РОЛЬ ПОЛИМОРФНЫХ МАРКЕРОВ В ГЕНАХ *TLR2, TLR4* И *TLR9* В РИСКЕ РАЗВИТИЯ АТОПИЧЕСКОГО ДЕРМАТИТА

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Резюме. Атопический дерматит – распространенное хроническое воспалительное заболевание кожи, ассоциированное со значительным снижением качества жизни. Считается, что атопический дерматит развивается у лиц с генетической предрасположенностью к атопии под воздействием факторов окружающей среды, иммунной дисрегуляции и изменением микробиома кожи. Толл-подобные рецепторы (TLR) являются неотъемлемой частью врожденной иммунной системы и участвуют в распознавании патоген-ассоциированных молекулярных паттернов. Целью исследования было оценить генетический риск развития атопического дерматита на основании изучения полиморфных маркеров в генах *TLR2, TLR4* и *TLR9*. В исследование были включены 100 пациентов с атопическим дерматитом средней (n = 56) и тяжелой (n = 44) степенью тяжести. Возраст варьировал от 18 до 65 лет. В группу контроля были включены 72 добровольца старше 18 лет, не имеющих в анамнезе каких-либо аллергических заболеваний кожи. В ходе исследования были проанализированы следующие маркеры: rs5743708 в гене *TLR2*, rs4986791 в гене *TLR4* и rs352140 в гене *TLR9*. Исследование генетических маркеров SNP rs5743708 в гене *TLR2* и rs4986791 в гене *TLR4* не выявило статистически значимых различий в распределении аллелей и генотипов. Изучение полиморфного маркера rs352140 в гене *TLR9* показало статистически достоверное различие между группой пациентов с атопическим дерматитом средней степени тяжести и контрольной выборкой. Частота встречаемости гомозиготы GG в группе с атопическим дерматитом составила 0,169, в то время как в группе контроля – 0,329 ($p < 0,05$; OR = 0,42; 95% CI = 0,18-0,97). На сегодняшний день изучение влияния полиморфных генетических локусов на риск развития различных заболеваний представляет особый интерес. Однако среди многочисленных

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исследований, направленных на поиск SNP-маркеров для диагностики повышенного риска развития заболеваний, встречаются лишь единичные публикации об ассоциации полиморфных маркеров в генах TLRs с атопическим дерматитом. Выбранные нами полиморфные маркеры rs5743708 в гене *TLR4* и rs4986791 в гене *TLR9* были изучены впервые при атопическом дерматите. В заключение, по результатам исследования был выявлен один полиморфный маркер, ассоциированный с риском развития атопического дерматита. Так, было показано, что гомозигота GG генетического маркера SNP rs352140 *TLR9* может быть предиктором относительно риска развития атопического средней степени тяжести. Таким образом, полученные данные могут быть использованы при оценке риска развития атопического дерматита у здоровых лиц с отягощенным семейным анамнезом.

Ключевые слова: атопический дерматит, TLR, SNP, маркер, атопические заболевания, врожденный иммунитет

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS OF *TLR2, TLR4* AND *TLR9* WITH ATOPIC DERMATITIS

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Abstract. Toll-like receptors (TLRs) are the most studied among all Pattern Recognition Receptors, the main function of which is to initiate innate immune response by recognizing pathogen-associated molecular patterns of various microorganisms on the skin surface. TLR-mediated recognition plays an important role in linking innate and adaptive immunity that ultimately leads to the production of key cytokines, chemokines and antimicrobial peptides. Today, there is growing interest in research on single nucleotide polymorphisms (SNPs) in *TLR* genes and its influence on susceptibility to inflammatory disease, including atopic dermatitis. The aim of the research was to study the association of the rs5743708 gene polymorphism in the *TLR2* gene, the rs4986791 gene polymorphism in the *TLR4* gene and the rs352140 gene polymorphism in the *TLR9* gene with the risk of developing severe cases of AD. A total of 100 patients with AD were included in the study (38 male and 62 female). The age range was from 18 to 65 years old. All participants were divided into 2 groups according to the SCORAD index (SCORing Atopic Dermatitis). The control group included 72 volunteers over 18 years old. The results of our study showed a statistically significant difference between the moderate AD group and healthy controls in the rs352140 gene polymorphism in the *TLR9* gene (Figure 1). The frequency of the GG genotype of SNP rs352140 in *TLR9* was 0.169 in the AD group versus 0.329 in the control group ($p < 0.05$; OR = 0.42; 95% CI = 0.18-0.97).

In conclusion, the results of our study showed that the *TLR9* rs352140 gene polymorphism may be linked to an increased risk of atopic dermatitis. Moreover, it was found that the GG genotype of SNP rs352140 in *TLR9* can be used as a predictor of the risk of developing moderate AD.

Keywords: atopic dermatitis, TLR, SNP, marker, atopic diseases, innate immunity

Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disease associated with the significantly decreased quality of life. The first signs of AD usually appear in infancy, which may precede the development of other allergic diseases, including asthma, allergic rhinitis or food allergy [2, 8]. Clinical presentations are diverse and may vary over time depending on age and the course of disease. Generally, the acute phase of AD is characterized by erythematous skin lesions and edema, whereas chronic stage features lichenification, generalized xerosis and

post-inflammatory hyperpigmentation. Although the pathophysiology of AD is still not completely understood, it is regarded as a complex interaction between immune dysregulation, altered skin microbiome and environmental factors in individuals with a positive family history of atopy [2, 4].

Skin microbiome research has highlighted its role in the development and exacerbation of AD. The composition of microbial communities in AD patients is usually characterized by loss of microbial diversity and predominance of *Staphylococcus aureus* (*S. aureus*) in both lesion and non-lesional skin [5]. That in turn induces altered immune responses by maintaining type 2 inflammation due to the elaboration of a wide range of staphylococcal enterotoxins (SEs) and other virulence factors by *S. aureus* strains [10]. Thus, these data suggest that the disturbed microbial composition and overgrowth of *S. aureus* are significantly involved in the clinical manifestation and pathogenesis of AD flares.

Toll-like receptors (TLRs) are the most studied among all Pattern Recognition Receptors (PRRs), the main function of which is to initiate innate immune response by recognizing pathogen-associated molecular patterns (PAMPs) of various microorganisms on the skin surface, such as lipopolysaccharide of Gram-negative bacteria, peptidoglycan and lipoteichoic acid of Gram-positive bacteria. TLR-mediated recognition plays an important role in linking innate and adaptive immunity that ultimately leads to the production of key cytokines, chemokines and antimicrobial peptides. These TLRs are expressed by both immune cells and non-immune cells, including keratinocytes. To date, there are 10 members of the TLR family (*TLR1-TLR10*) identified in humans, which are further divided based on their localization into extracellular TLRs (*TLR1,TLR 2, TLR4, TLR5, TLR6, TLR10*) and intracellular TLRs (*TLR3, TLR7, TLR8, TLR9*) [9, 10]. The latter recognize nucleic acids of viruses, bacteria as well as damaged host cells. Conversely, extracellular TLRs can detect a broad spectrum of structures of Gram-positive bacteria and also components of damaged host cells. In addition, *TLR4* is implicated in the recognition of lipopolysaccharide of Gram-negative bacteria. *TLR5* binds bacterial flagellin found in many Gram-negative and Gram-positive bacteria. *TLR2* and *TLR4* are known for their ability to bind endogenous ligands such as heat shock protein and hyaluronic acid [10, 11].

There is growing interest in research on single nucleotide polymorphisms (SNPs) in *TLR* genes and its influence on susceptibility to inflammatory disease. For instance, certain polymorphisms were associated with impaired *TLR2* and *TLR4* function in individuals with AD, which may contribute to the prevalence of Th2-mediated immune responses [1, 7].

The aim of the research was to study the association of the rs5743708 gene polymorphism in the *TLR2* gene, the rs4986791 gene polymorphism in the *TLR4* gene and the rs352140 gene polymorphism in the *TLR9* gene with the risk of developing severe cases of AD.

Materials and methods

A total of 100 patients with AD were included in the study (38 male and 62 female). All patients were admitted in the clinic of skin and venereal diseases named after V.A. Rakhmanov (Sechenov University). The age range was from 18 to 65 years old. The diagnosis of AD was established by using Hanifin and Rajka's criteria [3]. All participants were divided into 2 groups according to the SCORAD index (SCORing Atopic Dermatitis) results: moderate (n = 56) and severe (n = 44) [6]. The control group included 72 volunteers over 18 years old without atopic dermatitis and other allergic diseases. Peripheral blood samples were collected into EDTA tubes (VACUETTE® TUBE 4 ml K3E K3EDTA, Greiner Bio-One). Total RNA was extracted using the "AmpliPRIME RIBOsorb" kit (Amplisens, Russia). The following markers were analyzed by PCR-based techniques using "SYBR Green I RT-PCR" kit (Syntol, Russia) and DT Prime 5 Real-Time PCR device (DNA-Technology, Russia): *TLR2* rs5743708, *TLR4* rs4986791 and *TLR9* rs352140. Statistical analysis was performed by using the χ^2 test. Results were considered statistically significant if a p-value was less than 0.05 ($p < 0.05$).

Results and discussion

Genotyping of the rs5743708 gene polymorphism in the *TLR2* and rs4986791 gene polymorphism in the *TLR4* did not reveal any statistically significant differences in allele frequency and genotype distribution. However, the results of our study showed a statistically significant difference between the moderate AD group and healthy controls in the rs352140 gene polymorphism in the *TLR9* gene (Figure 1). The frequency of the GG genotype of SNP rs352140 in *TLR9* was 0.169 in the AD group versus 0.329 in the control group ($p < 0.05$; OR = 0.42; 95%CI = 0.18-0.97).

Although studies focused on SNP analysis have been of particular interest in recent decades, the data on potential associations between TLR polymorphisms and AD is limited to a few publications. To our knowledge, the investigated SNPs rs5743708 in the *TLR4* gene and rs4986791 in the *TLR9* gene were studied for the first time in patients with atopic dermatitis.

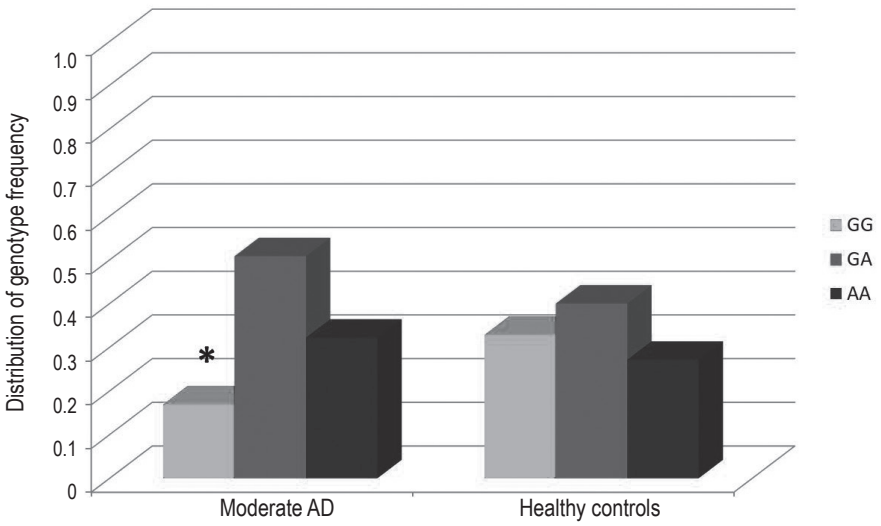

Figure 1. Distribution of TLR9 SNP rs352140 genotypes in patients with moderate atopic dermatitis compared to healthy controls

Note. *, $p < 0.05$.

According to a meta-analysis conducted by Zhang Y. et al. (2019), carriage of *TLR2* SNP rs5743708 GA genotype revealed a significantly higher risk for developing AD in the Caucasian race [12]. Nevertheless, the results of our study found no difference between patients with AD and healthy subjects.

It is notable that the scientific literature contains scarce data related to the analysis of *TLR4* gene polymorphisms and the susceptibility of AD. Zhao J. et al. (2017) conducted a meta-analysis that included more than 340 articles. It has been reported that rs4986791 in *TLR4* was significantly associated with the risk of developing asthma [13]. However, no data on AD was found.

According to the literature, the most common *TLR9* polymorphisms are rs5743836, rs187084, rs352140, and rs2066807. In particular, SNPs rs5743836 and rs187084 in the *TLR9* gene have been previously studied in patients with AD, but no significant association with AD was found [14]. And since there was previously detected a significant association of the rs352140 SNP in *TLR9* with regard to other diseases, we chose this particular SNP [15].

Conclusion

In conclusion, the results of our study showed that the *TLR9* rs352140 gene polymorphism may be linked to an increased risk of atopic dermatitis. Moreover, it was found that the GG genotype of SNP rs352140 in *TLR9* can be used as a predictor of the risk of developing moderate AD.

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