ORIGINAL ARTICLE

Effect of interferon λ3 gene polymorphisms, rs8099917 and rs12979860, on response to hepatitis B virus vaccination and hepatitis B or C virus infections among hemodialysis patients

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KEY WORDS

hepatitis B virus, hepatitis C virus, hemodialysis, IFN-λ3 gene, vaccination

ABSTRACT

INTRODUCTION The higher prevalence and risk of hepatitis B virus (HBV) or hepatitis C virus (HCV) infections are still observed in hemodialysis (HD) patients compared with healthy people. Interferons (IFNs) are known for their involvement in immune response. The addition of IFN-λ3 to immunization in animal models was shown to increase the immune response of T helper-1 cells.

OBJECTIVES We studied whether polymorphisms of the IFN-λ3 gene (*IFNL3*) might be associated with the development of antibodies to HBV surface antigen [anti-HBs] in response to the HBV vaccination or HBV infection as well as spontaneous resolution of HCV infection in HD patients.

PATIENTS AND METHODS The HD group consisted of 806 individuals without a history of HBV or HCV infection (of whom 672 developed anti-HBs in response to the HBV vaccination), 241 HBV-infected patients (of whom 186 developed anti-HBs), and 63 HCV-infected patients (including 39 HCV RNA-positive subjects). All patients were genotyped for *IFNL3* rs8099917 and rs12979860 polymorphisms using a high-resolution melting curve analysis.

RESULTS The comparison of responders and nonresponders to HBV vaccination revealed no significant differences in the *IFNL3* genotype distribution. In HBV-infected patients, the differences in the distribution of *IFNL3* variants between anti-HBs-negative and anti-HBs-positive patients were also nonsignificant. Spontaneous HCV clearance was significantly less common in the carriers of the rs8099917 allele G or rs12979860 allele T, while the CT rs12979860_rs8099917 haplotype was more frequent ($P = 0.02$) in patients showing spontaneous HCV clearance.

CONCLUSIONS In HD patients, the *IFNL3* polymorphisms do not affect anti-HBs development in response to HBV infection or vaccination, but might be involved in the resolution of HCV infection.

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INTRODUCTION Hemodialysis (HD) patients are at risk of hepatitis B and C infections owing to a greater exposure to the potential reservoirs of these viruses. Because of the impaired function of the immune system, dialysis patients are less likely to develop antibodies after immunization and tend to lose them faster than healthy vaccinated subjects.[1,2]

Interferons (IFNs) and interleukins (ILs) are known for their involvement in immune response. IFN-λ2 (also known as IL-28A) and IFN-λ3 (also known as IL-28B) were found to enhance response to immunizations in animal models. The addition of IFN-λ3 to immunization with DNA of Rhezus Macaques or mice boosted and sustained the T helper-1 (Th1) response.[3,4]

IFN-λ3 is a protein that is encoded by the IFN-λ3 gene (*IFNL3*) located on a chromosomal region mapped to 19q13.[5] *IFNL3* rs12980275 (C>T) and rs8099917 (T>G) are single nucleotide

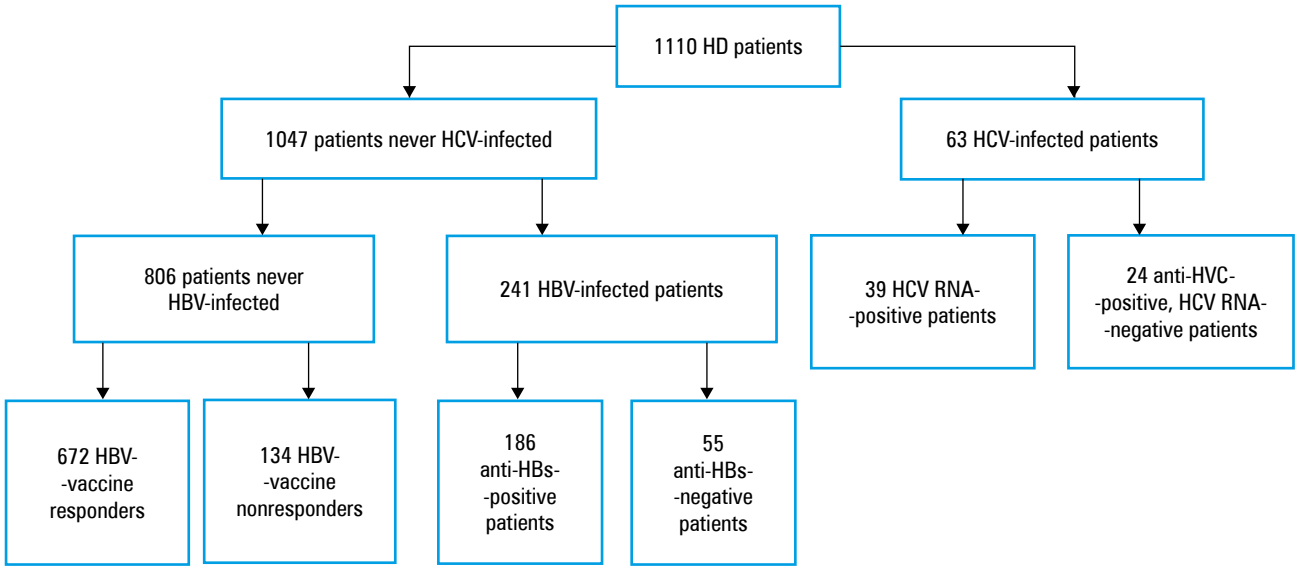

FIGURE 1 Schematic presentation of hemodialyzed patient subgroups divided according to the hepatitis B or hepatitis C status and response to hepatitis B vaccination

Abbreviations: anti-HBs, antibodies to the hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HD, hemodialysis

polymorphisms (SNPs) of *IFNL3*. The T allele of rs12980275 is associated with lower IFN-λ3 production[6] and a considerably increased expression of numerous IFN-stimulated genes when compared with CC carriers.[7] Lower IFN-λ3 mRNA levels were detected in association with the rs8099917 GG genotype,[8,9] whereas peripheral blood mononuclear cells of patients carrying the major alleles of *IFNL3* SNPs were found to have an increased expression of *IFNL3*.[8]

IFNL3 SNPs have attracted considerable attention due to their influence on the outcome of hepatic infections in the general population. The major alleles of *IFNL3* rs12980275 and rs8099917 promote hepatitis C virus (HCV) clearance.[10,11] Not only spontaneous clearance but also response to treatment of HCV-infected patients is dependent on the *IFNL3* SNPs.[12,13]

Less favorable results were obtained for patients infected with hepatitis B virus (HBV). Although in some studies, the CC genotype of rs12979860 was an independent predictor of HBsAg seroclearance by interferon therapy,[14] and patients with this genotype were found to have more frequently lower viral load levels,[15] a recent meta-analysis by Tang et al[16] has not found any association between *IFNL3* rs12979860 C/T and the risk of persistent HBV infection.

The above effects of *IFNL3* SNPs were established under nonuremic conditions. The uremic milieu with related altered immunocompetence decreases the response to vaccinations[17] and promotes the maintenance of HBV or HCV replication.[18,19] These uremia-related activities may diminish the expression of genetic effects on the clinical parameters tested in the present study. Moreover, the association between *IFNL3* polymorphisms and response to HBV vaccination was not studied in individuals under nonuremic or uremic conditions. Therefore, we aimed to show whether the SNPs of *IFNL3* might be associated with the development of antibodies to HBV surface antigen (anti-HBs) in response to HBV vaccination or infection as well as with spontaneous HCV clearance in HD patients.

PATIENTS AND METHODS

Patients and controls The study included 1110 patients on renal replacement therapy (RRT). They were recruited from 21 dialysis centers located in the Greater Poland region of Poland. The enrollment was started in January, 2009, and finished in May, 2014. All patients were treated with HD on enrollment; however, 28 subjects (2.5%) started RRT with peritoneal dialysis. Subjects dually infected with HBV and HCV or those infected with human immunodeficiency virus were excluded from the study. Of the whole group, 63 patients were HCV-infected (showed antibodies to HCV, anti-HCV) but only 39 of the 63 showed positive for HCV RNA. The remaining 1047 patients were anti-HCV negative, of whom 806 had no history of HBV infection, while 241 were HBV-infected (showed total antibodies to the core antigen of HBV, anti-HBc). In the latter group, 186 subjects developed anti-HBs. HD patient groups are shown in FIGURE 1.

Subjects were included into the group of never-HBV-infected patients (n = 806), if they met the following criteria: 1) had no history of hepatitis B and tested negative for anti-HBc; 2) underwent full hepatitis B vaccination series that is recommended for HD patients (4 doses of 40 μg each at 0, 1, 2, and 6 months)[20] and developed anti-HBs titer equal to or exceeding 10 IU/l in response to this primary vaccination or additional vaccine doses (this group was referred to as "vaccine responders"; n = 672, 83.4% of all patients);

and 3) received full hepatitis B vaccination series that is recommended for HD patients and at least 3 additional vaccine doses that are recommended for nonresponders to the primary vaccination[20] and did not develop anti-HBs titer of at least 10 IU/l (this group was referred to as "vaccine nonresponders"; n = 134, 16.6% of all patients).

All patients who were vaccinated against HBV used recombinant DNA yeast-derived vaccines, composed of the S protein of the HBV surface antigen (Engerix B, GlaxoSmithKline Biologicals, Belgium; Hepavax–Gene, BIOMED SA, Poland; Euvax B, LG Chemical, South Korea).

The group of HBV- or HCV-infected patients included only those who had never received relevant antiviral therapy. Patients with positive anti-HCV antibodies repeatedly showing HCV RNA below the detection limit of 20 IU/ml were recognized as those who underwent spontaneous HCV clearance (resolved HCV infection without antiviral treatment).

A control group, consisting of healthy volunteers without a history of viral hepatitis (n = 375), was enrolled to compare the distribution of *IFNL3* SNPs between HD subjects and healthy individuals. The group included 360 blood donors and 15 individuals from the medical staff. The control group underwent blood testing for anti-HBs and HBV DNA as well as for anti-HCV and HCV RNA (blood donors obligatorily[21] and medical staff members voluntarily). All blood samples were negative for the tested viral markers. The serum alanine aminotransferase activity in controls was not higher than twice the upper normal limit of the applied laboratory method according to the criteria of the Polish Ministry of Health for blood donation.[21]

All patients and controls were Caucasians of the Polish origin. The comparison of sex distribution and age of all patients and controls is presented in Supplementary material online, *Table S1*.

Blood sampling and data recording Blood samples for genotyping were collected on enrollment to the study. Leukocyte DNA was stored at –20°C before genotyping.

Patients meeting the inclusion criteria were assigned to individual study groups on enrollment. However, their HBV and HCV status was monitored every 10 to 12 months until the final data analysis. In sporadic cases of new HBV or HCV infection developing in enrolled patients, affected subjects were moved to the infected groups as appropriate when their infective status was established (ie, resolution, persistence).

Genotyping *IFNL3* rs8099917 and rs12979860 polymorphisms were genotyped using a high-resolution melting (HRM) curve analysis on the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany) with the use of 5× HOT FIREPol EvaGreen HRM Mix (Solis BioDyne, Tartu, Estonia). The polymerase chain reaction (PCR) program consisted of an initial step at 95°C for 15 minutes to activate HOT FIREPol DNA polymerase, followed by 50 amplification cycles of denaturation at 95°C for 10 seconds, annealing at 61°C for 10 seconds, and elongation at 72°C for 15 seconds. Amplified DNA fragments were then subjected to the HRM curve analysis with 0.1°C-increments in temperature ranging from 76°C to 96°C. The PCR primers were designed using the Primer3 program (http://bioinfo.ut.ee/primer3-0.4.0/). The primers used for the PCR with the subsequent HRM curve analysis were as follows: rs8099917F 5'TTTGTCACTGTTCCTCCTTTTG3', rs8099917R 5'AAGACATAAAAAGCCAGCTACCA3', rs12979860F 5'CGTGCCTGTCGTGTACTGAA3', and rs12979860R 5'AGGCTCAGGGTCAATCACAG3'.

For quality control, approximately 10% of the randomly chosen samples were regenotyped using the same genotyping method and the concordance rate was 100%. Samples that failed the genotyping were excluded from further statistical analyses.

Statistical analysis Descriptive statistics were shown as percentage values for categorical variables and as a mean with 1 standard deviation or as a median with range for normally and non-normally distributed continuous variables, respectively. Where applicable, the χ^2 test, *t* test, or Mann–Whitney test was applied to compare the data that described selected subgroups of HD patients. Other tests used were indicated in table footnotes.

The χ^2 test was applied to check the Hardy–Weinberg equilibrium. Polymorphisms were tested for associations using the χ^2 test for trend (P_{trend}). Genotype distributions were compared between cases and controls by the standard χ^2 test ($P_{genotype}$). The strength of associations between tested genotypes and selected categorical variables was evaluated by the comparison of a specific phenotype frequency in the carriers of tested genotype(s) with such a frequency in the reference group consisting of major homozygotes in the respective polymorphisms, with the odds ratio (OR) and associated 95% confidence intervals (CIs) as an output. The χ^2 test or Fisher exact test was used, as appropriate, for statistical evaluation of the OR. The results were adjusted, if possible, for parameters that significantly differed between the groups, using a logistic regression analysis.

The multivariate adaptive regression splines (MARSplines) model with generalized cross validation was applied to show the significance of genotypes among selected clinical factors used in HD patients as possible indicators of HCV persistence.

The software used for the above statistical calculations included GraphPadInStat 3.10, 32 bit for Windows, created July 9, 2009 (GraphPad Software, Inc., La Jolla, California, United States), Cytel Studio version 10.0, created January 16, 2013 (CytelStudio Software Corporation, Cambridge,

Massachusetts, United States), and Statistica version 10, 2011 (Stat Soft, Inc., Tulsa, Oklahoma, United States).

The power of the study was determined using the Quanto v.1.2.4 software[22] under the dominant, recessive, and log-additive models assuming an unmatched case-control study design and a 2-tailed α of 0.05. The power was calculated under different ranges of factors, such as the minor allele frequency of 0.18 and 0.34, disease population risk of 0.0012, 0.01, 0.016, and 0.05, and genetic effect (OR) from 0.5 to 8.0.

The pairwise linkage disequilibrium (LD) between the *IFNL3* rs8099917 and rs12979860 polymorphisms was determined using the genotype data from control samples and the Haploview 4.2 software package (http://www.broad.mit.edu/mpg/haploview/). Using r^2 values, the LD was interpreted as weak (<0.3), moderate (0.3–0.8), or strong (>0.8).

Haplotype frequencies were estimated using the Haploview 4.2 software. Statistical significance was assessed using the 1000-fold permutation test.

A *P* value of less than 0.05 was considered to be statistically significant. All probabilities were 2-tailed.

Ethical approval The research design was approved by the Institutional Review Board of the Poznan University of Medical Sciences, Poznań, Poland. All study participants gave their informed consent to participate in the study, and the study was conducted in accordance with the approved guidelines.

RESULTS In all tested groups, *IFNL3* genotypes were distributed in accordance with the HWE. The LD analysis revealed that the *IFNL3* rs8099917 and rs12979860 polymorphisms were in moderate LD (D' = 0.99; r^2 = 0.43; Supplementary material online, *Figure S1*).

There were no significant differences in the distribution of *IFNL3* polymorphic variants (Supplementary material online, *Table S2*) as well as *IFNL3* haplotypes (Supplementary material online, *Table S3*) between controls and all HD patients. Sample power was sufficient (>90%) to detect an association at an OR of 1.5 or higher in a dominant or log-additive mode of inheritance (Supplementary material online, *Table S4*). As HD subjects and controls significantly differed in age (Supplementary material online, *Table S1*), we additionally selected 255 individuals from each group, matched for sex and age as far as possible. Matched HD patients and controls (Supplementary material online, *Table S5*) also did not differ in the distribution of *IFNL3* rs8099917 and rs12979860 polymorphisms (Supplementary material online, *Table S6*) or *IFNL3* haplotypes (Supplementary material online, *Table S3*).

The characteristics of nonresponders and responders to hepatitis B vaccination are shown in TABLE 1. The comparison of these groups did not reveal significant differences in the distribution of *IFNL3* genotypes (TABLE 2; a power analysis in Supplementary material online, *Table S7*) and *IFNL3* haplotypes (Supplementary material online, *Table S3*).

Among HBV-infected patients (characteristics are presented in Supplementary material online, *Table S8*), there was no significant difference in the frequency distribution of polymorphic variants of *IFNL3* between anti-HBs-negative and anti-HBs-positive patients (TABLE 3, results of the power analysis are presented in Supplementary material online, *Table S9*). Statistical results were not influenced in a significant way by the adjustment for parameters significantly differentiating the tested groups (Supplementary material online, *Tables S10* and *S11*).

The entire HD group (n = 1110) included only 63 HCV-positive patients (5.7%). The main data of HCV-infected patients are shown in Supplementary material online, *Table S12*. This small group had to reveal an OR of at least 6.0 for a sufficient sample power (Supplementary material online, *Table S13*). HCV RNA-positive patients differed from the negative ones in the frequency distribution of *IFNL3* rs8099917 and *IFNL3* rs12979860 polymorphic variants (TABLE 4). HCV RNA-positive patients had a 3.5-fold higher risk of *IFNL3* rs8099917 allele G compared with patients in whom HCV infection resolved. A significance was shown in the dominant mode of inheritance for rs12979860 with an OR of 4.50 (95% CI, 1.52–13.4). In addition, the risk of rs12979860 allele T was over 3-fold higher in HCV RNA-positive patients than in negative ones. The CT rs12979860_rs8099917 haplotype was more frequent (*P* = 0.02) in HD patients showing spontaneous HCV clearance than in those who remained HCV RNA-positive (Supplementary material online, *Table S3*).

In the MARSplines model (generalized cross validation, 0.148; corrected R^2, 0.646), the strength of *IFNL3* genotypes as indictors of HCV persistence in the HCV-infected HD group was shown among other factors as follows: age at RRT onset (number of references to this predictor 6), serum alanine aminotransferase activity (6 references), *IFNL3* rs12979860 CC genotype (5 references), serum aspartate aminotransferase activity (4 references), *IFNL3* rs8099917 TT genotype (3 references), and diabetic nephropathy (3 references).

DISCUSSION To our knowledge, this study is the first to examine the effect of *IFNL3* rs8099917 and rs12979860 polymorphisms on response to hepatitis B vaccination and development of anti-HBs in HBV-infected HD patients. Both SNPs were in moderate LD. Our results showed that *IFNL3* polymorphic variants do not affect response to hepatitis B vaccination or development of anti-HBs after natural HBV infection among patients on RRT. The tested *IFNL3* polymorphisms affected spontaneous HCV clearance.

TABLE 1 Demographic, clinical, and laboratory characteristics of nonresponders and responders to hepatitis B vaccination among patients treated with maintenance hemodialysis

Parameter	Nonresponders (n = 134)	Responders (n = 672)	*P* value
demographic/clinical data			
age at RRT onset, y	70.0 (22.0–90.8)	60.8 (11.8–89.8)	<0.0001[a]
RRT vintage, y	3.4 (0.08–12.1)	4.3 (0.09–25.4)	<0.0001[a]
male sex	68 (0.51)	387 (0.58)	0.2[b]
main causes of end-stage renal disease			
diabetic nephropathy	40 (0.30)	200 (0.30)	1.0[b]
hypertensive nephropathy	19 (0.14)	147 (0.22)	0.05[b]
chronic glomerulonephritis	16 (0.12)	84 (0.13)	0.9[b]
chronic tubulointerstitial nephritis	15 (0.11)	70 (0.10)	0.9[b]
laboratory data			
total calcium, mg/dl	8.9 (7.3–12.8)	8.9 (6.0–12.25)	0.5[a]
phosphorus, mg/dl	5.1 (2.0–9.3)	5.1 (1.8–11.3)	0.1[a]
PTH, ng/l	329 (12.9–2.398)	373 (7.3–3.757)	0.1[a]
total ALP, U/l	91 (41–579)	94 (39–1.684)	0.1[a]
ALT, U/l	12 (0.6–107)	13 (2–209)	0.04[a]
AST, U/l	14 (5–56)	14 (3–177)	0.3[a]
GGT, U/l	31 (6–235)	26 (1–682)	0.2[a]

Data are presented as median and range (minimum–maximum) or the number (percentage) of patients.

Conversion factors to SI units are as follows: for calcium, 0.25; for phosphorus, 0.323.

a Mann–Whitney test; **b** χ^2 test

Abbreviations: ALT, alanine aminotransferase; ALP, total alkaline phosphatase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; PTH, parathyroid hormone; RRT, renal replacement therapy

The lack of association with anti-HBs formation might be due to the fact that IFN-λ3 plays a considerable role in immune response by activation of the Th1 pathway enhancing the function of cytotoxic lymphocytes.[23] Therefore, IFN-λ3 enhances cellular immune response, whereas the Th2 pathway primarily results in antibody formation. Additionally, animal studies showed that the addition of IFN-λ3 to immunizations either did not increase the antibody titers or increased only the titers of IgG2a antigen-specific antibodies, produced after the stimulation of the Th1 pathway.[4,24] Thus, IFN-λ3 as such and *IFNL3* polymorphisms would not affect the production of neutralizing antibodies also in humans, which is in line with our results. However, at least 2 aspects have to be emphasized. First, Th1/Th2 cytokines are functionally linked, and the abnormal expression of the Th1 cytokine pathway may alter Th2-cell activation. CCL23, a chemokine promoting Th1, was increased in nondialyzed patients with severe chronic kidney disease.[25] In dialysis patients, increased blood levels of Th1 cytokines involved in the IFN-γ production, such as IL-12[26] or free IL-18,[26] were noted. Genes encoding IL-18 (*IL18* rs360719) and IL-12B p40 (*IL12B* rs321227) were linked to the development of anti-HBs in HD patients.[27,28] On the other hand, the monocyte chemotactic protein 1 gene (*MCP1*-2518 A/G rs1024611) was not associated with anti-HBs formation in response to HBV vaccination,[29] although MCP1 is involved in Th2 polarization.[30] Secondly, our study was conducted in patients on RRT, who have impaired immune response. It is possible that the effect of the *IFNL3* SNPs is not pronounced in these patients and might have a different effect in healthy subjects.

TABLE 2 Distribution of *IFNL3* genotypes, rs8099917 and rs12979860, in responders and nonresponders to hepatitis B vaccination among hemodialysis patients

IFNL3 genotype	Nonresponders	Responders	Odds ratio (95% CI)	*P* value[a]	P_{trend}	$P_{genotype}$
rs8099917	n = 134	n = 671				
TT	95 (0.71)	432 (0.64)	reference	–	0.3	0.3
GT	34 (0.25)	222 (0.33)	0.696 (0.456–1.064)	0.09		
GG	5 (0.04)	17 (0.03)	1.337 (0.481–3.716)	0.6[b]		
GT+GG	39 (0.29)	239 (0.36)	0.742 (0.495–1.112)	0.2		
MAF	44 (0.16)	256 (0.19)	0.833 (0.587–1.183)	0.3		
HWE	*P* = 0.7	*P* = 0.2				
rs12979860	n = 134	n = 666				
CC	59 (0.44)	283 (0.42)	reference	–	0.7	0.9
CT	61 (0.46)	310 (0.47)	0.944 (0.637–1.398)	0.8		
TT	14 (0.10)	73 (0.11)	0.920 (0.487–1.740)	0.8		
CT+TT	75 (0.56)	383 (0.58)	0.939 (0.646–1.366)	0.7		
MAF	89 (0.33)	456 (0.34)	0.955 (0.723–1.262)	0.7		
HWE	*P* = 0.95	*P* = 0.7				

a χ^2 test; **b** Fisher exact test

Abbreviations: CI, confidence interval; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency

TABLE 3 Distribution of *IFNL3* genotypes, rs8099917 and rs12979860, among patients with a history of hepatitis B infection who were either positive or negative for anti-HBs

IFNL3 genotype	Anti-HBs negative	Anti-HBs positive	Odds ratio (95% CI)	*P* value[a]	P_{trend}	$P_{genotype}$
rs8099917	n = 55	n = 185				
TT	30 (0.54)	120 (0.65)	reference	–	0.4	0.1
GT	24 (0.44)	55 (0.30)	1.745 (0.935–3.260)	0.08		
GG	1 (0.02)	10 (0.05)	0.400 (0.049–3.249)	0.7[b]		
GT+GG	25 (0.45)	65 (0.35)	1.538 (0.835–2.834)	0.2		
MAF	26 (0.24)	75 (0.20)	1.217 (0.733–2.023)	0.4		
HWE	*P* = 0.3	*P* = 0.6				
rs12979860	n = 55	n = 181				
CC	23 (0.42)	77 (0.425)	reference	–	0.7	0.8
CT	22 (0.40)	77 (0.425)	0.957 (0.492–1.859)	0.9		
TT	10 (0.18)	27 (0.15)	1.240 (0.523–2.937)	0.6		
CT+TT	32 (0.58)	104 (0.57)	1.030 (0.559–1.899)	0.9		
MAF	42 (0.38)	131 (0.36)	1.089 (0.701–1.692)	0.7		
HWE	*P* = 0.5	*P* = 0.6				

a χ^2 test; **b** Fisher exact test

Abbreviations: see **FIGURE 1** and **TABLE 2**

TABLE 4 Distribution of *IFNL3* genotypes, rs8099917 and rs12979860, among HCV RNA-positive patients and anti--HCV-positive or HCV RNA-negative patients

IFNL3 genotype	HCV RNA positive	Anti-HCV positive/ HCV RNA negative	Odds ratio (95% CI)	*P* value[a]	P_{trend}	$P_{genotype}$
rs8099917	n = 39	n = 24				
TT	23 (0.59)	20 (0.83)	reference	–	0.03	0.097
GT	13 (0.33)	4 (0.17)	2.826 (0.793–10.075)	0.1		
GG	3 (0.08)	0 (0)	6.106 (0.297–125.45)	0.2		
GT+GG	16 (0.41)	4 (0.17)	3.478 (0.997–12.130)	0.05		
MAF	19 (0.24)	4 (0.08)	3.542 (1.125–11.154)	0.03		
HWE	*P* = 0.6	*P* = 0.7				
rs12979860	n = 39	n = 24				
CC	12 (0.31)	16 (0.67)	reference	–	0.006	0.02
CT	21 (0.54)	7 (0.29)	4.000 (1.283–12.468)	0.03		
TT	6 (0.15)	1 (0.04)	8.000 (0.847–75.597)	0.09		
CT+TT	27 (0.69)	8 (0.33)	4.500 (1.516–13.355)	0.009		
MAF	33 (0.42)	9 (0.19)	3.178 (1.354–7.457)	0.007		
HWE	*P* = 0.5	*P* = 0.8				

a Fisher exact test

Abbreviations: see **FIGURE 1** and **TABLE 2**

In our study, 38.1% of HD subjects showed HCV clearance, which is in line with a wide range of reported findings.[31] A recent study by Yu et al[32] showed that *IFNL3* rs8099917 affects spontaneous HCV clearance in Taiwanese HD patients (rs12979860 was not examined). Generally, our study confirmed this finding and, additionally, showed that *IFNL3* rs12979860 rather than *IFNL3* rs8099917 is the main factor considerably affecting the outcome of HCV infection among Polish HD patients. Additionally, the MARSplines analysis showed a more pronounced role of rs12979860, compared with rs8099917, among the indicators of HCV RNA positivity also among other clinical and laboratory variables. It is also noteworthy that HD patients showing the CT rs12979860_rs8099917 haplotype have a greater chance of spontaneous HCV clearance.

However, our HCV-positive population consisted of a limited number of subjects, and a statistical significance was not corrected for multiple testing. On the other hand, if the already

established associations are investigated in more detail in a more uniform cohort, the lack of such a correction seems to be justified.[33,34] Studies on a larger HCV-positive HD population, ensuring sufficient sample power at lower genetic effects, might provide more insight into this topic.

The mechanisms by which *IFNL3* polymorphisms exert their effect on the outcome of patients with hepatitis C have not been fully elucidated. Compared with TT genotype carriers, patients with rs12979860 major allele homozygosity were found to have higher inhibitory natural killer cell receptor levels among the subpopulation of natural killer cells with high cytotoxic properties, a decreased number of lymphocytes expressing tumor necrosis factor-related apoptosis-inducing ligand,[35] and higher production of antiviral IFN-λ3.[6] However, the IFN-λ3 production and its blood concentrations in HD patients remain unknown. *IFNL3* rs12979860 was also reported to affect the expression of genes associated with the HCV pathway.[36] The impact of *IFNL3* SNPs on HCV clearance might be also connected with the fact that another functional SNP, rs28416813, influences the expression of the downstream reporter gene due to its proximity to a binding site of NF-κB.[37] This SNP is in high LD with rs12979860, rs4803219, rs8103142, and rs4803217 polymorphisms,[37,38] whereas the rs8099917 SNP is in low LD with these 4 polymorphic variants.[38]

In summary, the current study has shown that the tested *IFNL3* polymorphisms are not associated with anti-HBs production in response to hepatitis B vaccination or HBV infection, but may affect spontaneous HCV clearance in HD patients. Our conclusion on the spontaneous resolution of HCV infection in HD patients has a broader significance, as it shows that *IFNL3* polymorphisms may play a role in HCV clearance not only in uremic patients, but also among other groups with compromised immune response. Moreover, because the HCV vaccine has not been elaborated for clinical use so far, HD patients not possessing favorable genotypes (*IFNL3* rs8099917 TT and *IFNL3* rs12979860 CC) could be under special supervision in HD facilities. Despite some previous data showing an association of *IFNL3* SNPs with HBV infection in individuals with preserved renal function, our study does not indicate such a relationship in HD subjects.

Contribution statement AEG and PPJ conceived the idea for the study. AEG and EJ were involved in data collection. AEG analyzed the data. AM performed genetic testing and statistical analysis. EJ drafted the manuscript. AEG prepared the final version of the manuscript. AEG and PPJ coordinated funding for the project. All authors edited and approved the final version of the manuscript.

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Supplementary material online Supplementary material is available with the online version of the article at www.pamw.pl.

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ARTYKUŁ ORYGINALNY

Wpływ polimorfizmów genu dla interferonu-λ3, rs8099917 oraz rs12979860, na odpowiedź na szczepienie przeciwko wirusowi zapalenia wątroby typu B oraz zakażenie wirusem zapalenia wątroby typu B lub C wśród pacjentów hemodializowanych

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SŁOWA KLUCZOWE

gen IFN-λ3, hemodializa, szczepienie, wirus zapalenia wątroby typu B, wirus zapalenia wątroby typu C

STRESZCZENIE

WPROWADZENIE Wśród pacjentów hemodializowanych nadal obserwuje się większą częstość występowania oraz większe ryzyko zakażenia wirusem zapalenia wątroby typu B (*hepatitis B virus* – HBV) lub C (*hepatitis C virus* – HCV) niż u osób zdrowych. Interferony (IFN) są znane ze swojego wpływu na odpowiedź immunologiczną. Dodatek IFN-λ3 do immunizacji na modelach zwierzęcych zwiększał odpowiedź immunologiczną limfocytów pomocniczych T typu 1.

CELE Wśród grupy pacjentów hemodializowanych zbadano wpływ polimorfizmów genu dla IFN-λ3 (*IFNL3*) na wytworzenie przeciwciał przeciw antygenowi powierzchniowemu HBV (anty-HBs) w odpowiedzi na szczepienie przeciwko HBV lub zakażenie HBV oraz samoistną eliminację HCV w przebiegu zakażenia tym wirusem.

PACJENCI I METODY Grupa pacjentów hemodializowanych składała się z 806 osób, które nigdy nie przebyły zakażenia HBV lub HCV (672 z nich wytworzyło przeciwciała anty-HBs w odpowiedzi na szczepienie), 241 pacjentów zakażonych HBV (186 spośród nich wytworzyło przeciwciała anty-HBs) oraz 63 pacjentów zakażonych HCV (w tym 39 HCV RNA-pozytywnych). U wszystkich pacjentów zbadano genotypy polimorfizmów rs8099917 oraz rs12979860 w *IFNL3* za pomocą analizy krzywych topnienia o wysokiej rozdzielczości.

WYNIKI Porównanie dystrybucji genotypów *IFNL3* u osób, które odpowiedziały i nie odpowiedziały na szczepienie przeciwko HBV, nie wykazało statystycznie istotnych różnic między grupami. Wśród pacjentów zakażonych HBV różnice w rozkładzie wariantów polimorficznych *IFNL3* również nie były istotne między pacjentami anty-HBs-ujemnymi i anty-HBs-dodatnimi. Samoistna eliminacja HCV występowała istotnie rzadziej u pacjentów mających allel G polimorfizmu rs8099917 albo allel T polimorfizmu rs12979860, natomiast haplotyp CT rs12979860_rs8099917 występował częściej ($p = 0,02$) u pacjentów wykazujących spontaniczną eliminację HCV.

WNIOSKI U pacjentów hemodializowanych polimorfizmy w *IFNL3* nie wpływają na wytworzenie przeciwciał anty-HBs w odpowiedzi na infekcję lub szczepienie przeciwko HBV, natomiast wydają się związane z samoistną eliminacją HCV w przebiegu infekcji.

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