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Research Article

Association of -590 C/T Il-4 Gene Promoter Polymorphism with Atopy in Polish Patients with Allergic Rhinitis

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Abstract

Interleukin 4 (IL-4) mediates allergic response. Polymorphisms of genetic loci on chromosome 5q can influence the immune response mediated by cytokines and promote atopy development. The aim of this study was to investigate the association of -590 C/T IL-4 gene promoter polymorphism and allergic rhinitis in a Polish population.

Materials and methods: 98 patients with allergic rhinitis and 87 controls were included in the study. Polymerase chain reaction-based analysis for -590 C/T IL-4 gene polymorphism was used for genotyping and detection. Atopic status was determined by positive skin prick tests (spts) and total and specific IgE levels. Statistica 9.0 software (Statsoft, Tulusa, USA) was used to perform analysis.

Results: Total IgE levels in patients with present IL-4 polymorphic variant were statistically higher than in patients without IL-4 polymorphism. There was no significant association between the IL-4 gene polymorphism and higher house dust mite and mixed grass pollen IgE levels. The IL-4 polymorphism was strongly associated with allergic rhinitis frequency in the investigated group of patients, chi^2=4,368; p<0.05.

Conclusion: The -590 C/T IL-4 gene promoter polymorphism may play an important role in the allergic rhinitis development in the investigated group and influence the clinical symptoms in allergic rhinitis patients.

Keywords: Allergic rhinitis, Atopy, IL-4 gene promoter polymorphism

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Introduction

Allergic Rhinitis (AR) is an inflammatory disorder characterized by nasal symptoms. The prevalence of allergic rhinitis is 24% in well developed populations [1]. IgE antibodies play an important role in allergic reaction. They bind to the high affinity IgE receptor on mast cells and basophils and initiate the cascade of inflammatory mediators including histamine, leukotrienes, and cytokines, like Interleukin 4 (IL-4). These mediators can induce acute and chronic symptoms of AR [2]. AR is known as a risk factor of asthma, rhinosinusitis and nasal polyposis, and sleep disorders [3,4]. Pathogenesis of AR is based on imbalance between T-helper (Th)1/Th2 immune response results in allergen-specific immunoglobulin E production. Th2 cells release interleukin 4, involved in IgE-mediated allergic inflammation [5,6]. IL-4 plays a crucial role in the development of allergic reaction by promoting immunoglobulin isotype class switching from IgM to IgE and modulating the differentiation of T cells to Th2 cells [7]. IL-4 may increase the adhesion of inflammatory cell to the endothelial cells by overexpression of Vascular Cell Adhesion Molecule 1 (VCAM-1) on endothelial cells [8]. Genetic factors may influence the development and severity of AR [9]. The polymorphism -590 C/T in the promoter region of IL-4 is associated with atopy. The aim of this study was to investigate the association of this polymorphism and allergic rhinitis in a Polish population.

Materials and Methods

The study included 98 patients with Allergic Rhinitis (AR) and 87 healthy controls. All subjects were recruited at the Medical University Hospital outpatient, Lodz, Poland. Recruited subjects have given their informed consents and that the study protocol has been approved by the Medical University of Lodz Ethics Committee on Human Research. The AR patients were sensitized to house dust mites and/or mixed grass pollen. All controls had no clinical features or family history of atopic diseases. Atopic status was confirmed by at least one positive skin prick test to house dust mite (hdm, including Dermatophagoides pteronyssinus (*Der p*) and Dermatophagoides farinae (*Der f*))/ mixed grass pollen (mgp) - *Artemisia, Timothy grass, Rye grass* allergens. Any helmints infection was excluded. The serum total IgE and specific IgE levels were measured with Pharmacia Cap System (Uppsala, Sweden). Total IgE and specific IgE concentrations were estimated: positive RAST (greater than 2) test to at least one allergen and elevated total IgE over 200 IU/ml were associated with a clinical manifestation of allergic rhinitis. Genomic DNA was isolated from leucocytes of venous blood with a commercial kit (Iso Quick, Microprobe Corporation, Garden Grove, USA). PCR reaction for the -590 C/T IL-4 gene promoter polymorphism was performed in a total volume of 15µl. PCR mixture consisted of 20µM of 3' and 5' primers: ACTAggCCTCACCTgATACg and gTTgTAATgCAgTCCTCCTg, 100ng of DNA template, 25 nM of each of dNTPs, PCR buffer, 2.5 U Taq polymerase and 100µl of mineral oil. Amplification performed in a thermal cycler was: preliminary cycle: 94°C for 5 min and next 32 cycles: 94°C for 30 sec, 60°C for 30 sec, 72°C for 5 min. Amplification products (5µl each) were digested with restriction

enzyme: 2U of *BsmF1* for the -590 C/T IL-4 gene promoter polymorphism. Restriction was performed for 2 hrs at 37°C and stopped by the agarose gel loading buffer (5µl). The digested products and DNA molecular weight marker were loaded onto agarose gel (2%) agarose/1xTBE gel), containing of 50ng/ml ethidium bromide. Electrophoresis was performed at 70V for 90 min. DNA fragments were visualised in UV light and photographed.

Statistical Analysis

The allelic frequencies were estimated by gene genotyping. The chi^2 test was used to compare the observed numbers of genotypes with expected ones for a population in the Hardy-Weinberg equlibrium and to test the significance of the differences of observed alleles and genotypes between groups. When calculating the probability, if the expected cell values were less than 5, Yates test was used. P-value of <0.05 was taken as statistically significant. Mann-Whitney test for non-normal distribution was used to compare each parameter between 2 groups. Statistica 9.0 software (Statsoft, Tulusa, USA) was ued to perform analysis.

Results

Total IgE levels were significantly greater in patients group to compare with controls, chi^2=8.460; p<0.001. In controls IgE ranges were not significant in both groups, chi^2=0.935; p>0.05. Total IgE levels in patients with present IL-4 polymorphic variant were statistically higher than in patients without IL-4 polymorphism, chi^2=2.896; p<0.01. The polymorphic variant of IL-4 gene was present in 15.3% of all patients. There was no significant association between the IL-4 gene poymorphism and higher house dust mite IgE levels, chi^2=0.406; p>0.05; however polymorphic variant was more frequent in patients with IgE level higher than 2. There was no significant association between the IL-4 gene poymorphism and higher mixed grass pollen IgE levels, chi^2=6.859; p>0.05; however polymorphic variant was more frequent in patients with IgE level higher than 2 (Table 1).

IL-4 gene polymorphism	IgE mgp classes										Total
	0		1		2		3		4		
	n	F	n	F	n	F	n	F	n	F	
Yes	4	0.07	1	0.11	6	0.38	3	0.21	1	0.33	15
No	52	0.93	8	0.89	10	0.62	11	0.79	2	0.67	83
Total	56	1.00	9	1.00	16	1.00	14	1.00	3	1.00	98

Table 1: Mixed grass pollen IgE level vs IL-4 gene polymorphism.

There was no significant association of IL-4 gene polymorphism and positive house dust mite skin prick tests, chi^2=0.221; p>0.05, but the frequency of positive house dust mite skin prick test was strongly correlated with polymorphic variant occurence (F=0.17) (Table 2). The frequency of positive mixed grass pollen skin prick test was correlated with polymorphic variant occurence (F=0.19) (Table 3).

IL-4 gene polymorphism	House dust mite skin prick test				Total
	Positive		Negative		
	n	F	n	F	
Yes	10	0.17	5	0.13	15
No	50	0.83	33	0.87	83
Total	60	1.00	38	1.00	98

Table 2: House dust mite positive/negative skin prick test vs IL-4 gene poylmorphism.

IL-4 gene polymorphism	Mixed grass pollen skin prick test				Total
	Positive		Negative		
	n	F	n	F	
Yes	14	0.19	1	0.04	15
No	60	0.81	23	0.96	83
Total	74	1.00	24	1.00	98

Table 3: Mixed grass pollen positive/negative skin prick test vs IL-4 gene poylmorphism.

The IL-4 polymorphism was strongly associated with allergic rhinitis frequency in the investigated group of patients, chi^2=4.368; p<0.05 (Table 4).

Polymorphism IL-4	Group			
	Allergic rhinitis		Controls	
	n	%	n	%
Yes	15	15.3	5	5.8
No	83	84.7	82	94.2
Total	98	100.0	87	100.0

Table 4: IL-4 gene polymorphism vs allergic rhinitis vs control group.

Discussion

Many authors underlay the close relation and influence between genetic factors, environment and lifestyle [10]. Davila et al., showed that AR patients had specific genes for cytokines important in the pathogenesis of AR [11]. IL-4, a cytokine playing a crucial role in allergic inflammation, regulates Th1/Th2 balance [12]. In 1995 Rosenwasser et al., reported a functional polymorphism -590 C/T in the promoter region of IL-4 They indicated influence of this genetic loci on overproduction of total IgE and overexpression of transcriptional factors in lymphocytes T resulting in a higher IL-4 level [13]. Other studies demonstrated an association of the polymorphism -590 C/T in the promoter region of IL-4 with asthma and atopy in different populations and ethnic groups [14]. Lu MP et al., investigated the association of the polymorphisms in IL4, IL13, and IL4RA with AR and the influence of house dust mites on its development in a Chinese population [15]. Their results suggest that the -590 C/T polymorphism in IL4 gene may contribute to the susceptibility to mite-sensitized AR. In a very recent study Movahedi et al., investigated effect of single nucleotide polymorphisms on expression of interleukin IL-4 and its receptor [16]. Their work was based on 4 genetic variants within genes of IL-4 and IL-4R (IL-4R variant (rs1801275) and three SNPs of IL-4 (rs2243248, rs2243250, and rs2070874). The patients group in this study was not large (98) and although the authors did not describe sufficiently atopic status, they found that SNPs in IL-4 was associated with AR. Our study showed the correlation of the polymorphism -590 C/T in the promoter region of IL-4 and allergic rhinitis in the investigated group. This genetic loci plays an important role in IgE-mediated allergic reactions and is one of the genes linked with atopy and may also regulate the development of allergic rhinitis. The results support other authors findings and indicate that genetic factors may play an important role in the allergic rhinitis development in the investigated group. The ranges of total IgE confirming atopic status differ in similiar papers (100IU-400IU or higher). In this paper we decided to choose a positive RAST as greater than 2 to at least one allergen and elevated total IgE, over 200 IU, to underlay the IgE overproduction. De Guia and Ramos found a significant relationship between the -590C/T IL-4 nucleotide plymorphism and very high

IgE levels (> 1000 IU/mL) and considered this loci as a potential risk factor correlated with atopy [17]. Micheal et al., in a recent study established a highly significant association between -590 C/T IL-4 and atopic asthma and allergic rhinitis [18]. Their findings were confirmed by Woitsch and Chiang [19,20]. Our study had some limitations. We had not very large study groups (98 cases and 87 controls). This can influence the final statistical data. However our and other authors findings indicate the need of large population-based prospective studies to clarify IL-4 polymorphisms -590C/T in the promoter region and AR susceptibility.

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