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Interferon-λ3 Gene Polymorphic Variants, rs4803217 and rs12980275, Responsiveness to HBV Vaccine and Outcome of HBV and HCV Exposure in Hemodialyzed Patients

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Abstract

Background: In non-uremic populations, rs4803217 in the *IFNL3* messenger RNA 3' untranslated region or rs12980275 downstream of *IFNL3* is connected with the spontaneous or therapeutic clearance of HCV and HBV, and rs12980275 is correlated with plasma IFN-λ3 levels. Moreover, rs12980275 is associated with the sustained virological response following antiviral therapy of chronic hepatitis C in hemodialysis patients.

Objectives: We investigated *IFNL3* polymorphisms, rs4803217 and rs12980275, for association with responsiveness to HBV vaccine and natural consequences of HBV and HCV exposure among hemodialyzed individuals.

Methods: The capacity to produce protective anti-HBs titers was recognized if they were $\geq$ 10 IU/L after vaccination or natural exposure. The *IFNL3* rs4803217 (G>T) and rs12980275 (A>G) genetic variants were analyzed using a high-resolution melting curve method in 1,337 hemodialysis subjects. Plasma IFN-λ3 was determined in 188 individuals using ELISA. The Kaplan-Meier method was applied for the analysis of survival probability.

Results: The tested polymorphisms did not show associations with the capacity to generate protective anti-HBs titers after HBV vaccination or exposition and self-limitation of HBV exposure. Natural HCV clearance was connected with the *IFNL3* rs4803217 GG genotype (OR: 3.036, 95% CI: 1.544 - 5.969, P = 0.001) and haplotypes comprising at least two more frequent alleles but without any variant allele of *IFNL3/IFNL4* genetic variants (P < 0.05). Plasma IFN-λ3 levels were not directly influenced by *IFNL3* rs4803217 and rs12980275, but differed concerning HBV/HCV serum markers (P = 0.00005) and firmly correlated with anti-HBs titers (r = 0.537, P = 4.15E-16). Both tested polymorphisms were not significantly associated with the survival of hemodialysis patients.

Conclusions: Genotyping *IFNL3* rs4803217 may be advantageous in the prognosis of natural HCV clearance but does not predict the self-limitation of HBV exposure, responsiveness to HBV vaccine, or hemodialysis patients' mortality.

Keywords: Genetic Polymorphisms, Hemodialysis, Hepatotropic Viruses, Interferon-λ3, Vaccination, Viral Clearance

1. Background

Exploration of factors contributing to the outcomes of hepatotropic viral infections and affecting prophylactic vaccination results are essential for the general population, especially subjects with altered immune competence. The latter group includes patients with end-stage renal disease (ESRD), mainly those receiving hemodialysis (HD).

Polymorphic variants of interferon (IFN)-λ genetic region (*IFNL*) are continuously explored for their associations with hepatitis C virus (HCV) clearance or hepatitis B virus (HBV) limitation. The *IFNL4* polymorphisms, rs12979860 and rs8099917, formerly *IFNL3* rs12979860 (1) and *IFNL3* rs8099917 (2), became known as the predictors of spontaneous (3) or INF-based regimen-induced HCV clearance (4-6). Less consistent are the results of the association between *IFNL* variants and HBV limitation (7, 8). Variants rs8099917 and rs12979860 show no association with the generation of antibodies to HBV surface antigen (anti-HBs) after HBV vaccination or exposition (9). In non-uremic populations, rs4803217 in the *IFNL3* messenger RNA 3' untranslated region (UTR) or rs12980275 downstream of *IFNL3*

was associated with spontaneous or therapeutic clearance of HCV (10-12) and HBV (13, 14), and rs12980275 was correlated with plasma IFN-λ3 levels (15). Moreover, rs12980275 was connected with the sustained virological response following antiviral therapy of chronic hepatitis C in HD patients (16). Both *IFNL3* and *IFNL4* are located on chromosome 19q13.2 at a distance of 1.3 kb (Appendix 11).

Besides, IFN-λ3, formerly interleukin-28B, is the protein product attributed to *IFNL3*. In a group containing chronic HBV-infected patients, self-limited HBV infection individuals, and healthy subjects, circulating IFN-λ3 levels were positively associated with major homozygosity in rs8099917, rs12979860, and rs12980275, located within *IFNL* on chromosome 19 (15). In HD patients treated with low permeable dialysis membranes, individuals showing rs8099917 major homozygosity (TT) presented higher IFN-λ3 levels than those harboring the G allele. Such a correlation was not revealed for rs12979860 (17).

In Chinese non-uremic individuals, higher levels of circulating IFN-λ3 were associated with HCV clearance (18) and self-limited HBV infection (15). However, chronic hepatitis C subjects from Japan demonstrated higher IFN-λ3 than healthy individuals before treatment (2). Our data on HD patients revealed that plasma IFN-λ3 was positively correlated with anti-HBs titers generated after vaccination or exposure to HBV (17, 19, 20). Plasma IFN-λ3 levels were positive predictors of survival in prevalent HD patients (20). The variant homozygosity (GG) of rs8099917 predicted a lower probability of survival in a prospective study of HD patients (21). The *IFNL3* polymorphic variants were not analyzed concerning the survival of HD subjects.

2. Objectives

This study aimed to investigate whether *IFNL3* rs4803217 and rs12980275 downstream of *IFNL3* are associated with the anti-HBs generation, self-limitation/spontaneous resolution of HBV/HCV infection, plasma IFN-λ3, and survival of HD patients. Additionally, we analyzed haplotypes of *IFNL3* and *IFNL4* concerning HCV spontaneous clearance.

3. Methods

3.1. Patients

The HD population (n = 1337), previously described concerning *IFNL4* polymorphic variants and anti-HBs generation (22), was qualified for genotyping *IFNL3* polymorphic variants. To be included, all patients not exposed to HBV had to undergo a completed vaccination series as proposed by the Advisory Committee on Immunization Practices Centers for Disease Control and Prevention and have the status of "responders" or "non-responders" to HBV vaccination (23). All patients were Caucasians.

The HD group included 942 patients with no evidence of exposition to HBV (having repeatedly negative results for total antibodies against HBV core antigen - anti-HBc) or HCV (having repeatedly negative results of HCV RNA and antibodies to HCV - anti-HCV), 241 patients with serological markers of exposition to HBV, 80 patients exposed to HCV, and 74 patients exposed to both of these viruses. Subjects with serological markers of HBV infection were included in the HBV-infected group (n = 315), and those exposed to HCV were assigned to the HCV-infected group (n = 154). Individuals showing the acute phase of both infections were excluded. The HBV-exposed patients (n = 315) included HBsAg-positive subjects (n = 31) and individuals who self-limited infection (HBsAg-negative, anti-HBc positive, n = 284). Patients who self-limited HBV infection included subjects generated anti-HBs (n = 252), and patients presenting isolated anti-HBc positivity (n = 32). Three HBsAg-positive patients were also able to generate anti-HBs.

The HBV vaccinated group was composed of 1,022 individuals. Vaccines containing the recombinant HBV surface antigen (HBsAg) (Engerix B, GlaxoSmithKline Biologicals, Belgium; Hepavax-Gene, BIOMED SA, Poland; and Euvax B, LG Chemical, South Korea) were administered for immunization. The HD subjects were diagnosed as being able to generate protective amounts of anti-HBs if their circulating anti-HBs titers gained at least once a value of $\geq$ 10 IU/L after HBV vaccination or exposure. The highest individual anti-HBs titers shown in the course of HD were analyzed for associations with tested polymorphisms. Anti-HBs titers exceeding 1000 IU/L were referred to as indicating overreacted response (24). The entire HD group included 154 HCV-exposed patients (11.5% of 1337). Eighty subjects were in the vaccinated group and 74 in the HBV-exposed group. The HCV RNA positivity was detected in 93 patients. Enrolled patients exposed to HBV or HCV were never treated with antiviral medications designated for infections with these viruses.

The plasma IFN-λ3 concentration was determined in 188 HD subjects in fixed clinical conditions. Blood probes for IFN-λ3 and standard chemistry were taken on an empty stomach before the HD procedure was performed in the middle of the week.

3.2. Survival Studies

All patients successfully genotyped for *IFNL3* single nucleotide polymorphisms (SNPs) were included in the retrospective longitudinal survival study. The tested period started at the beginning of renal replacement therapy (RRT) of each patient and ended in November 2019. Additionally, 402 patients tested for rs4803217 and 391 pa-

tients tested for rs12980275, who were prospectively observed since January 20, 2009 (22), were analyzed concerning survival in November 2019 (the period of the prospective study was 10.81 years).

3.3. Laboratory Determinations

The commercial ELISA kit specific for IFN-λ3 (Human Interleukin 28B (IL-28B) ELISA Kit, Sunred Biological Technology Co., Ltd., Shanghai, China) was applied for measuring the plasma IFN-λ3 level at 450 nm in an ELISA plate reader (Infinite F50, Tecan Group Ltd., Männedorf, Switzerland). The sensitivity of this kit was 0.65 ng/L. The intra-assay and the inter-assay coefficients of variation were below 10 and 12%, respectively. Microparticle enzyme immunoassay (MEIA) technology (ABBOTT, Germany) or chemiluminescent microparticle immunoassay (CMIA) method (ABBOTT, Ireland) was applied to estimate anti-HBs titers. Values of anti-HBs titers were presented numerically up to 1000 IU/L; values $\geq$ 1000 IU/L were shown as 1000 IU/L in result descriptions and statistical calculations.

3.4. Genotyping

Patients' blood probes for genotyping were obtained from December 30, 2008, to January 15, 2018. The DNA material was frozen. The *IFNL3* SNPs (rs4803217, rs12980275) were genotyped using high-resolution melting (HRM) curve analysis. Besides, *IFNL4* rs8099917 and *IFNL4* rs12979860 SNPs, previously tested in the examined patients, were also genotyped by HRM (9, 17, 22). Appendix 1 presents HRM conditions applied for the identification of tested SNPs. Successful genotyping was conducted in 1,327 patients for rs4803217 and 1,311 individuals for rs12980275. For quality control, about 10% of the probes were genotyped again using the same method; the concordance rate was 100%.

3.5. Statistics

We used the Shapiro-Wilk test to determine how continuous variables were distributed. The percentages for dichotomous variables and medians with ranges for continuous parameters are shown. The characteristics of HD patients were analyzed concerning *IFNL3* SNPs using dominant, recessive, and additive inheritance models. The Mann-Whitney test was used to compare continuous parameters. The Fisher's exact test was applied to check differences in dichotomous data. The chi-square test was used to evaluate the agreement with Hardy-Weinberg Equilibrium (HWE). The SNP frequencies were computed using Fisher's exact test or Cochran-Armitage test (P_{trend}) to show tendencies in associations. Distributions of genotypes were evaluated by the chi-square test ($P_{genotype}$).

Odds ratios (ORs) and their 95% confidence intervals (CIs) were computed using Fisher's exact test. Logistic regression was applied to demonstrate whether tested SNPs were significant predictors of spontaneous HCV clearance among demographic and clinical variables. Patient characteristics chosen for this analysis included age, RRT duration, and chronic glomerulonephritis because they were significantly different between groups with spontaneous HCV clearance and persistent HCV infection. All-cause, cardiovascular, infection-related, and neoplasm-related reasons for death were analyzed in dominant, recessive, and additive inheritance models. Additionally, survival analyses were performed separately in patients exposed to HCV or HBV. The Kaplan-Meier method with the log-rank test was applied to show dissimilarities in survival. The P values below 0.05 were accepted as presenting significance. The Bonferroni correction was applied in examinations of SNP results. All analyses mentioned above were computed using Statistica version 12 (Stat Soft, Inc., Tulsa, OK, USA).

As *IFNL3* and *IFNL4* show close positions on chromosome 19 (Appendix 11), pair-wise linkage disequilibrium (LD) between their polymorphisms was calculated. D' and r^2 were obtained using Haploview 4.2 software (http://www.broad.mit.edu/mpg/haploview/). We calculated haplotype frequencies with the same software. The 1000-fold permutation test was used to show the significance of the data.

3.6. Compliance with Ethical Standards

The Institutional Review Board of Poznan University of Medical Sciences, Poland, accepted the concept of our investigations. All patients or their parents, as appropriate, provided written informed consent for research.

4. Results

4.1. HD Patients' Data

Table 1 shows the characteristics of the examined patients. In the entire HD group, the distribution of *IFNL3* polymorphic variants followed HWE (P = 0.9 for rs4803217 and P = 0.7 for rs12980275). In analyses performed in inheritance models, patients' data did not show significant differences regarding *IFNL3* rs4803217 and rs12980275 variants if the Bonferroni correction was applied (Supplementary Tables 2 and 3, respectively). However, uncorrected data of all examined HD patients indicated that bearers of the rs4803217 variant allele (T) showed a higher susceptibility for HCV RNA positivity (OR: 2.024, 95% CI: 1.267 - 3.234, P = 0.003) (Appendix 2).

Table 1. Essential Characteristics of HD Patients Concerning Infections with HBV and HCV [a, b]

Parameter	HD Patients not Exposed to HBV/HCV, N = 942 (A)	HD Patients Exposed to HBV, N = 241 (B)	HD Patients Exposed to HCV, N = 80 (C)	HD Patients Exposed to HBV And HCV, N = 74 (D)	P-Value [c]	Subgroups Showing Significant Differences [d]
Male gender	521 (55.3)	134 (55.6)	39 (48.8)	40 (54.1)	0.7	
Age at RRT onset, y	62.2 (11.8 - 91.7)	61 (19 - 90.1)	53.3 (8.7 - 85.3)	46.2 (14.1 - 85.9)	< 0.000001	A-C, A-D, B-C, B-D
Diabetic nephropathy	292 (31.0)	74 (30.7)	15 (18.8)	18 (24.3)	0.09	
Chronic glomerulonephritis	119 (12.6)	29 (12.0)	24 (30.0)	18 (24.3)	0.0001	A-C, A-D, B-C, B-D
Hypertensive nephropathy	203 (21.5)	46 (19.1)	12 (15.0)	7 (9.4)	0.048	A-D
RRT duration, y	5.2 (0 - 28.3)	5.6 (0 - 28)	9.6 (0 - 30.8)	11.2 (0.2 - 32.3)	< 0.000001	A-C, A-D, B-C, B-D
Body mass index, kg/m^2	25.9 (14.3 - 59.2)	25.1 (15.7 - 38.3)	23.7 (15.4 - 39.5)	23.0 (15.2 - 35.5)	0.000006	A-C, A-D
HBsAg positivity	N/A	23 (9.5)	N/A	8 (10.8)	0.7	
Not generating anti-HBs after HBV vaccination or infection	148 (15.7)	44 (18.3)	11 (13.8)	19 (25.7)	0.1	
Anti-HBs titer, IU/l	161 (0 - 1000)	250.3 (0 - 1000)	259 (0 - 1000)	197.5 (0 - 1000)	0.4	
HCV RNA positivity	N/A	N/A	48 (60.0)	45 (60.8)	0.9	
ALT, IU/L	13 (0.6 - 131)	15.0 (2 - 95)	15.5 (2 - 135)	18 (2 - 195)	< 0.000001	A-B, A-C, A-D
AST, IU/L	15 (3 - 177)	17 (3 - 81)	18 (8 - 97)	18.5 (8 - 152)	< 0.000001	A-B, A-C, A-D
ALP, IU/L	97 (12.3 - 1684)	97.1 (13.5 - 1353.3)	102.3 (52 - 400.8)	107.5 (15 - 803.8)	0.9	
GGT, IU/L	30 (1 - 682)	25 (4 - 692)	32.3 (7 - 472)	34 (7 - 498)	0.1	
25(OH)D, ng/mL [e]	13.8 (3 - 42)	13.2 (4.9 - 37.6)	14.3 (8 - 18.6)	10.1 (5.7 - 20)	0.4	

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; Anti-HBc, antibodies against core antigen of hepatitis B virus; Anti-HCV, antibodies against hepatitis C virus; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HBsAg, surface antigen of hepatitis B virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HCV RNA, ribonucleic acid of hepatitis C virus; HD, hemodialysis; RRT, renal replacement therapy.

[a] Conversion to SI units: to change 25(OH)D to nmol/L, multiply by 2.496, alanine aminotransferase to μkat/L, by 0.0167, alkaline phosphatase to μkat/L, by 0.0167, aspartate aminotransferase to μkat/L, by 0.0167, gamma-glutamyltransferase to μkat/L, by 0.0167.

[b] Median and range or the number of individuals showing the tested variable with the percentage of all studied subjects are presented.

[c] χ^2 test for 2x4 or 2x2 contingency tables for dichotomous data and Kruskal-Wallis test for continuous data.

[d] χ^2 test with Bonferroni correction for dichotomous variables and post hoc test for continuous variables.

[e] n = 265.

4.2. IFNL3 Polymorphisms and Anti-HBs

In the HBV-vaccinated patients and HBV-exposed group, we tested the capacity to produce anti-HBs, titers of anti-HBs, the prevalence of anti-HBs titers $\geq$ 1000 IU/L, and anti-HBs titers when patients who did not generate protective levels of anti-HBs were excluded. Any differences in the frequency of ability to produce protective anti-HBV titers and the quantity of immunization after HBV infection were presented concerning *IFNL3* rs4803217 (Appendix 5) and *IFNL3* rs12980275 (Appendix 7) polymorphic variants. However, in HBV-exposed subjects, higher anti-HBs titers were shown in individuals bearing two G alleles of *IFNL3* rs4803217 (P = 0.02 without Bonferroni correction, Appendix 6).

4.3. IFNL3 Polymorphic Variants and Self-Limitation of HBV Infection

The HD patients who remained HBsAg positive after HBV exposition did not differ from HBsAg negative patients in the distribution of *IFNL3* polymorphic variants (Appendix 6). We also analyzed differences in the frequency of *IFNL3* variant distribution between HbsAg-positive individuals not generating anti-HBs and HBsAg negative subjects generating anti-HBs (Appendix 8) and patients with isolated anti-HBc positivity (Appendix 9). All of these analyses yielded no significant results.

4.4. IFNL3 Polymorphisms and Spontaneous HCV Resolution

In HCV-exposed HD patients, spontaneous HCV elimination was correlated with tested *IFNL3* polymorphic variants (Table 2). Individuals homozygous for the *IFNL3* rs4803217 major allele (G) showed over three-fold higher probability for spontaneous HCV clearance. There was also a trend for association between the homozygosity of the rs12980275 major allele (A) and spontaneous HCV elimination.

As expected, HCV RNA-positive patients showed higher serum activities of liver enzymes than HCV RNA-negative

Table 2. *IFNL3* Polymorphic Variants and HCV RNA Positivity in HD Patients Exposed to HCV [a]

Genotypes, MAF	HCV RNA Positive Patients	HCV RNA Negative Patients	Odds Ratio (95% CI), P-Value [b]
***IFNL3* rs4803217 (n = 153, P_{trend} [c] = 0.0009, $P_{genotype}$ [d] = 0.003)**			
GG	26 (28.6)	34 (54.8)	Reference
GT	50 (54.9)	24 (38.7)	2.724 (1.346 - 5.516), 0.005 [e]
TT	15 (16.5)	4 (6.5)	4.904 (1.454 - 16.53), 0.008 [e]
GT + TT vs GG	65 (71.4)	28 (45.2)	3.036 (1.544 - 5.969), 0.001
TT vs GG + GT	15 (16.5)	4 (6.5)	2.862 (0.902 - 9.081), 0.08
MAF	(0.44)	(0.26)	2.255 (1.371 - 3.708), 0.002
***IFNL3* rs12980275 (n = 149, P_{trend} [c] = 0.001, $P_{genotype}$ [b] = 0.03 [e])**			
AA	28 (31.8)	33 (54.1)	Reference
AG	45 (51.1)	22 (36.1)	2.411 (1.177 - 4.936), 0.02 [e]
GG	15 (17.0)	6 (9.8)	2.946 (1.008 - 8.61), 0.08
AG + GG vs AA	60 (68.1)	28 (45.9)	2.526 (1.287 - 4.957), 0.007 [e]
GG vs AA + AG	15 (17.0)	6 (9.8)	1.884 (0.686 - 5.168), 0.2
MAF	(0.43)	(0.28)	2.521 (1.504 - 4.223), 0.01 [e]

Abbreviations: HCV, hepatitis C virus; *IFNL3*, interferon λ3 gene; HD, hemodialysis; MAF, minor allele frequency; RNA, ribonucleic acid.
[a] Values are expressed as No. (%) unless otherwise indicated.
[b] Fisher's exact test.
[c] Cochran-Armitage Trend Test.
[d] Pearson's chi-squared test.
[e] Not significant after Bonferroni correction (P > 0.004).

individuals (Table 3). Additionally, the group with HCV RNA positivity was younger but longer on RRT and revealed chronic glomerulonephritis as a more frequent cause of ESRD than HCV RNA negative individuals. Therefore, we analyzed whether the GG homozygosity of rs4803217 was associated with spontaneous HCV clearance if age, RRT duration, and chronic glomerulonephritis were simultaneously used as possible prognostic factors for spontaneous resolution of HCV infection. A similar multivariate analysis was performed for the AA homozygosity of rs12980275. The rs4803217 GG homozygosity (OR: 2.91, 95% CI: 1.42 - 5.97, P = 0.003) and rs12980275 AA homozygosity (OR: 2.92, 95% CI: 1.42 - 6.00, P = 0.003) were positive predictors of spontaneous HCV clearance, whereas longer RRT duration was a negative predictor (OR: 0.94, 95% CI: 0.88 - 0.99, P = 0.03 together with rs4803217 and OR: 0.94, 95% CI: 0.88 – 1.00, P = 0.038 in the model with rs12980275).

4.5. Linkage Disequilibrium

We examined LD between rs12980275, rs4803217, rs12979860, and rs8099917 variants. The strongest LD was between rs4803217 and rs12979860 (r^2 = 0.81), and the weakest was shown between rs4803217 and rs8099917 (r^2 = 0.34) (Appendix 11).

4.6. IFNL3 and IFNL4 Haplotype Analysis

We analyzed haplotypes of *IFNL3* and *IFNL4* concerning the outcome of HCV infection (Table 4). Spontaneous HCV clearance was connected with haplotypes comprising two or more frequent alleles but without variant alleles of the examined IFNL SNPs. The most substantial relationship with spontaneous HCV clearance was demonstrated for haplotype rs12979860-C_rs8099917-T.

4.7. Plasma IFN-λ3

Circulating IFN-λ3 levels did not differ regarding tested *IFNL3* SNPs. An analysis in subgroups separated by HD modality (low-flux HD, high-flux HD, and hemodiafiltration) also did not reveal significant differences in plasma IFN-λ3 concentrations (Appendix 10). The exclusion of HCV RNA positive and HBsAg positive subjects from the analysis did not show any association between plasma IFN-λ3 and tested polymorphisms.

The HCV RNA positive (IFN-λ3 74.0, 4.9 - 240 ng/L) and HBsAg positive (IFN-λ3 17.0, 9.0 - 57.7 ng/L) patients showed significantly lower plasma IFN-λ3 concentrations than patients who composed a group of subjects not exposed to HBV/HCV infections, those who had self-limited HBV infection, and those who spontaneously resolved HCV infection (IFN-λ3 92.0, 0.24 - 240 ng/L). The latter three subgroups did not differ in plasma IFN-λ3 concentrations (Kruskal-Wallis test P-value = 0.2). The HBsAg positive patients (including five HBV DNA positive patients and one subject not currently tested for HBV DNA) had lower IFN-λ3 levels than HCV RNA positive subjects (Figure 1).

There were positive correlations between circulating IFN-λ3 concentrations and anti-HBs titers in all HD patients (r = 0.537, P = 4.15E-16), as well as HBV-vaccinated (r = 0.539,

Table 3. Demographic, Clinical, and Laboratory Data of HD Patients Concerning Spontaneous HCV Clearance [a, b]

Parameters	HD Patients Persistently HCV RNA Positive, n = 89	HD Patients Who Spontaneously Cleared HCV, n = 63	P-Value [c]
Male gender	48 (53.9)	30 (47.6)	0.5
Age at RRT onset, y	46.2 (8.7 - 79.5)	53.3 (14.1 - 85.9)	0.01
Diabetic nephropathy	15 (16.9)	17 (27.0)	0.2
Chronic glomerulonephritis	32 (36.0)	10 (15.9)	0.009
Hypertensive nephropathy	11 (12.4)	8 (12.7)	1.0
RRT duration, y	14.7 (1.1 - 31.2)	7.9 (0.2 - 32.4)	0.001
BMI, kg/m^2	22.9 (15.2 - 33.5)	23.6 (16.9 - 37.9)	0.1
HBsAg positivity	4 (4.5)	4 (6.3)	0.7
Not generating anti-HBs	15 (16.9)	15 (23.8)	0.3
Anti-HBs titer, IU/L	175.6 (0 - 1000)	289.5 (0 - 1000)	0.9
Anti-HBc positivity	44 (49.4)	30 (47.6)	0.9
ALT, IU/L	24 (2 - 195)	13 (3 - 63)	0.00003
AST, IU/L	23 (8 - 152)	15 (8 - 46)	0.00007
ALP, IU/L	113.2 (15 - 647.3)	88 (45 - 803.8)	0.01
GGT, IU/L	47 (7 - 498)	26.5 (7 - 261)	0.002

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; Anti-HBc, antibodies against core antigen of hepatitis B virus; Anti-HCV, antibodies against hepatitis C virus; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; HBsAg, surface antigen of hepatitis B virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HCV RNA, ribonucleic acid of hepatitis C virus; HD, hemodialysis; RRT, renal replacement therapy.

[a] Median and range or the number of individuals showing the tested variable with the percentage of all studied subjects are presented.

[b] SI conversion factors: to convert alanine aminotransferase to μkat/L, multiplying by 0.0167, alkaline phosphatase to μkat/L, by 0.0167, aspartate aminotransferase to μkat/L, by 0.0167, and gamma-glutamyltransferase to μkat/L, by 0.0167.

[c] Fisher's exact test for categorical results and Mann-Whitney U test for continuous variables.

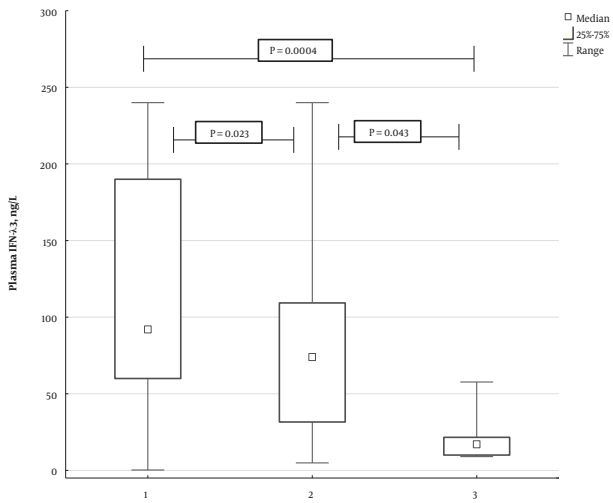

Figure 1. Plasma IFN-λ3 concentrations in HD patients concerning HBV/HCV status [Kruskal-Wallis test P-value = 0.00005; groups: 1, HBV/HCV-not exposed subjects and those who self-limited/spontaneously resolved these infections (n = 147); 2, HCV RNA-positive subjects (n = 35); 3, HbsAg-positive patients (n = 6)].

Table 4. Frequencies of *IFNL3* and *IFNL4* Haplotypes Concerning Spontaneous HCV Elimination [a, b, c]

SNPs/Haplotype	Frequency	Case, Control Frequencies	Chi-Square	P-Value	$P_{corrected}$ Value [c]	OR (95% CI); P-Value [d]	OR (95% CI); P-Value [e]
rs12980275_rs4803217							
AG	0.603	0.531, 0.705	9.402	0.002	0.005	Reference	0.465 (0.287 - 0.753); 0.002
GT	0.332	0.393, 0.244	7.358	0.007	0.02	2.146 (1.286 - 3.579); 0.003	1.996 (1.206 - 3.304); 0.007
AT	0.034	0.046, 0.018	1.833	0.2	0.5	3.747 (0.775 - 18.132); 0.08	2.884 (0.602 - 13.819); 0.1
GG	0.031	0.030, 0.033	0.018	0.9	1.0	1.405 (0.384 - 5.146); 0.6	1.052 (0.291 - 3.807); 1.0
rs4803217_rs12979860							
GC	0.617	0.549, 0.714	8.501	0.004	0.005	Reference	0.489 (0.300 - 0.795); 0.004
TT	0.349	0.422, 0.245	10.22	0.001	0.001	2.23 (0.1.344 - 3.697); 0.002	2.239 (1.356 - 3.699); 0.002
TC	0.017	0.017, 0.017	0.000	1.0	1.0	1.364 (0.223 - 8.351); 0.7	1.051 (0.173 - 6.385); 1.0
GT	0.017	0.012, 0.024	0.677	0.4	1.0	0.606 (0.099 - 3.712); 0.6	0.461 (0.076 - 2.799); 0.4
rs12979860_rs8099917							
CT	0.638	0.562, 0.746	10.49	0.001	0.003	Reference	0.438 (0.264 - 0.726); 0.001
TG	0.208	0.278, 0.107	12.92	3.0E-4	0.0000E0	3.47 (1.765 - 6.802); 0.0002	3.235 (1.667 - 6.278); 0.0003
TT	0.154	0.159, 0.148	0.074	0.8	1.0	1.430 (0.285 - 2.759); 0.3	1.093 (0.575 - 2.080); 0.8
rs12980275_rs4803217_rs12979860							
AGC	0.596	0.531, 0.689	7.664	0.006	0.007	Reference	0.479 (0.294 - 0.781); 0.003
GTT	0.325	0.388, 0.236	7.726	0.005	0.006	2.112 (1.259 - 3.545); 0.004	1.988 (1.193 - 3.311); 0.008
ATT	0.023	0.032, 0.009	1.847	0.2	0.7	5.432 (0.641 - 46.052); 0.08	4.221 (0.502 - 35.528); 0.2
GGC	0.020	0.016, 0.025	0.288	0.6	1.0	0.905 (0.178 -4.607); 1.0	0.680 (0.135 - 3.428); 0.6
ATC	0.011	0.013, 0.009	0.148	0.7	1.0	1.811 (0.161 - 20.335); 0.6	1.375 (0.123 - 15.343); 0.8
GGT	0.011	0.013, 0.008	0.173	0.7	1.0	1.811 (0.161 - 20.335); 0.6	1.375 (0.123 - 15.343); 0.8
rs4803217_rs12979860_rs8099917							
GCT	0.617	0.549, 0.714	8.501	0.004	0.006	Reference	0.462 (0.282 - 0.755); 0.002
TTG	0.200	0.264, 0.108	11.25	8.0E-4	0.002	3.12 (1.610 - 6.033); 0.0005	2.857 (1.496 - 5.457); 0.001
TTT	0.149	0.158, 0.137	0.261	0.6	1.0	1.497 (0.769 - 2.917); 0.2	1.159 (0.604 - 2.224); 0.7
TCT	0.017	0.017, 0.017	0.0	1.0	1.0	1.364 (0.223 - 8.351); 0.7	1.034 (0.170 - 6.283); 1.0
GTG	0.010	0.012, 0.008	0.087	0.8	1.0	1.818 (0.162 - 20.406); 0.6	1.382 (0.124 - 15.419); 0.8
rs12980275_rs4803217_rs12979860_rs8099917							
AGCT	0.596	0.532, 0.689	7.631	0.006	0.02	Reference	0.479 (0.294 - 0.781); 0.003
GTTG	0.198	0.261, 0.109	10.73	0.001	0.002	3.04 (1.564 - 5.906); 0.0008	2.768 (1.446 - 5.296); 0.002
GTTT	0.127	0.127, 0.126	0.001	1.0	1.0	1.301 (0.645 - 2.625); 0.5	0.983 (0.496 - 1.949); 1.0
ATTT	0.023	0.032, 0.010	1.649	0.2	0.8	5.432 (0.641 - 46.052); 0.08	4.221 (0.502 - 35.528); 0.2
GGCT	0.020	0.016, 0.025	0.309	0.6	1.0	0.905 (0.178 - 4.607); 0.9	0.680 (0.135 - 3.428); 0.6
ATCT	0.011	0.013, 0.009	0.151	0.7	1.0	1.811 (0.1612 - 20.335); 0.6	1.375 (0.123 - 15.343); 0.8
GGTG	0.011	0.013, 0.008	0.177	0.7	1.0	1.811 (0.1612 - 20.335); 0.6	1.375 (0.123 - 15.343); 0.8

[a] Cases, HD patients showing persistent HCV infection.
[b] Controls, HD patients showing spontaneous HCV clearance.
[c] Variant alleles are underlined.
[d] Empirical P-value based on 1,000 permutations.
[e] The most frequent haplotype was the reference.
[f] All other haplotypes pooled together were applied as the reference.

P = 3.22E-11) and HBV-infected (r = 0.553, P = 8.02E-06) HD subjects.

4.8. Survival and IFNL3 Polymorphisms

In the cross-sectional study, patients showing the *IFNL3* rs4803217 GG genotype had higher infection-related mortality (log-rank P = 0.021), but it was not observed in either the prospective study or Cox regression analysis, including age and gender. There were no other associations between tested *IFNL3* variants and survival, irrespective of study design, examined groups, or causes of death. Survival curves are shown in online resource 1.

5. Discussion

In the examined HD patients, the capacity to generate anti-HBs titers at the protective level in response to HBV inoculation or infection did not correlate significantly with *IFNL3* rs4803217 and rs12980275 SNPs. The highest anti-HBs titers or prevalence of overreacted responses appeared not to depend on the examined variants after Bonferroni correction. Neither *IFNL3* variants in the current study nor *IFNL4* variants in the already published research (9) were associated with anti-HBs generation.

In this study, IFN-λ3, a protein product of *IFNL3*, was not significantly related to tested *IFNL3* SNPs. Besides, IFN-λ3 showed higher circulating levels in HD patients than in healthy subjects by the same method, possibly due to the upregulation of production and/or lower degradation rate of IFN-λ3 in the uremic milieu (14). Human blood dendritic cell antigen 3 (BDCA3) (+) dendritic cells are robust IFN-λ3 generators in response to HCV stimulation (25). However, in chronic HBV or HCV infections, functional alterations of dendritic cell subsets were noticed (25, 26), so IFN-λ3 production could also be impaired. However, the determination of circulating IFN-λ3 in non-uremic subjects demonstrated not consistent results concerning differences in IFN-λ3 levels in patients resolving HCV infection or showing persistent HCV RNA positivity (2, 15, 18). Our data on HD patients showed that IFN-λ3 levels were lower in persistent HBV/HCV infections than in HD subjects not exposed to HBV/HCV or those who self-limited/spontaneously resolved these infections, which is in agreement with earlier data in non-uremic subjects (15, 18).

Circulating IFN-λ3 levels positively and strongly correlated with anti-HBs titers. The HBsAg positive patients, who did not generate anti-HBs, showed low IFN-λ3 levels. This finding confirms an earlier report by Li et al. (15). A lack of expected associations between *IFNL3* polymorphic variants and IFN-λ3 and anti-HBs possibly depends on intrinsic and extrinsic influences on transcription and translation processes. Determination of the *IFNL3* transcript in future studies could help approach this problem.

An association of *IFNL3* rs4803217 with spontaneous or therapeutic HCV clearance was shown in non-uremic patients (10, 11). Our study revealed the positive impact of major homozygosity (GG) of *IFNL3* rs4803217 on spontaneous HCV clearance in HD patients. In contrast, bearers of the rs4803217 variant allele (T) showed about two-fold higher susceptibility for persistent HCV RNA positivity. Significance was obtained at a P-value of 0.001, which was significant also after Bonferroni correction. As rs4803217 is in the strong LD with rs12979860 (0.81 in this study), and *IFNL3* rs12979860 was already associated with HCV clearance in GWAS analyses (4), the level of significance shown in our research in a specific smaller group of patients should be sufficient for the recognition of a true association between *IFNL3* rs4803217 and spontaneous HCV clearance. Logistic regression analysis, which included rs4803217, rs12980275, and clinical variables as possible predictors of spontaneous HCV clearance in HD patients, revealed similar predictivity of both SNPs concerning HCV resolution. Longer RRT duration diminishes the chance for spontaneous HCV clearance.

McFarland et al. (27) found that the rs4803217 T allele caused twice lower stability of the mRNA transcript acting through the AU-rich element (ARE)-mediated decomposition of *IFNL3* mRNA. AU-rich elements are connected with post-transcriptional gene regulation (28). The rs4803217 G allele mRNA sets up a well-defined 3'UTR form while the T allele mRNA is more active, which controls the effectiveness of mRNA translation. Non-translating mRNA levels were higher in the case of the rs4803217 T allele mRNA (29). Therefore, the unfavorable (T) allele bearers could show worse controlling of HCV infection. In our study, HD patients with persistent HCV infection revealed a higher frequency of the unfavorable rs4803217 allele and lower levels of plasma IFN-λ3 than subjects free from HCV RNA. However, although IFN-λ3 generation is putatively lower in the rs4803217 T allele possessors due to lower transcript stability (27, 29), our study did not demonstrate the association of rs4803217 with circulating IFN-λ3, even if it was analyzed in subgroups categorized by HD modality. High permeable HD membranes may influence plasma IFN-λ3 levels (17), causing a dialysate loss of IFN-λ3, which has a molecular weight of about 22 kDa (30) and can cross HD membranes to the dialysate.

Data reported by McFarland et al. (27) and Lu et al. (29) suggest that a functional SNP, rs4803217, has a causative role in HCV resolution. Simultaneously, other SNPs of the *IFNL* region may correlate with HCV resolution only because of LD with SNP rs4803217. The LD between rs4803217 and rs12979860 variants is very strong (r^2 = 0.92) (31). In

Polish HD patients, the LD between these variants seems to be weaker (r^2 = 0.81). In non-uremic patients with chronic hepatitis C living in the same region as the examined HD patients, the LD between rs4803217 and rs12979860 SNPs was even lower (r^2 = 0.75) (11). The rs4803217 and rs8099917 SNPs show weak LD [r^2 = 0.34 (this study), r^2 = 0.36 (11)]. The LD between two brands depends on intrinsic cellular components and extrinsic aspects of the human past (32). On the other hand, the association of haplotype rs12979860-C_rs8099917-T with spontaneous HCV clearance, the strongest one among other significant haplotypes concerning HCV resolution, suggests that the major rs4803217 allele may not be necessary for spontaneous HCV clearance. Moreover, the haplotype rs368234815-ΔG_ rs4803217-G, which includes the favorable rs4803217 G allele and unfavorable rs368234815 ΔG allele discovered by Prokunina-Olsson et al. (33), was associated with a poorer response to anti-HCV treatment compared to haplotype rs368234815-ΔG_ rs4803217-T, which is composed of the unfavorable rs4803217 T allele (31). These data suggest that the association between SNP rs4803217 and HCV clearance may occur due to its strong LD (r^2 = 0.90) with SNP rs368234815 in subjects of European ancestry (31). Therefore, although the major homozygosity (GG) of rs4803217 predicted spontaneous HCV clearance in HD patients in our study, the strength of this predictability may be weakened in possessors of *IFNL4* rs368234815 ΔG.

The associations of tested *IFNL3* SNPs with survival were not convincing in the examined HD patients. Neither all subjects nor subgroups categorized by HBV/HCV infection showed an influence of *IFNL3* SNPs on the death rate, possibly due to a lack of association with plasma IFN-λ3 levels (20, 21).

5.1. Limitations

A limitation of our study was the use of recombinant hepatitis B vaccines provided by three manufacturers. Engerix B and Euvax B contained HBsAg variant adw2, while Hepavax-Gene was composed of HBsAg variant adr. We analyzed vaccinated HD patients as one group because Euvax B and Hepavax-Gene vaccines were shown as clinically identical (equally effective) to the Engerix B reference vaccine (34, 35). Moreover, as all the three vaccines were recommended uniformly powerful, some HD patients during their long-term RRT were receiving booster vaccine doses different from those used for the primary immunization. Therefore, differences in anti-HBs generation resulting from the use of the above-mentioned vaccines should be minimal or do not exist.

Similarly, anti-HBs titers were measured using two methods: MEIA and CMIA. By the manufacturer (Abbott Ireland), quantitative anti-HBs values obtained by such assays may not be equivalent due to the heterogenicity of anti-HBs. Each HD facility used one method of anti-HBs determination (MEIA or CMIA). All patients dialyzed in the same center, independently of their anti-HBs results, had performed the anti-HBs titration with this one method. How discrepancies in anti-HBs measurements contributed to a lack of significant differences between the studied groups (Tables 1 and 3) remains unclear.

5.2. Conclusion

In HD patients, natural HCV clearance is connected with the *IFNL3* rs4803217 GG genotype and haplotypes comprising at least two more frequent alleles but without any variant allele of tested *IFNL3/IFNL4* SNPs. Therefore, genotyping *IFNL3* rs4803217 may be used for practical purposes in the prognosis of natural HCV clearance. Nevertheless, it does not forecast the self-limitation of HBV exposure, responsiveness to HBV vaccine, or patient mortality. Circulating IFN-λ3 levels are not straightly influenced by *IFNL3* rs4803217 and rs12980275, but show differences dependently on HBV/HCV seromarkers, as well as firmly correlate with anti-HBs titers. Both tested polymorphisms are not associated with the survival of HD patients.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

Acknowledgments

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Footnotes

Authors' Contribution: AEG conceived the idea for the study. AEG designed the research. AEG and WM were involved in data collection. WW was responsible for statistics. AM and PPJ were responsible for genotyping. AEG interpreted the data. AEG wrote the article. All authors edited and approved the final version of the manuscript.

Conflict of Interests: There is no conflict of interest in this study.

Ethical Approval: The Institutional Review Board of Poznan University of Medical Sciences, Poland, approved the concept of our investigations.

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