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Distribution of Allel Variants and Genotypes of Il4, Il10, Il12b, Tlr2 Genes in the Group of Patients with CPRS

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ABSTRACT

Due to the prevalence of polyposis rhinosinusitis (PRS) and its tendency to recurrence, the search for new pathogenetic mechanisms is still relevant. Despite the many classifications and attempts at endotyping this disease, the question remains open. The modern direction of personalized medicine is the study of immunological mechanisms and the search for genetic predictors. One of these targets is interleukin (IL) 10, which is considered a new key factor in the development of allergic diseases, including bronchial asthma, allergic rhinitis and ORS. Interest was aroused not only by this cytokine and its ST2 receptor, but also by single nucleotide polymorphisms of the IL-10 gene, which may determine the severity of the course of the disease.

KEYWORDS

Chronic Polyposis Rhinosinusitis, IL, Nasal Cavity, Eosinophil, Gene Polymorphism, Polyp.

Introduction

Chronic polyposis rhinosinusitis is a disease characterized by chronic inflammation of the nasal mucosa and sinuses. In the tissues of polyps and intranasal secretions, an increase in the concentration of various inflammatory mediators, in particular interleukins, is observed due to an increase in their de novo synthesis by effector cells [1,2,3].

Particular importance is attached to an increase in the concentration of cytokines involved in the development, recruitment and activation of eosinophils (IL-4, IL-12, IL-13, GM-CSF), the main pro-inflammatory (IL-1, IL-2, TNF-a, IL- 10), regulatory cytokines (IL-10, TLR2b), contributing to the chronicity of the inflammatory process in the nasal cavity [4].

A hereditary predisposition to the development of CPRS suggests the role of genetic factors in its determination, but is not direct evidence of it. However, numerous studies indicate the association of interleukin gene polymorphism with the development, severity and chronicity of ENT diseases [5].

The distribution of the allele frequencies of the resistance susceptibility genes that form the risk of development or resistance to multifactorial pathology is determined by the racial and ethnic origin of the studied group [6,7]. The cytokine system is a universal regulatory network of mediators designed to control proliferation, differentiation and functional activity of cellular elements in all homeostatic systems of the body, of which the immune system plays the most important role in the onset and development of diseases.

Cytokines - leukocyte inflammatory mediators are classified as pro- or anti-inflammatory regulatory peptides. IL-1 and TNF-a play the greatest role in pro-inflammatory processes. The mechanisms by which these cytokines are involved in the inflammatory response have much in common. They are able to enhance the expression of the COX-2 gene with a subsequent increase in the production of leukotrienes and prostaglandins and their involvement in the pathological process, thereby increasing the migration of leukocytes and inflammatory infiltration, and activating the endothelium.

IL-6 and IL-8 also have pro-inflammatory properties. Anti-inflammatory cytokines are active antagonists of IL-1 and

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TNF-a, the most active of which are IL-4 and IL-10. Their anti-inflammatory activity is manifested in the suppression of the production of IL-1 and TNF-a, colony-stimulating factors, and a decrease in the cytotoxicity of macrophages.

Earlier, a number of studies have demonstrated a different ability to bind to transcription factors of alternative allelic variants in position -308 (G-> A), which leads to an increase in the transcriptional activity of TNFA-308A compared to the TNFA-308G promoter. Substitution of guanine for adenine in this position increases gene expression and the level of TNF-a production in unstimulated monocyte and T-lymphocyte cell lines [8].

Thus, the presence of the TNFA AA genotype may be one of the immunogenetic factors of predisposition to the development of a chronic pathological process with a pronounced and long-term current inflammatory component. We studied the promoter polymorphism of the IL10 gene, the protein product of which is the most important cytokine, which negatively regulates the inflammation process by suppressing the production of major proinflammatory cytokines, such as IL-1, IL-2, IL-6, TNF-a and IFN-y [9].

The study of the ability of polyp tissue to produce this interleukin revealed an increase in the number of IL-10-producing cells [10], moreover, an increase in the content of IL-10 in the nasal secretion of CPRS patients was found [11]. An increase in the serum level of IL-6 and an increase in its production in cultures of epithelial cells of nasal polyps in patients with chronic rhinosinusitis [12] indicates its direct role in maintaining the inflammatory process in NIN. On the other hand, IL-6 is an atypical pro-inflammatory cytokine and is able to inhibit the production of TNF-a, as well as inhibit the proliferation of plasma cells that produce IgE during allergic inflammation [13,14].

The IL5 gene is localized in the 5q31-q33 region, in a cluster with genes for interleukins IL3, IL13, and GM-CSF, whose protein products also play an important role in the proliferation, activation, and recruitment of eosinophils [15]. For the first time, single nuclear polymorphism of the IL5 gene at position -703 was detected during screening of this region for the presence of functional polymorphisms that cause its linkage with autosomal dominant familial eosinophilia [].

Previously, associations of this polymorphism with atopic AD [16], serum IgE levels and blood eosinophils [17,18] were established. The functions of IL-4, the growth factor of B-lymphocytes, which enhances the production of IgE and IgGl, supports the proliferation of mast cells, and also increases the expression of adhesion molecules (VCAM-1) on the vascular endothelium, which increases the content of eosinophils in tissues, suggest its direct participation in pathogenesis of CPRS [19,20]. The IL4 gene is mapped to chromosomal region 5q23-31, for which associations with atopic signs and diseases, including diseases of ENT organs, have been repeatedly.

Material and Methods

In accordance with the purpose of the study and to fulfill the assigned tasks, clinical studies were carried out in 140 patients with CPRS and with chronic rhinosinusitis, who were examined and treated in the ENT department of the multidisciplinary clinic of the Tashkent Medical Academy in 2017-2019. The control group consisted of 20 healthy volunteers aged 19 to 60 years from among the employees of the multidisciplinary clinic of the Tashkent Medical Academy.

All volunteers included in the study, during the last month before the start of the study, did not tolerate acute diseases, primarily of an infectious nature, and did not have a chronic pathology of inflammatory genesis.

For real-time PCR, a commercial kit with SYBR Green I dye (Litekh, Russia) was used. The polymorphism of five positions of cytokine genes: IL4, IL10, IL12B, TLR2 was studied. Genotyping of the samples was carried out using allele-specific polymerase chain reaction (PCR) in real time on a DT-96 device (DNA-Technology) using the SYBR Green I intercalating dye. The reaction mixture corresponded to the manufacturer's recommendations. The reaction began with the activation phase of Taq polymerase (93 ° C, 1 min). The next 35 PCR cycles consisted of denaturation (93 ° C, 10 sec.), Annealing (64 ° C, 15 sec.), And elongation (72 ° C, 20 sec.) Phases. The signal was read at the elongation stage.

Results and Discussion

In order to study the genetic determination of CPRS disease on the part of interleukin genes, we investigated the distribution of allelic variants and genotypes of IL4, IL10, IL12B, TLR2 genes in the group of patients suffering

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from this disease. To assess the relationship of the allelic variant or genotype with the disease, the% 2 criterion was used or, if necessary, when the expected number of observations in at least one of the table cells was less than five, Fisher's exact test.

When analyzing the relationship between the dimorphic positions of the promoter regions of IL genes with a complex pathological state, it is difficult to expect a significant association; the achievement of a statistical significance level of p <0.10 indicates the establishment of a trend towards a positive or negative association of IL genotypes with the development of CPRS. In the group of patients with CPRS, a threefold increase in the frequency of carriers of the IL12B genotype was found in comparison with healthy individuals (OR = 3.09, p = 0.1816). Since the value of the odds ratio criterion is greater than one (OR 3.09), we can talk about a tendency towards the association of the AA genotype with CPRS. A similar trend characterizes allele A as a whole (OR 1.25, p = 0.500) (Table 1). Thus, the presence of the IL12B genotype may be one of the immunogenetic factors of a predisposition to the development of a chronic pathological process with a pronounced and long-term inflammatory component.

Table1. Distribution of allelic variants and genotypes of the IL12B gene among patients CPRS

Polymorphism genes interleukins	**Allele / Genotype**	**Allele frequency (h) and genotypes (%)**		**OR**	**P**
		Patients with CPRS	**Healthy donors**		
IL12B -A1188C (A—>C) Nl=71 N2=80	G	0.8648	0.8889	0.8	0.500
	A	0.1354	0.1111	1.25	
	GG	77.08	79.17	0.89	0.749
	AG	18.75	19.44	0.96	0.912
	AA	4.17	1.39	3.09	0.182

Note: OR is the criterion of the odds ratio, P is the level of statistical significance, N1 is the number of examined practically healthy persons, N is the number of examined patients with CPRS.

The study of the frequency distribution of alleles and genotypes of IL4 -589 (C-> T) revealed an increase in the frequency of the "minor" allele - 589T (OR 1.51, p = 0.122) and homozygous TT variant (OR 1.49, p = 0.582), which determine an increase in serum IgE, in patients with CPRS compared with the control group. Moreover, there was a tendency towards a decrease in the frequency of TT homozygotes among CPRS patients compared with the control group (OR 0.58, p = 0.100), which indicates a possible protective role of the “wild” IL4 CC genotype in the development of CPRS (Table 2).

Table2. Distribution of allelic variants and genotypes of the IL4 gene among patients CPRS

Polymorphism genes interleukins	**Allele / Genotype**	**Allele frequency (h) and genotypes (%)**		**Polymorphism genes interleukins**	**P**
		Patients with CPRS	**Healthy donors**		
IL4 -589 (C->T) N1=71 N2=80	C	0.7083	0.7857	0.66	0.122
	T	0.2917	0.2143	1.51	
	CC	47.92	61.42	0.58	0.100
	CT	45.83	34.29	1.69	0.125
	TT	6.25	4.29	1.49	0.582

Note: OR is the odds ratio criterion, P is the level of statistical significance, N is the number of examined practically healthy persons, N is the number of examined patients with CPRS.

Analysis of the frequency distribution of IL10 genotypes among patients with CPRS showed a more than twofold increase in the frequency of the IL10 TT genotype (14.58%) compared with the group of healthy individuals (6.57%). The odds ratio criterion OR in carriers of the homozygous IL10 TT variant was 2.59, which indicates its association with the development of this disease (p = 0.089) (Table 3). The established association of the IL10 -592 (C-> A) polymorphism with the development of CPRS is consistent with the important role of IL-10 in the pathogenesis of this disease. IL-10 is a key mediator in the pathogenesis of diseases characterized by eosinophilic inflammation, inducing the processes of eosinophil homing, their migration into tissues and degranulation, prolonging their survival in target tissues, blocking eosinophil apoptosis.

Table3. Distribution of allelic variants and genotypes of the IL10 gene among patients CPRS

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Polymorphism genes interleukins	Allele / Genotype	Allele frequency (h) and genotypes (%)		OR	P
		Patients with CPRS	Healthy donors		
IL10 -592 (C->A) N1=71 N2=80	C	0.6979	0.7445	0.79	0.374
	A	0.3021	0.2555	1.26	
	CC	54.17	55.47	0.95	0.875
	CA	31.25	37.6	0.74	0.405
	AA	14.58	6.7	2.43	0.089

Note: OR is the criterion of the odds ratio, P is the level of statistical significance, N is the number of examined practically healthy persons, N is the number of examined patients with CPRS.

The functional polymorphism of the TLR2 gene in the position - Arg753Gln (Arg->Gln) was studied. It is known that the wild type allele is associated with both increased expression of the TLR gene and a high serum content of this cytokine. Comparative analysis of the distribution of TLR2 genotypes among CPRS patients and healthy donors showed that in general in the group of patients it corresponded to the control and was characterized by a high frequency of the heterozygous CG variant (42.42%), and a twofold predominance of the frequency of Arg \ Arg homozygotes (39.39%) over Arg \ Gln homozygotes (18.18%). In patients with CPRS, there is a tendency towards a decrease in the frequency of occurrence of the homozygous Gln \ Gln genotype (OR 0.83, p = 0.7205), and an increase in the frequency of the Gln \ Gln genotype (OR 1.37, p = 0.4573) associated with an increased the level of secretion of TLR2 (table 4).

Table 4. Distribution of allelic variants and genotypes of the TLR2 gene among patients CPRS

Polymorphism genes interleukins	**Allele / Genotype**	**Allele frequency (h) and genotypes (%)**		**Polymorphism genes interleukins**	**P**
		Patients with CPRS	**Healthy donors**		
Arg753Gln (Arg—>Gln) N1=71 N2=80	Arg	0.61	0.56	1.23	0.4784
	Gln	0.39	0.44	0.81	
	Arg\Arg	39.39	32.22	1,37	0,4573
	Arg\Gln	42.42	46.66	0,84	0,6767
	Gln\Gln	18.18	21.11	0,83	0,7205

Note: OR is the criterion of the odds ratio, P is the level of statistical significance, N1 is the number of examined practically healthy persons, N2 is the number of examined patients with CPRS.

Conclusion

Thus, as a result of the study of interleukin gene polymorphism with the development of CPRS, the most pronounced association was established for functional polymorphisms IL4 - 589 (C -> C) and IL12B - A1188C (A -> A), in particular, the factor of predisposition to the development of CPRS is " minor "genotype IL10 CC, while the TLR2 Arg \ Arg genotype plays a protective role in the development of this disease. The results obtained are consistent with the leading role of these anti-inflammatory ILs in the pathogenesis of CPRS.

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