English version: THE ROLE OF GENES POLYMORPHISM OF TOLL-LIKE RECEPTORS 2,4 AND CLARA CELL PROTEIN IN THE DEVELOPMENT OF ASTHMA IN ADULTS*

Lyahovskaya N.V., Izmailova O.V., Shlykova O.A, Kaydashev I.P.

Research Institute for Genetic and immunological bases for the development of pathology and pharmacogenetics Supreme State educational institution of Ukraine "Ukrainian Medical Stomatological Academy", Poltava.

Genetic aspects of asthma and atopy have been widely studied. Candidate genes and loci of chromosomes probably responsible for the occurrence of bronchial asthma (BA) is defined by a large amount. The aim of our work was to study polymorphisms 2258G / A gene TLR2 (rs5743708) and 896A / G genaTLR4 (rs4986791), Clara cell protein gene (A38G), with a specific weight of 16 kDa (CC16) in the adult population. Materials and Methods. We examined 45 patients with asthma in the period without exacerbation. Diagnosis of asthma severity and installed in accordance with the approved criteria. Results: Patients with BA significantly more common had genotype GA (11,1%) gene TLR2 (p = 0.04) compared with the control group. In patients who are carriers of a mutant allele of a gene TLR2 A history pneumonia frequently observed (p = 0.046) and there were signs of candidiasis (p = 0.034) compared with patients with no polymorphism. In the study of polymorphism of TLR 4 found that genotype AG statistically more likely (p = 0.04) is found in the BA group (15.6%) than in the control group. Patients with polymorphism 896A / G TLR4 gene disease begins in childhood (p = 0.03) in the spectrum of sensitization were dietary factors (p = 0.02) and there were other manifestations of allergic diseases (p = 0.045). Polymorphic variant gene 38G CC16 significantly more common in patients with BA than in the control population (p = 0.019). Clinical manifestations in patients with BA who are carriers of the gene allele 38G CC16 are fungal sensitization, atopic dermatitis and history of tuberculosis, the need for frequent doses of glucocorticoids.

Key words: asthma, Toll Like receptor, polymorphism, Clara cell protein.

Bronchial asthma (BA) is a global problem [5]. Based on standardized methods for estimating the prevalence of asthma in adults and children, it can be argued that this figure in different countries ranges from 1 to 18% of the population has steadily increased. In particular, in Russia for the last 20 years, the prevalence of asthma increased by almost a factor of 2 and is now 10-15% of the population [18]. Multifactorial nature of the formation of asthma include genetic predisposition, environmental influences, immune and neurogenic level of nonspecific and specific hyperreactivity, the role of viral and microbial factor requires consideration of each additional component, capable of influencing the course of asthma [1]. Genetic aspects of asthma and atopy have been widely studied. Candidate genes and loci of chromosomes probably responsible for the occurrence of BA has defined a large number. Most often in the literature refer about the regions of chromosomes 5q23-31, 6p21.1-p23, 11q13, 12q14-24.33 and 13q11-32. In this regard, in the last decade of great interest associated with a change of the genetic regulation of Toll-like receptors (TLR) [8]. E.Gali et al. [4] found no association between eczema and food allergy mix-mutation TLR2, whereas other studies confirm the correlation between the concentration of IL4, IgE and violations in this gene [11]. Some authors point to a relationship between the level of IgE and genes TLR4 [14], other scholars argue that in some cases do not have the relationship with specific sensitization [7]. We can conclude that the presence of TLR4 gene polymorphisms in children with atopic dermatitis can promote hypersensitivity to viral infections and burden of the disease [15] define the changing nature of the course and severity of clinical manifestations of asthma in children. [13].

Currently, a limited number of studies conducted on the impact of genetic variants on the development of BA CC16, their results have been mixed (Baldini et al., 1998; Gui et al., 2003; Kalyoncu et al., 2003; Laing et al., 1998a; Laing et al., 1998; Sengler et al., 2003; Sharma and Ghosh, 2004). Most scientific research in small population group confirm the association between the polymorphism of CC16 and risk asthma (Candelaria et al., 2005; Kalyoncu et al., 2003; Laing et al., 1998; Mansur et al., 2002; Saadat et al., 2004).

The aim of our work was to study polymorphisms 2258G / A gene TLR2 (rs5743708) and 896A / G genaTLR4 (rs4986791), Clara cell protein gene (A38G), with a specific weight of 16 kDa (SS16) in the adult population of Poltava regions and determine the clinical course BA based on changes in the genome.

Materials and Methods. We examined 45 patients with the BA. Diagnosis of asthma and its severity will establish the approved criteria (international recommendations GINA, 2011) on the basis of Allergy and Pulmonology Poltava Regional Hospital. All patients with BA were held general clinical laboratory and instrumental examination of allergy (skin prick test). The survey was carried out in the absence of the patient's worsening primary or concomitant chronic, non-acute intercurrent infectious diseases and severe comorbidity, which could affect the results of the study. The control group included 90 DNA samples from healthy individuals without allergic history of DNA base CRI genetic and immunological bases for the development of pathology and pharmacogenetics of Ukrainian Medical Dental Academy. Identification of polymorphisms 2258G / A gene TLR2 and 896A / G gene TLR4, gene CC16 conducted by polymerase chain reac-

* *To cite this English version: Lyahovskaya N.V., Izmailova O.V., Shlykova O.A, Kaydashev I.P.. The Role of Genes Polymorphism of Toll-like Receptors 2,4 and Clara Cell Protein in the Development of Asthma in Adults / / Problemy ekologii ta medytsyny. - 2013. - Vol 17, № 5-6. - P. 76 -80.*

tion [12]. The phenotype of the lymphocytes was assessed by determining the expression levels of cell surface antigens using the monoclonal antibodies CD4 +, CD25 ("Sorbent", Russia), and Intracellular protein FoxP3 («eBioscience», USA) on a flow cytoflyuorometri EPIX LX-MCL (Beckman Coulter, USA) using a program System II TM software. Serum total IgE, IL-4, 10 (OOO "Ukrmed Don", Ukraine) in serum was determined by indirect IFA analysis.

Mathematical processing of the data was performed using the program «STATISTICA 6.0» (StatSoft Inc). The distribution of genotypes of polymorphic loci with the test checked for compliance with Hardy-Weinberg equilibrium using the criterion χ2. Comparison of genotype frequencies between the study groups was performed by analysis of contingency tables using Fisher's exact test. To compare the allele frequencies used criterion χ2. To assess the significance of differences between groups using two-tailed Fisher's exact (for small groups). For all types of analysis were considered statistically significant differences at p <0.05.

Results and discussion. Genetic determinism of BA recently regarded as one of the main factors in the etiopathogenesis of this disease [16]. Among surveyed in 3 (6.6%) patients had allergic reactions on the part of both parents, mother - in 16 people (35.5%), father - in 10 people (22.2%). Thus, the transmission of hereditary predisposition to allergic diseases often marked on the mother, which is consistent with literature data [17].

We analyzed the frequency of polymorphic variants of genes TLR 2, TLR4, CC16 gene among patients with BA and population control group (Table 1). Individuals who belonged to the control group, the frequency of TLR2 GG genotype was 97.8%, the frequency of heterozygous genotype GA - 2,2%, AA genotype was detected. Patients with BA results were : GG - 88,9%, GA - 11,11% and AA also was not identified, there was a statistically significant difference (p = 0.04) between the frequencies of genotypes in the control group and patients BA. Frequency of allele A of the control group was 1.1%, and among patients BA - 5.6%, which did not differ significantly (p = 0, 08) (Table 1).

In the study of polymorphism 896A / G TLR4 gene in the control group, the frequency of AA genotype was 95.6%, heterozygous genotype AG - 4,5%, GG genotype was not found. Patients BA respectively: AA - 84,4%, AG - 15,6%, GG - not found. Between the frequencies of genotypes in the population control group and patients with BA was significant difference (p <0.05), which can characterize this pathology is an inherited disorder of the immune response. The frequency of the mutant G allele in patients with BA was statistically higher (p = 0.064) and was 7.8%, compared with the control group (Table 1).

The control group for the study of gene polymorphism CC16 was selected 46 DNA samples from people who are not suffering from an allergic pathology. Within this group the results were as follows: the frequency of the homozygous AA genotype was 86.9% (40 people), GG genotype was not detected, the frequency of the heterozygous genotype AG was 13% (6 people). Patients BA relevant data were as follows: genotype AA - 64.4% (29 people), AG - 28,9% (13 people), GG 6.52% of patients (3 people), that is, between the frequencies of genotypes in the group control and patients BA noted a significant difference (p = 0.019). Frequency of allele A in the control group was 93.4% in patients with AAA - 78.9%. G allele frequency among patients with BA was 7.8% in the control group - 2.2%, which did not differ significantly (p = 0.06) (Table 1).

Table 1.
The Frequency Distribution of Genotypes and Alleles gene's TLR2, TLR4, CC16

Gene, polymorphism	Genotypes	Control group	Patients with asthma (n=45)	p*	Alleles	Control group	Patients with asthma (n=45)	χ^2, df=1	p**
TLR2 2258G/A	GG GA AA	97,8 (88) 2,2 (2) -	88,9 (40) 11,1 (5) -	0,04	G A	98,9 (178) 1,1 (2)	94,4 (85) 5,6 (5)	3,10	0,08
TLR4 896A/G	AA AG GG	95,6 (86) 4,5 (4) -	84,4 (38) 15,6 (7) -	0,04	A G	97,8 (176) 2,2 (4)	92,2 (83) 7,8 (7)	3,42	0,06
CC 16 A38G	AA AG GG	86,9 (40) 13 (6) -	64,4 (29) 28,9 (13) 6,52 (3)	0,019	A G	86 6	71 19	6,99	0,008

**p ≤ 0,05 in comparison with the control group*

In the analysis of intra-frequency distribution of all the above genotypes and alleles observed uneven distribution of alleles, as indicated by the analysis of index excluding rare alleles (a <2) and the proportion of rare alleles (h> 0). For all the studied loci in the control groups and patients ABA genotype distributions were as expected for the equilibrium of Hardy - Weinberg. Also found a match expected heterozygosity and heterozygosity, which is observed, indicating that the equilibrium genetic structure of the population. Heterozygous genotype GA gene TLR 2 (n = 5) was observed only in women (100%). Significantly more often carriers of allele A (p = 0.046) had a history of pneumonia (2 or more times during his life), and there were signs of candidiasis (p = 0.034) compared with patients without the polymorphism (Table 2). Due to changes in the TLR2 gene detection is a disturbance of infectious agents (including fungal), which leads to an imbalance of the innate immune system functioning and development of chronic inflammatory diseases. When comparing the levels of immunological parameters in patients with different BA TLR2 gene variants statistically significant difference was observed only in the concentrations of cytokines. Thus, high levels of IL-4 (63,7±8,7, pg /L) were observed in the group without exhibiting polymorphism (Mann-Whitney U (n1 = 40; n2 = 5) 2.79, p = 0.005) and IL-10 levels were significantly elevated in carriers of heterozygous genomic embodiment TLR2 (Mann-Whitney U (n_1 = 40; n_2 = 5) 33.0, p = 0.01) (Table 3).

Table 2
The Clinical Features of Asthma Depend on Genes Variants TLR2, TLR4, CC16

Attribute		Genotypes GG gene TLR2, (n=40)	Genotypes GA gene TLR2, (n=5)	p*
Polymorphism 2258G/A gene TLR2				
Frequent pneumonia (more than 2 time a life)	yes no	12 28	4 1	**0,046**
Symptoms of candidiasis and \ or fungal skin lesions	yes no	11 29	4 1	**0,036**
Polymorphism 896A/G gene TLR4				
		Genotypes AA gene TLR4, (n=38)	Genotypes AG gene TLR4, (n=7)	p*
Polysensibilisation that including food alergens	yes no	7 31	7 0	**0,013**
Comorbidities (rhinitis, conjunctivitis)	yes no	6 32	5 2	**0,045**
”Atopic march” in the history of deases	yes no	7 31	6 1	**0,029**
Polymorphism A38G gene CC16				
		Genotypes AA gene CC16, (n=29)	Genotypes AG gene CC16, (n=16)	p*
Comorbidities atopic dermatitis	yes no	4 24	6 10	**0,04**
More likely to use inhaled glucocorticoids	yes no	3 26	9 7	**0,02**
Funginal sensibilisation	yes no	3 26	8 8	**0,03**

Gene SNP TLR4, which encodes the extracellular structure ektodomenu receptor is replaced by a glycine amino acid aspartic Asp299Gly 1187 (rs4986790) and the final stage is connected with a suppressed phosphorylation of IkB-alpha following stimulation with LPS, which in turn leads to a decrease in translocation of NFκB nucleus and affects the synthesis of the corresponding inhibition of proinflammatory cytokines. Further disruption of NFκB activation signal is accompanied by an imbalance of Th1/Th2 synthesis and determines the severity of the clinical manifestations of the disease and the presence of comorbidity. Asp299 Gly TLR4 polymorphism with change of Asp to Gly allele was detected in 7 patients. In 6 persons in this group (p = 0.03) manifestations BA began in early childhood, and 4 patients underwent standard steps "atopic march." Significantly more often (p = 0.02) in these patients compared with patients without these genetic changes determined dietary factors sensitization. Clinical sign of the TLR4 SNP was also associated with allergic pathology (p = 0.045) and gastrointestinal diseases (Table 2). Thus, gene polymorphism Asp299 Gly TLR4 mainly affects the common manifestations of atopy patients with BA than signs bronchopulmonary difunktsii and severity of this disease, in contrast to the data obtained by other authors [10] showed a significant change in the level of $CD4^{+}$ / 25^{+} / $Foxp3^{+}$ (Mann- Whitney U (n = 38; n = 7) = 68,0; p = 0,04) and IL10 patients with polymorphism 896A / G gene TLR4 (Mann- Whitney U (n = 38; n = 7) = 60,5; p = 0,02) (Table 3). In reviewing the results of gene polymorphism CC 16 found that the vast majority of individuals had sensitization to two or more allergens feature of these patients had hypersensitivity to fungal allergens (often group Aspergillus), so in the group with heterozygotes gene CC16 these manifestations were 6 people mutant homozygotes in 2 people, which is statistically significant when compared with the AA genotype CC16 gene (Table 3). Our results concide with the scientific data [3] on the importance pathogenetic damaging effects on lung epithelium fungal allergens, but statistically significant indicators, unlike us, they have not received. Among comorbidities noteworthy significant difference on Fisher's exact test (Table 2) by the number of individuals with atopic dermatitis in comparison with groups polymorphism CC16. Thus, among the genotype GG2 persons who suffer from intermittent for atopic dermatitis among genotype AG - 4 people. In fact, the effect of the polymorphism of lung epithelial cells (CC16) on the skin damage is contradictory character, but there are authors [2], which indicate a direct relationship between gene polymorphism CC16 patients with atopic dermatitis in adults.

Table 3
Immunological parameters Dependency Genotypes

Immunological parameters	Genotypes GG gene TLR2, (n=40)	Genotypes GA gene TLR2, (n=5)	p*
Polymorphism 2258G/A gene TLR2			
IL-10, pg/l	0,42±0,02	0,7 ± 0,05	**0,013**
IL-4, pg/l	63,7±8,7	24,1 ±6,28	**0,03**
Polymorphism 896A/G gene TLR4			
	Genotypes AA gene TLR4, (n=38)	Genotypes AG gene TLR4, (n=7)	p*
CD $4^+/25^+/Foxp3^+$, G/l	0,07 ± 0,01	0,03 ± 0,01	**0,04**
IL-10, pg/l	0,45±0,02	0,35 ± 0,03	**0,02**
Polymorphism A38G gene CC16			
	Genotypes AA gene CC16, (n=29)	Genotypes AG gene CC16, (n=16)	p*
IgE, MOd/ml	123,7 ± 12,26	244,9 ± 30,33	**0,0013**

In the analysis of immunological data of patients with different variants of the BA genotypes CC16 found that a statistically significant difference was observed only at the level of total IgE. The carriers of the heterozygous gene CC16 figure was 244,9 ± 30,33 MOD / ml, homozygote GG 191,7 ± 13,0 MOD / ml, homozygote AA - 123,7 ± 12,26 MOD / ml, which is significantly (Kruskal-Wolisa method, p = 0.0013) (Table 3). The data on total IgE levels depending on the state of the genetic apparatus CC16. Perhaps this is due to located on chromosome 11q13, as Clara cell protein gene and FceRI-receptor - b (FcεRI-b) IgE.

In the analysis of drug treatment found that genotype AG or GG CC 16 gene significantly increased the use of glucocorticoids (Table 2). Low sensitivity to these agents can be explained by similar mechanism of action of Clara cell protein and glucocorticoids [9].

Another feature of the carriers of the mutant homozygous genotype (GG) is transferred active tuberculosis in 2 of 3 patients (8 and 18 years prior to our survey). It is known that tuberculosis is an infectious disease whose activity depends on many factors, among them, possibly, changes in gene SS16. However, there is evidence that activation of TLR2 leads to intracellular kiling M. tuberculosis macrophages [6]. To determine the possible combinations of different genotypes of all genes, which are determined by an analysis of haplotypes. For carrying out statistical processing of the data 46 DNA samples selected from the above control group TLR-receptors. Revealed that most often occurring haplotype GGAAAA as in the control group, and patients BA (Table 4). In our study revealed that one person with the GG genotype SS16 gene (haplotype - GAAAGG) and 1 man with the AA genotype (haplotype GAAAAA) SS16 gene variant were heterozygous (GA) TLR2 gene and had a history of manifestations of active TB. In the analysis of haplotypes and gene TLR4 CC16 found that 3 people from the heterozygous genotype SS16 gene (haplotype GGAGAG) and 1 person from homozygotes (haplotype GGAGGG) were heterozygous gene variant Tlr4, all these patients had frequent manifestation of SARS that were protracted and require the use of antimicrobials. In the analysis of immunological parameters of carrier haplotypes revealed that the level of a statistical trend ($r \leq 0,06$) different levels of $CD4^+ / 25^+ / Foxp3^+$ carriers haplotypes with mutant allele. Study of polymorphic gene haplotypes CC16, TLR2 and TLR4 is an important aspect in understanding the clinical manifestations of the BA.

Table 4
Gaplotypes of genes TLR2, TLR4, CC16

CC 16 TLR 4 TLR 2	GG AA AA	GA AA AA	AA AA AA	GG AA AG	GA AA AG	AA AA AG	GG AA GG	GA AA GG	AA AA GG	GG AG AA	GA AG AA	AA AG AA	GG AG AG	GA AG AG	AA AG AG	GG AG GG	GA AG GG	AA AG GG	GG GG AA	GA GG AA	AA GG AA	GG GG AG	GA GG GA	AA GG AG	GG GG AA	GA GG GG	AA GG GG
Control group (n= 46)	63,0 (29)	4,3 (2)	0	23,9 (11)	0	0	0	0	0	4,3 (2)	0	0	4,3 (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Patients with asthma (n=45)	51,1 (23)	6,7(3)	0	20,0 (9)	2,2 (1)	0	2,2 (1)	2,2 (1)	0	6,7 (3)	0	0	6,7 (3)	0	0	2,2 (1)	0	0	0	0	0	0	0	0	0	0	0	0

Conclusions:

Patients with BA significantly more common had genotype GA (11,1%) gene TLR2 (p = 0.04) compared with the control group. In patients who are carriers of a mutant allele of a gene TLR2 A history pneumonia frequently observed (p = 0.046) and there were signs of candidiasis (p = 0.034) compared with patients with no polymorphism.

In the study of polymorphism of TLR 4 found that genotype AG statistically more likely (p = 0.04) is found in the BA group (15.6%) than in the control group. Patients with polymorphism 896A / G TLR4 gene disease begins in childhood (p = 0.03) in the spectrum of sensitization were dietary factors (p = 0.02) and there were other manifestations of allergic diseases (p = 0.045).

Polymorphic variant gene 38G CC16 significantly more common in patients with BA than in the control population (p = 0.019). Clinical manifestations in patients

with AAA who are carriers of the gene allele 38G CC16 are fungal sensitization, atopic dermatitis and history of tuberculosis, the need for frequent doses of glucocorticoids.

Thus, the study of polymorphisms 896A / G TLR4 gene and 2258G / A gene TLR2, A38G CC16 gene is important in the diagnosis and treatment and prevention of the BA.

literature

1. Anne L. Wright Анализ эпидемиологических исследований: факты и артефакты // Аллергология. — 2003. — № 2.—P.26-28
2. Candelaria P.V. Association between asthma-related phenotypes and the CC16 A38G polymorphism in an unselected population of young adult Danes./ P.V. Candelaria, Backer V, et al // Immunogenetics. -- 2005.-- №57 -- P.25-32.
3. Claire de Burbure. Uteroglobin-Related Protein 1 and Clara Cell Protein in Induced Sputum of Patients With Asthma and Rhinitis/ Claire de Burbure, Patrizia Pignatti, Massimo Corradi // Chest.—2007.-- №131.—P.172-179.
4. Galli E., Ciucci A., Cersosimo S. Et al. Eczema and food allergy in an Italian pediatric cohort: no association with TLR-2 and TLR-4 polymorphisms. J. Immunopathol. 2010-№2- P. 671- 675.
5. Guyatt GH, Oxman AD,Kunz R. Going from evidence to recommendation.// BMJ- 2008.- P. 336
6. Long Xiao-Bo. Clara Cell 10-kDa Protein Gene Transfection Inhibits NF-kB Activity in Airway Epithelial Cells./ Xiao-Bo Long// April 2012.-- Volume 7.-- режим доступу: www.plosone.org
7. Michael S. D. Kormann, PhD. Toll-like receptor heterodimer variants protect from childhood asthma. J. Allergy and clinical immunology. 2008; Vol.122: 86-92.
8. Postma D., Koppelman G. Genetics of asthma: where are we and where do we go? The Proceedings of the American Thoracic Society. 2009; V. 6: 283-287.
9. Thoma-Uszynski S. Induction of direct antimicrobial activity through mammalian Toll-like receptor/ S. Thoma-Uszynski // Science.- 2001. – Vol 291. – P. 1544 – 1547.
10. Yang I.A. Toll-like receptor 4 polymorphism and severity of atopy in asthmatics/ IA Yang1, SJ Barton1, S Rorke1 and other// Genes and Immunity.-2004.- V5.-P. 41–45
11. Yoshika M., Fukuishi N., Iriguchi S., et al. Lipoteichoic acid downregulates FcåRI expression on human mast cells through Toll-like receptor 2. J. Allergy. Clin. Immunol. 2007.- V. 120.-P. 452-461.
12. Izmaylova O.V., Shlikova O.A., Bobrova N.O. ta in. Rol' polimorfizmu Toll-podibnogo rezeptora 4 Asp299Gly u rozvitku bakterial'nich infekziy, schope-redayut'sya statevim shlyachom / Problemi ekol. ta med. – 2009. – T.13, № 5-6. – S. 3-6.
13. Kryuchko T.O., Kaydashev I.P., Vovk Yu.O. i dr. Genetichniy polimorfizm Toll-podibnogo rezeptora 4 u ditey z atopichnoyu bronchial'noyu astmoyu / Klin. imunol. Alergol. Infektol. – 2011. – № 5. – S. 52-54.
14. Kuzenko N.L. Assoziaziya polimorfizma Toll-podobnogo rezeptora 4 Asp299Gly s povyshennym uro-vnem produkzii allergenspezificheskich immunoglo-bulinov E u pazientov s allergicheskimi zabolevani-yami./ N.L Kuzenko., O.V Izmaylova., L.E. Vesnina [ta in.] // Immunologiya. –2011.-- №6 -- S. 310-313.
15. Levchenko L. Yu. Polimorfizm 896 A/G genu TLR4, a ne genu 1196 S/T genu TLR4 ta 2258 G/A genu TLR2, viznachaє tyazhkiy perebig atopichnogo dermatitu u ditey. / L. Yu. Levchenko, O.V. Izmaylova, O. A. Shlikova [ta in.].// Zitologiya i genetika. – 2013-- №1.—C. 41-45.
16. Ochotnikova E.N. Allergicheskiy «marsh»: svyaz' pokoleniy i eskalaziya allergii u detey (lekziya) // Sovre-men. pediatriya. - 2008. - №4(21). - S.190-197.
17. Cherkashina I.I., Nikulina S.Yu. Kliniko-geneticheskie osobennosti bol'nych bronchial'noy astmoy i ich rodstvennikov // Problemy tuberkuleza i bolezney leg-kich. - 2008. – №9. – S. 47-50.
18. 18. Chuchalin A.G. Bronchial'naya astma i astma podobnye sostoyaniya // Pul'monologiya. – 2007. – №11. – S. 1-9.

Матеріал надійшов до редакції 3.12.2013.