

Original article

Two CD14 promoter polymorphisms and atopic phenotypes in Czech patients with IgE-mediated allergy

Background: Immunoglobulin E (IgE)-mediated allergy belongs to common chronic disorders resulting from an interaction between both genetic and environmental factors. The gene encoding CD14 is a positional candidate gene for allergic diseases as it is localized on chromosome 5q31.1, a region that is linked to asthma and bronchial hyperresponsiveness. Recently, several polymorphisms in the promoter region of this gene have been associated with atopic phenotypes in various populations.

Methods: We investigated relationship among atopic phenotypes and two polymorphisms [C(-159)T and G(-1359)T] in the promoter of the CD14 gene in the Czech population. Polymerase chain reaction with restriction fragment length polymorphism analyses was used to determine the CD14 genotypes in subjects with IgE-mediated allergic diseases ($n = 562$) and random controls ($n = 320$).

Results: The CD14 allele or genotype distributions were similar in patients and control group. However, the frequency of the C allele of the C(-159)T polymorphism was higher in patients with positive skin prick tests for moulds than in patients without reactivity to this antigen ($P < 0.002$, $P_{\text{corr}} < 0.01$). In addition, we found that patients with homozygous genotype (GG) of the G(-1359)T polymorphism had marginally lower percentage of positive skin prick tests compared with the other genotypes ($P < 0.029$, $P_{\text{corr}} > 0.05$).

Conclusions: Our study supports the idea that CD14 gene variants may act as disease modifiers of IgE-mediated allergic diseases.

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The prevalence of allergic diseases has increased significantly over the last few decades (1). There is evidence that the interindividual variability in immunoglobulin E (IgE)-mediated allergy depends on both environmental and genetic factors. The gene encoding CD14 is just one of many genes which appear to contribute to the expression of the allergic phenotype, as it is localized on chromosome 5q31.1, a region that is linked to both asthma and total serum IgE concentration (2). Cluster differentiation antigen (CD14), a 55-kD glycoprotein, is a pattern recognition factor that plays a central role in innate immunity through recognition of bacterial lipoglycans (LPS). According to Vercelli et al. (3), exposure to bacterial products, such as LPS, may modulate IgE regulation, possibly by activating innate immune pathways that promote Th1 differentiation and/or suppress Th2-dependent IgE responses.

Although CD14 exist as a single-copy gene, CD14 protein is found in two distinct forms: as a 55-kDa membrane molecule (mCD14), expressed primarily on the surface of monocytes/macrophages, dendritic cells and

neutrophils (4), and as a soluble form (sCD14) in serum. Regulation of CD14 gene expression appears to be important in many diseases and elevated serum levels of sCD14 were found in several states including atopic dermatitis (5). A genetic variant CD14 C(-159)T in the promoter region of the CD14 gene has been found to be associated with higher levels of sCD14 expression (6), and inversely associated with levels of total serum IgE (6–8). Koppelman et al. (8) reported an association of CC homozygotes of this polymorphism with higher number of positive skin prick tests compared with CT and TT genotypes. Recently, five single nucleotide polymorphisms (at positions -1619, -1359, -1145, -809, and -159) were characterized in the promoter of the gene encoding CD14. Vercelli et al. (9) showed that carriers of the -1359T/-1145A/-159C haplotype had the highest levels of IgE, and the lowest levels of sCD14, and conversely carriers of the -1359G/-1145G/-159T haplotype had the highest levels of sCD14 and the lowest IgE values.

The aim of this case-control study was to investigate an association of the two polymorphisms in CD14 gene

(at positions -1359, and -159) with phenotypes of IgE-mediated allergic diseases in the Czech, highly homogeneous population.

Material and methods

Characteristics of subjects

Caucasian subjects of exclusively Czech nationality ($n = 882$) were included in this study. All subjects were selected using a detailed questionnaire modified according to the American Thoracic Society respiratory questionnaire (10) regarding lifetime symptoms suggestive of asthma and rhinitis extended with additional questions on symptoms and therapy as well as on other diseases. Phenotype status was assigned without previous knowledge of genotypes by two investigators independently.

A total of 562 patients with clinically manifested IgE-mediated asthma and/or rhinitis, 280 men and 282 women, aged 27.9 ± 13.1 years (mean $\pm$ SD) were studied. A total of 320 reference subjects (162 men and 158 women), aged 36.2 ± 15.9 years, who were free of lung, skin and cardiovascular disease and had no clinical evidence of personal or familial allergic diseases, asthma, hay fever or eczema, were recruited as controls.

Asthma was defined by using the questionnaire together with a physician's diagnosis of asthma, according to the GINA criteria (11). IgE-mediated allergic disease was recognized by specific IgE (>0.35 kU/l) and/or a positive skin prick test to one or more allergens as described previously (12).

All the subjects gave written informed consent to participation in the study, which was approved by the Committee for the Ethics of Medical Experiment on Human Subjects, Medical Faculty, Masaryk University Brno.

Genotype identification

Genomic DNA was isolated from peripheral blood leukocytes by a standard method using the proteinase K digestion of cells.

Detection of the bi-allelic polymorphism C(-159)T in the promoter of the CD14 gene

The C(-159)T polymorphism was detected using a modification of a method described previously (6). A 497-bp polymerase chain reaction (PCR) product was generated using primers 5'-GTGCCAACAGATGAGGTTTAC-3' and 5'-GCCTCTGACAGTTTATGTAATC-3'. Commercially available AVAII endonuclease (New England Biolabs, Frankfurt am Main, Germany) is specific for the sequence GGTC, which was present in this PCR product only among carriers of the CD14/-159 T allele. The digested fragments were separated by electrophoresis in 2% agarose gel and visualized with ethidium bromide. The homozygous C allele of the CD14 gene appears as one 497-bp band and the homozygous T allele as 144 and 353 bp fragments. Heterozygotes exhibited all bands: 144, 353, and 497 bp.

Detection of the bi-allelic polymorphism G(-1359)T in the promoter of the CD14 gene

The G(-1359)T polymorphism was detected by newly developed PCR method and a subsequent restriction analysis with FokI endonuclease (New England Biolabs). Specific primer sequences (5'-GTTGCAGTGAGCCAAGATCA-3' and 5'-CCCTA

GACCTCTGGGGAAAG-3') were derived from the original sequence (GenBank, Accession No X74984). The PCR was performed in a final volume of 15 μ l, containing 50 mmol KCl, 10 mM Tris-HCl buffer (pH 8.4), 1.7 mM MgCl₂, 6 pM each primer, 200 μ M dNTP and 0.1 μ g of genomic DNA in the presence of 0.1 U of *Taq* polymerase (MBI Fermentas, Vilnius, Lithuania) to provide 120 bp products. After the initial denaturation step (95°C for 2 min), each cycle (of additional 30 cycles) consisted of a 94°C denaturation for 20 s, a 61°C annealing for 10 s, a 72°C extension for 15 s, with the final extension lasting 5 min at 72°C. The 12 μ l of the PCR product was digested with FokI for 2 h at 37°C. The digestion revealed fragments of 96 and 24 bp lengths for the T allele, and 120 bp for the G allele.

Statistics

All statistical analyses were performed using the program package Statistica v. 3.0 (StatSoft, Inc., Tulsa, OK, USA). The chi-square test and Fisher's exact test were used for comparison of differences in genotype or allele frequencies among groups. Odds ratio (OR), confidence intervals, and *P* values were calculated. Correction for multiple comparisons (Bonferroni adjustment method) was performed and only the values of P_{corr} (*P* corrected) <0.05 were considered to be significant.

Results

The genotype distributions for both polymorphisms were consistent with Hardy-Weinberg equilibrium in both allergic and control groups. There was a similar proportion of males/females among allergic patients as in the control group.

No significant differences between allergic patients and healthy controls were found in the genotype or allelic frequencies for the two polymorphisms of the CD14 genes. The (CC) genotype was present in 29.2% patients vs 31.3% controls, the heterozygous genotype (CT) in 47.8% patients vs 48.9% controls, and the (TT) genotype in 23.1% patients vs 19.7% controls for the C(-159)T polymorphism. The (GG) genotype was present in 59.8% patients vs 57.8% controls, the heterozygous genotype (CT) in 34.6% patients vs 35.9% controls, and the (TT) genotype in 5.5% patients vs 6.3% controls for the G(-1359)T polymorphism.

However, when the patients were subdivided in subjects with positive and those with negative skin prick tests, the CD14 C(-159)T polymorphism was significantly associated with reactivity to the moulds (Table 1). Patients with IgE-mediated asthma and/or rhinitis and positive prick test for this antigen were more often carriers of the C allele of the CD14 C(-159)T polymorphism ($P = 0.002$, $P_{\text{corr}} < 0.05$). In the patients with negative skin prick tests for moulds, there was a relative over-representation of the CD14 TT homozygotes ($P = 0.01$, $P_{\text{corr}} < 0.05$).

Concerning the G(-1359)T polymorphism, we detected a tendency towards a decreased percentage of positive skin prick tests in the patients to lumped GG genotype ($53 \pm 30\%$) compared with GT heterozygotes and TT homozygotes ($59.7 \pm 32.1\%$, $P = 0.029$, $P_{\text{corr}} > 0.05$).

Table 1. Distribution of genotypes and allele frequencies of the C(-159)T and G(-1359)T polymorphisms in IgE-mediated allergy group with negative and positive prick tests for moulds

C(-159)T polymorphism				
	Negative test for moulds (<i>N</i> = 101)		Positive test for moulds (<i>N</i> = 37)	
	<i>n</i>	%	<i>n</i>	%
CC	21	20.8	15	40.5
CT	51	50.5	19	51.4
TT	29	28.7	3	8.1
<i>P</i> _a = 0.011*				
Allele C	0.460		0.662	
Allele T	0.540		0.338	
<i>P</i> _b = 0.002**				

* The statistical significance for the comparison of genotype distributions (chi-square test).

** The statistical significance for the comparison of allele frequencies (Fisher's exact test).

Finally, there were no significant associations among the CD14 alleles and/or genotypes and several quantitative traits investigated in our study, including total IgE levels, selected specific IgE levels or pulmonary function tests.

Discussion

Allergic predisposition is regarded a multifactorial condition whose onset and severity are influenced by both genetic and environmental factors. Genome-wide screening for susceptibility to allergy has highlighted several candidate regions so far, including chromosome 5q, an area where linkage to Th2 prevalent phenotypes, such as high total serum IgE levels, eosinophilia and asthma was reported (12). CD14 is one of the many genes of this region and our study represents an investigation into the role of this gene in IgE-mediated allergic diseases in the Czech population.

First, we looked for a possible difference in the allele and genotype frequencies of two promoter polymorphisms in the CD14 gene between healthy and allergic subjects. Because these frequencies did not differ between both groups, this gene does not seem to constitute, *per se*, a risk for the development of the IgE-mediated allergic diseases.

Secondly, we detected an association of the C allele of the C(-159)T promoter polymorphism with the positive skin prick test to the moulds. Previously, in the popula-

tion of children as described by Baldini et al. (6), the TT genotype of the C(-159)T polymorphism in CD14 gene was associated with higher circulating sCD14 and with lower total serum IgE. Koppelman et al. (8) have reported a significantly higher total serum IgE levels and a higher number of positive skin tests in CC homozygotes in a population from the Netherlands. In our study as well, atopic predisposition, although selectively to molds, was associated with CC genotype. In contrast, association between atopic phenotypes and T allele of the C(-159)T polymorphism was found in a study by Ober et al. (13) in the Hutterites.

Thirdly, we examined the polymorphism G(-1359)T in the promoter of the CD14 gene. There was a trend towards lower percentage of positive skin prick tests in the patients with the GG variant compared with other genotypes. In contrast to Koppelman et al. (8), we used the overall percentage of skin tests positivity, as not all our patients were examined by the same set of skin prick tests.

In conclusion, our results confirm the importance of the polymorphisms in the CD14 gene in modulating expression and severity of the atopic phenotype. There are several plausible explanations of a possible role of CD14 in IgE-mediated allergic diseases. CD14 functions as a receptor for LPS on several types of cells, thereby inducing cytokine release (7). Complexes of CD14 and bacterial LPS may increase Th1-favored T cell differentiation and activate higher expression of sCD14, which may result in a reduction of the serum IgE levels (14). In addition, Jabara et al. (15) suggested a direct action of CD14 on monocytes, thereby inhibiting IgE production by B-lymphocytes.

Moreover, we showed the potential importance of complex interactions between the genetic background and the expression of the IgE-mediated allergic phenotype. However, because the difference in the genetic background influences allele frequencies of phenotype-associated genes, more studies in other ethnic populations should be undertaken in order to analyse the putative relevance of the CD14 gene in IgE-mediated allergy.

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