UDK [577.21:616.5-002]-053.3/.5

Sakevych Victoria D., Shlykova Oksana, Nelia A. Bobrova , Kaydashev Igor P.
Research Institute for Genetic and immunological bases of pathology and pharmacogenetics State Higher School of Ukraine» Ukrainian Medical Stomatological Academy» in Poltava

Prevalence of polymorphic alleles 2258G / A gene TLR2 and its relationship with some immunological parameters among patients with allergic rhinitis

Key words: *polymorphism, Toll-like receptors, allergic rhinitis.*

There is a tendency to increase in the prevalence of allergic diseases (EP) in different countries, one of the most common of which is allergic rhinitis (AR) [10]. AR problem still exists, still poses a serious issue because of the widespread, annual growth of widespread disease, frequent complications, as well as a sharp decline in performance and quality of life of patients.

Based on the results of epidemiological studies on AR affects about 20 % of the population of different age groups. Prevalence of disease in most European countries ranges from 10 to 32 % in Australia – 40 %, in 25 % of the population have symptoms of AR [10]. The incidence of AR in Ukraine is 113.0 per 100 thousand of the adult population [6].

According to modern concepts of AR multifactorial pathology, that occurs in the case of the interaction of various environmental factors and genetic predisposition. All allergens – infectious (bacterial, fungal viral) and noninfectious (herbal, household, alimentary, epidermal) is an activator of innate immune cells. The innate immune response (Tue) realized through family Toll-like receptors (Toll-like receptors; TLRs) [1]. TLR-mediated Tue seeks to identify pathogens and to determine its type, and immediate activation and stimulation of adaptive immunity. Defect innate immune response as well as violations of the functional unity of innate and adaptive immunity play an important role in the development of RA. According to modern concepts of Toll-like receptor 2 (TLR2) is an important structural element of the molecular pattern - distinctive receptors as responsible for identifying ligands wide range of microorganisms.

Recently there information on the identification of functional polymorphisms of genes TLR, due to the replacement of single nucleotides (from Eng. Single nucleotide polymorphism – SNP). As a result of these substitutions reduced ability to recognize appropriate ligands and efficiency of signal pulses, which leads to disruption of the activation of immune cells. A functional polymorphism of TLR2 regulation violates the innate immune response. An abnormal activation of Th2-type immune responses to harmless antigens environment, which plays a crucial role in the formation of chronic inflammation and attracted attention as a potential risk factor for the development of atopic diseases, including RA.

The positive correlation between SNP in TLR2 with atopic diseases and asthma [9, 11].

Pathogenetic role of polymorphisms of TLR2 and its practical value in proven, polypous rhinosinusitis [7], atopic eczema [12], urogenital pathology [2] and in patients with allergic diseases [4, 5].

The number of pathologies of disturbances in TLR increases [3]. In order to better study and understanding of genetic predisposition to the emergence of sound is the study of the prevalence of functional polymorphisms of TLR2 gene among patients with RA.

The aim of our study was to study the polymorphism 2258G / A gene TLR2 (rs5743708) among patients with RA, analysis of immunological parameters and clinical manifestations in patients with polymorphic variants studied genes.

Materials and methods

A survey of 45 patients with AR aged 19 to 65 years (35,6 ± 1,57) (men accounted for 51 % (23 patients), and women – 49 % (22 patients)). At the time of the survey, patients were in clinical remission stage and stopped receiving allergy

Table 1

Frequency distribution of genotypes of polymorphism 2258G / A gene TLR2 (rs5743708) among healthy individuals and patients with AR, %, (n)

Gene polymorphism	The frequency of genotype	Control group (n = 95)	Patients (n = 45)	p*	The frequency of allele	Control group (n = 95)	Patients with AR, (n = 45)	Pearson χ^2, df = 1	OR (95% CI)	p**
TLR2 2258G/A	GG GA AA	97,8 (93) 2,2 (2) -	93,3 (42) 6,6 (3) -	0,33	G A	98,9 (188) 1,1 (2)	96,6 (87) 3,4 (3)	0,74	4,59 (1,007-20,94)	0,34

*p – significance level obtained Fisher exact test.

medications 72 hours, the patients had severe comorbidity. Diagnosis is established based on the AR diagnostic criteria ARIA (2008) diagnostic algorithm adopted in Ukraine and approved by the Ministry of Health of Ukraine. Quality of life of patients was determined using generally recognized questionnaires (Adult Rhinoconjunctivitis Quality of Life Questionnaire).

Sensitization to allergens diagnosed on the basis of complex allergy diagnostic testing: collection allergic history, a positive skin scraping test to allergens using standard sets (of «Immunologist', Vinnitsa, Ukraine).

Getting the peripheral blood of patients was done by sampling blood from the vena cubitalis; fasting in volume 4 ml vacuum tube with EDTA (8.4 mg K3EDTA). Bold genomic DNA was carried out by phenol- chloroform extraction. Definition polymorphism 2258G / A gene TLR2 conducted by polymerase chain reaction [2].

According to the standard procedure was conducted to determine the number of white blood cells in the blood and counting of blood cells in smears. Lymphocyte phenotype was analyzed in venous blood using monoclonal antibodies to CD4, SD25 (production of «sorbent», Russia) and intracellular protein Foxp3 («Bioscience», USA) by flow cytofluorometry by EPIX LX-MCL (Beckman Coulter, USA) using a program called System II TM software.

The levels of total IgE, interleukin-4 (IL-4) and interleukin-10 (IL-10) were determined using ELISA test kits (of «Ukrmed Don», Ukraine) using ELISA analyzer «Stat-Fax 2100» (U.S.).

The control group was 95 healthy individuals from a database of genetic samples SRI Genetic and immunological bases of pathology and pharmacogenetics VDNZU» UMSA». The study was conducted in accordance with provided written consent to the inspection and conclusion of the Commission on ethical issues and bioethics Ukrainian Medical Dental Academy.

Mathematical analysis of the data was carried out using the program «STATISTICA 6.0» (StatSoft Inc). Comparison of genotype frequencies between the study groups was performed by analysis of contingency tables using Fisher's exact test. To compare allele frequencies used criterion χ^2. To assess the reliability of differences between groups using Fisher's exact two-sided test (for small groups). For all types of analysis considered differences statistically significant at $p < 0.05$.

Results and discussion.

In the study of family allergic history in patients with AR found various manifestations of allergy in the family in 76 %. The presence of allergic diseases in relatives and II degree relatives of the mother was found in 35 % of the father – in 30 % of both parents – 11 % of all patients examined in AR. There were no data on the burdened history of allergy in 24 % of patients with RA. The results are consistent with data indicating preferential relationship with atopic diseases of the mother.

As noted above, as a result of observation of the dynamics of the disease in patients with AR were installed AR severity: mild course – in 11 (25 %), medium-heavy – in 32 (71 %), heavy – in 2 (4 %). Also revealed the presence of genetic predisposition to allergic relatives and II degree relatives of the mother was found in 35 % of the father – in 30 % of both parents – 11 % of all patients examined in AR. In 44 % of the course AR has been associated with various nosological forms of allergic disease. In 20 % of the patients on concomitant AR was established diagnosis of asthma, 15 % present symptoms of AD, the full triad of atopy was found in 11 % of patients surveyed by us in AR. With Allergic examination of patients with AR in 89 % of patients were found positive skin tests to pollen, fungal, household, epidermal and food allergens. Moreover, 7 % occurred sensitization to one allergen group, 29 % – to two groups, 36 % – up to three groups, 13 % – to the four groups, and 4 % – of all five groups of allergens. In 11 % of patients had negative skin tests to all allergens used.

The analysis of frequency distribution of genotypes and alleles per polymorphic gene studied in groups of observations are shown in Table 1.

Individuals that were in the control group, the frequency of the «wild-type» genotype TLR2 GG was 97.8 %, the frequency of heterozygous genotype GA – 2,2 %, the mutant genotype AA was not detected. In patients with AR corresponding results were as follows: GG – 93,3 %, GA – 6,6 % and AA was also not detected. There was no significant relationship between the frequency of polymorphic alleles. In the control group and patients with AR ($\chi^2 = 0,74$; $p = 0.34$).

Genetic markers may determine susceptibility to disease or be associated with the relevant pathogenetic significant figures. Therefore, within the proposed research studied the effect of polymorphism 2258G / A gene TLR2 on the course and features of the clinical manifestations of AR (Table 2).

Analyzing the symptoms, differences in the incidence of seasonal and year-round flow shape ($p = 0.1690$) between

Table 2

A comparison group of patients examined for AR (n = 6), depending on the genotype at polymorphism 2258G / A gene TLR 2

Clinical features of AR		Patients with mutant AR alellyu TLR2 Asp 299 Gly (n = 3)	Patients with AR homozygous carriers of «wild» allele, (n = 42)	p*
Perennial AR	yes no	2 1	10 32	0,1690
Seasonal AR	yes no	1 2	32 10	0,1690
Concomitant asthma	yes no	2 1	7 35	0,0973
Additional AD	yes no	1 2	4 38	0,3037
Related asthma + AD	yes no	1 2	1 38	0,3037

* p – significance level obtained Fisher exact test.

the groups of patients with AR depending on genotype polymorphism 2258G / A gene TLR2 had no statistical significance. Nor a statistically significant association of the polymorphism 2258G / A gene with the development of TLR2 in patients with other atopic disease (p = 0.3037).

It is known that the main feature of allergic diseases is the imbalance T1/T2-helperiv of abnormal T2-activation of the immune response. Th-2 way of immune response in atopy is caused by many factors. In response to exposure to allergens in patients with AR – T2 is the release of cytokines that activate eosinophils and mast cells, affecting the orientation of production by B cells of total IgE. There is evidence that stimulation of Th-2 occurs indirectly through TLR2 [8]. In order to detect differences between patients with AR depending on genotype polymorphism 2258G / A gene for TLR2 immunological parameters was carried out by comparison of groups using the Mann – Whitney (Table 3).

As shown in Table 3, revealed significant differences between the groups of patients with AR with the presence of the mutant allele 2258G / A gene TLR2 and homozygous carriers of «wild» allele in terms of $CD4^+$ ($U_{(n = 42;\ n = 3)} = 12{,}00$ $p = 0.020$). It should be noted that the level of expression of CD4 + molecules in patients with mutant AR alleles 2258G / A gene TLR2 on average tended to increase, and in patients with AR Media «wild» alleles did not go beyond the parameters of healthy individuals.

Also a group of patients with mutant AR alleles 2258G / A gene TLR2 significantly higher values differed by lymphocytes ($U_{(n = 42;\ n = 3)} = 11{,}50$; $p = 0{,}019$) from a group of patients with AR homozygous carriers of «wild» allele.

Conclusions

1. In patients with AR frequency «wild-type» genotype TLR2 GG was 93.3 % , the frequency of heterozygous genotype GA – 6,6 %, the mutant genotype AA was not detected.

2. Revealed significant differences between the groups of patients with AR with the presence of the mutant allele 2258G / A gene TLR2 and homozygous carriers of «wild» allele in terms of $CD4^+$ ($U_{(n = 42;\ n = 3)} = 12{,}00$; $p = 0{,}020$).

3. Patients with mutant AR with alellyu 2258G / A gene for TLR2 differ significantly higher value of lymphocytes ($U_{(n = 42;\ n = 3)} = 11{,}50$; $p = 0{,}019$) from a group of patients with AR homozygous carriers of «wild» alleles.

Thus, polymorphism 2258G / A gene TLR2 is important in determining the course of the disease, confirming the pathogenetic relationship between innate and adaptive immunity in AR.

Table 3

Differences in the immunological parameters of patients with AR (n = 45), depending on genotype polymorphism 2258G / A gene TLR 2

index	Patients on AP s mutant alleles 2258G / A gene TLR2 (n = 3)	Patients on AP (n = 42) homozygous carriers
$CD4^+$, %	50,8±3,78	39,81± 1,19
U, p	**$U_{(n=42;n=3)} = 12{,}00$; p=0,020**	
Limfotsiti%	37,67±0,88	28,0± 1,14
U, p	**$U_{(n=42;n=3)} = 11{,}50$; p=0,019**	

U, p – Differences within the groups for Mann Uitni criterion

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РОЗПОВСЮДЖЕНІСТЬ ПОЛІМОРФНИХ АЛЕЛЕЙ 2258G/A ГЕНУ TLR2 ТА ЇХ ЗВ'ЯЗОК З ОКРЕМИМИ ІМУНОЛОГІЧНИМИ ПОКАЗНИКАМИ СЕРЕД ХВОРИХ НА АЛЕРГІЧНИЙ РИНІТ

Сакевич В. Д., Шликова О. А., Боброва Н. О., Кайдашев І. П.

Актуальність. *На сьогодні існує тенденція до зростання рівня розповсюдженості алергічних захворювань у різних країнах світу, одним із найбільш поширених серед яких є алергічний риніт (АР). За сучасними уявленнями АР мультифакторна патологія, що виникає в разі взаємодії різноманітних чинників навколишнього середовища та спадкової схильності. Кількість патологій з порушеннями в системі TLR зростає. Для кращого вивчення та розуміння генетичної схильності до виникнення обгрунтованим є вивчення поширеності функціонального поліморфізму гену TLR 2 серед хворих на АР.*

Метою *нашого дослідження стало вивчення поліморфізму 2258G/A гену TLR2 (rs5743708) серед хворих на АР, аналіз імунологічних показників та клінічних проявів у хворих з поліморфними варіантами досліджуваних генів. У дослідженні проаналізовано клінічні прояви, стан клітинного і гуморального імунітету та розповсюдженість поліморфних алелей 2258G/A гену TLR2 серед хворих на АР (n = 45) для вивчення механізмів патогенезу та розуміння генетичної схильності до виникнення цього захворювання. Обстеженно 45 хворих на АР віком від 19 до 65 років. Діагноз АР встановлювали на основі критеріїв діагностики ARIA (2008) за алгоритмом діагностики прийнятим в Україні та затвердженим МОЗ України.*

Сенсибілізацію до алергенів діагностували на підставі комплексу алергологічних методів обстеження: збір алергологічного анамнезу, позитивних шкірних скарифікаційних тестів на алергени з використанням стандартних наборів (ТОВ «Імунолог», Вінниця, Україна). Виділення геномної ДНК здійснювали методом фенол-хлороформної екстракції. Визначення поліморфізму 2258G/A гену TLR2 проведено методом полімеразної ланцюгової реакції.

За стандартною методикою проведене визначення числа лейкоцитів в крові та підрахунок формених елементів крові в мазках. Фенотип лімфоцитів аналізували у венозній крові, використовуючи моноклональні антитіла до CD4,CD25 (виробництво «Сорбент», Росія) та внутрішньоклітинного білку Foxp3 («Bioscience», США) методом проточної цитофлюориметрії за допомогою проточного цитофлюориметра EPIX LX-MCL (Beckman Coulter, США), використовуючи програму System II TM software. Рівні загального IgE, інтерлейкіну-4 (ІЛ-4) та інтерлейкіну-10 (ІЛ-10) визначали за допомогою тест-систем ІФА (ТОВ «Укрмед-Дон», Україна) з використанням імуноферментного аналізатора «Stat- Fax 2100» (США). Групу контролю становили 95 практично дорових осіб з бази генетичних зразків НДІ Генетичних та імунологічних основ розвитку патології та фармакогенетики ВДНЗУ «УМСА». Математичну обробку отриманих даних здійснювали з використанням програми «STATISTICA 6.0» (StatSoft Inc).

Результати досліджень. *При дослідженні поліморфізму гену Asp299Gly TLR4 хворих на АР частота «дикого типу» генотипу TLR2 GG становила 93,3 %, частота гетерозиготного генотипу GA – 6,6 %, мутантний енотип AA не був виявлений. В результаті проведених досліджень виявлена достовірна різниця між групами хворих на АР з наявністю мутантної алелі 2258G/A гену TLR2 та гомозиготними носіями «дикої» алелі за показником $CD4^+$ ($U_{(n=42;n=3)}$ = 12,00; p = 0,020). Група хворих на АР з мутантною алеллю 2258G/A гену TLR2 відрізнялась за достовірно вищим значенням лімфоцитів ($U_{(n=42;n=3)}$ = 11,50; p = 0,019) від групи хворих на АР гомозиготних носіїв «дикої» алелі. Проведене дослідження, дає можливість висунути припущення поліморфізм 2258G/A гену TLR2 має важливе значення в визначенні перебігу захворювання, що підтверджує патогенетичний взаємозв'язок між вродженим та адаптивним імунітетом при АР.*

Ключові слова: *поліморфізм, Toll-подібні рецептори, алерічний риніт.*

Науково-практичний журнал «Астма та алергія», 2013, №3

І. П. Кайдашев
«Українська медична стоматологічна академія», професор,
36024, Україна, м. Полтава, вул. Шевченка, 23
Тел./факс:: 38053 227 0539
e-mail: kaidashev@yandex.ru

THE RELATIONSHIP OF CLINICAL AND IMMUNOLOGICAL PARAMETERS WITH MUTANT ALLELES OF TOLL-LIKE RECEPTOR 4 (2258G/A TLR2) IN PATIENTS WITH ALLERGIC RHINITIS

Sakevich Victoriia Dmytrivna, Shlykova Oxana Anatoliyivna, Kaydashev Igor Petrovych

Urgency. *There is a tendency to increase in the prevalence of allergic diseases in different countries, one of the most common of which is allergic rhinitis (AR). According to modern concepts AR multifactorial pathology that occurs in the case of the interaction of various environmental factors and genetic predisposition . Number of pathologies with impaired TLR are already growing. For better learning and understanding of genetic predisposition to the examiner is to examine the prevalence of functional TLR 2 gene polymorphism among patients with RA.*

The aim of our study was to study the polymorphism 2258G/A gene TLR2 (rs5743708) among patients with AR, analysis of immunological parameters and clinical manifestations in patients with polymorphic variants studied genes. The study analyzed the clinical manifestations, the state of cellular

and humoral immunity and prevalence of polymorphic alleles 2258G/A TLR2 gene among patients with AR (n = 45) to study the mechanisms of pathogenesis and understanding the origin of genetic predisposition to the disease. Examination 45 patients with AR aged 19 to 65 years. The diagnosis of AR establish diagnostic criteria based on ARIA (2008) algorithm for diagnosis adopted in Ukraine and approved by the Ministry of Health of Ukraine.

Sensitization to allergens diagnosed on the basis of complex allergy survey methods : collection allergic history, positive skin tests to allergens skaryfikatsiynyh using standard kits (LLC «Immunologist', Vinnitsa, Ukraine). Bold genomic DNA was performed by phenol- chloroform extraction. Identification of polymorphism 2258G / A gene TLR2 conducted by polymerase chain reaction.

According to the standard procedure was conducted to determine the number of leukocytes in the blood and counting of blood cells in smears. Lymphocyte phenotype was analyzed in venous blood using monoclonal antibodies to CD4, SD25 (production of «sorbent», Russia) and intracellular protein Foxp3 («Bioscience», USA) by flow tsytoflyuorymetriyi by flow tsytoflyuorymetra EPIX LX-MCL (Beckman Coulter, USA) using a program called System II TM software. Levels of total IgE , interleukin -4 (IL- 4) and interleukin-10 (IL-10) were determined using ELISA kits (LLC «Ukrmed-Don», Ukraine) using ELISA analyzer «Stat-Fax 2100» (U.S.). The control group was 95 healthy individuals from a database of genetic samples Institute of Genetic and immunological basis of disease and pharmacogenetics VDNZU «UMSA.» Mathematical treatment of the data was performed using the program «STATISTICA 6.0» (StatSoft Inc).

Studies. *In the studied polymorphisms Asp299Gly TLR4 gene of patients with AR frequency of «wild-type» TLR2 GG genotype was 93.3 %, the frequency of heterozygous genotype GA–6,6 %, mutant genotype AA was not detected. As a result of the studies found significant differences between the groups of patients with AR with the presence of mutant alleles 2258G / A TLR2 gene and homozygous carriers of «wild» alleles in terms of $CD4^+$ ($U_{(n = 42; n = 3)} = 12,00; p = 0,020$). Group of patients with mutant AR alellyu 2258G / A gene for TLR2 differ significantly higher value of lymphocytes ($U_{(n = 42; n = 3)} = 11,50; p = 0,019$) from a group of patients with AR homozygous carriers of "wild" alleles. This study makes it possible to speculate polymorphism 2258G/A gene TLR2 is important in determining the course of the disease, confirming the pathogenetic link between innate and adaptive immunity in AR.*

Keywords: *polymorphism, Toll-like receptors, allergic rhinitis.*

Theoretical and practical J. «Athma and allergy», 2013, 3.

I. P. Kaidashev,
Higher State Educational Establishment «Ukrainian Medical Stomatological Academy», MD, Professor
36024, Ukraine, Poltava, Shevchenko str., 23
tel./fax: 38053 227 0539
e-mail: kaidashev@yandex.ru