

***IFN-λ-3 (IL28B)* genotyping by restriction fragment length polymorphism method: detection polymorphism of rs12979860**

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***Aim.** The goal of our study was to develop an accurate detection of the SNP rs12979860 by RFLP-based method and to evaluate the polymorphic genotype distribution for this SNP among individuals with unknown HCV status from Ukraine. **Methods.** The SNP rs12979860 was tested by PCR RFLP-based method in 99 individuals from Ukraine. **Results.** The method of accurate detection of the SNP rs12979860 was developed. The genotypes distributions were: CC – 56 %, CT – 34 %, TT – 10 %. **Conclusions.** Due to the high incidence of CC genotype, found in our study, the SNP rs12979860 analysis may be useful for Ukrainian patients to predict responses to the treatment considering the HCV genotype and viral load.*

Keywords: *IFN-λ-3 (IL28B) gene, SNP rs12979860, PCR-RFLP method, hepatitis C.*

Introduction. Hepatitis C virus (HCV) infects about 3 % of the world population [1]. Only 20–30 % of HCV-infected individuals recover spontaneously, with remaining 70–80 % developing chronic infection [2]. It was shown that regardless decreasing the incidence of acute hepatitis C, the number of people chronically infected with HCV increases in Ukraine [3]. Chronic HCV infection can lead to the development of cirrhosis and hepatocellular carcinoma [4]. The current standard of care for chronic HCV infection is 24 or 48 weeks of therapy with pegylated interferon-alfa (PEG-IFN) and ribavirin (RBV). A response to the therapy is variable: host and viral characteristics determine whether patients develop a sustained virological response (SVR). The patients infected with HCV genotype 1, who were treated for 48 weeks with PEG-IFN and RBV, have a 50 % possibility of developing SVR. Patients with

HCV genotype 2 or 3 have SVR rate 80 % after 24 weeks of the PEG-IFN and RBV therapy [5]. Other factors predictive of response are hepatitis C viral load, patient age, sex, weight, and liver fibrosis stage. Patient's genetic ancestry is also an important factor in the treatment outcome. The patients of African ancestry with chronic HCV have an almost 50 % reduction in SVR rates at the PEG-IFN and RBV therapy compared with the patients of European ancestry [6].

Recently, four research groups have independently identified single nucleotide polymorphisms (SNPs) linked to the interferon lambda gene (*IFN-λ-3*, also known as *IL28B*) that are associated with response to the PEG-IFN and RBV treatment of HCV-infected individuals of European, Asian and African ancestry [7–10]. Ge et al. identified a SNP 3 kb upstream of the *IFN-λ-3 (IL28B)* gene, rs12979860, that was strongly associated with SVR. It was shown that CC genotype of rs12979860 was associated with an approximately

2-fold greater rate of SVR compared with the TT genotype, both among patients of European ancestry and African Americans. Ge et al. sequenced the *IFN-λ-3* (*IL28*) gene in 96 individuals and found two variants in linkage disequilibrium with rs12979860: a G > C transition 27 upstream of the translation initiation codon (rs28416813), and a non-synonymous coding SNP (213A > G, K70R, rs8103142). However, the tests for independence were not possible owing to the high degree of correlation among three SNPs, and it could not be determined which, if any, of the SNPs is uniquely responsible for the association with SVR [7]. Thomas et al. genotyped the rs12979860 SNP in more than 1000 people with spontaneous clearance of HCV or viral persistence and found that CC genotype was strongly associated with HCV clearance [11].

Because the CC genotype was substantially more frequent in European than African populations, Ge et al. (2009) estimated that rs12979860 could explain approximately half of the difference in SVR between African Americans and the patients of European ancestry [7].

The goal of our study was to develop simple Restriction Fragment Length Polymorphism (RFLP)-based method for the detection of polymorphism rs12979860 and to evaluate the polymorphic genotype distribution in population of Ukraine.

Materials and methods. Genomic DNA was extracted from the peripheral leukocytes by standard methods in 99 individuals with unknown HVC status from different regions of Ukraine after informed consent [12].

Published sequence information of the rs12979860 was used to design a pair of primers spanning this SNP [http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=12979860]. The polymerase chain reaction (PCR) was carried out under standard conditions. After an initial denaturation step at 94 °C for 4 min, the samples were processed through 5 cycles of amplification consisting of: 94 °C temperature for 1 min, primer annealing of 62 °C for 1 min, and extension at 72 °C for 1 min, 10 cycles of amplification consisting of: 94 °C for 1 min, primer annealing of 58 °C for 1 min, and extension at 72 °C for 1 min, 25 cycles of amplification consisting of: 94 °C for 1 min, primer annealing of 55 °C for 1 min, and extension at 72 °C for

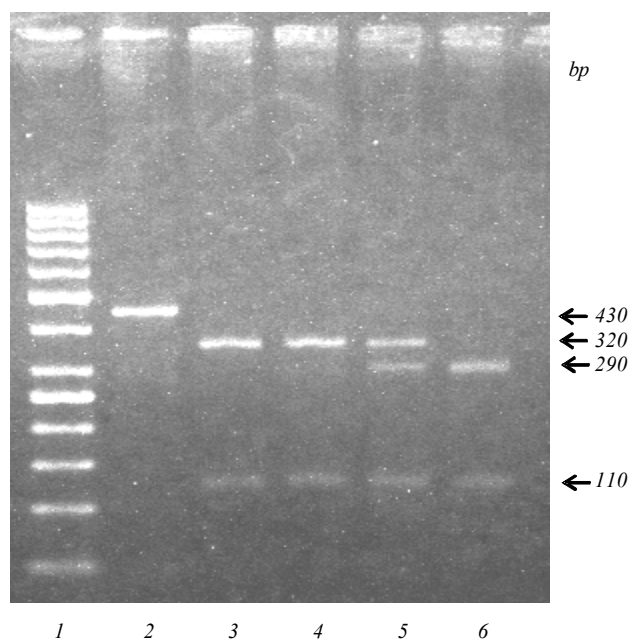
1 min. The final extension step was prolonged to 7 min at 72 °C.

The C to T transition (rs12979860) creates the site for *Hpy8I* restriction enzyme. Amplified DNA (10 μl) was digested in a 20 μl reaction mixture, using the buffers and temperatures recommended by the manufacturers («Fermentas», Lithuania). The amount of restriction enzyme required for digestion was 5 U, samples were incubated during overnight. Digested products were fractionated in 2 % agarose gel and visualized by the ethidium bromide staining and transillumination with ultraviolet light. The size marker was GeneRuler 50 bp DNA Ladder («Fermentas»). Statistic analysis was performed using χ^2 [13].

Results and discussion. In 2003 Sheppard et al. by genomic sequence analysis identified the *IFN-λ-1*, *IFN-λ-2*, *IFN-λ-3* genes and described human genes in the type-III interferon family having high conserved sequences (including exons, introns and flanking DNA) and deduced that 200-amino acid *IFN-λ-3* protein was 96 % identical to *IFN-λ-2* and 81 % identical to *IFN-λ-1* [14, 15]. The SNP rs12979860 analysis was complicated due to the existence of site for the restriction enzyme *Hpy8I* in highly homologous sequence of *IFN-λ-2* gene. To avoid the analysis miscarriage the primers design based on minor sequence mismatch between *IFN-λ-2* and *IFN-λ-3* genes was performed.

The results of SNP rs12979860 genotyping are shown in Figure. The product length after amplification is 430 bp. *Hpy8I* digestion of the amplification fragment produces a constant 110 bp fragment. The C to T transition was detected due to the existence of an additional restriction site for *Hpy8I*: the T allele is defined by the presence of 290 bp band, whereas the allele C is defined by the presence of 320 bp band.

The genotypes and allelic distributions among 99 healthy controls were: CC – 56 %, CT – 34 %, TT – 10 %; C – 73 %, T – 27 %. The genotype distribution was in Hardy-Weinberg equilibrium. Thomas et al. reported the allelic frequencies of rs12979860 in different ethnic groups. By genotyping more than 2000 healthy individuals from different countries they observed that the protective allele C was most highly prevalent in Asian populations, of intermediate frequency in Caucasian individuals, and presented at low



Detection of the SNP rs12979860 by the PCR-RFLP method. 2-% Agarose gel. PCR product was digested by *Hpy8I*: 1 – marker of molecular weight GeneRuler 50 bp DNA Ladder; 2 – 430 bp undigested PCR product; 3, 4 – individual with CC genotype; 5 – individuals with CT genotype; 6 – individual with TT genotype

frequency in African populations [11]. The allelic frequencies for Ukrainian population analyzed do not statistically differ from frequencies of other European populations reported by Thomas et al. and statistically differ ($P < 0.05$) from Asian and African populations.

Conclusions. We have described here a new, rapid, and relatively inexpensive method for the *IFN- λ -3 (IL28B)* genotyping, using PCR-RFLP approach. Due to the high incidence of CC genotype, revealed in our study and taking into consideration the numerous data, showing that this genotype was associated with high rate of SVR, the rs12979860 analysis may be useful in Ukraine to predict patients' responses to the treatment considering HCV genotype and viral load.

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Генотипування *IFN- λ -3 (IL28B)* методом поліморфізму довжини рестрикційних фрагментів: визначення поліморфізму rs12979860

Резюме

Мета. Метою даної роботи була розробка простого методу детекції поліморфізму rs12979860 на основі ПДРФ-аналізу та проведення генотипування за даним поліморфізмом серед індивідів з невизначеним статусом хронічного гепатиту С в популяції України. **Методи.** SNP rs12979860 проаналізовано методом ПЛР/ПДРФ

ПДРФ серед 99 індивідів з України. **Результати.** Розроблено точний метод детекції rs12979860 та виявлено такий розподіл генотипів: CC – 56 %, CT – 34 %, TT – 10 %. **Висновки.** Зважаючи на визначену високу частоту генотипу CC у нашому дослідженні, аналіз поліморфізму rs12979860 можна застосовувати в Україні для прогнозу відповіді пацієнта на лікування з урахуванням генотипу вірусу С і вірусного навантаження.

Ключові слова: ген *IFN- λ -3 (IL28B4)*, SNP rs12979860, метод ПЛР/ПДРФ, гепатит С.

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Генотипирование *IFN- λ -3 (IL28B)* методом полиморфизма длины рестрикционных фрагментов: определение полиморфизма rs12979860

Резюме

Цель. Цель данной работы состояла в разработке простого метода определения полиморфизма rs12979860 на основе ПДРФ-анализа и проведение генотипирования по данному полиморфизму среди индивидов с неустановленным статусом хронического гепатита С из Украины. **Методы.** SNPrs12979860 проанализирован методом ПЦР/ПДРФ среди 99 индивидов из Украины. **Результаты.** Разработан точный метод детекции rs12979860 и выявлено следующее распределение генотипов в популяции Украины: CC – 56 %, CT – 34 %, TT – 10 %. **Выводы.** Принимая во внимание высокую частоту генотипа CC, определенную в нашем исследовании, можно сделать вывод о том, что анализ полиморфизма rs12979860 целесообразно применять в Украине для прогноза ответа пациента на лечение с учетом генотипа вируса С и вирусной нагрузки.

Ключевые слова: ген *IFN- λ -3 (IL28B4)*, SNP rs12979860, метод ПЦР/ПДРФ, гепатит С.

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