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# *IFNL4* Polymorphism as a Predictor of Chronic Hepatitis C Treatment Efficiency in Ukrainian Patients[1]

**A. M. Kucherenko[a], V. M. Pampukha[a], K. Yu. Romanchuk[b], S. Yu. Chernushyn[a], I. A. Bobrova[c], L. V. Moroz[b], and L. A. Livshits[a]**

[a] *Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine, Department of Human Genomics, 150 Zabolotnogo str., 03680 Kyiv, Ukraine*

[b] *National Pirogov Memorial Medical University, Department of Infectious Diseases with Epidemiology Course, 56 Pyrogova str., 21018 Vinnytsya, Ukraine*

[c] *Ukrainian Treatment and Diagnostic Center, 6D Mykilsko-Slobidska Str., 02002 Kyiv, Ukraine*

*e-mail: kucherenko.a.m@gmail.com*

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**Abstract**— The aim of this study was to examine association between *IFNL4* gene ss469415590 and treatment efficiency in group of Ukrainian PEG-interferon/ribavirin-treated chronic hepatitis C patients. Study group consisted of 92 unrelated hepatitis C virus genotype 1 mono-infected patients: case group – 29 patients with late or absent virological response; control group – 63 patients with sustained virological response. Study material was genomic DNA. Genotyping was performed using amplification-refractory mutation system PCR. Statistical analysis was performed using GenePop and OpenEpi statistical packages. Obtained results show that ss469415590 $\Delta G/\Delta G$ genotype is associated with poor virological response (OR = 3.62; CI 95%: 1.12–11.67) in PEG-interferon/ribavirin-treated chronic hepatitis C patients from Ukraine.

*Keywords: IFNL4*, pharmacogenetic marker, hepatitis C, treatment efficiency

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## INTRODUCTION

Chronic hepatitis C (CHC) is a worldwide healthcare problem due to the increasing number of affected individuals [1]. Interferon-based therapy schemes prove the most effective in CHC treatment [2, 3]. The discovery of impact of host *IFNL3* (*IL28B*) genotype in combination with virus genotype on treatment outcome was a milestone in the development of antiviral therapy strategies [4–6]. The wide range of studies has proven the association of *IL28B* gene polymorphic variants rs12979860 and rs8099917 with the antiviral therapy efficiency in patients with chronic hepatitis C (virus genotype 1) as well as spontaneous viral clearance [7, 8]. Interestingly, this association shows ethnical diversity [9]. However, the obscurity of exact molecular mechanism underlying such association was the significant drawback for further investigations. The discovery of a previously unknown transcript which expression in hepatocytes was activated by hepatitis C virus exposure has lead to the significant progress in the field [10]. A recently discovered dinucleotide polymorphic variant ss469415590 was shown to cause a frame-shift mutation creating an open reading frame – *IFNL4* (interferon lambda 4) gene [10, 11]. This polymorphism was reported to be in linkage disequilibrium with rs12979860 in some populations [12] and is being thoroughly investigated for association with sustained virological response (SVR) in interferon-treated chronic hepatitis C patients [13–16].

The aim of the current study was to examine possible association between genotypes for ss469415590 and rs12979860 in group of Ukrainian CHC patients.

## MATERIALS AND METHODS

The data for this study were collected from 92 Ukrainian CHC patients. All of them were HCV RNA positive for more than 6 months and mono-infected with chronic hepatitis C with no evidence of HIV or hepatitis B infection. All participants were untreated patients with no evidence of any associated liver diseases other than chronic viral hepatitis C. The pretreatment HCV RNA level was detected by quantitative real time polymerase chain reaction (PCR) technique with a lower limit of detection of 75 IU $mL^{-1}$. The high viral load (RNA HCV > 600000 IU $mL^{-1}$) was observed in 46.73% of patients. In all the patients enrolled in this study the HCV genotype 1 infection was detected. Qualitative PCR by TaqMan assay as

[1] The article was translated by the authors.

**Table 1.** Genotype frequency of studied polymorphic variants

| rs12979860 | ss469415590 | | | $D'$ | $r^2$ |
|---|---|---|---|---|---|
| | *TT/TT* | *TT/ΔT* | *ΔG/ΔG* | | |
| *CC* | 35 | 0 | 0 | 1 | 0.976 |
| *CT* | 0 | 43 | 0 | | |
| *TT* | 0 | 0 | 14 | | |

$D'$—standardized linkage disequilibrium coefficient; $r^2$—correlation coefficient.

well as ELISA was used for detection of viral load at weeks 4, 12, 24 and 48.

The treatment protocol specified duration of 48 weeks. The majority of patients (55.43%) received PEG-IFNα2a (Pegasys) at a dose of 180 μg per week and 44.57% received PEG-IFNα2b (Pegintron) as a subcutaneous injection at a dose of 1.5 μg $kg^{-1}$ per week. All patients received a 15 mg $kg^{-1}$ daily oral dose of RBV (Copegus or Rebetol). Under futility-stopping rule, the treatment was halted if there was <2 log10 HCV RNA decline at week 12 or persistent viremia at week 24.

According to the viral load changes all the patients were distributed into two groups: case group – 29 patients with late or absent virological response, and control group – 63 patients with sustained virological response. Informed consent was obtained from all participants prior to the collection and storage of blood samples for testing. The study has been approved by The Bioethical Committee of Institute of Molecular Biology and Genetics of NAS of Ukraine.

The material of the study was genomic DNA extracted from peripheral blood samples using standard phenol–chloroform technique. Genotyping for *IFNL4* gene ss469415590 was performed using the amplification-refractory mutation system (ARMS) PCR [17]. Primer design was conditioned by several factors. Firstly, high sequence homology between *IFNL* gene subfamily needed to be taken into the consideration. Secondly, the sequence of ss469415590 flanking region is able to form hairpin loops and dimers with ΔG up to −4.7 Kcal $mol^{-1}$. Therefore, additional mismatches were introduced in the target-specific primers to avoid dimer formation. The primers sequences were IFNL4ΔG: TCC TTT ACA CGG TGA TCG CAG C, IFNL4TT: TCC TTT ACA CGG TGA TCG CAG AA, and IFNL4com: TGA TTG ACC CTG AGC CTG CG. Conditions for amplification were as follows: initial denaturation at 95°C for 5 minutes, 30 cycles of 30 seconds at 95°C, 30 seconds at 62°C, and 30 seconds at 72°C, followed by 5 minutes of final extension at 72°C. The amplification products of 299 bp were visualized on 2% agarose gel with ethidium bromide staining. In order to confirm the accuracy of the developed genotyping method, genotypes of random samples were re-determined via Sanger sequencing. DNA sequencing was performed by standard dideoxynucleotide chain-termination method using BigDye® Terminator v3.1 Cycle Sequencing Kit and ABI Prism 3110 Genetic Analyser (Applied Biosystems, USA). Genotyping for *IL28B* gene rs12979860 was performed by a PCR-based restriction fragment length polymorphism assay using *Hpy8*I restriction enzyme as described previously [18].

Statistical analysis has been performed using GenePop and OpenEpi statistical packages [19, 20]. The χ2 test was used to detect deviations from Hardy-Weinberg equilibrium in genotype distribution. The likelihood-ratio test has been performed to estimate the linkage disequilibrium between ss469415590 and rs12979860. A P-value of less than 0.05 was regarded as significant.

## RESULTS AND DISCUSSION

The results of genotyping for both studied polymorphic variants in CHC patients group are presented in Table 1.

Genotype frequencies for both studied variants showed no significant deviation from the ones expected according to Hardy-Weinberg equilibrium. The $\chi^2$ values for ss469415590 and rs12979860 equaled 0.91 and 0.42 respectively (df = 2). The likelihood-ratio test was performed to estimate the genotypic linkage disequilibrium between ss469415590 and rs12979860. The results indicated that the studied variants are tightly linked ($p > 0.0001$), alleles ss469415590 TT and rs12979860 C are in phase. These results are consistent with our previous research conducted on a group representing general population of Ukraine [17].

Regarding the distribution of studied polymorphisms' genotypes, the results are presented in Table 2. As can be seen, genotype and allele distributions in group of patients with SVR differed significantly compared to group without SVR. The analyses show positive associations of ss469415590 *TT*/*TT* genotype with SVR, and ss469415590 *ΔG*/*ΔG* genotype with poor virological response to treatment. This association fits well with an additive model of inheritance.

Several studies on different populations have shown that IFN-λ4 is linked with the failure to clear hepatitis C virus (HCV) infection in response to treatment [11, 14]. IFN-λ4 is generated only by individuals who carry the *IFNL4*-ΔG allele, which was thus chosen as promising host factor for predicting clearance of HCV. IFN-λ4 most closely resembles IFN- λ3, but these proteins share only 29% amino-acid identity, and, compared to IFN-λ3, IFN-λ4 is only weakly secreted [10, 21, 22].

There are some crucial characteristics in the antiviral activities induced by type III (λ) IFNs. First, in contrast to type I IFNs, which receptors are broadly expressed in virtually all somatic cells, type III IFN receptors are predominantly expressed by non-hema-

**Table 2.** Genotype and allele distribution in studied groups

| ss469415590/ rs12979860 | Patients with sustained virological response | | Patients without sustained virological response | | Odds ratio | | |
|---|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *p* | OR | 95% CI |
| *Genotypes* | | | | | | | |
| *TTTT/CC* | 31 | 49.2 | 4 | 13.8 | 0.0006 | 0.17 | 0.05 – 0.53 |
| *TTΔG/CT* | 26 | 41.3 | 17 | 58.6 | | 2.02 | 0.83 – 4.92 |
| *ΔGΔG/TT* | 6 | 9.5 | 8 | 27.6 | | 3.62 | 1.12 – 11.67 |
| *Total* | 63 | | 29 | | | | |
| *Alleles* | | | | | | | |
| *TT/C* | | 69.8 | | 43.1 | 0.0005 | 0.33 | 0.17 – 0.62 |
| *ΔG/T* | | 30.2 | | 56.9 | | 3.06 | 1.61 – 5.82 |

CI—confidence interval; OR—odds ratio.

tologic cell types, especially cells of epithelial origin such as bronchial epithelium, gastrointestinal epithelium, and keratinocytes [12]. Second, viral infection studies in several mouse models have shown that type III IFNs play a more prominent role than type I IFNs in mediating antiviral protection against certain types of viruses which preferentially infect gastrointestinal and/or respiratory epithelium [23]. Early clinical trials of recombinant IFN-λ1 for the treatment of chronic hepatitis C indicate that its antiviral effects are similar to recombinant IFN-α, but it induces fewer adverse effects due to the more limited range of cells which express IFN- λ R1 [24, 25].

The exact mechanism of IFN-λ4 level influence on hepatitis C antiviral treatment efficiency is yet to be discovered. However, several hypotheses call for further investigation. According to the first hypothesis IFN-λ4 production may have an impact on interferon-stimulated genes (ISG) expression. IFN-λ4 signaling is proven to occur through the IFN-λ receptor complex, consequently inducing ISGs expression via the Janus kinase-signal transducer and activator of transcription signaling pathway [22]. Individuals who carry *IFNL4-ΔG* allele may express low levels of IFN-λ4 protein that induces low, but persistent ISGs expression in the liver, which makes hepatocytes refractory to stimulation by IFN-α [10, 22]. It was shown, that these individuals have higher basal levels of ISG expression and are less likely to respond efficiently to PEG-IFNα and ribavirin therapy [11, 14]. These findings show the importance of discovering new treatment targets for hepatitis C therapy, avoiding IFN-α pathway.

On the other hand, there is evidence of an impaired secretion of IFN-λ4, probably due to inefficient post-translational glycosylation [22]. It is possible that intracellular accumulation of non-glycosylated IFN – λ4 could be cytotoxic and result in hepatocyte death, deteriorating patients' state and promoting further infection damage [12, 22].

## CONCLUSIONS

In this study we present for the first time the evidence of ss469415590 being a valuable pharmacogenetic marker of CHC treatment efficiency in Ukrainian patients of Eastern European origin. While rs12979860 is in tight linkage disequilibrium with ss469415590 and is proven to be informative for our population as well, the wide range of functional changes promoted by IFN-λ4 production makes ss469415590 more favorable and feasible genetic marker.

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