable donor's genotype (also low ISG in hepatocytes). On the whole, low levels of antiviral proteins would contribute to HCV replication and liver injury. The hypothetical model described would clearly require confirmation in studies examining correlation between the IFNL3 genotype with ISG expression in hepatocytes and macrophages. Nevertheless, it well explains the constellation of viral load and ALT activity in the study presented.

In this paper, also the relationship between IFNL3 polymorphism and acute rejection rates was examined. The rates of acute rejection following liver transplantation were lower among recipients with the most unfavorable (from the point of view of treatment efficacy) IFNL3 variants: rs12979860 TT and rs8099917 GG. This finding is surprising: few reports about a relationship between IFNL3 polymorphism and AR either failed to point the differences in rejection rates between SNPs or pointed to lower rejection rates in carriers of the favorable CC variant [17, 20]. However, when the results are analyzed according to the model, which is based on the studies by Chen *et al.*, one may assume that recipients of the unfavorable B-TT variant have low expression of pro-inflammatory cytokines in macrophages and other immune cells, which is why there may be lesser readiness to reject the donor's tissue, especially in the setting of immunosuppression. Taking such view explains also the lack of impact of the donor's genotype on the rejection process. Evidently, the concept presented should be considered with caution not only because of different findings of other authors, but

also due to relatively low AR rates in the study group. On the other hand, its confirmation might have significant clinical implications: unfavorable polymorphism in the recipient at the same time promotes higher viral load and possibly more severe inflammation and greater damage to the liver graft. Therefore, more bold reduction of immunosuppression, without an increase of the rejection risk, might prove beneficial in this group of patients.

This paper, however, points to a tendency towards higher incidence of HCC in the liver of patients with unfavorable homozygotic variants of IFNL3: rs12979860 TT and rs8099917 GG, even though the differences were not statistically significant. A similar relationship between HCC and unfavorable SNPs has been reported by other authors [21, 22]. At the cellular level, neogenesis to HCC is caused by chronic HCV-induced inflammation leading to overproduction of free oxygen radicals in hepatocytes, excited hepatic macrophages and the cells of inflammatory infiltrate. Chronic oxidative stress causes damage to cellular DNA and promotes neogenesis. It has been demonstrated that patients with unfavorable IFNL3 variants paradoxically have a higher expression of ISGs in hepatocytes, and therefore have more severe inflammation, which may increase the risk of neogenesis. It is worth considering whether variants TT for rs12979860 and GG for rs8099917 should be regarded as additional risk factors for HCC in patients with HCV-induced cirrhosis of the liver, and should increase oncological awareness when treating carriers of unfavorable SNPs. It is particularly important in light of the recent reports of higher incidence of hepatocellular carcinoma in patients after successful HCV eradication using new therapeutic regimes based on DAAs [23].

Conclusions

The IFNL3 rs12979860 polymorphism may be considered a predictor for IFN/RBV effectiveness following liver transplantation. Determination of the role of rs8099917 polymorphism in the Polish population requires further studies involving larger group of patients.

The course of HCV reinfection following liver transplantation may be more aggressive if an unfavorable variant in the recipient coexists with a favorable variant in the donor. Specific indications for urgent antiviral therapy after the transplantation should be considered in this group of patients.

Particularly careful oncological surveillance for HCC should be considered in patients with liver cirrhosis of HCV etiology, who are carriers of unfavorable IFNL3 variants.

Renata Wieczorek-Godlewska, Rafał Płoski, Agnieszka Perkowska-Ptasińska, Olga Tronina, Anna Sadowska, Marek Pacholczyk, Wojciech Lisik, Magdalena Durlik

Disclosure

Authors report no conflict of interest.

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