

Relationship Between the IL-12B Promoter Polymorphism and Allergic Rhinitis, Familial Asthma, Serum Total IgE, and Eosinophil Level in Asthma Patients

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■ Abstract

Background: IL-12B is a strong candidate gene for asthma.

Objectives: We investigated the relationship between IL-12B and asthma, allergic rhinitis, familial asthma, and levels of eosinophils and total immunoglobulin (Ig) E in the serum of asthma patients.

Methods: The study group consisted of 53 asthma patients and 60 control patients. Serum total IgE levels, eosinophil count, and the presence of allergic rhinitis and familial asthma were determined, and the IL-12B polymorphism was analyzed. Both patients and controls were divided into 3 groups based on their genotypes—homozygous for allele 1 (A1), homozygous for allele 2 (A2), and heterozygous patients, ie, alleles 1 and 2 (A1A2). Each genotype was compared with the other genotypes and the control genotypes.

Results: The rates for genotypes A1, A1A2, and A2 were 17%, 40%, and 43%, respectively. Male and female total IgE levels were not different between the groups ($P > .05$), but they were higher than in the controls ($P > .05$). The frequencies of allergic rhinitis and familial asthma were not different between the groups ($P > .05$), although allergic rhinitis in the A1A2 genotype and familial asthma in the A2 genotype were higher than in the controls ($P < .05$).

Conclusions: Our comparison of asthma patients and controls showed that familial susceptibility to asthma may be related to the A2 genotype, whereas coexistence of asthma and allergic rhinitis may be related to the A1A2 genotype. In asthmatic patients, the effects of the IL-12B polymorphism on asthma, allergic rhinitis, familial asthma, eosinophilia and total IgE levels, are controversial. We think that there is a need to investigate this hypothesis in larger series.

Key words: Allergic rhinitis. Asthma. Familial asthma. IL12B polymorphism. Total IgE.

■ Resumen

Antecedentes: La IL-12B es un importante gen candidato para el asma.

Objetivos: Investigamos la relación entre la IL-12B y el asma, la rinitis alérgica, asma familiar, niveles de eosinófilos e inmunoglobulina (Ig) E en el suero de pacientes asmáticos.

Métodos: El grupo de estudio consistía en 53 pacientes asmáticos y 60 pacientes controles. Se han determinado los niveles séricos de IgE total, el recuento de eosinófilos, y la presencia de rinitis alérgica y asma familiar y se analizó el polimorfismo IL-12B. Tanto los pacientes como los controles fueron divididos en 3 grupos basados en su genotipo-homocigotos para el alelo 1 (A1), homocigotos para el alelo 2 (A2), y pacientes heterocigotos p.e. alelo 1 y 2 (A1A2). Cada genotipo fue comparado con los otros genotipos y con los genotipos control.

Resultados: Los porcentajes de los genotipos A1, A1A2, y A2 fueron 17%, 40%, y 43%, respectivamente. Los niveles de IgE de varones y mujeres entre los grupos no fueron diferentes ($P > .05$), pero eran mayores que en los controles ($P < .05$). La frecuencia de rinitis alérgica y asma familiar no era diferente entre los grupos ($P > .05$), aunque la rinitis alérgica en el genotipo A1A2 y el asma familiar en el genotipo A2 fueron más frecuentes que en los controles ($P < .05$).

Conclusiones: La comparación que hemos realizado entre pacientes asmáticos y controles mostró que la susceptibilidad familiar a presentar asma podría estar relacionada al genotipo A2, mientras que la coexistencia de asma y rinitis alérgica podría estar relacionada con el genotipo A1A2. En pacientes asmáticos, los efectos del polimorfismo IL-12B en el asma, la rinitis alérgica, el asma familiar, la eosinofilia y los niveles de IgE total, son controvertidos. Pensamos que se necesita investigar esta hipótesis en muestras mayores.

Palabras clave: Rinitis alérgica. Asma. Asma familiar. Polimorfismo de la IL12B. IgE total.

Introduction

Allergic sensitization to 1 or more allergens (atopy) is one of the most important risk factors for asthma, as are family history and environmental exposure in early life [1]. This complex and heterogeneous etiology has made genetic studies challenging. Not surprisingly, an association with many regions has been reported, and at least 25 genes are involved in the pathogenesis of asthma [2]. Positional cloning studies have recently identified genes in 6 of these regions, and these genes confer susceptibility to asthma, bronchial hyperresponsiveness, and atopy [3,4]. Large-scale genome screening has identified several regions, including 5q31-33, that are linked to asthma and asthma-related phenotypes such as elevated serum immunoglobulin (Ig) E levels and bronchial hyperresponsiveness [5]. The cytokine gene cluster located in 5q31-33 contains several potential candidate genes for asthma, including interleukin (IL) 3, IL-4, IL-5, IL-9, IL-12B, IL-13, IL-14, and the β_2 -adrenergic receptor [6,7].

The immunomodulatory cytokine IL-12 is secreted by activated phagocytes and dendritic cells and induces interferon γ (IFN- γ) production by natural-killer and T lymphocytes [8-10]. It is the primary inducer of the development of T helper 1 (T_H1) cells, with downregulation of T_H2 cytokines that are associated with asthma [9,11]. It consists of 2 subunits, p35 and p40, which are encoded by IL-12A and IL-12B, respectively [8-10], and functional polymorphisms of IL-12B can affect production of these proteins in several tissues [11]. An association has been reported between heterozygosity for an IL-12B promoter polymorphism and the severity of asthma in atopic and nonatopic individuals. Therefore, IL-12B is a strong candidate gene for asthma [9].

Two polymorphisms are mapped to the IL-12B gene, and their biological role may be significant. These polymorphisms are a 4-bp insertion-deletion within the promoter region and an A \rightarrow C single nucleotide polymorphism located in the 3' untranslated region at position 1188 [12]. IL-12B promoter heterozygosity has also been reported to be strongly

associated with a predisposition to severe asthma [13]. Our objective was to explore the relationship between the IL-12B promoter polymorphism and asthma susceptibility, serum total immunoglobulin E (IgE) level, eosinophilia, and the presence of allergic rhinitis and familial asthma in asthmatic patients.

Methods

Participants

Our study population was composed of 53 adult patients (13 males and 40 females) admitted to our respiratory medicine department with a minimum 3-month history of moderate persistent asthma diagnosed according to the criteria of the Global Initiative for Asthma (GINA) [14]. All the patients were selected and evaluated by a pulmonologist according to the GINA report. To qualify for inclusion in the study, patients had to be aged between 18 and 60 years, and nonsmokers, with no significant comorbid medical conditions. The control group consisted of 60 healthy nonsmoking volunteers (29 males and 31 females) with no asthma, asthmatic symptoms, or eosinophilia (Table 1). All patients were treated according to the GINA guidelines, all members of the patient and control groups were informed about the study and provided written informed consent, and approval was obtained from the local ethics committee. Patients with other active diseases were excluded from the study.

Predicted forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), and age of asthma patients and control subjects are compared in Table 1.

Design and Clinical Assessment

Participants with allergic rhinitis or allergic rhinosinusitis diagnosed by an ear, nose and throat (ENT) specialist were selected. The others were referred to an ENT specialist to determine the presence or absence of allergic rhinitis or rhinosinusitis. Patients whose first-degree relatives had a history of asthma (diagnosed by a pulmonologist) were

Table 1. Ages and Baseline Spirometric Measurements^a

	Male				Female			
	n	Age	FEV_1^b	FVC ^b	n	Age	FEV_1^b	FVC ^b
Patients	13	38.00 (7.91) (n=13)	67.67 (14.94) (n=12)	63.42 (13.37) (n=12)	40	43.45 (11.74) (n=40)	68.08 (12.44) (n=37)	71.53 (11.67) (n=36)
Controls	29	39.35 (11.98) (n=29)	92.24 (10.08) (n=29)	98.52 (11.03) (n=29)	31	36.48 (9.95) (n=31)	95.30 (12.79) (n=30)	95.70 (13.86) (n=27)
<i>P</i> value		<i>P</i> > .05	<i>P</i> < .05	<i>P</i> < .001		<i>P</i> < .05	<i>P</i> < .05	<i>P</i> < .001

^a Values are expressed as the mean (SD)

^b Predicted

Abbreviations: FEV_1 , forced expiratory volume in 1 second; FVC, forced vital capacity.

interviewed and their data recorded.

Spirometric tests, serum total IgE measurements, eosinophil counts, and analysis of arterial blood gas values and IL-12B gene promoter polymorphisms were carried out in addition to routine tests.

Peripheral blood eosinophil counts were estimated using a Technicon-H1 blood cell counter (Bayer AG, Leverkusen, Germany) and expressed as the number of cells/ μ L. Peripheral blood eosinophilia was defined as $> 275/\mu$ L [15].

Total serum IgE concentrations were determined using the CAP system (Pharmacia, Woerden, The Netherlands) and expressed in arbitrary kilounits per liter (kU_A/L). High serum total IgE levels were defined as $> 100 kU_A/L$.

IL-12B Genotyping

DNA extracted from peripheral blood [16] was genotyped to identify a previously reported IL-12B CTCTAA/GC promoter polymorphism by polymerase chain reaction (PCR) amplification of the region containing the polymorphism. CTCTAA (allele 1) was 196 bp in length, 4 bp longer than allele 2 (insertion), and GC (allele 2) was also 192 bp in length. PCR amplicons of 196 bp (allele 1) and/or 192 bp (allele 2) were generated using the primers 5'-TACAGCCTGTCTCCGAGAGAA-3' and 5'-GAGGAAG TGGTTCTCGTACTTTAGC-3' [13]. PCR reactions were carried out in a total volume of 25 μ L using approximately 100 ng DNA, 2.5 mmol/L MgCl₂, 200 mol/L dNTPs, 12.5 ng of each primer, and 0.5 units of Taq DNA polymerase (Promega, Madison, Wisconsin, USA) in the buffer provided by the manufacturer (10 mmol/L Tris-HCl, pH 9.0, and 50 mmol/L KCl). PCR involved an initial denaturation step at 94°C for 5 minutes, followed by 35 denaturation cycles at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. Amplification was confirmed by electrophoresis of 5 μ L of the PCR product on a 3% agarose gel containing ethidium bromide and subsequent visualization under UV illumination. After amplification, 5 μ L of the amplicon was digested in a 10- μ L reaction containing 1.5 units of Alu I (Promega, Madison,

Wisconsin, USA) at 37°C for at least 6 hours. The fragments resulting from the digestion were separated on 3% agarose gel and visualized as before. Digestion at 24 bp from the 3' end served as an internal control to indicate complete digestion. Alu I also digested 22 bp from the 5' end of the amplicon in the presence of allele 1 to produce a 150-bp fragment. As allele 2 lacks the second restriction site and is 4 bp shorter than allele 1, digested amplicons with this allele produce a 168-bp fragment.

Patients were separated into 3 groups according to their IL-12B promoter genotypes: patients who were homozygous for allele 1 (A1), patients who were homozygous for allele 2 (A2), and patients who were heterozygous, ie with both alleles 1 and 2 (A1A2) [10,13]. Once the allelic variants were established in the patient and control groups, the parameters of each allele group were examined separately.

Statistical Analysis

SPSS for Windows (SPSS Inc, Chicago, Illinois, USA) was used for the statistical analysis and the results were expressed as the mean (SD). Patient genotypes were compared with each other and with the control genotypes. The chi-square test was used to analyze the percentage distribution of genotypes. The independent samples test and the Mann-Whitney U test were used to compare age, mean percentage of predicted (FEV₁ and FVC), and the mean ages of the genotypes. The independent samples test was used to compare mean baseline PaO₂, SaO₂, and genotypes with respect to total IgE levels. Coexistence of asthma and allergic rhinitis, eosinophilia, and familial asthma between genotypes were compared using the chi-square test. Statistical significance was set at a *P* value of less than .05.

Results

Group 1 (A1) was composed of 1 male patient and 8 female patients with a mean age of 41.56 years, group 2 (A2) was composed of 4 male and 19 female patients with a mean age of 41.39 years, and group 3 (A1A2) was composed of 8 male and 13 female patients with a mean age of 43.14 years.

Table 2. Distributions of Genotypes and Age in the Study Groups^a

IL-12B Promoter Genotypes	Patient Group			Control Group		
	n	% Patients	Age	n	% Controls	Age
A1 ^b	9	16.98	41.56 (12.18) ^e	–	–	–
A2 ^c	23	43.39	41.39 (12.67) ^e	38	63.33	37.45 (10.88) ^e
A1A2 ^d	21	39.62	43.14 (9.11) ^e	22	36.67	38.59 (11.3) ^e
<i>P</i> value	–	–	$> .05$	–	–	$> .5$

^a Values are expressed as the mean (SD)

^b Homozygous for allele 1

^c Homozygous for allele 2

^d Heterozygous

^e No statistically significant difference ($P > .05$).

Table 3. Comparison of Genotypes in the Patient and Control Groups for Total IgE Levels^{a,b}

IL-12B Promoter Genotypes	Total IgE Levels in Patient			Total IgE Levels in Controls			P Value		
	Male, n	Female, n	Total, n	Male, n	Female, n	Total, n	P (Males)	P (Females)	P (Total)
A1 ^c	1 50 (-)	8 399.25 (388.81)	9 360.44	0	0	0	-	< .05	
A2 ^d	4 295.25 (362.43)	19 255.45 (282.06)	23 262.37 (288.52)	18 19.39 (9.18)	20 24.10 (10.14)	38 21.63 (10.04)	< .05	< .05	< .05
A1A2 ^e	8 214.50 (192.41)	13 385.65 (413.11)	21 320.45 (350.15)	11 20.09 (6.43)	11 25.45 (8.64)	22 22.77 (7.92)	< .05	< .05	< .05

Abbreviation: Ig, immunoglobulin.

^a Total IgE expressed as kU_A/L in serum

^b Values expressed as the mean (SD)

^c Homozygous for allele 1

^d Homozygous for allele 2

^e Heterozygous

Table 4. The Relationship Between Genotypes and the Presence of Allergic Rhinitis, Eosinophilia, and Familial Asthma in the Patient and Control Groups

IL-12B Genotypes	Patients			Controls			P Value		
	Allergy ^a n (%)	FAs n (%)	Eosinophilia, n (%)	Allergy ^a n (%)	FAs n (%)	Eosinophilia, n (%)	P (Allergy)	P (FAs)	P (Eosinophilia)
A1 ^b	5 (55%)	2 (22%)	4 (44%)	-	-	-	-	-	-
A2 ^c	12 (52%)	8 (35%)	11 (48%)	11 (29%)	5 (13%)	-	<i>P</i> > .05	<i>P</i> < .05	
A1A2 ^d	13 (62%)	8 (38%)	14 (67%)	3 (13.6%)	4 (18%)	-	<i>P</i> < .05	<i>P</i> > .05	

Abbreviation: FAs, familial asthma.

^a Allergic rhinitis or allergic rhinosinusitis

^b Homozygous for allele 1

^c Homozygous for allele 2

^d Heterozygous

In both the patient and control groups, the A2 genotype was more common than the other 2 genotypes. In the patient group, the least common genotype was homozygote A1. However, no A1 genotypes were found among the controls. The rates for the A1A2 genotype in both the patient and control groups were similar. It seems that the increase in the rate of the A2 genotype is the result of the increasing absence of the A1 genotype in the control group. With the exception of the higher frequency of the A1 genotype in the patient group, there were no significant differences between genotypes (*P* > .05). The distribution of the genotypes by presence of IL-12B polymorphisms and the mean (SD) ages of patients with asthma and control subjects are presented in Table 2.

Total IgE levels, which are accepted as indicators of atopy, were significantly higher in all genotypic groups of female and male asthmatic patients than in the controls (*P* > .05) (except

the A1 genotype for males); however, among the genotypic groups for both male and female patients, there were no statistically significant differences (*P* > .05). Additionally, there were no significant differences in total IgE levels between the genotypic groups overall (*P* > .05). The genotypes of the control groups for the rates of total IgE levels were statistically similar (*P* > .05) (Table 3).

As for atopic symptoms of the upper respiratory tract, familial asthma susceptibility, and characteristics of eosinophilia, no differences were found in the IL-12B genotype groups in the asthmatic patients (*P* > .05). The genotypes of the control groups for the same parameters (atopic symptoms of the upper respiratory tract, familial asthma susceptibility, and characteristics of eosinophilia) were also statistically similar (*P* > .05). However, the symptoms of allergic rhinitis or allergic rhinosinusitis in the A1A2 genotype and the presence

of familial asthma in the A2 genotype were higher than in the controls ($P > .05$) (Table 4).

For A1, A2, and A1A2, the mean (SD) SaO₂ (94.44% [1.67], 95.41% [1.87], and 94.05% [3.47], respectively) and PaO₂ (88.63 [9.11], 88.30 [10.16], and 80.14 [14.36] mm Hg, respectively) were not significantly different ($P > .05$).

Discussion

The expression of IL-12B is much more regulated than that of IL-12A, although expression of both IL-12A and of IL-12B is regulated by activation of the producing cells [17]. IL-12 p40 has been shown to play an essential role in the etiology of asthma and the induction of T_H2-type allergic inflammation in an animal model [18].

Several studies have analyzed the IL-12B gene as a potential candidate gene for asthma [5,13]. It has been mapped to chromosome 5q31-33, and this region has been associated with circulating eosinophil levels, T_H2 CD4 differentiation, and quantitative phenotypes associated with asthma, atopy, and total serum IgE levels [19].

Khoo et al [5] reported that the IL-12B promoter polymorphism was not associated with asthma susceptibility, severity, or atopy at ages 7 to 42 years in an Australian population. Similarly, we did not detect significant differences in asthma susceptibility between genotypic variants of the patient group. However, in contrast to the other genotypes, susceptibility to familial asthma was significantly more frequent in the A1 genotype than in the controls. Interestingly, no members of the control group had the A1 genotype.

The IL-12B promoter polymorphism may have a moderate effect on total IgE levels [5]. Khoo et al [5] reported that male patients with the A1 and A1A2 genotype had higher levels of total IgE than those with a A2 genotype, although no differences were detected in female subjects. Hirota et al [11] confirmed the association between the IL-12B promoter variant and asthma susceptibility and elevated serum IgE levels. In our study, however, there were no associations between genotypes of the IL-12B promoter polymorphism and serum IgE levels or peripheral blood eosinophil counts in either female or male asthma patients. Noguchi et al [20] also failed to find an association between this polymorphism and serum IgE levels in asthma and allergic rhinitis patients. It has been reported that this outcome might be due to other cytokines, such as IL-4, IL-5, and IL-13, which influence serum IgE levels and peripheral eosinophil counts in asthma and atopic diseases [10]. Although we could not demonstrate conclusively the influence of the IL-12B polymorphism on total serum IgE level and eosinophil count in asthmatic patients, the mean total IgE of patients in all genotypes was markedly higher than that of the controls. In other words, this result suggests that the IL-12B polymorphism may not be the only factor in the development of atopy. Furthermore, in vitro conditions might not reflect in vivo conditions. Indeed, it has been argued that it is necessary to compare serum IgE levels and peripheral blood eosinophil counts in healthy individuals or to perform larger-scale studies to exclude the effects of these cytokines and determine whether they are associated with serum IgE levels or peripheral blood eosinophil counts [10].

Failure to replicate genetic associations in a complex disease is not uncommon [21]. The functional role of IL-12B, which is involved in immune responses against environmental antigens, might be affected by the number of microbes. Furthermore, social interactions may reflect contradictory interethnic results [11]. Other authors have reported that preferential transmission of IL-12B genotypes to asthma was not observed [22]. In Japan, no associations were noted for IL-12B between asthma and allergic rhinitis [20]. Similarly, no significant associations were found between asthma susceptibility or allergic rhinitis and asthma and the IL-12B promoter polymorphism among the 3 genotypes in our patient population. Although genotypic differences do not seem to affect the coexistence of asthma and allergic rhinitis, the frequency of allergic rhinitis with the A1A2 genotype was higher in patients than in controls. This result may suggest that the coexistence of allergic rhinitis and asthma is related to the A1A2 genotype.

Our study failed to find any association between patient genotypes and familial susceptibility to asthma, but it did indicate that familial susceptibility to asthma could be higher in the A2 genotype than in healthy individuals.

We were unable to show any effect of IL-12B in phenotypic characteristics related to atopy in the asthmatic patient group. However, a comparison of the patient group with the control group suggests that predisposition to familial asthma may be associated with the A2 genotype. The coexistence of allergic symptoms and asthma phenotype may be related to the A1A2 genotype.

The lack of agreement between our results and those of other authors may be due to unrecognized differences in environmental exposures. Another plausible explanation may be that sample size has an effect on the contradictory result. We believe that it would be beneficial to test this assumption in advanced studies with larger samples and multiple variables.

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