ORIGINAL RESEARCHES

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RESEARCH OF THE ROLE OF POLYMORPHIC OPTIONS OF INTERLEUKINS GENES IN DEVELOPMENT OF ASTHMA IN THE YAKUT POPULATION

ABSTRACT

The purpose of this study was to investigate the polymorphic loci of interleukins genes *IL4 (C589T, G/C3'UTR), IL4R (Q551R, I50V), IL5 (C703T), IL5RA (G80A), IL9 (T113M)* in patients with asthma and in the control group of individuals. As a result of the analysis, we showed that the markers of an increased risk of developing asthma in the children-Yakuts are the *C* allele and the *CC* genotype *(G/C 3'UTR)* of the *IL4* gene, the *T* allele and the genotype *TT (C589T)* of the *IL4* gene, the allele of the *R (Q551R)* gene *IL4RA*, allele *I (I50V)* of *IL4RA* gene, allele *M (T113M)* of *IL9* gene. Thus, this study for the first time shows the association of polymorphic variants of I*L4, IL4RA, IL9* genes with the development of asthma in the Yakut population.

Keywords: asthma, association analysis, genes, Yakut population.

INTRODUCTION

Comparative epidemiological researches of the last years constantly confirm the high prevalence of the bronchial asthma (BA) around the world which is followed by broad variability [9] that is connected not only with problems in diagnostics, but also with structure of susceptibility to this disease in various populations. In view of the fact that the crucial role in development of inflammation at BA belongs to cytokine system, namely *IL4*, *IL5, IL9*, being initiators of the cascade of the reactions leading the most active researches to emission of mediators and migrations of cells in the center of atopic inflammation [2, 10] are conducted in the field of studying of genes of *IL4, IL5, IL9* interleukins and their receptors of *IL4RA, IL5RA* which are carrying out transmission of signals of these ligands in cells targets [3, 5, 14]. In many works on mapping of genes – candidates of BA close coupling of a disease with a locus located on a chromosome 5q31 – 33 [6, 7, 8, 13] is shown. Despite visible progress on studying of the BA molecular mechanisms, the reproduced results are received only for a half of them. The increasing number of publications indicates value of ethnic specifics in determination of allergic diseases, there are suggestions that these specifics can be the cornerstone of interpopulation variability of incidence BA [4], and it assumes need of further studying of genes of interleukins as BA predictors taking into account ethnic origin.

Identification of biological predictors of BA susceptibility allows forming reasonably risk groups for realization in them of actions for prevention of development of this disease. Use of the molecular predictors associated with clinical and also laboratory - functional indicators of BA allows carrying out therapy taking into account ethnic and genetic features of patients.

In our research we analyzed association of one-nucleotide polymorphisms of genes of *IL4 (C589T, G/C3'UTR), IL4R (Q551R, I50V), IL5 (C703T), IL5RA (G80A), IL9 (T113M)* with bronchial asthma in the Yakut population.

Materials and methods

Work consisted of a one-stage open research in the comparative groups created by the principle "case - control". The research has included 150 patients suffering BA (group 1, 2) and 289 conditionally healthy people (group 3, 4). For a weight influence exception BA on differences in structure of genetic susceptibility ad hock the analysis is carried out, the group 5 corresponding on weight BA to group 2 is created from group 1(Tab. 1).

The group 1 has included patients of pulmonary office of the PC of RHN№1 (Yakutsk) in group 2 patients randomized of the DISPAN database of regional children's hospital (Tomsk), at the age of 4 - 15 years, the blood having the level of the general IgE in serum 100ME/ml with the confirmed diagnosis easy, medium-weight, heavy BA for 12 months until inclusion in a research. The diagnosis was verified on the basis of the following criteria: existence of the anamnesis characteristic of BA, typical clinical symptoms of a disease (short wind, cough, suffocation), data of FVD (the proved reversibility of a bronchial obstruction), an atopiya (the atopic anamnesis, positive skin allergic test (SAT), level of the general IgE>gt; of 100 ME/ml). The severity of a disease was established according to classification, all patients received basic therapy of a disease with use of inhalation glucocorticosteroids according to the document GINA 2010 [11].

Groups 3 and 4 included conditionally healthy children who are living in Yakutsk and Tomsk respectively, not having allergic diseases with the level of the general IgE in blood serum <100 ME/ml.

The protocol of a research is approved by local ethical committee at YSC KMP. Parents (trustees) of all included children have signed the informed consent to par-

Table 1

Comparative characteristics of the groups of research

Major group (sick BA)	Monitoring group (conditionally healthy)	The group further statistical analysis (ad hoc)
Group 1 The Yakuts, n = 103, The average age of 9,9±0,2 years Boys, n = 67 (65%), Girls, n = 36 (35%)	Group 3 The Yakuts, n = 223 The average age of 10.1±2.0 years Boys, n = 135 (60.5%), Girls, n = 88 (39.5%)	Group 5 The Yakuts, n = 47 The median age of 9.8±1.6 years Boys, n = 30 (63,8%) Girls, n = 17 (36, 2%)
Group 2 Russian living in the city of Tomsk and Tomsk region, n = 47 The average age of 9.21±0,4 years Boys, n = 30 (63,8%), Girls, n = 17 (36, 2%)	Group 4 Russian living in the city of Tomsk and Tomsk region, n = 66 The average age of 21.2±2.0 years Men, n = 40 (60.6%), Women, n = 24 (39.4%)	

ticipation in the real research.

Clinical examination was conducted on the basis of pulmonary office of PC of RHN№1 (Yakutsk) and the Children's center of clinical immunology and allergology (Tomsk). To all participants of a research allergological examination with application of a method of scarification skin tests with extracts household, the epidermal, pollen allergens, determination of content of the general IgE in blood serums by method of the solid-phase immunofermental analysis and also assessment of maintenance of eosinophils in a nasal secret by L.A. Matveeva [1] technique, assessment of the function of external breath (FEB) by a standard technique (the analysis a curve stream volume and spirometry indicators) on the device MasterScope ("Erih Jaeger GMBH", Germany), the molecular and genetic analysis of 7 polymorphisms of 5 genes of interleukins (*C589T, G/C3'UTR* of a gene of *IL4, Q551R, I50V* of a gene *IL4RA, C703T* of a gene of *IL5, G80A* of a gene *IL5RA, T113M* of a gene of *IL9*) is conducted.

For genotyping used samples of the genomic DNA emitted from blue blood with method phenol - chloroformic extraction [12]. Genotyping was carried out by the analysis of the polymorphism of lengths of restriction fragments (PLRF) of products of amplification of the polymerase chain reaction (PCR) of specific sites of a genome [5].

Statistical processing was carried out by means of the software package of "Statistica for Windows 13.0". Data are presented in the form by X ± x where X – an arithmetic average, x – an average error. Applied Mann-Whitney's U-criterion to assessment of difference of averages in in pairs not the connected selections, considered a difference of values significant at p<0,05. Distribution of genotypes on the studied polymorphisms was checked for compliance to the Hardy-Weinberg's balance (HWB) by exact test of Fischer. For comparison of frequencies of alleles between various groups we used χ2 criterion with Yates's amendment on continuity. For assessment of probability of development of an event the method of the relation of chances with use of the "Statcalc" software product is used.

Results and discussion

By results of ad-hock testing it is revealed that the BA demonstration at the Yakuts children arose later, than at the Russians (Tab. 2). Frequency of the burdened heredity significantly didn't differ and met in 30–40% of cases. The raised indicators of the general IgE in serum of blood were noted both at Yakuts, and at the Russian patients, but the analysis of his level has shown significant distinctions. So, for Yakuts the average level of IgE in serum of blood was 523 ± 40,36 ME/ml whereas for Russians it was twice lower.

Indicators of OFV_1 were within norm both at Yakuts with BA, and at russians that is connected with application of basic therapy and confirms controlled and partially controlled course of a disease. When comparing level within two populations it is revealed that lower OFV_1 level is characteristic of Yakuts. The comparative analysis of level of eosinophils between Yakuts and Russians has shown that at the Russian patients he was twice higher, than at Yakuts. The analysis of the frequency BA which is combined with atopic dermatitis, allergic rhinitis and an allergic conjunctivitis hasn't shown differences between Yakutы and Russians. Comparable results are gained in the analysis of triggers developments of BA symptoms. So, at Yakuts the sensitization household allergens (93,6%) prevailed, it occurred among the Russian patients less often (p = 0,001). Among triggers for the Russian patients epidermal allergens (53,2%), a sensitization pollen allergens also more often were significant met among Russians (p = 0,04).

The analysis of the studied genes of interleukins at Yakuts (group 1) has allowed to establish BA association and its clinical-functional manifestations with all polymorphic options of genes. The probability of BA formation is 3 times higher at carriers of a genotype of a TT of polymorphism *C589T* of a gene of *IL4* (OR = 2,81; CI:95 of % 1,69 - 4,68), is twice higher at carriers of alleles of *T* of polymorphism *C589T* of a gene of *IL4* (OR = 1,97; CI:95 of % 1,35 - 2,87) and *M* of polymorphism of *T113M* of a gene of *IL9* (OR = 1,92; CI:95 of % 1,23 - 2,98) and also genotype of the *CC* polymorphism of *G/C 3'UTR* of a gene of *IL4* (OR = 2,28; CI:95 of % 1,38 - 3,79), is one and a half times higher at carriers of alleles. From polymorphism of *G/C 3'UTR* of a gene of *IL4* (OR = 1,62; CI:95 of % 1,1 - 2,39), *R* polymorphism of *Q551R* of a gene of a receptor of *IL4RA* (OR=1,66; CI:95 of % 1,15 - 2,4) and *I* polymorphism of *I50V* of a gene of a receptor of *IL4RA* (OR = 1,61; CI:95 of % 1,14 - 2,28). Genotypes of the *CC* polymorphism of *G/C 3'UTR* (p = 0,001) a gene of *IL4, GG* (p = 0,012) polymorphism of *G80A* of a gene of *IL5RA* and *MM* (p = 0,019) polymorphism of *T113M* of a gene of *IL9* are BA associated with heavy. Higher level of the general IgE was noted at patients with genotypes of *CC* (p = 0,001) polymorphism of *G/C 3'UTR* of a gene of *IL4, II* (p = 0,015) polymorphism of *I50V* of a gene of a receptor of *IL4RA* and *MM* (p = 0,013) polymorphism of *T113M* of a gene of *IL9*. Lower indicators of OFV1 are registered at carriers of genotypes of a *TT* (p = 0,002) polymorphism of *C703T* of a gene of *IL5*, QR (p = 0,03) polymorphism of *Q551R* of a gene of a receptor of *IL4RA* and *MM* (p = 0,04) polymorphism of *T113M* of a gene of *IL9*. The analysis of distribution of alleles and genotypes of the studied genes of interleukins in groups 5 and 2 has revealed significant distinctions for polymorphic *C589T* and *G/C* options *3'UTR* of a gene of *IL4, Q551R* of a gene of *IL4RA* and *G80A* of a gene *IL5RA*. So in group of Yakuts with carriers allele *T* of polymorphism of *C589T* (p =0,01), allele from polymorphism of *G/C 3'UTR* of a gene of *IL4* (p =0,03), and allele and polymorphism of a gene of *IL5RA* BA prevailed (p =0,01). An allele *Q* polymorphism of *Q551R* of a gene of *IL4RA* prevailed both at Yakuts, and at Russians with BA, but with a different frequency (p =0,04).

Conclusion

It is established what BA at children of Yakuts is characterized by heavier current, a late demonstration, lower indicators of quantity of eosinophils of a nasal secret and OFV1, higher level of the general IgE in blood serum that is caused by clinical-functional effect of the alleles prevailing in this population. So the genotype of the *CC (G/C 3'UTR)* gene of *IL4*, a genotype of the *II (I50V)* gene of *IL4RA*

Table 2

Comparative clinico-laboratory and functional characteristics of groups additional statistical (ad hock) analysis

Test Parameters	Group 5, n = 47	Group 2, n = 47	p
Age (years)	9,87 ± 0,23	9,21 ± 0,39	0,161
Age of manifestation (years)	6,16 ± 0,1	3,93 ± 0,3	0,001
** Weighed down heredity, n (%)	29,8%	38,3%	0,386
IgE, (IU / ml)	523 ± 40,36	243 ± 35,19	0,001
FEV1 (% of due)	99,5 ± 1,86	125 ± 6,95	0,002
Eosinophils of nasal secretion, (%)	1,14 ± 0,06	2,8 ± 0,37	0,001

Note. To assess the differences in pairwise unrelated samples, the Mann-Whitney U test was used, * - to evaluate the difference, a two-sided exact Fisher test was used.

and a genotype of *MM (T113M)* of a gene of *IL9* is associated with higher level of the general IgE in serum of blood. The genotype of a *TT (C703T)* of a gene of *IL5,* a genotype of *MM (T113M)* of a gene of *IL9* and a genotype of *QR (Q551R)* of a gene *IL4RA* is associated with lower indicator of OFV1. Genetic markers of the increased probability of development heavy BA at Yakuts is the genotype of the *CC (G/C 3'UTR)* gene of *IL4*, a genotype of *GG (G80A)* of a gene *IL5RA* and a genotype of *MM (T113M)* of a gene of *IL9*.

Thus, we have defined for the first time in children of the Yakut ethnicity genetic structure of BA susceptibility, but markers of the increased probability of asthma development in the Yakuts are the genotype of *CC* and an allele of *C (G/C 3'UTR)* of a gene of *IL4*, a genotype of a *TT* and an allele of *T(C589T)* of a gene of *IL4*, an allele of *R (Q551R)* of a gene of *IL4RA*, an allele of the *I (I50V)* gene of *IL4RA*, an allele of the *M (T113M)* gene of *IL9.*

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