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Single nucleotide polymorphisms of TLR-2, TLR-3, TLR-4 and susceptibility to inflammatory diseases of the respiratory tract

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Key words: genotype, respiratory tract diseases, Arg753Gln polymorphism of TLR-2 gene, Leu412Phe of TLR-3 gene, Asp299Gly of TLR-4 gene.

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The aim of the research is to establish an association between Arg753Gln polymorphism of TLR-2 gene, Leu412Phe of TLR-3 gene, Asp299Gly of TLR-4 gene and susceptibility to inflammatory diseases of the upper and lower respiratory tract and the complicated forms of ARI development.

Materials and methods. 98 healthy subjects distributed according to the genotype of TLR-2, TLR-3, TLR-4 were enrolled in the study of association between Arg753Gln polymorphism of TLR-2, Leu412Phe of TLR-3 and Asp299Gly of TLR-4 genes and inflammatory diseases of the upper and lower respiratory tract. The polymorphic site of TLR-2 Arg753Gln, TLR-3 Leu412Phe and TLR-4 Asp299Gly genotyping was performed by polymerase chain reaction using oligonucleotide primers. The relative risk of the disease and complications development was estimated using the odds ratio with 95 % confidence interval. The statistical significance of differences in qualitative characteristics was evaluated using Fischer's exact test.

Results. It has been revealed that individuals with polymorphic TLR-2, TLR-3 and TLR-4 genes have an increased susceptibility to ARI with frequent episodes during the year that are complicated by lower respiratory tract inflammation as well as chronic inflammatory diseases of the upper respiratory tract. It has been shown that the risk of bronchitis and pneumonia development in ARIs is higher in subjects with polymorphic genotypes of TLR-2, TLR-3 and TLR-4 as compared to the carriers of normal alleles distribution: 2.9 times with Leu/Phe genotype of TLR-3, 20.0 times with Phe/Phe of TLR-3 and 12.8 times with combinations of polymorphic genotypes of TLR-2, TLR-3, TLR-4.

Conclusions. The results of the study indicate that the presence of single-nucleotide polymorphisms TLR-2 Arg753Gln, TLR-3 Leu412Phe and TLR-4 Asp299Gly is the marker of high susceptibility to respiratory diseases. Individuals with polymorphic status of TLR-2, TLR-3 and TLR-4 genotypes have an increased susceptibility to inflammatory diseases of the upper and lower respiratory tract with ARI frequency of 4 or more episodes during the year.

Ключові слова: генотип, запальні захворювання верхніх і нижніх дихальних шляхів, поліморфізм Arg753Gln гена TLR-2, Leu412Phe гена TLR-3, Asp299Gly гена TLR-4.

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Однонуклеотидні поліморфізми TLR-2, TLR-3, TLR-4 та сприйнятливість до запальних захворювань дихальних шляхів

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Мета роботи – простежити зв'язок поліморфізму Arg753Gln гена TLR-2, Leu412Phe гена TLR-3, Asp299Gly гена TLR-4 зі схильністю до запальних захворювань верхніх і нижніх дихальних шляхів і розвитком ускладнених форм ГРІ.

Матеріали та методи. Зв'язок поліморфізму генів Arg753Gln TLR-2, Leu412Phe TLR-3, та Asp299Gly TLR-4 із запальними захворюваннями верхніх і нижніх дихальних шляхів вивчили у 98 практично здорових осіб, яких поділили залежно від генотипу TLR-2, TLR-3, TLR-4. Генотипування поліморфної ділянки Arg753Gln гена TLR-2, Asp299Gly гена TLR-4, Leu412Phe гена TLR-3 здійснили методом полімеразної ланцюгової реакції з використанням олігонуклеотидних праймерів. Відносний ризик розвитку захворювання та ускладнень оцінювали за допомогою показника відношення шансів із довірчим інтервалом 95 %. Статистичну значущість відмінностей для якісних ознак оцінювали з використанням точного тесту Фішера.

Результати. Встановили, що особи з поліморфно зміненими генотипами TLR-2, TLR-3, TLR-4 мають підвищену схильність до ГРІ з частими епізодами протягом року, які ускладнюються запальними процесами нижніх дихальних шляхів, а також до хронічних запальних захворювань верхніх дихальних шляхів. Доведено, що ризик розвитку бронхіту та пневмонії при ГРІ вищий в осіб із поліморфно зміненими генотипами TLR-2, TLR-3, TLR-4 порівняно з носіями нормального розподілу алелей: з генотипом Leu/Phe TLR-3 – у 2,9, Phe/Phe TLR-3 – у 20,0, комбінаціями поліморфно змінених генотипів TLR-2, TLR-3, TLR-4 – у 12,8 раза.

Висновки. Результати дослідження свідчать, що маркером високої сприйнятливості до респіраторних захворювань є наявність однонуклеотидних поліморфізмів Arg753Gln гена TLR-2, Leu412Phe гена TLR-3, Asp299Gly гена TLR-4. Особи з поліморфно зміненими генотипами TLR-2, TLR-3, TLR-4 мають підвищену схильність до запальних захворювань верхніх і нижніх дихальних шляхів, ГРІ з частотою епізодів 4 і більше протягом року.

Ключевые слова: генотип, воспалительные заболевания верхних и нижних дыхательных путей, полиморфизм Arg753Gln гена TLR-2, Leu412Phe гена TLR-3, Asp299Gly гена TLR-4.

Однонуклеотидные полиморфизмы TLR-2, TLR-3, TLR-4 и восприимчивость к воспалительным заболеваниям дыхательных путей

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Цель работы – проследить связь полиморфизма Arg753Gln гена TLR-2, Leu412Phe гена TLR-3, Asp299Gly гена TLR-4 со склонностью к воспалительным заболеваниям верхних и нижних дыхательных путей и развитием осложненных форм ОРИ.

Материалы и методы. Связь полиморфизма генов Arg753Gln TLR-2, Leu412Phe TLR-3, и Asp299Gly TLR-4 с воспалительными заболеваниями верхних и нижних дыхательных путей изучили у 98 практически здоровых лиц, которые были распределены в зависимости от генотипа TLR-2, TLR-3, TLR-4. Генотипирование полиморфного участка Arg753Gln гена TLR-2, Asp299Gly гена TLR-4, Leu412Phe гена TLR-3 проводили методом полимеразной цепной реакции с использованием

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олигонуклеотидных праймеров. Относительный риск развития заболевания и осложнений оценивали с помощью показателя отношения шансов с доверительным интервалом 95 %. Статистическую значимость различий для качественных признаков оценивали с использованием точного теста Фишера.

Результаты. Установлено, что лица с полиморфно измененными генотипами TLR-2, TLR-3, TLR-4 имеют повышенную склонность к ОРИ с частыми эпизодами в течение года, которые осложняются воспалительными процессами нижних дыхательных путей, а также к хроническим воспалительным заболеваниям верхних дыхательных путей. Доказано, что риск развития бронхита и пневмонии при ОРИ выше у лиц с полиморфно измененными генотипами TLR-2, TLR-3, TLR-4 по сравнению с носителями нормального распределения аллелей: с генотипом Leu/Phe TLR-3 – в 2,9, Phe/Phe TLR-3 – в 20,0, комбинациями полиморфно измененных генотипов TLR-2, TLR-3, TLR-4 – в 12,8 раза.

Выводы. Результаты исследования свидетельствуют, что маркером высокой восприимчивости к респираторным заболеваниям является наличие однонуклеотидных полиморфизмов Arg753Gln гена TLR-2, Leu412Phe гена TLR-3, Asp299Gly гена TLR-4. Лица с полиморфно измененными генотипами TLR-2, TLR-3, TLR-4 имеют повышенную склонность к воспалительным заболеваниям верхних и нижних дыхательных путей, ОРИ с частотой эпизодов 4 и более в течение года.

Introduction

Acute respiratory infections (ARIs) are the most common infectious diseases affecting all age groups. 10–14 million people in Ukraine suffer from ARIs every year, which accounts for 25–30 % of overall morbidity and about 75–90 % of infectious diseases incidence in the country. According to the WHO experts report, ARIs rank first among the causes of temporary disability and third among the main causes of death, yielding only to coronary heart disease and cerebrovascular pathology. It is also noted that this group of diseases is constantly replenished by new representatives and a negative tendency of ARIs pathomorphism to the protracted course and the complicated forms development [1] are observed.

Therefore, understanding the mechanisms of the respiratory tract nonspecific protection from infectious agents becomes of particular relevance. The variety and abundance of infectious pathogens, contacted by the respiratory tract mucous membrane involve the existence of complex multifactorial induction of the respiratory tract local protection. According to modern concepts, Toll-like receptors (TLRs) are the central link of the multi-level system for recognition of pathogen-associated molecular structures, whose activation when the respiratory tract is infected leads to the expression of genes involved in the inflammatory process regulation, the innate mechanisms of protection against infectious agents and acquired immunity.

Respiratory tract epitheliocytes express all known TLRs, most intensively TLR-2, TLR-3, and TLR-4 [2]. The ability of these TLRs to recognize a wide range of ligands (gram-positive and gram-negative bacteria, viral structural proteins) indicates their key role in the pathogenesis of respiratory diseases of both viral and bacterial etiology.

In recent years, more and more information has been collected about TLR dysfunction. One of such dysfunctions causes may be the substitution in genomic DNA (single nucleotide polymorphism), which leads to changes in the TLR structure, thus disrupting the pathogens recognition and congenital immunity system function and as a result – predisposition to a variety of diseases, as well as the severity of their course [3].

At present, it has been found that Asp299Gly polymorphism of TLR-4 gene is closely linked to the development of sexually transmitted bacterial infections [4], respiratory syncytial infection in infants and newborns [5], sepsis induced by gram-negative bacteria [6]. Arg753Gln polymorphism of TLR-2 gene is associated with increased susceptibility to tuberculosis [7], staphylococcal infections [8]. The variant of Leu412Rhe polymorphism of TLR-3 gene is associated with the development of subacute sclerosing panencephalitis with the cortex affection [9], myocarditis and dilated cardiomyopathy in case of enterovirus infection [10], the severe and complicated course of influenza and influenza-associated pneumonia [11].

Thus, the data of scientific literature indicate that susceptibility to infectious agents is genetically determined, and the association study between Arg753Gln polymorphism of TLR-2, Leu412Phe of TLT-3 and Asp299Gly of TLR-4 genes and susceptibility to inflammatory diseases of the upper and lower respiratory tract and complicated forms of ARI development is an urgent and challenging task, which became the subject of our study.

The aim

The aim of the research is to establish an association between Arg753Gln polymorphism of TLR-2, Leu412Phe of TLR-3 and Asp299Gly of TLR-4 genes and susceptibility to inflammatory diseases of the upper and lower respiratory tract and the complicated forms of ARI development.

Materials and methods

The study of association between Arg753Gln of TLR-2, Leu412Phe of TLR-3 and Asp299Gly of TLR-4 genes polymorphism and inflammatory diseases of the upper and lower respiratory tract included 98 subjects (women – 55 (56.1 %), men – 43 (43.9 %) aged from 18 to 59 (average age – 32.47 ± 1.25) who did not have generally recognized risk factors for influenza and other acute respiratory infections (pregnancy, obesity, diabetes mellitus, immunosuppressive disorders, chronic diseases of the lungs, heart, kidneys, liver, etc.). By polymorphic variants of TLR genes they were distributed as follows: Leu/Phe of TLR – 34, Phe/Phe of TLR-3 – 11, Arg/Gln of TLR-2 – 5, Asp/Gly of TLR-4 – 4, combinations of polymorphic genotypes in the studied TLRs – 8. The results were compared with the data of gender- and age-matched 36 individuals with normal distribution of TLR-2, TLR-3 and TLR-4 genes.

Genotyping of the polymorphic sites of TLR-2 Arg-753Gln, TLR-3 Leu412Phe and TLR-4 Asp299Gly was carried out at the Research Institute of Genetic and Immunological Foundations of Pathology and Pharmacogenetics of "Ukrainian Medical Stomatological Academy" by polymerase chain reaction using oligonucleotide primers.

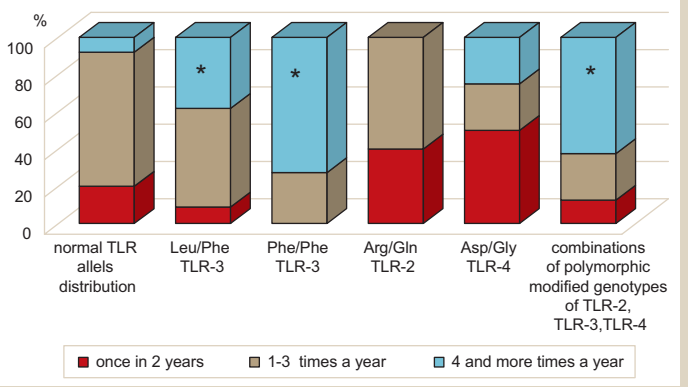

Fig. 1. Frequency of acute respiratory infections episodes during a year in people with polymorphic variants of genotypes and normal distribution of TLR-2, TLR-3 and TLR-4 alleles.

*: P < 0.05 in comparison to those with normal distribution of TLR-2, TLR-3, TLR-4 alleles (the significance level was obtained by Fischer's exact test).

The amplification was performed using the Tertsik amplifier ("DNK-Tekhnologiya", Russia).

The material for the research comprised the outpatient medical records and case histories. The frequency of acute respiratory infections and inflammatory diseases of the upper and lower respiratory tract during the year, the severity of their course and the complications development were particularly detailed.

The statistical analysis of data was carried out using the method of variation statistics using the computer software Microsoft Office Excel 2010 and Statistica 7.0. The relative risk of the disease and complications development was estimated using the odds ratio OR. The indicator OR = 1 was considered as the lack of association; OR >1 – as a positive association ("predisposition"), OR <1 – as a negative association of allele or genotype with the disease. For the analysis of qualitative parameters correlations the Pearson's contingency ratio was determined. The statistical significance of differences in qualitative characteristics was evaluated using Fischer's exact test. The differences were considered significant for all types of analysis with the error probability, generally accepted in medical and biological studies – P < 0.05.

Results and discussion

According to the study results, individuals with polymorphic genotypes in TLR-2, TLR-3 and TLR-4 showed a high susceptibility to ARIs with frequent episodes during the year which were complicated by inflammatory processes of the lower respiratory tract (LRT), as well as chronic inflammatory diseases of the upper respiratory tract.

Thus, it was found that sustained ARI were more often in people with TLR-3 Leu/Phe (91.2 %), TLR-3 Phe/Phe (100.0 %) genotypes and combinations of TLR-2, TLR-3 and TLR-4 (100.0 %) genes polymorphic variants as compared with carriers of normal TLR genotypes (69.4 %, P < 0.03, P < 0.04, P < 0.05, respectively).

In these categories of people the percentage of those suffered from ARI 4 or more times a year was also significantly higher: 4.6 times with TLR-3 Leu/Phe (38.2 %, P < 0.003), 8.7 times with TLR-3 Phe/Phe (72.7 %, P < 0.00006) genotypes, 7.8 times with combinations of polymorphic variants of TLR-2, TLR-3 and TLR-4 genes (62.5 %, P < 0.002) (in the normal alleles of TLR genes distribution – 8.0 %) (*Fig. 1*).

It should be noted that one third (30.6 %) of the subjects with normal distribution of TLR gene alleles suffered from ARI infrequently – once every 2 years or less, which was practically not observed in individuals with polymorphic variants of TLR-2, TLR-3 and TLR-4 genes (in TLR-3 Leu/Phe – 8.8 %, P < 0.03, TLR-3 Phe/Phe – 0.0 %, P < 0.04).

In patients with TLR-2, TLR-3 and TLR-4 polymorphic variants of genotypes complications of respiratory tract infections were found in 42 out of 62 cases (67.7 %) (with normal distribution of TLR alleles in 12 out of 34 (35.3 %, P < 0.001), in particular: at genotype TLR-3 Leu/Phe - in 21 (61.8 %, P < 0.05), TLR-3 Phe/Phe - in 10 (90.9 %, P < 0.001), TLR-4 Asp/Gly - in 4 (100.0 %, P < 0.02) and combination s of mutations in TLR-2, TLR-3 and TLR-4 genes - in 7 (87.5 %, P < 0.01). Bronchitis and pneumonia were prevalent among the complications of the LRT lesions.

Thus, the development of bronchitis against the background of ARI was observed in 64.5 % (P < 0.03) of subjects with TLR-3 Leu/Phe genotype, in 81.8 % (P < 0.01) with TLR-3 Phe/Phe, in 75.0 % (P < 0.05) with TLR-4 Asp/Gly, in 87.5 % (P < 0.01) with variant genotypes of TLR-2, TLR-3 and TLR-4 combined (with normal distribution of TLR alleles in 32.0 %); pneumonia – in 23.5 % (P < 0.04) of people with TLR-3 Leu/Phe genotype, in 45.5 % (P < 0.01) with TLR-3 Phe/Phe and in 50.0 % (P < 0.02) with variant genotypes of TLR-2, TLR-3 and TLR-4 combined (with normal distribution of TLR alleles in 8.0 %). The obtained results were confirmed by the calculated odds ratio according to which individuals with polymorphic variants of TLR-2, TLR-3 and TLR-4 genotypes had an increased risk of the LRT inflammatory processes development in ARI: 2.9 times with TLR-3 Leu/Phe genotype (OR = 2.9; 95 % CI: 1.1–7.94), 20.0 times with TLR-3 Phe/Phe (OR = 20, 95 % CI: 2.29–175.05), 12.8 times with variant genotypes of TLR-2, TLR-3 and TLR-4 combined (OR = 12.8; 95 % CI: 1.41–117.01) as compared to carriers of normal distribution of TLR genes alleles.

In addition, it turned out that people with TLR-2, TLR-3 and TLR-4 genes polymorphism were more likely to suffer from inflammatory diseases of the LRT that were not associated with respiratory viral infection. Thus, according to anamnesis and outpatient medical records, bronchitis affected 32.4 % of subjects with TLR-3 Leu/Phe genotype; 45.5 % with TLR-3 Phe/Phe; 62.5 % with combinations of TLR-2, TLR-3 and TLR-4 polymorphism (with normal distribution of TLR genes alleles in 11.1 %, P < 0.04, P < 0.02, P < 0.03, respectively); pneumonia – 27.3 % with TLR-3 Phe/Phe, 37.5 % with combinations of TLR-2, TLR-3 and TLR-4 polymorphism (with normal distribution of TLR genes alleles in 2.8 %, P < 0.03).

Chronic inflammatory diseases of the upper respiratory tract were also more likely to occur in people with TLR-2, TLR-3 and TLR-4 genes polymorphism and were mainly represented by tonsillitis and sinusitis and partially by pharyngitis (*Fig. 2*).

As can be seen from *Fig. 2*, tonsillitis was 2.3 times (44.1 %, P < 0.03) more likely to be diagnosed in carriers of TLR-3 Leu/Phe genotype in comparison to those with

normal distribution of TLR gene alleles (19.4 %), 2.8 times (54.4 %, P < 0.04) in TLR-3 Phe/Phe, 4,1 times (80.0 %, P < 0.01) in TLR-2 Arg/Gln, 3.9 times (75.0 %, P < 0.04) in TLR-4 Asp/Gly and 3.2 times (62.5 %, P < 0.02) in combinations of TLR-2, TLR-3 and TLR-4 polymorphism. Sinusitis was more likely to be observed in individuals with combinations of TLR-2, TLR-3 and TLR-4 polymorphism (37.5 %) (with normal distribution of TLR gene alleles 5.6 %, P < 0.03).

Consequently, the analysis showed that individuals with polymorphic variants of TLR-2, TLR-3 and TLR-4 genotypes have an increased susceptibility to inflammatory diseases of the upper and lower respiratory tract with ARI frequency of 4 episodes or more during the year, which are consistently complicated by inflammatory processes of LRT. The obtained data were confirmed by the results of correlation analysis, due to which we detected the significant direct correlations between the polymorphic genotypes Leu/Phe and Phe/Phe of TLR-3 and their combinations with Arg/Gln of TLR-2 and Asp/Gly of TLR-4 and ARI (φ = 0.371, P < 0.05, φ = 0.305, P < 0.05, φ = 0.332, P < 0.05 respectively) with frequent (more than 4 times a year) episodes throughout the year (φ = 0.390, P < 0.05, φ = 0.536, P < 0.01, φ = 0.508, P < 0.05 respectively), complicated course of LRT inflammatory processes (φ = 0.384, P < 0.05, φ = 0.478, P < 0.01, φ = 0.421, P < 0.01 respectively), tonsillitis (φ = 0.570, P < 0.05, φ = 0.654, P < 0.05, φ = 0.654, P < 0.05 respectively), bronchitis (φ = 0.383, P < 0.05, φ = 0.525, P < 0.05, φ = 0.531, P < 0.05, respectively), pneumonia (φ = 0.356, P < 0.05, φ = 0.547, P < 0.05, φ = 0.499, P < 0.05, respectively).

Thus, our findings showed that polymorphisms Arg-753Gln of TLR-2, Leu412Phe of TLR-3 and Asp299Gly of TLR-4 genes are associated with increased susceptibility to inflammatory diseases of the upper and lower respiratory tract and ARI with 4 or more episodes during the year. The obtained data agree with the results of other studies that indicate the key role of TLR-2, TLR-3 and TLR-4 in the pathogenesis of ARI and their complications, since these receptors recognize viral structural proteins, gram-positive and gram-negative bacterial ligands (TLR-2 and TLR-4), dsRNA - RNA replication and transcription product and DNA-genomic viruses (TLR-3) [12,13]. Furthermore, nowadays it is determined that one of the main causes influencing changes in the immune response of TLR in infectious pathology is the polymorphism of single nucleotides, which makes an important contribution to the individual peculiarities of protective reactions development, as well as susceptibility to a variety of diseases by forming alleles-specific gene [14,15]. It has been shown that these genetic defects lead to inadequate functions of TLR accompanied by disruption of nuclear transduction (NF-κB) and discoordinated synthesis of proinflammatory and anti-inflammatory cytokines, including those that are essential to the development of IL-1β inflammation [16]. The study of Russian scientists has established the association of the mutant 299Gly allele with high susceptibility of children to respiratory viral infections. Increases in IL-10 and IL-1RA (IL-1β receptor antagonist) and lower production of immunoglobulins (IgA, sIgA, IgM, IgG) were detected in carriers of mutant genotypes TLR-4 (Asp299Gly, Gly299Gly), as compared to those with

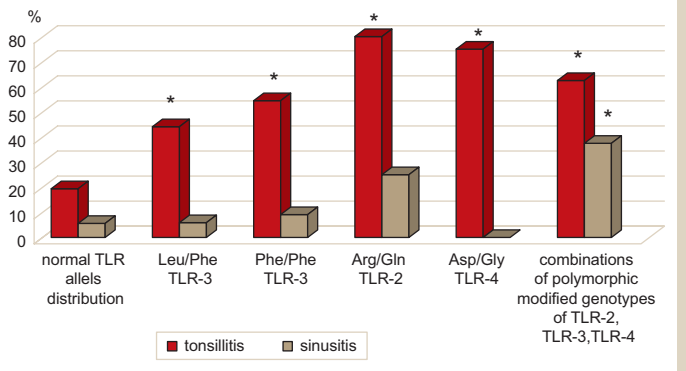

Fig. 2. Frequency of chronic upper respiratory tract pathology in people with polymorphic genotypes and normal distribution of TLR-2, TLR-3 and TLR-4 alleles.

*: P < 0.05 as compared to those with normal distribution of TLR-2, TLR-3 and TLR-4 alleles (the significance level was obtained by Fischer's exact test).

normal allele distribution. IL-10 and IL-1RA cytokines are known to induce an immunosuppressive effect by inhibiting the Th1-cell response and, as a consequence, disrupting the adaptive immune response and synthesis of immunoglobulins. The obtained results indicate the genetically determined restriction of antibodies response in carriers of the Asp299Gly polymorphism of TLR-4 gene as one of the possible causes of anti-infective protection deficiency in children who frequently catch a cold [17].

A number of scientific studies link the Arg753Gln polymorphism of TLR-2 with viral infections. An association has been established between the Arg/Arg homozygous genotypes carriage and CMV-infection development in patients after liver transplantation. Another study indicates a linkage between the mutant Arg/Arg genotype and development of graft failure after liver transplantation in patients with chronic hepatitis C, which caused the death of all TLR-2 mutation carriers [18,19]. The participation of TLR-2 in the immunopathogenesis of HCV infection has been proven in studies conducted in vitro using cells that contain the Arg753Gln mutation of TLR-2 gene, which showed the inability to recognize the nuclear and NS3 proteins of HCV. As a result, the antiviral immune response has been disrupted [20].

Another study found that blood cells collected from carriers of the TLR-2 gene Arg753Gln polymorphism had significantly lower TNF-α and IFN-g production in response to B. burgdorferi lysate as compared to samples with no mutation [21].

The study of Leu412Phe polymorphism functional significance was performed in several in-vitro studies using cells that contain mutations in TLR-3 gene and WT (wild-type) by analyzing the interferon-induced response. The experiments showed that Leu412Phe cells reduced NF-κB and IFN-α signaling by 30 % as compared to WT cells in response to polyinosinic-polycytidylic acid [poly(I:C)] stimulation [10]. The obtained data allowed scientists to assume that the polymorphism Leu412Phe of TLR-3 gene has a definite influence on the course of the infectious process.

Today, the association of SNP Leu412Phe of TLR-3 with the development of subacute sclerosing panencephalitis in measles, myocarditis and dilated cardiomyopathy has been proven in enterovirus infection [10]. Thus, in the exa-

mined patients with enterovirus infection and diagnosed with polymorphism Leu412Phe of TLR-3, the researchers recorded significantly lower levels of INF-α and higher viral load as compared to those who had no mutations in TLR-3 gene. Uncontrolled viral replication led to altered expression of proinflammatory cytokines and chemokines, and their damaging effects on the heart. The findings of Chinese scientists revealed the association between the carriage of Leu412Phe missense mutation in the TLR-3 gene and the severe course of atypical pneumonia with coronavirus-induced ARDS development [22]. In the study, conducted by A. Nahum et al. [23] using mononuclear cells and fibroblasts derived from TLR-3 Leu412Phe mutation carriers, significantly lower levels of IFN-γ, IFN-λ, IFN-β and TNF-α in response to stimulation by Candida Albicans ligands, CMV and synthetic analog of dsRNA poly (I:C) were found compared with cells that had a normal genotype. The identified immune response alterations in the TLR-3 gene polymorphism allowed the scientists to explain the susceptibility to the chronic course of candidiasis and recurrent viral infections.

Consequently, active research of genetic variability of TLR in the last decade shows that polymorphism of single nucleotides makes an important contribution to the individual peculiarities of the protective reactions development, as well as susceptibility to a variety of diseases by specific gene alleles variations.

Conclusions

1. It has been established that the presence of single-nucleotide polymorphisms Arg753Gln of TLR-2, Leu412Phe of TLR-3 and Asp299Gly of TLR-4 genes is the marker of high susceptibility to respiratory diseases.

2. Individuals with polymorphic status of TLR-2 (Arg/Gln), TLR-3 (Leu/Phe and Phe/Phe) and TLR-4 (Asp/Gly) display high susceptibility to inflammatory diseases of the upper and lower respiratory tract with ARI frequency of 4 or more episodes during the year.

3. The risk of bronchitis and pneumonia development in patients with ARIs was higher in subjects with polymorphic status of TLR-2, TLR-3 and TLR-4 genotypes as compared with carriers of normal distribution of alleles: 2.9 times ($P < 0.05$) with TLR-3 Leu/Phe genotype, 20.0 times ($P < 0.001$) with TLR-3 Phe/Phe, 12.8 times ($P < 0.01$) with combinations of TLR-2, TLR-3 and TLR-4 polymorphic genotypes.

Prospects for further research. The results of the conducted analysis indicated the presence of increased susceptibility to ARI complicated by inflammatory processes of the LRT in persons with TLR-2, TLR-3 and TLR-4 polymorphic genotypes. In our previous study we found the association between TLR-2 Arg753Gln, TLR-3 Leu412Phe and TLR-4 Asp299Gly genes polymorphisms and severe influenza and influenza-associated pneumonia with the development of acute respiratory distress syndrome and multiple organ failure [11]. The obtained data allows us to classify individuals with Arg753Gln of TLR-2, Leu412Phe of TLR-3 and Asp299Gly of TLR-4 genes polymorphism as a high risk group for influenza-associated complications development, which requires their specific prophylaxis necessity. Since it is known that genetic variability of TLR by influencing the recognition of PAMP is able to change the immune response to both infection and vaccination, and data on the efficacy of specific influenza prevention in individuals with polymorphic genotypes is rather limited, this needs to study the issue further.

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References

[1] Khobzei, M. K., Holubovska, O. A., Lishchyshyna, O. M., Andreichyn, M. A., Batsyura, H. V., Hradil, H. H., et al. (2015). Nakaz Ministerstva okhorony zdorovia Ukrainy «Unifikovanyi klinichnyi protokol pervynnoi medychnoi dopomohy doroslym ta ditiam "Hostri respiratorni infektsii"» vid 16.06.2014 roku №499 [Order of the Ministry of Public Health of Ukraine «Unified clinical protocol of primary care for adults and children "Acute respiratory infections"» of June 16, 2014, №499]. *Klinichna imunolohiia. Alerholohiia. Infektolohiia*, 5–6(84–85), 33–40 [in Ukrainian].

[2] Abaturov, A. Ye., Volosovec, A. P., Yulish, Ye. I. (2012). Rol' TOLL-podobnykh receptorov v rekognicii patogen-associirovannykh molekulyarnykh struktur infekcionnykh patogennykh agentov i razvitii vospaleniya. Chast' 1. Semejstvo TLR [The role of Toll-like receptors in the recognition of pathogen-associated molecular structures of infectious pathogenic agents and the development of inflammation. Part 1. Family TLR]. *Zdorov'ye rebenka*, 5(40), 116–121 [in Russian].

[3] Trejo-de la, O. A., Hernández-Sancén, P., & Maldonado-Bernal, C. (2014). Relevance of single-nucleotide polymorphisms in human TLR genes to infectious and inflammatory diseases and cancer. *Genes and Immunity*, 15(4), 199–209. doi: 10.1038/gene.2014.10.

[4] Izmailova, O. V., Shlykova, O. A., Bobrova, N. O., & Kaidashev, I. P. (2009). Rol polimorfizmu Toll-podibnoho retseptora 4 Asp299Gly u rozvytku infektsii, shcho peredaiutsia statevym shliakhom [The role of Toll-like Asp299Gly receptor polymorphism in the development of sexually transmitted infections]. *Problemy ekolohii ta medytsyny*, 13(5–6), 3–6 [in Ukrainian].

[5] Paulus, S. C., Hirschfeld, A. F., Victor, R. E., Brunstein, J., Thomas, E., & Turvey, S. E. (2007). Common human Toll-like receptor 4 polymorphisms: role in susceptibility to respiratory syncytial virus infection and functional immunological relevance. *Clin. Immunol*, 123(3), 252–7. doi: 10.1016/j.clim.2007.03.003.

[6] Holmes, C. L., Russell, J. A., & Walley, K. R. (2003). Genetic Polymorphisms in Sepsis and Septic Shock: Role in Prognosis and Potential for Therapy Free To View. *J. CHEST*, 124(3), 1103–1115.

[7] Ogus, A. C., Yoldas, B., Ozdemir, T., Ugus, A., Olcen, S., Keser, I., et al. (2004). The Arg753Gln polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. *Eur Respir* J, 23(2), 219–223. doi: 10.1183/09031936.03.00061703.

[8] Lorenz, E., Mira, J., Cornish, K., Arbour, N. C., & Schwartz, D. A. (2000). A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. *Infect Immun*, 68(11), 6398–6401. doi: 10.1128/IAI.68.11.6398-6401.2000.

[9] Ishizaki, Y., Takemoto, M., Kira, R., Kusuhara, K., Torisu, H., Sakai, Y., et al. (2008). Association of Toll-like receptor 3 gene polymorphism with subacute sclerosing panencephalitis. Journal of *NeuroVirology*, 14(6), 486–491. doi: 10.1080/13550280802298120.

[10] Gorbea, C., Makar, K. A., Pauschinger, M., Pratt, G., Bersola, J. L., Varela, J., et al. (2010). Role for Toll-like Receptor 3 Variants in Host Susceptibility to Enteroviral Myocarditis and Dilated Cardiomyopathy. *Journal of Biological Chemistry*, 285(30), 23208–23. doi: 10.1074/jbc.M109.047464.

[11] Dubinskaya, G., Pryimenko, N., Kaidashev, I., Pokhylko, V., & Chub, K. (2014). Rol' polimorfizma genov TLR-2, TLR-3, TLR-4 pri grippe [The role of tlr-2, tlr-3, tlr-4 genes polymorphism of grippe]. *Gergian medical news*, 7–8(232–233), 51–55 [in Russian].

[12] Drutskaya, M. S., Nedospasov, S. A., & Belousov, P. V. (2011). Vrozhdennoe raspoznavanie virusov [Innate mechanisms of viral recognition]. *Molekulyarnaya biologiya*, 45(1), 7–19. [in Russian].

[13] Kumar, H., Kawai, T., & Akira, S. (2009). Toll-like receptors and innate immunity. *Biochem. Biophys. Res. Commun.*, 388(4), 621–5. doi: 10.1016/j.bbrc.2009.08.062.

[14] Bairakova, A. L., Voropaeva, E. A., Afanasiev, S. S., Aleshkin, V. A., Nesvizhsky, Yu. V., Karaulov, A. V., et al. (2008). Rol' i biologicheskoe znachenie toll-podobnykh receptorov v antiinfekcionnoj rezistentnosti organizma [The role and biological significance of the Toll-like receptors in the anti-infective resistance of the organism]. *Vestnik Rossijskoj akademii medicinskikh nauk,* 1, 45–54. [in Russian].

[15] Koval'chuk, L. V., Khoreva, M. V., Varivoda, A. S., Nikolaeva, I. N., Gracheva, L. A., Galukhina, Ye. R. et al. (2008). Oposredovannye cherez Toll-podobnye receptory vyrabotka citokinov i e'kspressiya poverkhnostnykh markerov lejkocitami cheloveka. [Mediated via Toll-like receptors production of cytokines and the expression of surface markers by human leukocytes]. *Immunologiya*, 4, 223–227. [in Russian].

[16] Kono, H., Karmarkar, D., Iwakura, Y., & Rock, K. L. (2010). Identification of the Cellular Sensor That Stimulates the Inflammatory Response to Sterile Cell Death. *J. Immunol.*, 184(8), 4470–4478. doi: 10.4049/jimmunol.0902485.

[17] Malezhik, L. P., & Karpov, N. I. (2011). Vliyanie polimorfizma receptorov Toll-4 (Asp299Gly) i Toll-6 (Ser249Pro) na produkciyu citokinov u detej, chasto boleyushchikh ostrymi respiratornymi virusnymi infekciyami [Effect of Toll-4 (Asp299Gly) and Toll-6 (Ser249Pro) receptor polymorphism on the production of cytokines in children who frequently suffer from acute respiratory viral infections]. *Vrach-aspirant*, 6(49), 125–131. [in Russian].

[18] Brown, R. A., Gralewski, J. H., Eid, A. J., Knoll, B. M., Finberg, R. W., & Razonable, R. R. (2010). R753Q Single Nucleotide Polymorphism Impairs Toll-like Receptor 2 Recognition of Hepatitis C Virus Core and Nonstructural 3 Proteins. *Transplantation*, 89(7), 811–815. doi: 10.1097/TP.0b013e3181cbac18.

[19] Eid, A. J., Brown, R. A., Paya, C. V., & Razonable, R. R. (2007). Association between toll-like receptor polymorphisms and the outcome of liver transplantation for chronic hepatitis C virus. *Transplantation*, 84(4), 511–6. doi: 10.1097/01.tp.0000276960.35313.bf.

[20] Dolganiuc, A., Oak, S, Kodys, K., Golenbock, D. T., Finberg, R. W., Kurt-Jones, E., & Szabo, G. (2004). Hepatitis C core and nonstructural 3 proteins trigger toll-like receptor 2-mediated pathways and inflammatory activation *Gastroenterology*, 127(5), 1513. doi: 10.1053/j.gastro.2004.08.067.

[21] Schröder, N. W., Diterich, I., Zinke, A., Eckert, J., Draing, C., von Baehr, V., et al. (2005). Heterozygous Arg753Gln polymorphism of human TLR-2 impairs immune activation by Borrelia burgdorferi and protects from late stage Lyme disease. *J. Immunol.*, 175(4), 2534–2540. doi: 10.4049/jimmunol.175.4.2534.

[22] Tsai, Wan-Yu, & Ho, Mei. (2005). Polymorphisms of Toll-like Receptor 3 (TLR3), TNF Receptor-associated Factor 6 (TRAF6), and Heme Oxygenase-1 (HO-1) are Associated with Clinical Severity of Severe Acute Respiratory Syndrome. Retrieved from http://www. http://handle.ncl.edu.tw/11296/ndltd/87497652997228105336.

[23] Nahum, A., Dadi, H., Bates, A., & Roifman, C. M. (2012). The biological significance of TLR3 variant, L412F, in conferring susceptibility to cutaneous candidiasis, CMV and autoimmunity. *Autoimmunity Reviews.*, 11(5), 341–347. doi: 10.1016/j.autrev.2011.10.007.