

Interleukin-10 gene promoter region polymorphism is associated with eosinophil count and circulating Immunoglobulin E in adult asthma

J. Karjalainen*, J. Hulkkonen†, M. M. Nieminen*, H. Huhtala‡, A. Aromaa¶, T. Klaukka** and M. Hurme†§

*Department of Respiratory Medicine and §Centre for Laboratory Medicine, Tampere University Hospital, †Department of Microbiology and Immunology, University of Tampere Medical School, ‡School of Public Health, University of Tampere, Tampere, ¶National Public Health Institute and **Social Insurance Institution, Helsinki, Finland

Summary

Background IL-10 has several functional effects relevant to asthma. It can modulate IgE production and induce apoptosis in eosinophils. Polymorphisms of IL-10 gene have been shown to affect IL-10 production.

Objective To establish whether IL-10 polymorphisms are associated with asthma and phenotype-related characteristics.

Methods The frequency of three single base exchange polymorphisms (at positions –1082, –819 and –592) and corresponding haplotypes of the IL-10 gene were analysed in 245 adult asthmatic subjects and 405 controls using PCR and restriction fragment length polymorphism (RFLP). The data were assessed for correlations with the eosinophil count, serum IgE and lung function.

Results The IL-10 haplotype frequencies were similar in asthmatics and controls. Eosinophil count median was 2.0- to 3.2-fold higher among asthmatics with rare ATA/ATA genotype than in asthmatics with other genotypes. No such difference was seen in the control group. When analysed by IL-10 haplotype carrier state and gender, male asthmatics with ATA haplotype had 2.8-fold higher serum IgE than those without ATA. A converse association was found in male controls with ATA haplotype, who had 1.9-fold lower serum IgE than their ATA-negative counterparts. The high IL-10-producing GCC haplotype was associated with impaired lung function in smoking male controls while in asthmatics no clear effect on lung function was found with any of the haplotypes studied.

Conclusion These results suggest that the eosinophil counts and serum IgE are differently regulated by IL-10 genotype in asthmatic and in normal subjects. However, IL-10 polymorphism is not related to susceptibility in asthma.

Keywords asthma, eosinophil, gene, IgE, IL-10, lung function, polymorphism

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Introduction

Airway inflammation is one of the major characteristics of asthma [1]. IL-10 is a mediator involved in the inflammatory process in this disease [2]. IL-10 down-regulates antigen-dependent activation of most of the cell types primarily involved in allergic reactions and prevents eosinophils from homing to the lung in experimental models of asthma [3]. Some observations suggest that IL-10 production is defective in patients with asthma. Borish and co-workers found that, when compared with non-atopic control subjects, patients with atopic asthma had diminished concentrations of IL-10 in bronchoalveolar lavage fluid as a result of inhibition of transcription [4]. Production of IL-10 was found in a study by John and associates to be decreased in alveolar macrophages and peripheral blood mononuclear cells (PBMCs) in patients with

mild asthma [5]. However, contrary associations have also been demonstrated [6, 7].

Twin and family studies have suggested that approximately 75% of the variation in IL-10 production is genetically determined [8]. IL-10 production would appear to be mainly controlled at transcriptional level [9]. Three single-base exchange polymorphisms (SNPs) sited at positions –1082 (G/A), –819 (C/T) and –592 (C/A) in the promoter region of IL-10 gene form haplotypes, of which three (GCC, ACC and ATA) have been described in Caucasian populations [9]. The GCC haplotype has been associated with high IL-10 production in PBMC cultures [9]. The ATA haplotype has been associated with lower transcriptional activity than the GCC haplotype and the ATA/ATA genotype, with lower IL-10 production in whole blood cultures than other haplotypes or genotypes [10]. These findings are consistent with our recent observation concerning the effects of IL-10 polymorphism on circulating IL-10 in primary Sjögren's syndrome [11].

In this present study we analysed IL-10 promoter region SNPs in 245 asthmatic and 405 control subjects. The eosinophil

Correspondence: Jussi Karjalainen, University of Tampere Medical School, FIN-33014 University of Tampere, Finland. E-mail: jussi.karjalainen@uta.fi

counts, IgE levels and lung function measurements were correlated with the genetic data in order to ascertain whether the IL-10 polymorphisms have an effect on these parameters.

Materials and methods

Subjects

Asthmatic and control subjects were participants in a Finnish population-based case-control study aimed at identifying risk factors and predictors of the outcome of adult asthma. Inclusion criteria for asthmatic subjects were age over 30 years and entitlement to special reimbursement for asthma medication from the Social Insurance Institution of Finland. Entitlement is granted if the criteria for persistent asthma are fulfilled as certified by a chest specialist. Typical history, clinical features and course of asthma must be documented in the medical certificate. At least one of the following physiological criteria is required for diagnosis: (i) a variation of $\geq 20\%$ in diurnal peak expiratory flow (PEF) recording (reference to maximal value); (ii) an increase of $\geq 15\%$ in PEF or forced expiratory volume of 1 s (FEV₁) with B₂-agonist; (iii) a decrease of $\geq 15\%$ in PEF or FEV₁ in exercise testing. Moreover, a period of at least 6 months of continuing regular use of asthma medication must have elapsed by the time of the decision. This method of case ascertainment has recently been described in detail and evaluated [12]. One to two controls who did not have asthma or chronic obstructive pulmonary disease were initially selected for each subject through a register covering the entire population. The controls were matched with cases for age, gender and area of residence. The ethnic origin of patients and controls was the same (Finnish Caucasian). Demographic and clinical characteristics of the asthmatic and control subjects at study entry are summarized in Table 1.

Lung function measurements. Lung function was measured with the Vitalograph® (Vitalograph Ltd, Buckingham, Buckinghamshire, UK) spirometer, and the results were corrected for BTPS (body temperature and pressure, saturated with water vapour). Results of two technically faultless measurements were recorded, and the highest readings for FEV₁ and forced expiratory capacity (FVC) were taken as the final results, whether obtained at the same measurement or not. The only reason for rejecting a measurement was a technically incomplete or otherwise unsuccessful blow. Calibration was performed according to manufacturer's instructions. Finnish reference values [13] were used when converting results to percentage of predicted form.

Table 1. Demographic characteristics of asthmatic and control group

Variable	Asthmatics (<i>n</i> = 245)	Controls (<i>n</i> = 405)
Age, years (mean $\pm$ SD)	59 $\pm$ 11	60 $\pm$ 11
Gender, <i>n</i> (female/male)	152/93	254/151
Smoking, <i>n</i> (Non/ex/current)	123/80/42	237/89/79
FEV ₁ , % predicted (mean $\pm$ SD)	86.3 $\pm$ 25	102.5 $\pm$ 20
Atopy*, <i>n</i> (yes)	137	154

*One or more positive reactions in skin prick test.

Laboratory analyses

Total serum IgE determinations were carried out by the immuno-noluminometric method (Ciba Corning Diagnostics, Halsted, UK). Eosinophil counting was performed using Technicon H3 analysers (Bayer Diagnostica, Tarrytown, NY, USA).

Genotype analyses

DNA was isolated from blood samples by the salting out method [14]. The C/T base exchange polymorphism at -819 of the IL-10 gene was analysed by PCR, restriction fragment length polymorphism (RFLP) and gel electrophoresis as previously described [15]. The -1082 G/A polymorphism and -592 C/A polymorphism of the IL-10 gene were analysed by PCR and RFLP and gel electrophoresis according to Edwards-Smith and associates [16]. By these methods, IL-10 genotyping was achieved for 242 asthmatic (99%) and for 395 control subjects (98%).

Statistical analyses

Concentrations of serum IgE, eosinophil counts and lung function measurements were compared by Kruskal-Wallis ANOVA. Interaction terms for FEV₁% predicted values and log-transformed IgE values were calculated using two-way ANOVA. The post-hoc comparisons were carried out by the least significant difference (LSD) test of means. Genotype frequencies were compared using $6 \times 2 \chi^2$ test (5 d.f.) and haplotype frequencies by $2 \times 2 \chi^2$ (1 d.f.) statistics. Findings were considered statistically significant at $P < 0.05$. Statistical calculations were carried out with Statistica software (ver. Win.5.1D, StatSoft Inc., Tulsa, OK, USA). Hardy-Weinberg equations were calculated by Arlequin [17] and power calculations by PS software [18]. The sample size was sufficient to show a significant effect on asthma susceptibility with a power > 0.80 if the difference in any of the haplotype frequencies between asthmatics and controls had been ≥ 0.12 .

Results

IL-10 allele and genotype frequencies

After determining the individual single-base exchange polymorphisms of -1082, -819 and -592 for each subject, the IL-10 genotypes were formed. As expected, the distributions of individual IL-10 alleles followed the Hardy-Weinberg equation. The genotype distribution and haplotype frequencies were similar in asthmatics and controls (Table 2). This finding proved consistent when the genders were analysed separately.

IL-10 polymorphisms and eosinophil count

The IL-10 genotype data were correlated with the laboratory parameters of patients and controls. In these analyses we observed that asthmatics with ATA/ATA genotype had markedly higher eosinophil counts than those with other genotypes (Fig. 1, $P = 0.0267$, 5 d.f.). A converse trend of association was noted in controls ($P = 0.0983$, 5 d.f.). Furthermore, there were no differences in eosinophil counts when asthmatic subjects and controls were categorized on the basis of GCC, ACC or ATA haplotype carrier status.

IL-10 polymorphisms and serum IgE levels

The IL-10 genotype had no statistically significant effect on serum IgE in the asthmatic or control group. In the control group ($n = 395$), the subjects carrying ATA haplotype ($n = 170$)

Table 2. Individual IL-10 genotypes, haplotype carrier rate and haplotype carrier frequencies in healthy controls and in patients with asthma

Genetic marker	Asthmatics (<i>n</i> = 242)	Controls (<i>n</i> = 395)	<i>P</i> -value χ^2 test
Genotype, <i>n</i> (%)			
GCC/GCC	49 (20)	71 (18)	0.9710
GCC/ACC	70 (29)	110 (28)	
GCC/ATA	51 (21)	91 (23)	
ACC/ACC	25 (10)	44 (11)	
ACC/ATA	38 (16)	65 (16)	
ATA/ATA	9 (4)	14 (4)	
Haplotype frequency			
GCC	0.452	0.434	0.5231
ACC	0.326	0.333	0.8118
ATA	0.221	0.233	0.6252

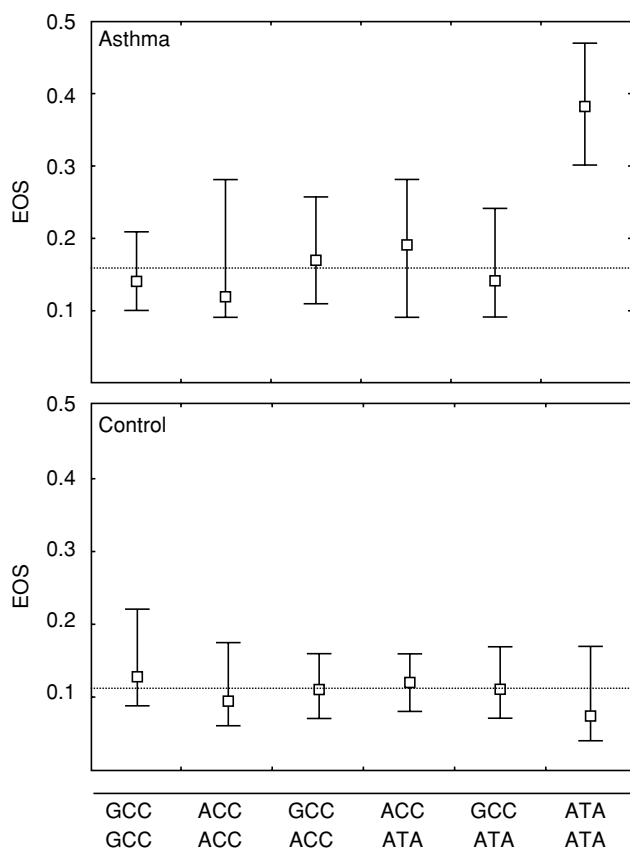


Fig. 1. Eosinophil counts in asthmatic patients (upper panel) and control subjects (lower panel) with different IL-10 genotype. Values are median ($\square$) and quartile range (whiskers), given in $10^9/L$. Dashed horizontal line indicates overall median value in asthmatic group (0.16) and in control group (0.11).

had 1.6-fold lower serum IgE (median 16; quartile range 8–42 IU/L) than their ATA-negative counterparts (median 26; quartile range 9–80 IU/L, $n = 225$, $P = 0.0231$, 1 d.f.). Moreover, ACC-positive had higher IgE levels (median 26; quartile range 9–77 IU/L) than ACC-negative controls (median 17; quartile range 7–49 IU/L, $n = 176$, $P = 0.0424$, 1 d.f.). In the unfractionated asthma group, the IL-10 haplotype carrier state had no effect on IgE ($n = 242$).

As expected, smoking and gender were significantly associated with IgE (data not shown). Therefore, haplotype effects were further analysed separately by gender and the confounding effect of smoking was assessed using two-way ANOVA. The male asthmatics with ATA haplotype ($n = 33$) had clearly (2.8-fold) higher IgE levels than ATA-negative male asthmatics ($n = 59$, $P = 0.0252$, 1 d.f., Fig. 2). In two-way ANOVA this effect of ATA carrier state was confirmed ($P = 0.0112$, 1 d.f.), and smoking had no effect. Moreover, no combined effect of smoking and ATA was found. Surprisingly, an opposite association was found in ATA-positive control males ($n = 60$), who had significantly (1.9-fold) lower IgE levels than their ATA-negative counterparts ($n = 88$, $P = 0.0323$, 1 d.f., Fig. 2). Also in this case, the effect of ATA carrier state was confirmed by two-way ANOVA ($P = 0.0420$, 1 d.f.) and no effect of smoking or of combined effect was found. In females, ATA carrier state had

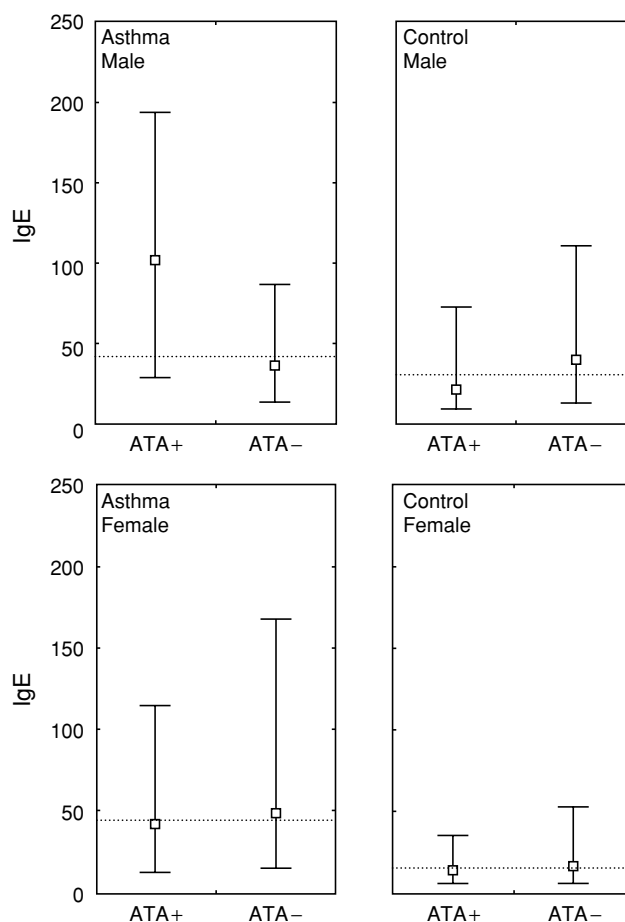


Fig. 2. Serum IgE in subjects categorized by ATA haplotype carrier state, disease status and gender. Values are median ($\square$) and quartile range (whiskers), given in IU/L. Dashed horizontal lines indicate overall median values in corresponding groups (i.e. asthmatic male 41, control male 31, asthmatic female 44.5 and control female 16).

no effect on IgE while smoking had an independent effect ($P=0.0065$ for asthmatics and $P=0.0257$ for controls).

When the effect of ACC carrier state and smoking was analysed by gender using two-way ANOVA, the ACC haplotype carrier state ($P=0.0210$, 1 d.f.) and smoking ($P=0.0060$, 2 d.f.) both had effect on IgE levels in female controls (current smokers vs. non-smokers, $P=0.0014$, current smokers vs. ex-smokers $P=0.0326$; ACC carriers vs. ACC non-carriers $P=0.0016$, LSD test of means). However, no combined effect of smoking and ACC carrier state on IgE level was found. Moreover, the ACC haplotype carrier state had no effects on IgE in male controls or male or female asthmatics. However, smoking had an increasing effect on IgE among female asthmatics ($P=0.0101$, 2 d.f., two-way ANOVA. Current smokers vs. non-smokers $P=0.0025$, current smokers vs. ex-smokers $P=0.0318$, LSD test of means).

No differences were observed in any of the analyses when the effect of the GCC haplotype was explored.

IL-10 polymorphisms and lung function tests

There were no significant differences in lung function (as measured with FEV₁% predicted) between determined IL-10 genotypes in the asthma and control groups (data not shown). This was also the case when subjects were categorized by IL-10 haplotype carrier status. As smoking was significantly associated with lung function in male asthmatics ($P=0.0038$, 2 d.f.) and male controls ($P=0.0012$, 2 d.f.), the genders were further studied separately and subjects were divided into groups of non-smokers, ex-smokers and current smokers. Smoking male controls with GCC haplotype ($n=20$) had a lower median FEV₁ (% predicted) value than those without GCC haplotype (95%, quartile range 76–105% vs. 110%, 93–123%, $n=15$, $P=0.0492$, 1 d.f.). In two-way ANOVA there was a clear interaction between smoking and GCC haplotype ($P=0.0138$, 2 d.f.) and the GCC haplotype carrier state had no independent effect on lung function. Post-hoc testing confirmed that the GCC-positive smoking control males differed significantly from all other groups. Neither other haplotype carrier state (ACC or ATA) nor IL-10 genotype was associated with lung function when smoking and sex were taken into account in the analyses.

Discussion

In this study we showed that the three SNPs in the promoter region of IL-10 gene alone and the putative haplotypes derived from them have no impact on asthma susceptibility. The positive predictive value of our asthma definition is high [12]. As our definition only includes persistent cases of asthma, our results apply to susceptibility to this disease form. Our finding is well in line with the results of Lim and co-workers [19], who observed that the low IL-10-producing haplotype was more common in patients with severe asthma; however, no clear effect on asthma susceptibility was noted.

Most allergic and non-allergic asthmatics have bronchial eosinophilia and there is a significant association between eosinophil activation and asthma severity [1]. The number of eosinophils is increased in asthma in consequence of reduced apoptosis [1]. Besides several other anti-inflammatory effects,

IL-10 is also known to induce eosinophil cell death [20]. In a recently published study, Immervoll and co-workers found an association of eosinophil cell count with the IL-10-592 polymorphic site of the IL-10 gene in asthma family material [21]. As mentioned in the introduction, the ATA/ATA genotype has been linked to lower IL-10 production than other genotypes [10, 11]. It is possible that the high eosinophil counts observed here in ATA/ATA genotypic asthmatics are an end result of genetically determined low IL-10 production capacity and diminished IL-10-mediated eosinophil cell apoptosis.

As these effects were not seen in healthy control subjects, the results suggest that IL-10 genotype effects are enhanced whenever there is disease stress which drives towards eosinophilia. This kind of disease-specific association of IL-10 genotypes seems to be typical likewise for circulating IL-10, as IL-10 polymorphism primarily regulates IL-10 levels in patients with primary Sjögren's syndrome but not in healthy control subjects [11]. It would thus appear that the basal and induced IL-10 effects are differently regulated.

Independent of allergic status, elevated serum IgE is a risk factor for asthma [22, 23]. Humbert and colleagues have shown that there is also similar cytokine [24] and high-affinity IgE receptor [25] expression in bronchial biopsies in both allergic and intrinsic asthmatics. Moreover, IgE plays a critical role in the development of bronchial hyper-responsiveness in asthmatics [26]. The role of IgE in asthma is now further evidenced by the promising results reported with the new anti-IgE monoclonal antibody treatment [27].

IL-10 is known to inhibit IL-4-induced IgE synthesis by PBMC from healthy non-atopic donors [28] and from allergic patients [29]. On the other hand, IL-10 has been shown to increase IgE production in B cells which are already IgE-switched [29]. Hobbs and co-workers have previously studied the IL-10-571 polymorphic site of the IL-10 gene in asthmatic subjects (site -571 is the same as site -592 here) [30]. They showed an association with allele A at this site and high IgE levels in asthmatics. Our finding concerning the association of the ATA haplotype with high IgE in asthmatic is consistent with their data. Surprisingly, we found that this association was the opposite in non-asthmatic control subjects.

Lung function measurements, mainly by spirometry, are of vital importance when diagnosing asthma and assessing its severity. FEV₁ (%predicted) is a reliable measure of airway obstruction. We found no previous studies reporting an association (or lack of association) between IL-10 and lung function. This could have been expected on the basis of findings reported by Lim and colleagues [19]. Moreover, Makela and associates demonstrated in mice that IL-10 has an important role in airway hyper-responsiveness [31]. We observed no clear connection between IL-10 and airway obstruction in asthmatics. Regarding smoking status our study population was fairly heterogeneous, but the above result held good when smoking status was taken into account.

One interesting association in male smokers was seen, which could possibly play a role in chronic obstructive pulmonary disease. There is some evidence that polymorphisms of cytokine genes exert an effect on the pathogenesis of airway obstruction. Recently, IL-1 haplotypes have been associated with lung function decline in healthy smokers [32] and non-smoking asthmatics [33]. Taking into account that IL-10 is usually considered an anti-inflammatory cytokine, it was interesting that smoking

male controls with the high IL-10-producing GCC haplotype had impaired lung function.

We consider that the earlier reported effect of the ATA haplotype on asthma severity [19] may be transmitted by eosinophils and IgE. Although the asthmatic subjects with the rare ATA/ATA genotype were clearly distinct in respect of their eosinophil counts, we did not observe any distinct associations with the ATA/ATA genotype and clinical phenotype in these patients. This may be explained by the fact that the number of cases with the ATA/ATA genotype was somewhat small for reliable frequency analysis. It is also likely that due to the subtle nature of allelic effects larger materials and longitudinal studies are needed to find this kind of phenotypic differences.

In summary, the IL-10 genotype does not carry asthma susceptibility, nor has it any significant effect on lung function in asthmatics. Our results suggest that the eosinophil counts and serum IgE are differently regulated by the IL-10 genotype in asthmatic and in normal subjects. When combined with findings from other studies, these results indicate that the IL-10 genotype becomes important in cases in which disease stress affects the immune system.

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