

A variant in the gene for GM-CSF, *I117T*, is associated with atopic asthma in a Swiss population of asthmatic children

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Asthma is a phenotypically heterogeneous inflammatory disorder clinically characterized by reversible airway obstruction. Atopy, the production of a sustained high-level IgE response to common environmental allergens, and bronchial hyperresponsiveness (BHR) are commonly found in asthmatic subjects. Both asthma and atopy are based on strong genetic components inherited in a complex pattern. Evidence for linkage between asthma and atopy and markers in the IL-4 cytokine gene cluster on chromosome 5q31-q33 have been reported.¹ The proinflammatory cytokines (IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF) encoded on 5q31-33 are expressed by T_{H2} cells orchestrating the allergic inflammatory response. Although IL-4 and IL-13 are likely to be involved in IgM-to-IgE isotype switching and the upreg-

Abbreviation used

BHR: Bronchial hyperresponsiveness

ulation of IgE biosynthesis, IL-5, IL-3, and GM-CSF stimulate proliferation and maturation of eosinophils. GM-CSF, rather than IL-3 or IL-5, has been described as associated with eosinophil survival-enhancing activity in asthmatic subjects.² In addition, GM-CSF has been shown to assist eosinophil adhesion to vascular cell adhesion molecule-1 and eosinophil migration into tissues, a characteristic of allergic diseases.³ It also stimulates the maturation of antigen-presenting cells. However, the expression of GM-CSF, a 4 α -helix glycoprotein, seems to be increased in atopic subjects with asthma compared with control subjects.⁴ Thus although previous research with the candidate gene approach has concentrated on IL-4 and IL-5, *GM-CSF* represents an encouraging candidate gene for atopic asthma.

We investigated whether any sequence polymorphisms in the 4 exons of the gene for GM-CSF were associated either with IgE or BHR in asthmatic children compared with control subjects.

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TABLE I. Genotypes of *GM-CSF* mutation in each population

Group	TT	TC	CC	Frequency of C allele	P values
A: Control subjects (n = 157)	104	52	1	0.17	—
B: Total asthmatic subjects (n = 214)	129	71	14	0.23	.046
C: Atopic subjects (n = 135)*	74	49	12	0.27	.0040
C.1: Elevated IgE (n = 87)	48	30	9	0.27	.0075
C.2: With BHR (n = 43)	17	22	4	0.34	.00063
C.3: With MEF ₅₀ (n = 36)	25	10	1	0.17	.826
D: Nonatopic asthmatic subjects (n = 78)†	54	23	1	0.16	.529

Genotypes in the asthma groups (B, C, and D) were compared with the control group (A) by means of the nonparametric χ^2 likelihood test with Yates correction.

MEF₅₀, Maximal expired flow at 50% of vital capacity.

*Family history of atopy and increased levels of total or specific IgE.

†Nonatopic asthma (exercise or infection induced).

METHODS

Two hundred fourteen asthmatic subjects (mean age, 8.3 years; SD, 3.3) were recruited from the Pediatric Pulmonary Out-Patient Clinic at the University Children's hospital of Berne. Asthma was defined, on the basis of the recommendation of the American Thoracic Society, as consisting of recurrent reversible airway obstruction with cough, dyspnea, or wheezing and wheezing by auscultation (ie, subjects with atopic asthma, exercise-induced asthma, or infection-induced asthma) and excluding other causes of dyspnea. Of 214 asthmatic subjects, 135 (mean age, 8.5 years; SD, 3.4) were given a diagnosis of atopy on the basis of family history of atopy (parental asthma, hay fever, or neurodermatitis) or elevated total (mean, 820 kU/L; median, 535 kU/L; SD, 1107) or specific IgE levels against one or more tested allergens; conversely, 78 were nonatopic (mean age, 8.1 years; SD, 3.2). Serum total IgE (kilounits per liter) was measured by using the CAP system fluoroimmunoassay (Diagnostics CAP FEIA, Kabi Pharmacia, Sweden), and IgE levels for the most common allergen was determined by RAST (Diagnostics CAP FEIA). An elevated IgE level was defined as being greater than the published normal values for children (Diagnostics CAP FEIA). In 43 of these atopic children, we found BHR defined as an increase of interrupter resistance at a cumulative provocation dose of carbachol of less than 480 mg⁵ (mean, 260 mg; median, 250 mg; SD, 90), and 36 had impaired lung function (maximal expired flow at 50% of vital capacity <80%). Lung function testing was performed with whole-body plethysmography by using an air-conditioned, constant volume, variable pressure, whole-body plethysmograph (Jaeger, Würzburg, Germany). The study was approved by the Ethics Committee of the University of Berne. Informed written consent was obtained from all parents. One hundred fifty-seven healthy individuals of the same local population were used as a control group. They had no family history of atopy and did not have recurrent reversible airway obstruction with cough, dyspnea, or wheezing in either childhood or adulthood.

Individuals were genotyped by using PCR amplification of all 4 exons, including intron/exon boundaries, followed by single-strand conformation polymorphism analysis and fluorescence-based DNA sequencing.

We found a T-to-C substitution in exon 4 of the *GM-CSF* gene at nucleotide 2600 (*I117T*). The data in Table I demonstrate that allele C is significantly associated with atopic asthma ($P = .004$) and even

more strongly with BHR ($P = .00063$), but not with nonatopic asthma (exercise- or infection-induced asthma). Genotype distribution fulfills the Hardy-Weinberg equilibrium in each asthma group, as well as in the control group.

DISCUSSION

In conclusion, the *I117T* mutation, located in close proximity to the fourth α -helix of the *GM-CSF* gene is involved in the development of atopic asthma and BHR in this asthmatic population of Swiss children. The mutation is either the disease-causing defect or at least it is in strong linkage disequilibrium with a not yet detected pathogenic sequence variant. Because *GM-CSF* is involved in maturation of antigen-presenting cells and migration of eosinophils, the *I117T* mutation may play a significant role in the pathogenesis of atopic asthma and BHR. In addition, future studies on the effect of this mutation on transcript and protein level may be of particular interest, providing more insight into the mechanisms involved.

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