



# Association between polymorphisms in genes encoding 2'-5'-oligoadenylate synthetases and the humoral immune response upon vaccination against tick-borne encephalitis

N.S. Yudin<sup>1, 2, 3</sup>✉, A.V. Igoshin<sup>1</sup>, S.L. Lutova<sup>4</sup>, Ya. Gon<sup>2</sup>, M.I. Voevoda<sup>1, 2, 3</sup>, V.A. Belyavskaya<sup>5</sup>

<sup>1</sup> Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

<sup>2</sup> Novosibirsk State University, Novosibirsk, Russia

<sup>3</sup> Research Institute of Internal and Preventive Medicine – Branch of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

<sup>4</sup> State Budgetary Health Care Institution of the Novosibirsk Region "City Hospital № 14", Novosibirsk, Russia

<sup>5</sup> State Research Center of Virology and Biotechnology "Vector" of the Federal Service for Surveillance in Consumer Rights Protection and Human Well-being, Novosibirsk region, Koltsovo, Russia

Vaccination forms active immunity and represents an effective way of preventing tick-borne encephalitis (TBE). However, excessive vaccination is unjustified in terms of economics and medical ethics. One of the individualized approaches to vaccines is the selection of vaccine doses depending on the expected levels of immune response. Therefore, there is a need for new methods for assessing potential human immune responses prior to vaccination. The aim of this study was to determine possible association between single nucleotide polymorphisms (SNPs) located within *OAS2* and *OAS3* genes, which have been previously associated with the development of severe forms of TBE, and the formation of antibodies and cytokines upon vaccination against TBE. The study involved 97 volunteers of both sexes who had not previously been vaccinated against TBE and had no contact with ticks. Venous blood samples were collected one month after vaccination against TBE using the EnceVir vaccine. Levels of specific IgG antibodies against tick-borne encephalitis virus and interleukin 4 (IL-4) were analyzed. Genomic DNA samples were genotyped for the SNPs rs2285932, rs2072136, rs1293762, rs15895 and rs1732778 in genes encoding 2'-5'-oligoadenylate synthetases *OAS2* and *OAS3*. Antibody production in response to vaccine administration was significantly associated with SNP rs1732778 in the regulatory region of the *OAS2* gene. This indicator was significantly higher in people with heterozygous genotypes G/A as compared to people with homozygous genotypes G/G and A/A. Carriers of the A allele (G/A or A/A genotypes) of the same SNP had reduced IL-4 levels as compared to the homozygous G/G individuals. Thus, the data obtained indicate that SNP rs1732778 in the regulatory region of the *OAS2* gene correlates with the formation of antiviral IgG antibodies and changes in IL-4 levels upon vaccination. Evidently, the genetic polymorphism in *OAS2* gene should be considered when performing individualized TBE vaccinations.

Key words: tick-borne encephalitis; vaccination; IgG antibodies; gene; *OAS2*; *OAS3*; single nucleotide polymorphism; association.

## Ассоциация полиморфизма генов 2'-5'-олигоаденилатсинтетаз с уровнем гуморального иммунного ответа после вакцинации против клещевого энцефалита

N.S. Yudin<sup>1, 2, 3</sup>✉, A.V. Igoshin<sup>1</sup>, S.L. Lutova<sup>4</sup>, Ya. Gon<sup>2</sup>, M.I. Voevoda<sup>1, 2, 3</sup>, V.A. Belyavskaya<sup>5</sup>

<sup>1</sup> Федеральное исследовательское учреждение Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия

<sup>2</sup> Новосибирский национальный исследовательский государственный университет, Новосибирск, Россия

<sup>3</sup> Научно-исследовательский институт терапии и профилактической медицины – филиал Федерального исследовательского центра Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия

<sup>4</sup> Государственное бюджетное учреждение здравоохранения Новосибирской области «Городская поликлиника № 14», Новосибирск, Россия

<sup>5</sup> Государственный научный центр вирусологии и биотехнологии «Вектор» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека, Новосибирская область, р. п. Кольцово, Россия

Вакцинация является эффективным средством профилактики клещевого энцефалита, формирующим активный иммунитет. Однако избыточная иммунизация при вакцинации неоправдана с точки зрения экономики и медицинской этики. Одним из подходов к индивидуализации вакцинации может быть подбор доз вакцины в зависимости от ожидаемого уровня иммунного ответа пациента. Поэтому возникает необходимость разработки методов оценки потенциального уровня иммунологических реакций человека до проведения вакцинации. Цель работы – поиск возможных ассоциаций однонуклеотидных полиморфных маркеров (ОНП) в генах *OAS2* и *OAS3*, для которых ранее была найдена корреляция с развитием тяжелых форм клещевого энцефалита, а также с образованием антител и цитокинов после вакцинации против клещевого энцефалита. В исследовании приняли участие 97 добровольцев обоего пола, ранее не вакцинированных и не имевших контактов с клещами. Через один месяц после иммунизации вакциной «ЭнцеВир» у них брали пробы венозной крови. Анализировали уровни специфических антител IgG против вируса клещевого энцефалита и интерлейкина 4 (ИЛ-4). Генотипировали

ОНП rs2285932, rs2072136, rs1293762, rs15895 и rs1732778 в генах 2'-5'-олигоаденилатсинтетаз *OAS2* и *OAS3*. Выработка антител в ответ на введение вакцины была достоверно ассоциирована с ОНП rs1732778 в регуляторном районе гена *OAS2*. Этот показатель оказался существенно выше у людей с гетерозиготным генотипом G/A, чем у субъектов с гомозиготными генотипами G/G и A/A. Индивиды-носители аллеля A в составе генотипов G/A и A/A этого же ОНП имели сниженный уровень ИЛ-4 по сравнению с гомозиготными индивидами G/G. Полученные нами результаты свидетельствуют о том, что ОНП rs1732778 в регуляторной области гена *OAS2* ассоциирован с образованием противовирусных антител IgG и уровнем ИЛ-4 после вакцинации. По-видимому, генетический полиморфизм в гене *OAS2* следует принимать во внимание при индивидуализации вакцинопрофилактики клещевого энцефалита.

Ключевые слова: клещевой энцефалит; вакцинация; IgG антитела; ген; *OAS2*; *OAS3*; однонуклеотидный полиморфизм; ассоциация.

#### HOW TO CITE THIS ARTICLE:

Yudin N.S., Igoshin A.V., Lutova S.L., Gon Ya., Voevoda M.I., Belyavskaya V.A. Association between polymorphisms in genes encoding 2'-5'-oligoadenylate synthetases and the humoral immune response upon vaccination against tick-borne encephalitis. Vavilovskii Zhurnal Genetiki i Selektcii = Vavilov Journal of Genetics and Breeding. 2018;22(4):445-451. DOI 10.18699/VJ18.381

Tick-borne encephalitis (TBE) is a natural focal viral infection that manifests as fever, intoxication and damage to the gray matter of the brain (encephalitis) and/or meninges (meningitis and meningoencephalitis) (Jerusalimsky, 2001). The disease can lead to long-lasting neurological and psychiatric complicating disorders or even death of the infected patient. TBE agent is the RNA-containing virus (TBEV) of the family Flaviviridae. The main vectors of disease and natural reservoir for the agent to survive are scale ticks *Ixodes persulcatus* and *Ixodes ricinus*. The cases are being detected more and more often in new areas as the evidence of the virus migration (Valarcher et al., 2015). Vaccination is the efficient way of preventing TBE by active immunity forming (Bilalova, 2009). Constant growth of people mobility and intensification of migration process make vaccination more and more urgent.

As new information is accumulated on immune response development mechanisms, its prediction and potential side effects, the differentiated approach towards preventive, or so called individualized vaccination, becomes actual (Medunitsin, 2004). Individualized vaccination aims to create necessary immune protection for the organism avoiding excessive immunization, which is unjustified and inappropriate in terms of economics and medical ethics. One of the individualized approaches is the vaccine dose selection depending on its potency, vaccination process stage and the expected levels of immune response. Thus, the methods should be developed to assess the potential human immune responses prior to vaccination.

Individual variability of antibodies formation and long-term post-vaccinal presence is mainly determined by the inherited peculiarities of cell-mediated and humoral immunity, type of the vaccine, vaccination schedule and some environmental factors, like nutrition (Poland et al., 2008). Genes to encode the immune system proteins and DNA regulatory regions near them comprise a large number of single nucleotide polymorphisms (SNPs) (Poland et al., 2011). In the main

complex of histocompatibility genes, cytokine genes and their receptors, SNPs have been established to correlate with the individual immune response (or its absence) to the vaccination (Linnik, Egli, 2016).

2'-5'-oligoadenylate synthetase protein family (*OAS*) are important factors of innate antiviral immunity. In humans, there are known four genes encoding the proteins of this family. They are *OAS1*, *OAS2* and *OAS3* located as a cluster in q24 region of the chromosome 12 and *OASL* localized in chromosome 12. In the cell, *OAS1*, *OAS2* and *OAS3* proteins are in monomeric form. dsRNA of the virus causes activation of enzymes which use ATP as a substrate and catalyze AMP polymerization producing 2'-5'-oligoadenylates. Their reaction with latent endoribonuclease L leads to its dimerization and activation, thus to both cell RNA and vRNA degradation and consequently, virus reproduction is suppressed. mRNA transcription of the *OAS* family genes is induced by interferons (Hovanessian, Justesen, 2007; Kristiansen et al., 2011; Yudin et al., 2018).

In groups with severe TBE cases (central nervous system damages as meningo-encephalitic form of the disease) and milder ones without central nervous system damages (meningeal form, fever) and/or population control, analysis of 23 SNPs within *OAS1*, *OAS2*, *OAS3* and *OASL* genes has previously revealed statistically significant distinction between genotype, allele and/or haplotype frequencies by five SNPs; those were rs1293762 (intron2), rs15895 (3'-UTR), rs1732778 (3'-flanking region) of *OAS2* genes and rs2285932 (exon6, Ile438Ile), rs2072136 (exon8, Ser567Ser) of *OAS3* genes (Barkhash et al., 2010). SNPs within the genes of *OAS* family are associated with susceptibility to other diseases caused by flaviviruses, such as West Nile fever (Yakub et al., 2005; Lim et al., 2009; Bigham et al., 2011; Danial-Farran et al., 2015) and Dengue fever (Alagarasu et al., 2013; Thamizhmani, Vijayachari, 2014), or with the response to the hepatitis C antiviral interferon therapy (Imran et al., 2014). I.H. Haralambieva et al. (2010) established the association

of 23 SNPs in the *OAS* cluster with the level of antibodies and interleukin-4, interleukin-6 and interleukin-10 secretion upon rubella vaccination.

The study targets to determine possible association between SNPs located within *OAS2* and *OAS3* genes being previously associated with the development of severe forms of TBE, and the formation of antibodies and cytokines upon vaccination against TBE.

## Materials and methods

The study involved 97 volunteers (56 men and 41 women) aged from 15 to 30 years old (median age 26) who had not previously been vaccinated against TBE and had no contact with ticks recorded in their surveys. The EnceVir vaccine against TBE (SPA “Microgen”, Tomsk) was used for vaccination. It is a purified concentrated sterile suspense of inactivated by formalin and aluminum hydroxide adsorbed TBE virus grown in suspension culture of chicken embryos. Vaccination was performed according to the scheme proposed by the manufacturer. Venous blood samples were collected one month upon the vaccination. The project was approved by the ethics committee of the Research Institute of Internal and Preventive Medicine, the branch of the Federal Research Center Institute of Cytology and Genetics SB RAS. The informed consent to participate in the investigation was received from all the patients.

Levels of specific IgG antibodies against tick-borne encephalitis virus (TBEV) and interleukin 4 (IL-4) were analyzed by immune-enzyme assay using test-systems VectoTBE-IgG-strip and Interleukin-4-IEA-BEST (JSC “Vector-Best”, Koltzovo, Novosibirsk region).

The genomic DNA was isolated by peripheral blood proteolysis followed by phenol extraction (Sambrook, Russel, 2006). Genotyping for the SNPs rs2285932, rs2072136, rs1293762, rs15895 and rs1732778 in genes encoding 2'-5' -oligoadenylate synthetases *OAS2* and *OAS3* was performed in accordance with techniques proposed by A.V. Barkhash et al. (2010). Genotype deviation of distribution from Hardy – Weinberg equilibrium was determined by Hardy – Weinberg equilibrium calculator (<http://www.oege.org/software/hwe-mr-calc.shtml>).

The Kolmogorov–Smirnov test was used to assess the distribution normality of the traits under the study. As the distribution of the characteristics was not normal, the medians were calculated and 25–75 % quartiles in the groups being compared. The gender effect was assessed by Mann-Whitney *U* test. Gender turned not to affect the analyzed traits significantly and it was not further taken into account. For genotype effect assessment, general linear model was used. For statistical analysis of the dependent variable (specific IgG antibodies level), logarithmic transformation to normality was applied. Our earlier study of the same sample has established the certain influence of the age on this indicator (Lutova et al., 2016). Thus for the model, genotype was taken as a fixed factor, while age as a covariate. Paired comparison was made by post hoc analysis, *F*-test being applied. Critical value of statistical significance for null hypothesis was taken as 0.05. For the data treatment the software package STATISTICA 8.0 was used.

## Results

We have registered the protective immune response against TBE in 85.8 % of women and 86.3 % of men, the IgG level being > 100 IU/ml. Analogous results were earlier obtained by other researchers while performing preventive vaccination of adult population (Bilalova, 2009). In all the individuals involved, IL-4 level was found to be within the range (0.37–25.95 mg/ml) testifying the normal course of the vaccination process (JSC “Vector-Best”, 2017).

In all the 97 samples, SNPs within the analyzed genes were genotyped successfully, but for rs2072136, rs1293762 and rs15895 being not revealed in one sample. Aside from rs1732778 ( $p < 0.05$ ), there was no significant deviation of genotype distribution from Hardy–Weinberg equilibrium for all SNPs. For rare alleles, the frequencies were as follows: 0.309 for SNP rs2285932 in *OAS3* (T allele); 0.313 for SNP rs2072136 in *OAS3* (A allele); 0.411 for SNP rs1293762 in *OAS2* (T allele); 0.302 for SNP rs15895 in *OAS2* (A allele) и 0.361 for SNP rs1732778 in *OAS2* genes (A allele). Those results are in agreement with the frequencies of the involved alleles being previously revealed in the population of Novosibirsk: 0.251, 0.294, 0.420, 0.281 and 0.273, correspondingly (Barkhash et al., 2010).

The results of analysis of SNPs in genes encoding 2'-5'-oligoadenylate synthetases association with IgG against TBEV and IL-4 levels are presented in Table. Antibody production in response to vaccination was significantly associated with SNP rs1732778 in the regulatory region of the *OAS2* gene ( $p < 0.05$ ). In people with heterozygous genotypes G/A, this indicator appeared to be significantly higher by comparison to people with homozygous genotypes G/G and A/A ( $p < 0.01$ ). Carriers of the A allele (G/A or A/A genotypes) of the same SNP had a reduced IL-4 levels as compared to the homozygous G/G individuals ( $p < 0.004$ ). Moreover, the distinctions held true upon application of Bonferroni correction for multiple comparisons. SNP rs15895 association with IL-4 titers can be considered as a tendency ( $p < 0.06$ ). However, paired comparisons revealed the individuals with the homozygous A/A genotype to have significantly reduced IL-4 as opposed to the carriers of the G allele ( $p < 0.04$ ).

## Discussion

Immune response upon vaccination is affected by numerous factors including individual genetic peculiarities. To work out the individual approach to preventive vaccination, it is essential to identify the genetic markers which control the immune response development (Medunitsin, 2004). Specific antibody production upon the vaccine administration is significantly governed by the genes encoding the main histocompatibility complex proteins, genes of cytokines and their receptors and of Toll-like receptors and others (Linnik, Egli, 2016). However, genetic factors mediating the immune response to vaccination against the flaviviral infections remain hardly studied. There is only one published case of vaccine-associated viscerotropic disease in 64-years old patient with mutations in *CCR5* chemokine receptor and its ligand RANTES genes after administration of the attenuated vaccine against yellow fever (Pulendran et al., 2008). In the sample involved in the present paper, deletion in the *CCR5* gene was earlier shown to offer promise as a potential marker of

Association of SNPs in genes encoding 2'-5'-oligoadenylate synthetases with levels of specific IgG antibodies against TBEV (IU/ml) and IL-4 (mg/ml) upon vaccination against tick-borne encephalitis

Gene	Position	Region	SNP	Genotype	Median*	25–75 % quartiles	p**
IgG							
OAS3	112 949 145	Exon 6, p.Ile438Ile	rs2285932	C/C (n = 44)	221.5	156.0–260.0	0.846
				C/T (n = 46)	219.0	101.0–260.0	
				T/T (n = 7)	210.0	81.0–260.0	
OAS3	112 961 114	Exon 8, p.Ser567Ser	rs2072136	G/G (n = 43)	210.0	121.0–260.0	0.988
				G/A (n = 46)	227.5	101.0–260.0	
				A/A (n = 7)	216.0	39.0–260.0	
OAS2	112 993 031	Intron 2	rs1293762	G/G (n = 35)	191.0	71.0–245.0	0.412
				G/T (n = 43)	237.0	145.0–260.0	
				T/T (n = 18)	199.0	94.0–238.0	
OAS2	113 010 483	Exon 10, p.Ter720Trp	rs15895	G/G (n = 48)	198.0	110.5–255.0	0.859
				G/A (n = 38)	236.0	101.0–260.0	
				A/A (n = 10)	199.0	136.0–243.0	
OAS2	113 019 120	3'-region, 7 397 bp	rs1732778	G/G (n = 35)	191.0 <sup>a</sup>	90.0–260.0	<b>0.047</b>
				G/A (n = 54)	235.5 <sup>b</sup>	169.0–260.0	
				A/A (n = 8)	112.0 <sup>a</sup>	31.0–201.0	
IL-4							
OAS3	112 949 145	Exon 6, p.Ile438Ile	rs2285932	C/C (n = 40)	9.89	4.47–15.65	0.835
				C/T (n = 40)	10.06	5.36–15.21	
				T/T (n = 5)	7.76	5.83–12.58	
OAS3	112 961 114	Exon 8, p.Ser567Ser	rs2072136	G/G (n = 37)	11.16	6.04–15.78	0.605
				G/A (n = 40)	8.31	4.35–14.84	
				A/A (n = 7)	10.0	4.56–14.28	
OAS2	112 993 031	Intron 2	rs1293762	G/G (n = 32)	9.21	5.83–15.21	0.926
				G/T (n = 38)	10.52	4.39–14.40	
				T/T (n = 14)	8.04	4.82–16.67	
OAS2	113 010 483	Exon 10, p.Ter720Trp	rs15895	G/G (n = 43)	8.42 <sup>a</sup>	4.39–14.28	<b>0.061</b>
				G/A (n = 33)	13.67 <sup>a</sup>	8.09–16.67	
				A/A (n = 8)	6.80 <sup>b</sup>	4.69–9.39	
OAS2	113 019 120	3'-region, 7 397 bp	rs1732778	G/G (n = 25)	14.40 <sup>a</sup>	7.76–16.80	<b>0.004</b>
				G/A (n = 53)	8.42 <sup>b</sup>	4.56–12.46	
				A/A (n = 7)	4.56 <sup>b</sup>	3.36–12.58	

\* The same letters of superscripts indicate absence of significant differences between medians for different genotype according to post hoc analysis by *F*-test.

\*\* Significance of SNP genotypes influences assessment by general linear model.

successful vaccination against TBE in the Russian population of West Siberia (Lutova et al., 2016).

This paper presents the investigations of polymorphisms in five SNPs located within *OAS2* and *OAS3* genes, which have previously been associated with the development of severe forms of TBE (Barkhash et al., 2010). To our knowledge, this is the first study on the influence of SNP loci in genes encoding 2'-5'-oligoadenylate synthetases on the specific antibody production upon vaccination against TBE. To eliminate possible influence of foreign genetic and environmental factors, the sample was formed mainly of young Russian volunteers, who had not previously been vaccinated against TBE and had no contact with ticks though having lived in the endemic TBE region over a long period.

The frequencies of rare SNP alleles under the study correspond well to those alleles frequencies previously revealed in the Russian population of Novosibirsk (Barkhash et al., 2010). The significant deviation of genotype distribution from Hardy–Weinberg equilibrium was observed only for SNP rs1732778 within the *OAS2* gene. Other researchers also noted the deviation of genotype distribution for SNPs rs1732778, rs2285932 and rs2072136 in the samples of Novosibirsk Russian residents with different forms of TBE (Barkhash et al., 2010). Hence, the reason of the deviation of SNP rs1732778 genotype distribution from Hardy–Weinberg equilibrium may be explained not by genotyping errors, but panmixia absence within the analyzed population, though the population stratification or the influence of selection should not be excluded either. It is notable that studying in seven ethnic groups of Northern Eurasia has detected the lowest frequencies of the G/G genotype for SNP rs2072136 within *OAS3* gene (the gene associated with predisposition to severe forms of TBE development) in the Altaians, Khakasses, Tuvinians and Shorians who probably have intensive contacts with ticks in the places of residence (Barkhash et al., 2010). The authors suggested that the tick-borne encephalitis virus might have become the selection factor of certain variations of *OAS* genes in Central Asia mongoloid people.

We have established the association of SNP rs1732778 in *OAS2* gene with the level of IgG upon vaccination against TBE (see Table). Antibody levels in individuals with the G/A heterozygous genotype were significantly higher in comparison to the ones with the homozygous genotypes G/G or A/A. Such superiority of heterozygotes over homozygotes can be observed rather often. For example, cellular immune response to the pertussis toxin and filamentous hemagglutinin was superior in the carriers of the A/G genotype of SNP rs1800896 in the *IL-10* gene promoter in comparison to the one of the individuals with G/G or A/A genotypes (Gröndahl-Yli-Hannuksela et al., 2016). We have found the only published paper aiming to investigate the role of *OAS* genes in the immune response development upon vaccination. I.H. Haralambieva with colleagues (2010) demonstrated that minor alleles of SNPs rs1732778 and rs2464288 located within the *OAS2* gene are related to the higher level of specific antibodies upon rubella immunization. SNP rs1732778 is associated with sensibility to the flaviviral diseases: TBE (Barkhash et al., 2010) and Dengue fever (Alagarasu et al., 2013; Thamizhmani, Vijayachari, 2014). It should be pointed that SNP rs1732778 is located at a distance of 7397

bp from the 3'-region of the *OAS2* gene (UCSC Genome Browser Gateway, 2017). It is not inconceivable that not this SNP directly affects the trait, but the other one, which is in linkage disequilibrium. In the chromosome 12 region of about  $\pm 250,000$  bp, 11 genes are located in the vicinity of rs1732778 (*RPH3A*, *OAS1*, *OAS2*, *OAS3*, *DTX1*, *RASAL1*, *CFAP73*, *DOX54*, *RITAI*, *IQCD*, *TPCNI*). However, only three of them (namely, the genes encoding 2'-5'-oligoadenylate synthetases *OAS1*, *OAS2* and *OAS3*) participate in the immune response controlling.

Our investigations have established that SNP rs1732778 located within *OAS2* gene is significantly associated with the IL-4 level upon vaccination against TBE. The result is intensified, as association between the IL-4 level and SNP rs15895, which is located in exon 10 of *OAS2* gene at a distance of 8637 bp from SNP rs1732778 has also appeared to be a tendency (see Table). IL-4 is an important regulatory cytokine which is produced by mast cells, Th2 cells, eosinophils and basophils (Gadani et al., 2012). IL-4 controls Th2-type cellular immunity activation (Zamorano et al., 2003), IgE production and secretion by B cells (Geha et al., 2003) and the alternative activation of macrophages, which differs from usual anti-inflammatory activation (Gordon, 2003). As the IL-4 level upon rubella vaccination is extremely low, other scientists did not reveal any SNP associations within *OAS* gene cluster (Haralambieva et al., 2010). The role of IL-4 in humoral immune response development has not been clarified yet. It can be assumed that innate antiviral immunity, which is regulated by *OAS* family genes, is involved to lymphocyte stimulation control thus modeling the structure and amplitude of specific (adaptive) response to the inactivated-virus vaccine.

This proposal is confirmed by the results of several investigations. Of all the *OAS* gene family in humans, *OAS2* has the highest level of induction by interferons (Sanda et al., 2006). By a proteomics approach the direct physical interaction between *OAS2* and *NOD2* proteins was shown in THP-1 human cell line (Dugan et al., 2009). *NOD2* protein is a member of Nod1/Apaf-1 family; being expressed in leucocytes of the peripheral blood it plays an important role in the immune response to bacterial lipopolysaccharides as it binds to bacterial muramyl dipeptide and activates NF $\kappa$ B protein (Ogura et al., 2001). *NOD2* protein may probably participate in the innate antiviral immunity formation. Another protein from *NOD*-like receptor family, *NLRP3* is known to be capable to interact with influenza A virus (Allen et al., 2009).

Thus, the results we have obtained testify the fact that SNP rs1732778 located in the regulatory region of the *OAS2* gene is associated with antiviral IgG antibodies and with the IL-4 level upon vaccination against tick-borne encephalitis. Genetic polymorphism in the *OAS2* gene should probably be taken into consideration to perform individualized preventive vaccination against this disease. However, due to the limited sample size, the results from the study should be interpreted with caution. Further investigations are needed to confirm those associations for large independent population samples and to analyze the mechanisms of the influence of polymorphisms in genes encoding 2'-5'-oligoadenylate synthetases on the immune response to vaccination against flaviviral diseases.

## Acknowledgements

This work was supported by Russian Science Foundation grant (project 16-15-00127).

## Conflict of interest

Authors state the absence of conflict of interest.

## References

- Alagarasu K., Honap T., Damle I.M., Mulay A.P., Shah P.S., Cecilia D. Polymorphisms in the oligoadenylate synthetase gene cluster and its association with clinical outcomes of dengue virus infection. *Infect. Genet. Evol.* 2013;14:390-395. DOI 10.1016/j.meegid.2012.12.021.
- Allen I.C., Scull M.A., Moore C.B., Holl E.K., McElvania-TeKippe E., Taxman D.J., Guthrie E.H., Pickles R.J., Ting J.P. The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. *Immunity.* 2009;30:556-565.
- Barkhash A.V., Babenko V.N., Kobzev V.F., Romashchenko A.G., Voevoda M.I. Polymorphism in the human 2'-5'-oligoadenylate synthetase genes (OAS), associated with predisposition to severe forms of tick-borne encephalitis, in populations from North Eurasia. *Molekulyarnaya Biologiya = Molecular Biology (Moscow).* 2010;44:985-993. (in Russian)
- Barkhash A.V., Perelygin A.A., Babenko V.N., Myasnikova N.G., Pilipenko P.I., Romashchenko A.G., Voevoda M.I., Brinton M.A. Variability in the 2'-5'-oligoadenylate synthetase gene cluster is associated with human predisposition to tick-borne encephalitis virus-induced disease. *J. Infect. Dis.* 2010;202(12):1813-1818. DOI 10.1086/657418.
- Bigham A.W., Buckingham K.J., Husain S., Emond M.J., Bofferd K.M., Gildersleeve H., Rutherford A., Astakhova N.M., Perelygin A.A., Busch M.P., Murray K.O., Sejvar J.J., Green S., Kriesel J., Brinton M.A., Bamshad M. Host genetic risk factors for West Nile virus infection and disease progression. *PLoS One.* 2011;6(9):e24745. DOI 10.1371/journal.pone.0024745.
- Bilalova G.P. Issues of the practical use of Encevir vaccine. *Sibirskiy Meditsinskiy Zhurnal = Siberian Medical Journal.* 2009;2:86-91. (in Russian)
- Danial-Farran N., Eghbaria S., Schwartz N., Kra-Oz Z., Bisharat N. Genetic variants associated with susceptibility of Ashkenazi Jews to West Nile virus infection. *Epidemiol. Infect.* 2015;143:857-863. DOI 10.1017/S0950268814001290.
- Dugan J.W., Albor A., David L., Fowlkes J., Blackledge M.T., Martin T.M., Planck S.R., Rosenzweig H.L., Rosenbaum J.T., Davey M.P. Nucleotide oligomerization domain-2 interacts with 2'-5'-oligoadenylate synthetase type 2 and enhances RNase-L function in THP-1 cells. *Mol. Immunol.* 2009;47(2-3):560-566. DOI 10.1016/j.molimm.2009.09.025.
- Gadani S.P., Cronk J.C., Norris G.T., Kipnis J. IL-4 in the brain: a cytokine to remember. *J. Immunol.* 2012;189:4213-4219. DOI 10.4049/jimmunol.1202246.
- Geha R.S., Jabara H.H., Brodeur S.R. The regulation of immunoglobulin E class-switch recombination. *Nat. Rev. Immunol.* 2003;3:721-732.
- Gordon S. Alternative activation of macrophages. *Nat. Rev. Immunol.* 2003;3:23-35.
- Gröndahl-Yli-Hannuksela K., Vahlberg T., Ilonen J., Mertsola J., He Q. Polymorphism of IL-10 gene promoter region: association with T cell proliferative responses after acellular pertussis vaccination in adults. *Immunogenetics.* 2016;68:733-741. DOI 10.1007/s00251-016-0923-0.
- Haralambieva I.H., Dhiman N., Ovsyannikova I.G., Vierkant R.A., Pankratz V.S., Jacobson R.M., Poland G.A. 2'-5'-Oligoadenylate synthetase single-nucleotide polymorphisms and haplotypes are associated with variations in immune responses to rubella vaccine. *Hum. Immunol.* 2010;71:383-391. DOI 10.1016/j.humimm.2010.01.004.
- Hovanessian A.G., Justesen J. The human 2'-5'-oligoadenylate synthetase family: unique interferon-inducible enzymes catalyzing 2'-5' instead of 3'-5' phosphodiester bond formation. *Biochimie.* 2007;89:779-788.
- Jerusalimsky A.P. Tick-Borne Encephalitis: Manual for Physicians. Novosibirsk: State Medical Academy Publ., 2001. (in Russian)
- Imran M., Manzoor S., Khattak N.M., Tariq M., Khalid M., Javed F., Bhatti S. Correlation of OAS1 gene polymorphism at exon 7 splice acceptor site with interferon-based therapy of HCV infection in Pakistan. *Viral Immunol.* 2014;27:105-111. DOI 10.1089/vim.2013.0107.
- Kristiansen H., Gad H.H., Eskildsen-Larsen S., Despres P., Hartmann R. The oligoadenylate synthetase family: an ancient protein family with multiple antiviral activities. *J. Interferon Cytokine Res.* 2011;31:41-47. DOI 10.1089/jir.2010.0107.
- Lim J.K., Lisco A., McDermott D.H., Huynh L., Ward J.M., Johnson B., Johnson H., Pape J., Foster G.A., Krysztof D., Follmann D., Stramer S.L., Margolis L.B., Murphy P.M. Genetic variation in OAS1 is a risk factor for initial infection with West Nile virus in man. *PLoS Pathog.* 2009;5:e1000321. DOI 10.1371/journal.ppat.1000321.
- Linnik J.E., Egli A. Impact of host genetic polymorphisms on vaccine induced antibody response. *Hum. Vaccin. Immunother.* 2016;12:907-915. DOI 10.1080/21645515.2015.1119345.
- Lutova S.L., Tumanov Yu.V., Voevoda M.I., Belyavskaya V.A. Tick-borne encephalitis and vaccines: genetic and physiological factors influencing the functional activity of specific antibodies. *Proceedings of the Scientific and Practical Conference. Novosibirsk,* 2016;191-193. (in Russian)
- Medunitsin N.V. *Vaccinology. Moscow: Triada-X Publ., 2004.* (in Russian)
- Ogura Y., Inohara N., Benito A., Chen F.F., Yamaoka S., Nunez G. Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *J. Biol. Chem.* 2001;276:4812-4818. DOI 10.1074/jbc.M008072200.
- Poland G.A., Kennedy R.B., Ovsyannikova I.G. Vaccinomics and personalized vaccinology: is science leading us toward a new path of directed vaccine development and discovery? *PLoS Pathog.* 2011;7:e1002344.
- Poland G.A., Ovsyannikova I.G., Jacobson R.M. Vaccine immunogenetics: bedside to bench to population. *Vaccine.* 2008;26:6183-6188.
- Pulendran B., Miller J., Querec T.D., Akondy R., Moseley N., Laur O., Glidewell J., Monson N., Zhu T., Zhu H., Staprans S., Lee D., Brinton M.A., Perelygin A.A., Vellozzi C., Brachman P. Jr., Lalor S., Teuwen D., Eidex R.B., Cetron M., Priddy F., del Rio C., Altman J., Ahmed R. Case of yellow fever vaccine – associated viscerotropic disease with prolonged viremia, robust adaptive immune responses, and polymorphisms in CCR5 and RANTES genes. *J. Infect. Dis.* 2008;198:500-507. DOI 10.1086/590187.
- Sambrook J., Russell D.W. *The condensed protocols from Molecular cloning: a laboratory manual.* Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2006.
- Sanda C., Weitzel P., Tsukahara T., Schaley J., Edenberg H.J., Stephens M.A., McClintick J.N., Blatt L.M., Li L., Brodsky L., Taylor M.W. Differential gene induction by type I and type II interferons and their combination. *J. Interferon Cytokine Res.* 2006;26:462-472.
- Thamizhmani R., Vijayachari P. Association of dengue virus infection susceptibility with polymorphisms of 2'-5'-oligoadenylate synthetase genes: a case-control study. *Braz. J. Infect. Dis.* 2014;18:548-550.
- UCSC Genome Browser Gateway. 2017. Available at [http://genome-euro.ucsc.edu/cgi-bin/hgGateway?hgsid=225788287\\_h9n14RnxWsBd3VHZFAl6jALan0UJ](http://genome-euro.ucsc.edu/cgi-bin/hgGateway?hgsid=225788287_h9n14RnxWsBd3VHZFAl6jALan0UJ).

- Valarcher J.F., Hägglund S., Juremalm M., Blomqvist G., Renström L., Zohari S., Leijon M., Chirico J. Tick-borne encephalitis. *Rev. Sci. Tech.* 2015;34:453-466.
- Vector-Best Company. Reagents for cytokine analysis. 2004. Available at <http://www.cytokines.ru/2004/4/Art12.php>. (in Russian)
- Yakub I., Lillibridge K.M., Moran A., Gonzalez O.Y., Belmont J., Gibbs R.A., Tweardy D.J. Single nucleotide polymorphisms in genes for 2'-5'-oligoadenylatesynthetase and RNase L in patients hospitalized with West Nile virus infection. *J. Infect. Dis.* 2005;192:1741-1748.
- Yudin N.S., Barkhash A.V., Maksimov V.N., Ignatieva E.V., Romaschenko A.G. Human genetic predisposition to diseases caused by viruses from Flaviviridae family. *Molecular Biology (Moscow)*. 2018;52:165-181.
- Zamorano J., Rivas M.D., Pérez M. Interleukin-4: A multifunctional cytokine. *Immunologia*. 2003;22:215-224.