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Influence of *OASL* gene polymorphisms on host response to interferon therapy in chronic hepatitis C virus patients

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Abstract

Hepatitis C virus (HCV) infection becomes a major public health problem and leading cause of chronic liver disease and liver failure. Pegylated interferon-alfa and ribavirin are currently the standard treatment for chronic hepatitis C (CHC). It is indicated that genes that trace the interferon signaling could be associated with the host response to the therapy.

In order to investigate the influence of these genes on host related response, we have analyzed seven single nucleotide polymorphisms (rs59248852, rs74390571, rs12811390, rs1169279, rs3213545, rs1083129 and rs2859398) in 2-5-Oligoadenylate-Synthetase-Like (*OASL*) gene in CHC cases from Republic of Macedonia. A simple and easy to use SNaPshot method was developed.

A cohort of 100 HCV RNA positive patients - non responders and 109 patients with achieved virological response after the standard treatment were included in this study. We have found significant association in five of the seven studied SNP`s: rs59248852 [$p = 6.5x10^{-31}$, OR=55.7 (20.0-155.3)]; rs12811390 [$p = 2.2x10^{-11}$, OR=4.3 (2.3-6.7)]; rs2859398 [$p=1.34x10^{-9}$, OR=3.4 (2.2-5.0)]; rs74390571 [$p=4.3x10^{-7}$, OR=2.9 (1.9-4.4)], and rs1083129 [p=0.0139, OR=2.0 (1.1-3.5)].

The results from this study can be used as a predictive factor of future patient's selection for the standard therapy, having an important cost benefit for the health insurance system.

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Introduction

Hepatitis C Virus (HCV) infection is arising as global health problem with more than 177 million people affected worldwide or 2.5% of the population (1). Acute HCV infection is mainly asymptomatic, but with a time it is leading to persistent HCV infection associated with the development of liver cirrhosis, hepatic cancer, liver failure, and death (2). Hepatitis C virus (HCV) is genetically highly diverse, displaying regional variations in genotype prevalence. Genotypes 1 and 3 dominate in most countries, while less frequent are genotypes 2, 4, 5 and 6 (3). Today, the standard treatment for HCV infection, accepted worldwide, includes a combination of pegylated interferon (PEG-IFN-α) and ribavirin –RBV (4). This therapy is introduced in the Republic of Macedonia in 2006. Administration of this therapy is physically and economically highly demanding, but with low success rate and highly dependable on HCV genotype (5). A sustained virological response (SVR) is achieved in only a half of genotype 1-infected patients, genotype 4 is also resistant to IFN-α therapy, while higher success rate can be achieved in treatment of patients with genotypes 2 and 3 (6). Furthermore, this therapy is associated with considerable toxicity and various side effects (flu-like syndrome or hematologic abnormalities), often requiring a dose reduction. In more than 15% a discontinuation in administration of the therapy is needed (7, 8). Therefore, the prediction of a patient's response before the treatment is of great clinical importance for avoiding the side effects of the therapy as well as to prevent the cost of treatment in those patients that do not respond.

The treatment outcome is associated with several viral and host factors such as viral load, obesity, age, male sex, ethnicity or genotypes (9, 10). The rate of HCV-RNA decline during treatment and virological responses at week 4 (rapid virologic response –RVR) and week 12 (early virologic response-EVR) are predictive factors of the treatment as well (11, 12).

Host genetic factors, mainly regulating the interferon metabolism have also been associated with the response to PEG-IFN-α /RBV therapy. Up to date, a number of candidate genes such as type 1 interferon receptor 1 (*IFNAR1*) (13), MAPK-Activated Protein Kinase 3 *(MAPKAPK3*) (14, 15), *(IL-28B)* gene coding for IFNL-3 (16-19), interferon signaling (*STAT1, STAT2, OAS1, OAS3, OAS2* and *OASL*) and interferon stimulated genes (*G1R2, G1P3, PKR, IP10, IRF7, IRF9, IFI35, IFNAR2, IFNAR1 MH2* and *MH1*) (20) have shown an association with treatment response and spontaneous clearance after HCV infection. IN28B or IFNL3 variant rs12979860 was proved to have a strong influence on host response to interferon therapy, with CC as a favorable response genotype and since 2014 it is recommended to be perform as a predictor of response to PEG-IFN-α /RBV containing regiment (21).

The effective response and clearance of HCV is closely related to understanding interferon pathways and its downstream targeted genes. Many studies demonstrated that the polymorphisms in IFN signaling genes, including *OASL* gene, are related to a treatment response (22-24). Oligoadenylate Synthase-Like Protein (*OASL)* with its 2'-5' oligoadenylate catalytic activity is recognized as a significant factor that influence antiviral response induced by the interferon (25, 26). Interferon is proved as a molecule that regulates the early phase of viral infection by degrading viral RNA, and/or inhibition of viral replication (27). The possibility that the host-related genetic variations imply the response rates of patients to IFN therapy is still in a field of research. In order to determine the influence of *OASL* polymorphisms on patient's viral response, seven single nucleotide polymorphisms (SNP) in this gene were investigated (rs59248852, rs74390571, rs12811390, rs1169279, rs3213545, rs1083129 and rs2859398). For that purpose, a new fast and reliable SNaPshot method for simultaneous determination of these polymorphisms was introduced.

Materials and Methods

Patient samples

This study was conducted on 209 HCV RNA positive patients included in the program of supplemental therapy with pegylated interferon / ribavirin (Pegasys®, Hofman La Roche) in a period of 24 or 48 weeks for patients with genotype 3 or genotype 1, respectively, carried out at the Clinic of Gastroenterohepatology and Clinic of Infectious Diseases and Febrile Conditions, Medical Faculty, Skopje. The success of the administered therapy was followed at RCGEB by HCV-RNA determination at 4th, 12th, 24th and 48th week after starting the therapy and in addition 24 weeks after the end of therapy. Patients that have completed the therapy in a period between 2010 and 2012, were invited to participate in the study. All subjects have given written informed consent for participation in the study. The study was approved by the Macedonian Academy of Sciences and Arts Ethical Committee.

Patients were separated in two main groups: 100 patients without response to antiviral therapy (NVR) and 109 cases with sustained viral response (SVR). Genotype was determined in all CHC patients. The group of NVR patients consisted of 77 Macedonian and 23 Albanian patients. Sixty eight patients were males while 32 were females. Mean age of analyzed patients in this group was 46.16± 16.47. Depending on the type of transmission, more than half (55%) were patients on hemodialysis, while the rest were intravenous drug users (45%). Regarding the genotype distribution 21 patients had genotype 3 while 79 patients had genotype 1. In the group of SVR patients 86 patients (78.9%) were Macedonians while 23 patients (21.1%) were of Albanian ethnic origin. Seventy eight percent were males while 28% were females. Mean age of analyzed patients in this group was 31.08± 8.01. This group predominates with CHC patients that were intravenous drug users (81.6%), while the rest (18.4%) were patients on hemodialysis. Regarding the genotype distribution, 73 patients had genotype 3 while 36 patients had genotype 1.

Peripheral blood samples were collected and genomic DNA was extracted using Proteinase K digestion, phenol/chloroform extraction and ethanol precipitation method. The concentration and purity of isolated DNA samples was determined using NanoVue- UV/VIS spectrophotometer (GE Healthcare, Little Chalfont, UK).

Detection and Quantification of HCV RNA

HCV RNA detection and quantification was performed using AMPLICOR HCV v2.0 kit (Roche Molecular Diagnostics, Pleasanton, CA). All operational steps were carried out according to the instructions given in the manuals provided by the manufacturer. The lower limit of detection of AMPLICOR HCV v2.0 was 50 IU ml (28).

Multiplex SNaPshot method for detection of 7 polymorphisms in *OASL* gene

The variant types of 7 different polymorphisms in *OASL* gene: rs59248852, rs74390571, rs12811390, rs1169279, rs3213545, rs1083129 and rs2859398 were analyzed using the primer extension-based method or SNaPshot. Typing of seven different polymorphisms in the *OASL* gene was done by two multiplex PCR reactions for each patient. The first reaction included polymorphisms (rs2859398, rs1083129, rs74390571 and rs59248852), while the second one encompassed the remaining three (**Table 1**). Briefly, the first PCR amplification was conducted in total volume of 20 μL containing: 0.5 μg genomic DNA, buffer (50 mM Tris-HCl, pH 9.2, 16 mM $(NH4)_2SO4$, 0.1% Tween 20), 4.0 pmol of specific primers, 200 μM mix of each dNTP, 1.75 mM $MgCl_2$, and 0.5U of enzyme Tth DNA polymerase. The PCR conditions were as follows: 2 min initial denaturation,

Table 1. Specific oligonucleotide primers used for amplification of seven polymorphisms in the *OASL* gene.

No.	Polymorphisms	Name of primer	Primer sequence (5'->3')	Location in OASL gene	PCR product (bp)
1	rs2859398	PCR For	GCCCTTTGCCACACTTGTAA	promoter	3kb
2	rs10083129	PCR Rev	AAGCAGATTCACCTGGCACT		
3	rs74390571				
4	rs59248852				
5	rs12811390	PCR For	CAATACTCATAGGGAGGCCG	intron 2	367
		PRC Rev	CTCAGTGATGTGTACCATGGAACT		
6	rs3213545	PCR For	GAAAACCATGTGGCAAAGC	exon 2	150
		PCR Rev	GCTCTGTAGGCAGGCACAAT		
7	rs1169279	PCR For	ATCACCTTGGGAGTAAGGACTTTT	3'end	117
		PCR Rev	TGCGGATTTTAGGAACTTCTTG		

10 cycles of denaturation for 15 sec at 95°C, annealing 30 sec at 60°C, and elongation 3 min at 68°C, followed by 25 cycles of 15 sec at 95°C, annealing 30 sec at 60°C, and elongation 3 min at 68°C plus 5 second at each cycle, and final extension was at 68°C for 7 min. The final PCR product was 3 kb.

The second PCR amplification reaction contained 0.1-0.5 µg genomic DNA, 10xB2 buffer (Solis BioDyne, Tartu Estonia), 4.0 pmol of specific oligonucleotide primers, 200 µM dNTPs, 2.5 mM $MgCl_2$, and 0.4U Hot Firepol DNA polymerase (Solis BioDyne, Tartu Estonia) in a total volume of 20 µl. The PCR conditions were as follows: 10 min initial denaturation, 38 cycles of 95°C for 30 sec, 60°C for 45 sec, 72°C for 1 min, and final extension was at 72°C for 5 min. Three PCR products were obtained: 150, 117 and 367 bp. ExoSAP-IT (USB, Cleveland, OH) was used for purifying the PCR products at at 37°C for 60 min followed by 86°C for 15 min for deactivation of the enzyme.

The SNaPshot PCR reaction was performed in multiplex format by adding specific primers for each polymorphism in concentration of 1 pM each (**Table 2**). The reaction was performed on a thermal cycler using the following conditions: 25 cycles of 95°C for 10 s, 55°C for 5 s, and 60°C for 30 s. The SNaPshot reaction was cleaned up with one unit of Shrimp alkaline phosphatase (SAP; GE Healthcare) for an hour at 37°C, followed by capillary electrophoresis on 3500 Genetic analyzer using GeneMapper Software Version 4.0 (Life Technologies). 0.5 µl GeneScan120 LIZ was used as a Size Standard ladder (Life Technologies Corporation, Carlsbad, CA, USA). The representative electrophoretic pattern for the seven analyzed *OASL* polymorphisms is given in **Fig. 1**.

Direct sequencing was used as a gold standard in order to optimize and validate the SNaPhot assay. Genomic DNA from 10 CHC patients, with previously determined *OASL* gene polymorphisms, was sequenced using BigDye terminator cycle sequencing reaction kit v1.1 (Applied Biosystems, Foster City, CA) and the same forward and reverse primers (**Table 1**). The amplified products were purified using 1-step enzymatic PCR and clean up system -Illustra ExoStar 1-step kit (GE Healthcare, Life Science, Little Chalfont, UK). Capillary electrophoresis was conducted on an ABI Prism 3130 Genetic analyzer (Life Technologies, USA), according the manufacturer protocol. Data were analyzed using GeneMapper analysis software version 4.0. These samples were also used to determine the reproducibility and performance (specificity and sensitivity) of the single-nucleotide primer extension method.

Table 2. Specific oligonucleotides used for the SNaPshot analysis of the seven *OASL* gene polymorphisms

Polymorphisms	SNaPshot primer sequence (5'->3')	Primer orient.	Nucl.change (N/M)*	Fragm. lenght N (bp)	Fragm. lenght M (bp)
rs2859398	CAGGAGGCAAAGCTTGCAGTGAGCCAAGA	F	T/C	50	52
rs12811390	CCCCCCCGAACTGGTTCCTGGTTCTCCCTAT	R	G/A	44	45
rs3213545	ATGGAGCAGAGAGTCCCCGATGCTCT	F	C/T	38	40
rs1169279	GACAGGCATTTGTTAGGGTTTGACATAAGTGA	R	C/T	32	34
rs10083129	CAAACCCCATCTCTACTAAAAGTA-CAAAAAATTAGCCGGGC	F	G/A	31	34
rs74390571	TGCCACCCTTTACTACTTAAACC	R	G/A	23	25
rs59248852	TGAAACCTAAATACATGCCCCCAA	R	C/T	24	26

*N=normal, M=mutant

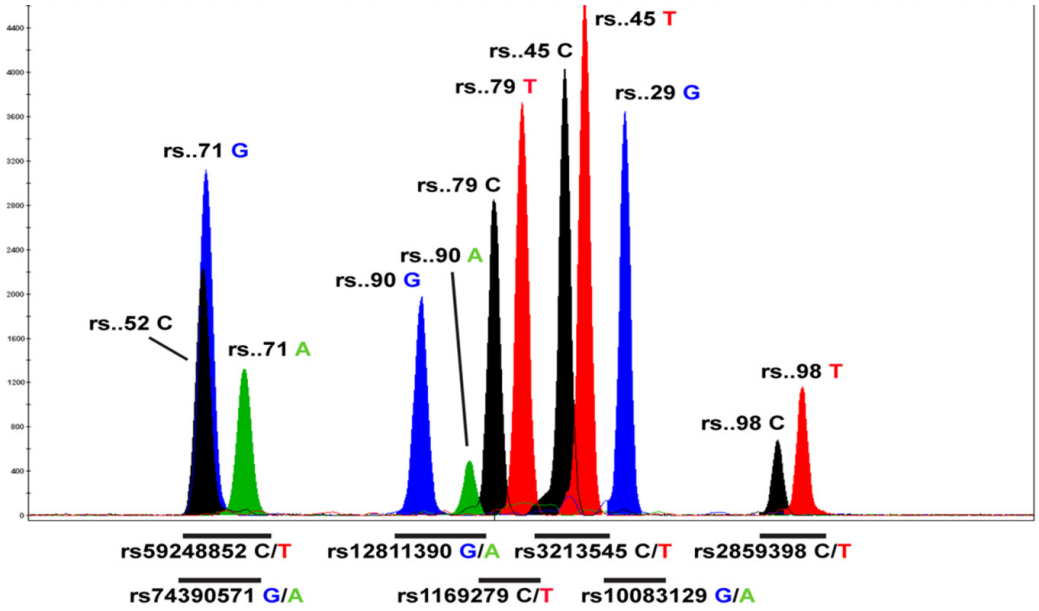

Figure 1. Representative electrophoreogram of the seven *OASL* gene polymorphisms analyzed with SNaPshot method.

Statistical analysis

We have performed all statistical calculations with SPSS 19.0 (SPSS, Chicago, IL, USA) software. Variables with categorical data were analyzed either with Fisher's exact test or the χ^2 test when appropriate. We have considered all *P*-values smaller than 0.05 as statistically significant. Odds ratios with their 95% confidence intervals for calculated χ^2 test were also presented. Stepwise multivariate logistic regression was performed in order to investigate the potential use of the studied polymorphisms for prediction of the therapeutic response. We have used Hosmer and Lemeshow test as test for the goodness of fit of the model to the data.

Results

In order to determine their association with the host's response to Peg-IFN-α plus ribavirin therapy in CHC patients, seven single nucleotide polymorphisms (rs59248852, rs74390571, rs12811390, rs1169279, rs3213545, rs1083129 and rs2859398) in *OASL* gene were analyzed using a multiplex SNaPshot method. A total of 209 CHC patients on pegylated interferon / ribavirin therapy of 24 / 48 weeks (for patients with genotype 3 / genotype 1, respectively) were included in the study. Patients who were negative for viral RNA at fourth week of therapy were considered with rapid virological response (RVR). Patients who showed no decline of the viral load after twelve weeks of treat-

Table 3. *OASL* gene polymorphisms in 10 DNA samples used for validation of the SNaPshot method

Patient	*OASL* gene polymorphisms determined with SNaPshot method							Concordance with BigDye standard sequencing method
	rs59248852	rs74390571	rs12811390	rs1169279	rs3213545	rs1083129	rs2859398	
HCV 2	C/C	A/A	A/A	C/C	C/C	G/G	T/T	100%
HCV 8	C/C	G/G	G/G	C/T	C/T	G/G	C/T	100%
HCV 22	C/C	A/A	G/A	C/T	C/T	A/A	C/C	100%
HCV 24	C/C	G/A	G/A	C/C	C/C	G/G	T/T	100%
HCV 60	C/C	G/G	G/G	T/T	C/T	G/A	C/T	100%
HCV 73	C/C	G/A	G/G	C/T	T/T	G/G	C/T	100%
HCV 81	T/T	A/A	A/A	T/T	C/T	A/A	T/T	100%
HCV 86	C/C	G/A	G/A	C/T	C/T	G/G	C/T	100%
HCV 96	T/T	A/A	G/G	C/C	C/C	G/G	T/T	100%
HCV 63	C/T	G/A	G/A	C/T	C/T	G/G	C/T	100%

Table 4. Distribution of allele frequencies of 7 polymorphisms in *OASL* gene analyzed among patients - non-responders to administered antiviral therapy (NVR) and patients with sustained virological response (SVR)

SNP	Alelle	NRs		SVR		p; OR (95% CI)
		No	%	No	%	
rs59248852	C	196	98,00	102	46,79	p = 6.5 x 10^{-31}; 55.7 (20.0-155.3)
	T	4	2,00	116	53,21	
	Total	200		218		
rs74390571	G	153	76,50	115	52,75	p = 4.3 x 10^{-7}; 2.9 (1.9-4.4)
	A	47	23,50	103	47,25	
	Total	200		218		
rs12811390	G	161	80,50	107	49,08	p = 2.2x10^{-11}; 4.3 (2.3-6.7)
	A	39	19,50	111	50,92	
	Total	200		218		
rs1169279	C	131	65,50	141	64,68	p = 0.8603 ; 1.0 (0.7-1.6)
	T	69	34,50	77	35,32	
	Total	200		218		
rs3213545	C	137	68,50	149	68,35	p = 0.9734 ; 1.0 (0.7-1.6)
	T	63	31,50	69	31,65	
	Total	200		218		
rs1083129	G	178	89,00	175	80,28	p = 0.0139 ; 2.0 (1.1-3.5)
	A	22	11,00	43	19,72	
	Total	200		218		
rs2859398	T	130	65,00	77	35,32	p = 1.34x10^{-9}; 3.4 (2.2-5.0)
	C	70	35,00	141	64,68	
	Total	200		218		

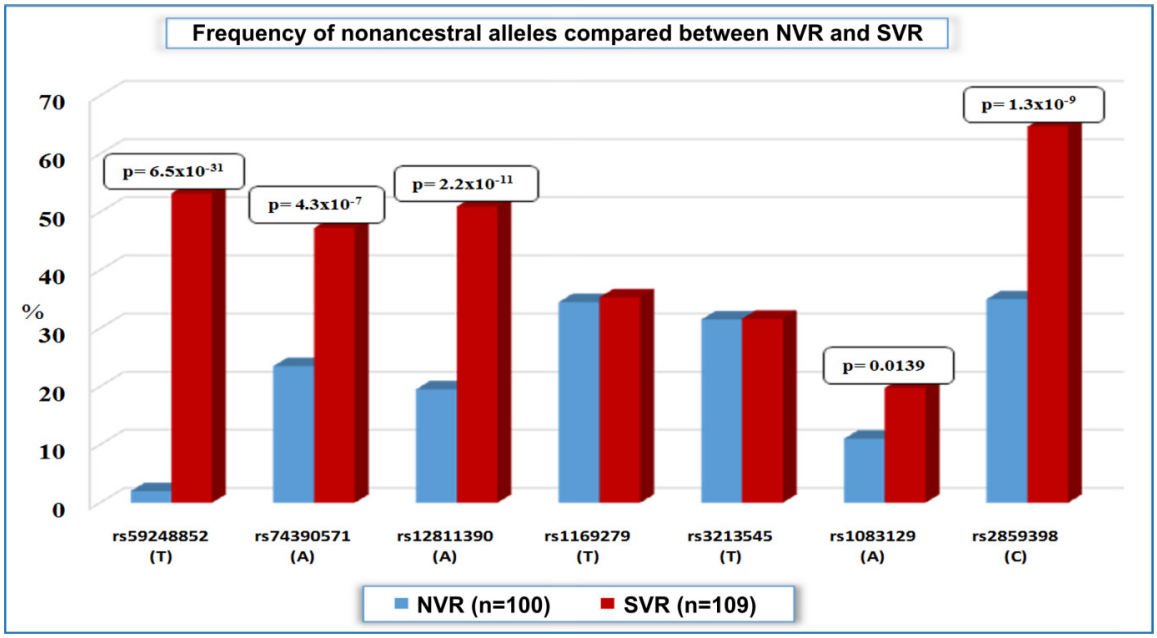

Figure 2. Allele distribution of 7 analyzed polymorphisms in *OASL* gene among patients with non-viral response (NVR) and patients with sustained viral response (SVR).

Table 5. Genotype distribution and frequencies of the analyzed OASL polymorphisms and 2-by-3 contingency table analyses

SNP	Genotype	NRs (n=100)		SVR (n=109)		Comparisons performed (SVR vs NVRs)		
						Comparisons using genetic models		Genotype test (2x3 table)
		No	%	No	%	Model	p; OR (95% CI)	p; χ^2
rs59248852	C/C	98	98.00	48	44.04	Dominant	$p=2x10^{-17}$; 0.02 (0.01-0.07)[a]	$p = 2.1 \times 10^{-16}$; $X^2 = 72.15$
	C/T	0	0.00	6	5.50			
	T/T	2	2.00	55	50.46	Recessive	$p=3.9x10^{-15}$; 49.9 (11.8-212.6)[b]	
	Total	100		109				
rs74390571	G/G	60	60.00	32	29.36	Dominant	$p= 8.3 \times 10^{-6}$; 0.3 (0.1-05)	$p=1.0 \times 10^{-5}$; $X^2 = 23.0$
	G/A	33	33.00	51	46.79			
	A/A	7	7.00	26	23.85	Recessive	p= 0.0008; 4.2 (1.7-10.1)	
	Total	100		109				
rs12811390	G/G	65	65.00	41	37.61	Dominant	$p= 7.6x10^{-5}$;0.3 (0.2-0.5)	$p=5.3 \times 10^{-9}$; $X^2 = 38.1$
	G/A	31	31.00	25	22.94			
	A/A	4	4.00	43	39.45	Recessive	$p= 8.7x10^{-10}$;15.2 (5.2-44.3)[c]	
	Total	100		109				
rs1169279	C/C	39	39.00	49	44.95	Dominant	p= 0.3838; 1.3 (0.7-2.3)	p = 0.0804; $X^2 = 5.1$
	C/T	53	53.00	43	39.45			
	T/T	8	8.00	17	15.60	Recessive	p= 0.0909; 2.1 (0.9-5.2)	
	Total	100		109				
rs3213545	C/C	42	42.00	55	50.46	Dominant	p= 0.2206; 1.4 (0.8-2.4)	p = 0.0143; $X^2 = 8.5$
	C/T	53	53.00	39	35.78			
	T/T	5	5.00	15	13.76	Recessive	p= 0.0315; 3.0 (1.0-8.7)	
	Total	100		109				
rs1083129	G/G	80	80.00	80	73.39	Dominant	p= 0.2602; 0.7 (0.4-1.3)	p = 0.0117; $X^2 = 8.9$
	G/A	18	18.00	15	13.76			
	A/A	2	2.00	14	12.84	Recessive	p= 0.0032; 7.2(1.6-32.6)[d]	
	Total	100		109				
rs2859398	T/T	42	42.00	19	17.43	Dominant	$p= 9.5 \times 10^{-5}$;0.3 (0.2-0.6)	$p = 6.6 \times 10^{-8}$; $X^2 = 33.1$
	C/T	46	46.00	39	35.78			
	C/C	12	12.00	51	46.79	Recessive	$p= 4.4 \times 10^{-8}$;6.5 (3.2-13.1)	
	Total	100		109				

[a]) Fisher exact test $p=1.1x10^{-19}$
[b]) Fisher exact test $p=7.7x10^{-17}$
[c]) Fisher exact test $p=3.9x10^{-9}$
[d]) Fisher exact test p=0.0034

ment or who remained HCV-RNA positive at week 24 were considered as non-responders (NVR). The sustained virological response (SVR) was obtained if a patient remains negative 24 weeks after ending therapy. Patients included in this study were separated in two groups: 100 patients without response to antiviral therapy (NVR) and 109 cases with sustained viral response (SVR). In the group of NVR, 21 patients were genotype 3 and 79 patients were with genotype 1, while in the group with SVR, 73 patients were genotype 3 and 36 patients with HCV genotype 1.

The validation of the assay was carried out on 10 DNA samples with *OASL* gene polymorphisms previously determined with SNaPshot method, demonstrating that it could accurately detect the 7 tested *OASL* gene polymorphisms (**Table 3**). Our results showed 100% concordance with the Sanger sequencing method confirming that the new assay was highly accurate. Reproducibility of the assay was ascertained by testing one sample in triplicate.

Allele frequencies distribution in both NVR and SVR patients groups for all the analyzed SNP's are presented in **Table 4**. Genotype frequencies for all analyzed SNP's together with the results from dominant and recessive genetic models used for comparison between NVR and SVR patients groups are presented in **Table 5**.

Table 6. Variables retained as predictors for SVR after performing stepwise multivariate logistic regression

Variables	B[a]	S.E.[b]	Wald	p value	Odds ratio (OR)
G3 genotype[c]	2,82	0,50	31,85	1,66E-08	16,844
rs59248852 - C/C (reference)			9,60	0,0082	
rs59248852 - C/T	21,77	13996,46	0,00	0,9988	2,84E+09
rs59248852 - T/T	3,27	1,06	9,60	0,0019	26,338
rs74390571 - G/G (reference)			8,16	0,0169	
rs74390571 - G/A	1,80	0,66	7,52	0,0061	6,037
rs74390571 - A/A	0,37	1,01	0,14	0,7116	1,453
rs12811390 - G/G (reference)			15,01	0,0006	
rs12811390 G/A	-2,11	0,73	8,28	0,0040	0,121
rs12811390 A/A	1,17	0,84	1,97	0,1603	3,235
rs2859398 - T/T (reference)			5,28	0,0712	
rs2859398 - C/T	0,60	0,48	1,52	0,2171	1,816
rs2859398 - C/C	1,51	0,66	5,16	0,0231	4,529
Constant	-2,80	0,55	25,96	3,49E-07	0,061

[a]) Regression coeficient
[b]) Standard error of B
[c]) G1 genotype as a reference

When comparing allele frequencies between NVR and SVR patients we found significant association for five of the studied *OASL* gene SNP`s: rs59248852 [p = $6.5x10^{-31}$, OR=55.7 (20.0-155.3)]; rs74390571 [p=$4.3x10^{-7}$, OR=2.9 (1.9-4.4)], rs12811390 [p = $2.2x10^{-11}$, OR=4.3 (2.3-6.7)], rs1083129 [p=0.0139, OR=2.0 (1.1-3.5)] and rs2859398 [p=$1.34x10^{-9}$, OR=3.4 (2.2-5.0)]. For two SNP`s (rs1169279 and rs3213545) we didn't find any significant association between the analyzed groups. Comparison of *OASL* allele frequencies between NVR and SVR patients is shown in **Fig. 2**. Alleles rs59248852-T, rs74390571-A, rs12811390-A, rs1083129-A and rs2859398-C were more common in the group of patients with SVR in comparison to those who did not respond to the standard therapy.

Analyzing genotype distribution in rs59248852 polymorphism we found TT genotype in only 2% of analyzed NVR patients, while 50.46% of patients in SVR group have TT genotype. Statistical analysis indicated that patients with TT genotype for the rs59248852 polymorphism have almost 50 times higher chances to respond to the interferon therapy than CC or CT genotypes [p=$3.9x10^{-15}$; OR (95%CI)= 49.9 (11.8-212.6)].

Genotype distribution of rs74390571 polymorphism shows AA genotype in seven patients (7%) of NVR group, and 26 (23.85%) patients in SVR group. Statistical analysis indicated that patients with AA genotype for the rs74390571 polymorphism have 4.2 times higher chances to respond to the interferon therapy than GG or GA genotypes [p= 0.0008; OR (95%CI)= 4.2 (1.7-10.1)].

AA genotype in rs12811390 polymorphism was detected in four patients (4%) of NVR group, while 43 (39.45%) patients in SVR group. Statistical analysis indicated that patients with AA genotype for the rs12811390 polymorphism have 15 times higher chances to respond to the interferon therapy than GG or GA genotypes [p= $8.7x10^{-10}$; OR (95%CI)=15.2 (5.2-44.3)].

AA genotype in rs1083129 polymorphism is also favorable for respond to the therapy with two patients with AA genotype (2%) in NVR group and 14 (12.84%) in SVR group. Statistical analysis indicated that patients with AA genotype for the rs1083129 polymorphism have 7.2 times higher chances to respond to the interferon therapy than GG or GA genotypes [p= 0.0032; OR (95%CI)=7.2(1.6-32.6)].

Genotype distribution in rs2859398 polymorphism was as follows: TT genotype was determined in 42 patients without viral response or 42%, CT genotype was observed in 46 (46%), while 12 patients in this group have CC genotype (12%). In the group of patients with SVR 19 or 17.43% were with genotype TT, 39 or 35.78% were heterozygous CT, while 51 (46.79%) patients were with CC genotype. Statistical analysis indicate that patients with CC genotype for the rs2859398 polymorphism have 6.5 times more chances to respond to the interferon therapy than CT or TT genotypes [p= 4.4×10^{-8}; OR (95%CI)=6.5 (3.2-13.1)].

In stepwise logistic regression analysis we have included the genotype data for seven studied polymorphisms (homozygous genotypes for major alleles as references) and HCV genotype (G1 genotype as a reference) when building the model for prediction of the SVR outcome (NVR as a reference) (**Table 6**).

The model retained 4 (rs59248852, rs74390571, rs12811390 and rs2859398) out of 7 studied SNPs as predictors. The p value for the Hosmer and Lemeshow test at final step was p=0.8791, suggesting that the model is a good fit to the data. When the classification cut-off was set at 0.6, the model correctly classified the outcome in 84.6% of the cases.

Discussion

Seven single nucleotide polymorphisms (rs59248852, rs74390571, rs12811390, rs1169279, rs3213545, rs1083129 and rs2859398) in *OASL* gene were analyzed in two groups of CHC patients (NVR and SVR) in order to determine their influence on host response to Peg-IFN-α and RBV therapy. For that purpose a multiplex SNaPshot method was introduced. It is accurate, simply to perform, cost effective and time consuming in comparison with PCR-RFLP or dot blot hybridization.

Of the seven SNP's evaluated, a clear statistical association was found in five of them. A strong significant association was determined in four: rs59248852, rs74390571, rs12811390 and rs2859398 ($p=6.5x10^{-31}$, $p=4.3x10^{-7}$, $p=2.2x10^{-11}$ and $p=1.34x10^{-9}$ respectively), while in one rs1083129, a significant association was found with p=0.0139. The rs59248852-T allele, rs74390571- A allele, rs12811390-A allele, rs1083129-A allele and rs2859398-C allele were significantly associated with obtaining SVR after interferon administered therapy (Table 5). The logistic regression showed that SNPs with strong significant association for SVR analyzed with the genotype test (Table 5) were also significant predictors for SVR when analyzed together (**Table 6**).

With this study we confirm the previous reports that polymorphisms in *OASL* gene could be host related factors that determine the success rate of the interferon therapy. The human OAS family is located on 12q24.1 region on chromosome 12. It consists of four genes: *OAS1, OAS2, OAS3* and *OASL* gene. It is already defined that some single nucleotide polymorphisms (SNPs) in this genes have influence on the host response to administered interferon-based therapy in patients infected with HCV. Su et al. in 2008 (20) found three *OASL* SNPs (rs2859398; rs3213545; rs1169279) to be associated with sustained virological response and they suggested the possible mechanism of the influence of these polymorphisms on the response to interferon therapy. For example they speculate that change in the sequence C/T in rs3213545, leads to change in SF2/AFF consensus ESE site, thus leading to reduction of the amount of full-length transcripts of the corresponding proteins. Their explanation was that the carrier of the mutant allele for those SNPs may have lower expression of *OASL* gene as compared with normal allele carriers, a condition that lead to increased antiviral activity of OAS isozymic forms, and viral clearance (29).

Other studies also indicate the influence of *OASL* gene polymorphisms on the outcome of interferon therapy. Yakub et al. 2005 analyzing the influence of 23 SNP's in *OASL* gene on outcome of therapy in patients infected with West Nile Virus infection, also identified a strong relationship between SNP rs3213545 and susceptibility to severe WNV disease (30-32). In the cohort of 23 analyzed SNP's that Yakub included in his study was a rs3213545, and supporting our results, he also did not found an association between this SNP and the outcome of the administered therapy.

Domagalski et al. analyzing the influence of IL28B and OAS polymorphisms, find an association between *OASL* gene polymorphism (rs1083129) and response to interferon therapy in pediatric cases with Hepatitis B infection (33).

The prediction of an individual's response before the treatment is of great clinical importance for avoiding the side effects of the therapy as well as the substantial cost of PEG-IFN-α / RBV treatment in those patients that do not respond. The results of this study suggest that CHC patients with HCV Genotype 3 and OASL polymorphisms rs59248852 - T/T, rs74390571 - A/A, rs12811390 A/A and rs2859398 - C/C are significantly associated with SVR and might be used as predictors for successful administration of interferon therapy.

In conclusion, an inexpensive multiplex SNaPshot method was introduced for rapid determination of OASL polymorphisms that can be used as predictive factors of patient's response to the standard interferon therapy still used in many developing countries, thus having an important cost benefit for their health insurance systems.

Declaration of Interest

The authors declare that there is not any conflict of interest and that they alone are responsible for the content and writing of this article.

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