Online Journal of Health and Allied Sciences
Peer Reviewed, Open Access, Free Online Journal
Published Quarterly : Mangalore, South India : ISSN 0972-5997
Volume 16, Issue 1; Jan-Mar 2017

Original Article:

The Association Analysis of Immune System Genes Allelic Variants With Embryonic Infection of Newborns.

Authors
Elena Vladimirovna Mashkina, Department of Genetics, Southern Federal University, Rostov-on-Don, Russia,
Alena Vladimirovna Dolgopolova, Pediatric neurologist, Department of Childhood Diseases, Rostov State Medical University, Rostov-on-Don, Russia,
Svetlana Aleksandrovna Hukazova, Department of Genetics, Southern Federal University, Rostov-on-Don, Russia,
Olga Georgievna Dolgopolova, Assistant, Department of Childhood Diseases, Rostov State Medical University, Rostov-on-Don, Russia,
Ekaterina Grigorevna Derevyanchuk, Senior Researcher, Department of Genetics, Southern Federal University, Rostov-on-Don, Russia,
Rozaliya Aleksandrovna Belovolova, City Hospital No 20, Rostov State Medical University, Rostov-on-Don, Russia,
Tatiana Pavlovna Shkurat, Professor, Dr, Department of Genetics, Southern Federal University, Rostov-on-Don, Russia.

Address for Correspondence
Ekaterina Grigorevna Derevyanchuk,
Senior Researcher, Department of Genetics,
Southern Federal University,
Rostov-on-Don, Russia,
194/1, Stachki avenue,
Rostov-on-Don, 344090,
Russian Federation.
E-mail: biolab2008@yandex.ru

Citation
Mashkina EV, Dolgopolova AV, Hukazova SA, Dolgopolova OG, Derevyanchuk EG, Belovolova RA, Shkurat TP. The association analysis of immune system genes allelic variants with embryonic infection of newborns. *Online J Health Allied Scs.* 2017;16(1):3. Available at URL: http://www.ojhas.org/issue61/2017-1-3.html

Submitted: Feb 26, 2017; Accepted: April 3, 2017; Published: May 15, 2017

Abstract: To investigate the association of marker genes and their polymorphisms with increased risk of embryonic infection (EI)- of the fetus and of the nervous system lesions development of different severity. Methods: The TLR2, TLR6, IL1ß, TNFa, IL10 genotypes and alleles frequencies were studied in three groups of infants of Rostov region with EI followed by hypoxic lesions of the central nervous system. Results: In our study the allelic variants of IL1ß and TNFa genes are characterized by a high level of expression, while the allelic variant of the IL10 gene results in a decrease in the corresponding mRNA level. Conclusion: The importance of the Ser249Pro (TLR6), -308G/A (TNFa) and -31C/T (IL1ß) interactions in changing the risk of the EI development was established.
Key Words: Embryonic infection, Central nervous system (CNS) abnormalities, TLR 2, TLR 6, IL 1ß, TNF a, IL10, Polymorphism.

Introduction:
Embryonic infections (EI) are currently one of the leading pediatric pathologies. Profound disability due to congenital malformations and chronic diseases becomes the result of infection. Of the 2.8 million neonatal deaths in 2013, 0.43 million deaths (uncertainty range: 0.22–0.66) were estimated to be caused by sepsis and other severe infections.(1)
Mechanisms of infectious process development in the fetus depend on many factors, mainly on the genetically determined immune response of an organism. According to modern concepts, the pregnancy develops in the background of the mother immune response suppression and is compensated by the innate immunity activation. Its key parts are toll-like receptors, which activation by microbial products leads to the production of proinflammatory cytokines that determine the further course of the inflammatory response. There are also evidences pointed out the association of these genes polymorphisms with the EI development.(2,3)
The aim of this work was to investigate the association of marker genes and their polymorphisms with increased risk of EI of the fetus and of the nervous system lesions development of different severity.

Materials and Methods
Venous blood samples were collected from 129 infants of Rostov region, having EI followed by hypoxic lesions of the central nervous system, depending on the severity of which 3 equal groups (43 children) were formed: mild (MP), average (AP) and heavy (HP) pathology. The biomaterial was provided by Municipal hospital No. 20 of Rostov-on-don (2013-2014). Genes polymorphisms analysis was performed at the Southern Federal University (Center for collective use «High technologies», Human and Animal Genetics Laboratory of Biology Research Institute and Genetics Department).
The genomic DNA extraction from the peripheral blood leukocytes was carried out by thermocoagulation method with use of «DNA-Express» reagents set (Litech, Moscow). Allelic variants Arg753Gln of *TLR2* gene, Ser249Pro of *TLR6* gene, -31*C/T* of *IL1ß* gene, -308*G/A* of *TNFa* gene, -592*C/A* of *IL10* gene were investigated using «SNP-Express»

sets of reagents (Litech, Moscow). The amplification products separation was performed by horizontal electrophoresis in 3% agarose gel. The electrophoregram analysis was carried out on GelDoc transilluminator (BioRad).

Statistical analysis: The accordance of genotype frequencies distribution to Hardy?Weinberg equilibrium was determined using «Calculator for statistics calculating in «case-control» studies» in the program http://gen-exp.ru/calculator_or.php. The differences evaluation of the allelic variants of genes distribution in the examined groups was performed by the χ^2 criterion also with the help of the abovementioned program. The risk of EI and the nervous system pathologies development was estimated by the odds ratio (OR). OR is specified with a 95% confidence interval (CI). The intergenic interactions analysis was performed using the algorithm of dimensionality reduction (Multifactor Dimensionality Reduction, MDR).

Results and Discussion

The genotypes and alleles frequencies of the studied genes polymorphisms are presented in Table 1. It was established in the present study that the genotypes frequency distribution of *TLR2* gene was similar to that for healthy newborns in Rostov-on-Don.(4) In all groups the homozygotes prevalence (91–95%) by normal *753Arg* allele and the complete absence of polymorphic *GlnGln* homozygotes were observed, that can be explained by a small sample size. The polymorphic allele frequency varied from 2 to 5% for different groups that corresponds to general population frequency from 2 to 3% for the European population.(5)

The genotyping of the *TLR6* gene showed the homozygotes predominance for *249Pro* allele polymorphic variant (42–58%). Its frequency ranged from 61 to 72%. Thus, there is a slight increase in the alleles frequencies in comparison to that for the European population (52–66%).(5)

The genotypes frequencies distribution of *TNF-a* gene polymorphism for the healthy women of Rostov-on-Don was the same (6) with the prevalence of homozygotes by normal *308G* allele in all studied groups (61–79%). The frequency of polymorphic *308A* allele was 11–22%. Population studies indicate that 27–33% of white-skinned Europeans have this allele.(7) The obtained data show a reduced frequency of this allele. There is the tendency of frequency decrease with the progression of the disease. The statistically significant difference in the *308A* allele occurrence frequency was found in the AP group indicating a slight protective effect, OR=0.41 (0.17–0.97). TNF affects the lipid metabolism, coagulation processes, endothelial function. It was shown that TNF may have an impact on the secretion of some matrix metalloproteinases.(8) TNF induces the production of cytokines that activate the expression of adhesion molecules and neutrophils activity.(9)

Statistically significant differences in the *IL1ß* gene genotypes distribution between groups and in comparison with healthy individuals (6) were not detected. The highest frequency of occurrence in equal measure was noted for homozygotes by normal *31S* allele (40–47%) and heterozygotes by polymorphic *31T* allele (40–49%). The polymorphic allele frequency was 34–40%. Its value for healthy women of Rostov-on-Don lies in a given interval of nearly 39%.(6)

The similar situation was observed for the *IL10* gene polymorphism. There was a slight difference in the polymorphic *592A* homozygotes frequency: from 0–4.7% in newborns with EI up to 10% in healthy women from Rostov-on-Don (6). The frequency of polymorphic allele was 24–28%, what also did not differ from values for healthy individuals from Rostov-on-Don - 27%.(6)

Table 1: The distribution of studied genes polymorphisms frequencies

Genotypes	Newborns groups, ab. (%)			The differences significance, X^2 (*p*)	
	HP	**AP**	**MP**	**HP**	**AP**
TLR2 Arg753Gln					
AgrArg	41 (95.3)	40 (93)	39 (90.7)	0.72 (0.7)	0.16 (0.93)
ArgGln	2 (4.7)	3 (7)	4 (9.3)		
GlnGln	0 (0)	0 (0)	0 (0)		
Gln allele frequency	0.023	0.035	0.047	0.69 (0.41)	0.15 (0.7)
HWE (*p*)	0.02 (0.88)	0.06 (0.81)	0.10 (0.75)	-	
TLR6 Ser249Pro					
SerSer	7 (16.3)	5 (20.9)	6 (14)	2.42 (0.3)	2.31 (0.31)
SerPro	18 (41.9)	19 (37.2)	12 (27.9)		
ProPro	18 (41.9)	23 (41.9)	25 (58.1)		
Pro allele frequency	0.628	0.605	0.721	1.69 (0.19)	2.60 (0.11)
HWE (*p*)	0.47 (0.49)	2.11 (0.15)	4.04 (0.04)	-	
TNF G-308A					
GG	34 (79.1)	34 (79.1)	26 (60.5)	3.53 (0.17)	4.57 (0.1)
GA	8 (18.6)	9 (20.9)	15 (34.9)		
AA	1 (2.3)	0 (0)	2 (4.7)		
-308A allele frequency	0.116	0.105	0.221	3.36 (0.07)	**4.27 (0.04)**
HWE (*p*)	0.88 (0.35)	0.59 (0.44)	0.01 (0.93)	-	
IL1ß -31C/T					
CC	17 (39.5)	18 (41.9)	20 (46.5)	0.56 (0.76)	0.93 (0.63)
TC	18 (41.9)	21 (48.8)	17 (39.5)		
TT	8 (18.6)	4 (9.3)	6 (14)		
-31C allele frequency	0.395	0.337	0.337	0.63 (0.43)	0.00 (1)
HWE (*p*)	0.67 (0.41)	0.37 (0.54)	0.57 (0.45)	-	
IL10 C-592A					
CC	23 (53.5)	21 (48.8)	22 (51.2)	2.25 (0.32)	2.05 (0.36)
CA	18 (41.9)	20 (46.5)	21 (48.8)		
AA	2 (4.7)	2 (4.7)	0 (0)		
-592A allele frequency	0.256	0.279	0.244	0.03 (0.86)	0.27 (0.6)
HWE (*p*)	0.43 (0.51)	1.05 (0.31)	4.49 (0.03)	-	

HWE – Hardy-Weinberg equilibrium; X^2 - Pearson fitting criterion; *p* - the coefficient of reliability (Fisher criterion). Bold are allele frequencies with significant differences.

The combined analysis of the investigated polymorphisms frequencies showed a significant interaction models for 3 of the 5 studied genes (Table 2). For the development of the EI there must be at least 1 polymorphic allele in one of the studied genes. Comparison of HP and AP groups helped to identify the

genotypes combination that determines the central nervous system pathology progression: polymorphic homozygotes for the TNFα gene and the TLR6 gene polymorphic allele carriers; TNFα and Il1β genes polymorphic alleles carriers; polymorphic homozygotes for the genes TLR6, TNFα and Il1β.

Table 2: The intergenic interactions analysis of *TLR2, TLR6, IL1ß, TNFa, IL10* genes

Genes combinations	Training balanced accuracy	Cross-validation consistency	X^2 (*p*)	OR, (CI = 95%)
TLR6, TNFa for AP group	0.66	10/10	10.12 (=0.002)	6.86 (1.90–24.82)
TLR6, TNFa, IL1ß for AP group	0.75	10/10	20.81 (<0.0001)	12.32 (3.77-40.27)
TLR6, TNFa, IL1ß for HP group	0.71	8/10	13.09 (=0.0003)	5.76 (2.16-15.33)

Choosing the highest coefficient of cross-validation from the obtained intergenic interactions models there can be allocated optimal interactions of *TLR6* and *TNFa*, as well as *TLR6*, *TNFa*and *IL1ß*. Two locus model of intergenic interaction characterized by the cross-validation coefficient 10/10 and the genes interaction degree – 0.655. Three locus model of intergenic interaction characterized by the cross-validation coefficient 10/10 and the genes interaction degree – 0.749 (Table 3).

Table 3: The intergenic interactions analysis in newborns with heavy pathologies of central nervous system

Genes combinations	Training balanced accuracy	Cross-validation consistency	X^2 (*p*)	OR, (CI = 95%)
TLR6	0.59	5/10	2.78 (=0.095)	2.19 (0.87–5.54)
TLR6, TNFa	0.66	10/10	10.12 (=0.002)	6.86 (1.90–24.82)
TLR6, TNFa, IL1b	0.75	10/10	20.81 (<0.0001)	12.32 (3.77-40.27)

Figure 1 shows the frequency distribution of two locus combinations of *TNFa* and *TLR6* genes genotypes among newborns with heavy nervous system pathologies compared to control. According to this model, all combinations have an increased risk except: homozygotes for the *TLR6* gene and carriers of at least one polymorphic *TNFa*gene allele, and homozygotes for the *TLR6* gene polymorphism and carriers of one polymorphic allele of *TNFa* gene.

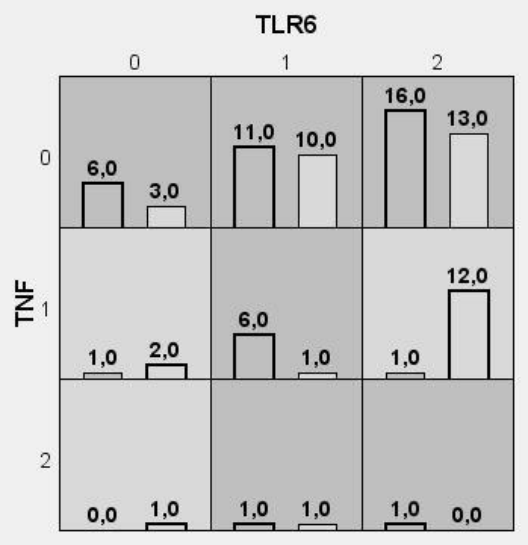

Figure 1: The frequency distribution of two locus combinations of *TNFa* and *TLR6* genes genotypes among newborns with heavy and mild central nervous system pathologies.

Dark-grey cells – high risk genotypes; light-grey cells – low risk genotypes; the left columns in the cells - newborns with heavy pathology, the right columns in the cells – control; 0 – homozygotes for the normal allele, 1 – heterozygotes, 2 – homozygotes for the polymorphism

Figure 2 shows the frequency distribution of three locus combinations of *TLR6, TNFa* and *IL1ß* genes genotypes among newborns with heavy nervous system pathologies compared to control. According to this model, the combination of homozygous carriage of polymorphic *IL1ß* gene allele with the studied changes in other genes determines fewer cases of observed pathology. Thus, the heterozygous carriage of the *TLR6* gene is necessary for the normal homozygotes in the *TNFa* gene or it is needed the combination of two polymorphic homozygotes. For the remaining cases more frequent the homozygous carriage of the wild alleles was observed.

The three locus model of intergenic interaction between *TLR6, TNFa* and *IL1ß*genes is characterized by the highest coefficient of cross-validation (8/10); the degree of genes interaction is 0.705 (Table 4).

Table 4: The intergenic interactions analysis in newborns with average pathologies of central nervous system

Genes combinations	Training balanced accuracy	Cross-validation consistency	X^2 (*p*)	OR, (CI = 95%)
TLR6	0.59	7/10	3 (=0.084)	2.31 (0.89–5.99)
TLR6, TNFa	0.66	6/10	10.12 (=0.002)	3.91 (1.49–10.29)
TLR6, TNFa, IL1b	0.71	8/10	13.09 (=0.0003)	5.76 (2.16-15.33)

Figure 3 shows the frequency distribution of three locus combinations of *TLR6, TNFa* and *IL1ß* among newborns with average pathologies of central nervous system compared to control. As well as for the heavy pathology a small number of pathology development cases is observed for homozygotes for the *Ser249Pro* polymorphism of *TLR6* gene in combination

with other allelic variants of the genes. The similarities were found in the genotypes distribution for two different degrees of disease severity. It can be noted that a greater number of increased risk genotypes combinations (11 vs 8) was identified in cases with heavy pathologies compared to that with average degree of disorder. Greater number of genotypes variants had not been met in the mild pathology development (10 vs 6). They appear in cases with heavy pathology as the high risk genotypes. They may determine the progressive development of this pathology. Such genotypes are combination of polymorphic homozygotes for all three genes; one polymorphic allele of the *TLR6* gene and two polymorphic alleles of the *TNFa*gene with normal homozygous *IL1ß* genotype; heterozygotes for the polymorphic *TNFa* and *IL1ß* genes with normal homozygous TLR 6 genotype.

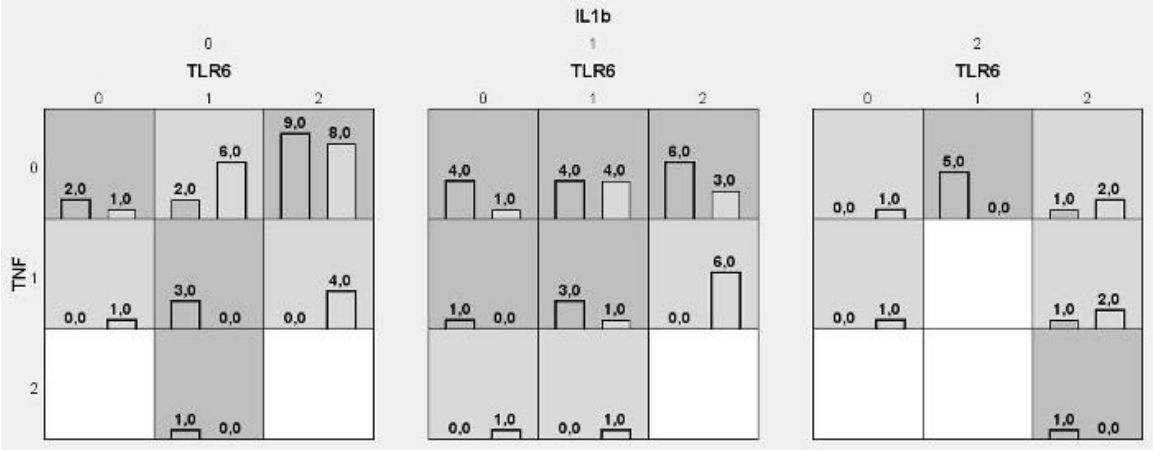

Figure 2: The frequency distribution of three locus combinations of *TNFa, TLR6* and *IL1ß* genes genotypes among newborns with heavy and mild central nervous system pathologies.

Symbols are as in Figure 1

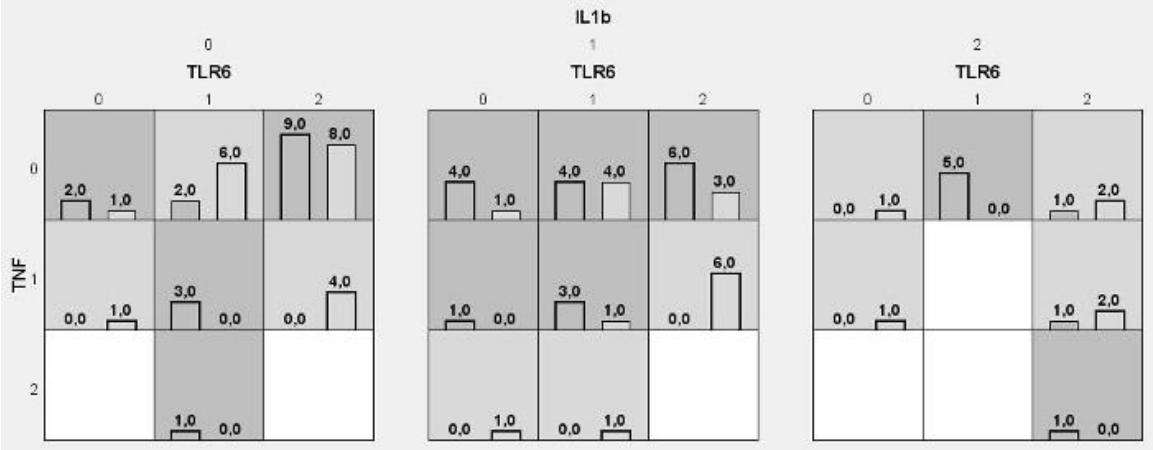

Figure 3: The frequency distribution of three locus combinations of *TNFa, TLR6* and *IL1ß* genes genotypes among newborns with average and mild central nervous system pathologies.

Symbols are as in Figure 1

Thus, EI with further central nervous system pathology development is characterized by the polymorphisms combination in the genes such as genes of innate immunity factors, for example *TLR* gene and cytokine network genes. Functional polymorphism of toll receptors implements its action at the initial stages of inflammation, on the course character of the protective responses and susceptibility to some diseases. The changes in the *TLR6* gene structure can affect the intracellular domain, leading to an aberrant response in response to the interaction with pathogens. Excessive *TLR* activation and the uncontrolled production of proinflammatory cytokines (TNFa and IL1ß) can contribute to the development of systemic inflammatory reaction, tissue damage, underlying disease complications.

Earlier studies showed that the EI implementation is accompanied by a ratio breach of pro– and anti-inflammatory cytokines, a reliable increase in the TNFa and IL1ß content and decrease of IL10 in blood serum of pregnant women.(10) In our study the allelic variants of *IL1ß* and *TNFa* genes are characterized by a high level of expression, while the allelic variant of the *IL10* gene results in a decrease in the corresponding mRNA level. The importance of the *Ser249Pro* (*TLR6*), *-308G/A* (*TNFa*) and *-31C/T* (*IL1ß*) interactions in changing the risk of the EI development was established.

Funding:
The study was performed on the equipment of the Center of collective use «High technologies» (Southern Federal University) in the framework of the basic part of the Ministry of education and science of the Russian Federation state task on the theme: «The study of the genetic polymorphisms and microRNAs functional role in the genomes of humans and animals», project No. 6.6762.2017/BT.
Conflict of Interest: None.

References

1. Oza S, Lawn JE, Hogan DR, et al. Neonatal cause-of-death estimates for the early and late neonatal periods for 194 countries: 2000–2013. *Bulletin of the World Health Organization*. 2015;93(1):19-28.
2. Gankovskaya OA. Molecular-genetic mechanisms of innate immunity at the mucous membranes level in the infectious genesis pathology. *An abstract of the medical sciences doctor dissertation(Russian).* 2010: 51 p.
3. Mironenko MM, Dolgikh TI. The role of allelic polymorphism of *CD14, TLR2, TLR6* genes in the development of intrauterine infection caused by Herpesviridae family viruses. *Medical immunology (Russian).* 2010;12(1-2):151-154.
4. Afonin AA. The role of the TLR2, TLR6 expression and polymorphism of their genes in the development of a generalized cytomegalovirus and herpes infections in newborn infants. *Medical Bulletin of the South of Russia*. 2015;3:24-27.
5. The Exome Aggregation Consortium (ExAC). URL: http://exac.broadinstitute.org/about (accessed 2.4.2016).
6. Mashkina EV, Kovalenko KA, Fomina NV. Shkurat TP. The study of the association of cytokine genes polymorphic variants with early embryonic loss. *Human ecological genetics (Russian).* 2014;12(1):19-27.
7. Rydlovskaya AV, Simbirtsev AS. TNFA gene functional polymorphism and pathology. *Cytokines and inflammation (Russian).* 2005;4(3):4-10.
8. Bischof P. Endocrine, paracrine and autocrine regulation of trophoblastic metalloproteinases. *Early Pregnancy*. 2001;5(1):30-31.
9. V?ss?lli P. The pathophysiology of tumor necrosis factor. *Annual review of immunology*. 1992;10:411-452.
10. Tirskaya YI. Immunological features of pregnant women at risk of intrauterine infection. *Medicine and education in Siberia (Russian).* 2014;2:16-17.