ORIGINAL ARTICLE

A Prospective Study in Children With a Severe Form of Atopic Dermatitis: Clinical Outcome in Relation to Cytokine Gene Polymorphisms

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Abstract

Background and Objective: The course of atopic dermatitis (AD) in childhood is characterized by typical changes in phenotype, including a shift from skin involvement to respiratory allergy usually around the third year of age. We thus designed a prospective study to monitor the outcome of severe AD and to investigate the association between cytokine gene polymorphisms and clinical manifestations.
Methods: Clinical and laboratory follow-up of 94 patients with severe AD and 103 healthy controls was performed using routine methodology. Allele, genotype, and haplotype frequencies of single nucleotide polymorphisms of 13 selected cytokine/receptor genes were analyzed using PCR with sequence-specific primers.
Results: In our study, genotypes of 7 polymorphisms—IL-4 -1098G/T and -590C/T, IL-6 -174C/G and nt565A/G, and IL-10 -1082A/G, -819C/T, and -592A/C were significantly associated with atopic AD ($P<.05$). A significant association was also found for TNF-α AA and IL-4 GC haplotypes and AD.
We confirm the progressive clinical improvement of AD together with a decrease in the severity index SCORAD (SCORing atopic dermatitis) during childhood ($P<.05$). We found significant differences between IL-4Rα +1902 A/G and positivity of tree pollen–specific IgE ($P<.05$) in the AD group. Moreover, a weak association was also found between IL-10 -819C/T and IL-10 -590A/C and the appearance of allergic rhinitis ($P<0.1$).
Conclusions: We confirmed a clinical shift in allergic phenotype in the first 3 years of life, and showed an association between *IL-4*, *IL-6*, and *IL-10* polymorphisms and AD. Our data indicate that *IL-4α* and *IL-10* polymorphisms may be considered predictive factors of respiratory allergy in children with AD.

Key words: Allergic rhinitis. Allergy. Atopic dermatitis. Single nucleotide polymorphism. Cytokines.

Resumen

Antecedentes y objetivo: La evolución de la dermatitis atópica (DA) en la infancia se caracteriza por alteraciones típicas en el fenotipo, entre ellas el paso de afectación cutánea a alergia respiratoria, que se produce habitualmente alrededor de los tres años de edad. Por tanto, se diseñó un estudio prospectivo para evaluar el desenlace de la DA grave e investigar la asociación entre los polimorfismos genéticos de las citocinas y las manifestaciones clínicas.

Métodos: Se realizó un seguimiento clínico y de laboratorio de 94 pacientes con DA grave y 103 controles, utilizando los métodos habituales. Se analizaron las frecuencias alélicas, genotípicas y haplotípicas de polimorfismos de un solo nucleótido de 13 genes de receptores/citocinas seleccionados, mediante PCR con cebadores de secuencia específica.

Resultados: En el estudio, los genotipos de 7 polimorfismos (-1098G/T y -590C/T en *IL-4*, -174C/G y nt565A/G en *IL-6* y -1082A/G, -819C/T, y -592A/C en *IL-10*) mostraron una asociación significativa con la DA atópica ($p < 0,05$). También se encontró una asociación significativa entre los haplotipos AA en TNF-α y GC en *IL 4* y la DA.

Se confirma una mejoría clínica progresiva de la DA junto con una disminución del índice de gravedad SCORAD (SCORing atopic dermatitis) en la infancia ($p < 0,05$). Se encontraron diferencias significativas entre +1902 G/A en *IL-4R*α y la positividad de IgE específica para el polen de árboles ($p < 0,05$) en el grupo con DA. Además, se halló una asociación débil entre -819C/T en IL-10 y -590A/C en *IL-10* y la aparición de rinitis alérgica ($p < 0,1$).

Conclusiones: Se confirmó un cambio clínico en el fenotipo alérgico durante los tres primeros años de vida, y se encontró una asociación entre los polimorfismos en *IL-4*, *IL-6* e *IL-10* y la DA. Los datos indican que los polimorfismos en *IL-4R*α e *IL-10* pueden considerarse factores predictivos de alergia respiratoria en niños con DA.

Palabras clave: Rinitis alérgica. Alergia. Dermatitis atópica. Polimorfismo de un solo nucleótido. Citocinas.

Introduction

The prevalence of allergic disease in Western countries has increased steadily in recent decades. In developed countries, the prevalence of asthma and atopic dermatitis (AD) is 10% and 15%, respectively, and is substantially higher among children [1]. Genetic predisposition has been proven to play a substantial role in the etiopathogenesis of AD [2]. The risk of a child having AD is 50% if 1 parent has an atopic disease (AD, asthma, or allergic rhinitis [AR)] and 75% if both parents do [2, 3]. Several genome screens for AD have been reported in the literature and have determined genome-wide significant linkage on chromosomes 1q, 3q, 3p, and 17p. A substantial number of candidate gene studies have revealed that multiple genes are involved in allergic inflammation (reviewed in [4,5]). Multiple single nucleotide polymorphisms (SNPs) in candidate genes and chromosomal regions that consistently show association with AD, asthma, and the severity of these diseases have been identified [1,5,6].

The immunopathogenesis of AD is a very complex process that involves an array of molecules, but it is evident that cytokines play a key role in the development of the clinical presentation of AD. AD is characterized by an overexpression of type 2 helper (T_H2) cytokines, such as interleukin (IL) 4, IL-5, IL-13, and the immunoregulatory cytokine IL-10, which have been observed in a significant proportion of acute AD skin lesions compared with the skin of healthy individuals [2,7]. Several studies have reported a population-dependent association between cytokine gene polymorphisms that affect cytokine production and chronic inflammatory disease; the association between gene polymorphisms in certain cytokines and allergic diseases in particular has been well documented [6,8-11]. Functional polymorphisms in cytokine-encoding genes may lead to defective or excessive cytokine production, resulting in disorders in T_H polarization. A strong genetic link to bronchial asthma and AD was described for the cytokine cluster on chromosome 5q34, including IL-4, IL-13, granulocyte-macrophage colony stimulating factor, and IL-9 [1]. Certain polymorphisms in the genes encoding IL-4, IL-4 receptor alpha chain (IL-4Rα), IL-5, and IL-13 are associated with the development of asthma, inhalation allergies, and AD, and with elevated serum immunoglobulin (Ig) E levels [12-16]. This association has not been observed in all populations, and some population studies have not detected any association between AD, asthma, and cytokine polymorphisms [2]. The exact role of cytokines and cytokine polymorphic variants in the course and long-term outcome of AD therefore remains unclear, with many possible effects described in the studies cited above.

AD is predominantly a disease of childhood. The first clinical symptoms usually appear early. Sometimes, and particularly in severe forms, AD lesions may already be present during the neonatal period. The typical feature of AD is its diversified course during childhood. The turning point is usually around the third year of age, when clinical expression of the disease can change. First, the infantile stage can change to the childhood stage, with involvement of flexor surfaces, or symptoms can become less severe and even completely disappear. Second, the children can develop respiratory allergies, such as bronchial asthma and AR. Furthermore, AD is often associated with food allergies, which can subside during childhood or persist and increase in variety and intensity.

We designed a prospective study with the goal of correlating the genetic pattern of the inherited cytokine set with the outcome of initially severe AD in very young infants. The association between a particular cytokine/cytokine receptor gene polymorphism and clinical status was analyzed after the third year of life. First, we investigated the polymorphic frequencies of selected cytokine/cytokine receptor genes in patients with AD and healthy controls. Second, we focused on a set of polymorphic variants of the alpha chain of the receptor

for the *IL-4* and *IL-10* genes and examined the association between these changes and clinical status and laboratory markers of AD during infancy and childhood.

Patients and Methods

Patients, Controls, and Study Design

Ninety-four pediatric patients (32 girls and 62 boys) from the Czech population with severe forms of AD were evaluated at the departments of dermatology and immunology at Charles University, 2nd Medical School, and University Hospital Motol, in Prague in the Czech Republic. The mean age of the patients at the time of diagnosis was 3.12 months (range, 0.5-27 months). There was a higher prevalence of boys (66%).

The diagnosis of AD, AR/asthma bronchiale, and food allergy was based on the SCORAD (SCORing atopic dermatitis) index (50-80), on clinical manifestations, and on levels of specific IgE and positive skin prick tests, respectively. The frequency of follow-up was dependent on clinical status, with 1 obligatory control during the first 3 years of life. Changes in the course of AD and food allergy, and the appearance of asthma or AR have been reported during childhood. In addition to the clinical manifestations of AD and respiratory allergy, we also evaluated total IgE serum levels, and serum levels of specific IgE against food allergens (particularly cow milk protein and egg white) and respiratory allergens, grass pollen, and tree pollen according to genotype. These immunologic parameters were investigated during the follow-up visits. We compared clinical status and laboratory markers at the time of diagnosis (3-6 months of age) and in the third year of life. Seventy-four patients (26 girls and 48 boys) older than 3 years of age were available for statistical analysis in prospective settings.

The control group for the genetic study consisted of 103 unrelated healthy blood donors who were recruited for an anthropological study at the Institute of Clinical and Experimental Medicine (IKEM) in Prague. Males and females were represented equally. The absence of allergy was a major criterion for inclusion in the control group; the controls were further matched for ethnicity (Caucasian population).

Informed consent was obtained prior to inclusion in the study from the patients' parents/guardians and from healthy donors.

Detection of Cytokine SNPs

The SNPs of genes encoding 13 selected cytokines and cytokine receptors were detected using polymerase chain reaction with sequence-specific primers (PCR-SSP) designed for the 13th IHWC (Cytokine Typing Tray Kit, Collaborative Transplant Study at the University Heidelberg, Heidelberg, Germany). Each PCR mix also contained an internal positive control primer pair for the *ß-globin* gene or for C-reactive protein (CRP) at a concentration 5 times lower than that of the specific primers. Genomic DNA was isolated from the peripheral blood cells of the patients using a DNA kit (Quiagen), and PCR reactions were performed in a 96-well plate (volume of 10 μL) containing genomic DNA, Taq DNA polymerase (Promega), 200nM dNTP (Promega, UK), 1.5 mM MgCl2 (Invitrogen), and primers (Cytokine Typing Tray Kit). PCR fragments were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

We analyzed the following SNPs: IL-1α -889C/T (rs1800587); IL-1*β* -511C/T (rs16944) and +3962C/T (rs1143634); IL-1R pst1 1970C/T (rs2234650) and IL-1Rα mspa1 11100C/T (rs315952); IL-2 -330G/T (rs2069762) and +166G/T (rs2069763); IL-4 -1098T/G (rs2243248), -590C/T (rs2243250), and -33C/T (rs2070874); IL-4Rα +1902G/A (rs1801275); IL-6 -174G/C (rs1800795) and nt565A/G (rs1800797); IL-10 -1082A/G (rs1800896), -819C/T (rs1800871), and -592A/C (rs1800872); IL-12 -1188A/C (rs3212227); TGF-*β*1 codon 10 C/T (rs1982073) and codon 25 G/C (rs1800471); TNF-α -308G/A (rs1800629) and -238G/A (rs361525); and INF-γ UTR 5644 A/T (rs2430561).

Allele, genotype, and haplotype frequencies of the above polymorphisms were also determined.

Analysis of Total Serum IgE Levels and Allergen-Specific IgE Levels

Total serum IgE and specific IgE antibodies levels against cow milk protein, egg white, grass pollen, and tree allergens of the patients were analyzed using the Immulite system (DPC Biermann, Germany). We chose to investigate grass and trees pollen as these are the most prevalent inhalant allergens in our region.

Statistical Analyses

All statistical analysis were performed using MS Excel and R software [17]. Statistical significance was set at $P<.05$. Where necessary, *P* values were adjusted using the Benjamini and Hochberg (BH) multiple correction method [18].

Statistical Analysis of Clinical Changes

The age groups (equidistant intervals, in years) were defined as follows: group 1, 0.50-1.49 years, group 2, 1.50-2.49 years, group 3, 2.50-3.49 years, group 4, 3.50-4.49 years, and group 5, 4.50–5.49 years. To test mean differences in the SCORAD index between 1) the reference age group (group 1) and the other age groups, and 2) stochastically ordered age groups (group 1 vs 2, group 2 vs 3, group 3 vs 4, and group 4 vs 5), the Mann-Whitney test for independent samples was applied.

Frequency of Cytokine Gene Polymorphisms in Patients With AD and in Healthy Donors

Allele, genotype, and haplotype frequencies of SNPs were calculated using the gene-counting method. The distributions of these frequencies in patients and healthy donors were compared using the χ^2 test. To compare the observed and expected genotype frequencies among controls, deviation from Hardy-Weinberg equilibrium (HWE) was analyzed using the χ^2 test. The associations between genotypes and haplotypes and the risk of AD were estimated by computing odds ratio (ORs) and 95% CIs from 3×2 or 4×2 contingency tables estimated using generalized linear models (GLM, binomial family, logit

link) separately for particular polymorphisms (for details, see Results), with haplotypes or genotypes as rows, and patients and controls as columns. *P* values were also adjusted using the BH multiple correction method.

Association Between IL-4Rα *and* IL-10 *Gene Polymorphisms and Changes in Clinical Status in AD Patients*

We used the χ^2 test of homogeneity (equality) for the rows in the 3×3 contingency tables for AD, AR, asthma, food allergy, total IgE, and specific IgE against egg white, cow milk, and tree and grass pollens [19] (Table not shown). The rows were represented by type of change, specifically: 1) negative to positive values; 2) no change; and 3) positive to negative values. The columns were represented by genotype, specifically the *IL-4R*α polymorphism +1902 (AA, GA, and GG) and the *IL-10* polymorphisms -1082 (AA, GA, and GG), -819 (CC, CT and TT), and -592 (AA, CA, and CC) (Table not shown). The choice of SNPs was directly related to significant genotype and haplotype results from the previous section (for details, see Results). Changes in clinical status and laboratory markers were compared by time of diagnosis (3-6 months of age and third year of life). The null hypothesis was defined as the probability distributions of +1902 (AA, GA, GG) for all types of change are equal, and the alternative hypothesis was that at least 2 probability distributions of +1902 (AA, GA, GG) would not be equal (and likewise for other polymorphisms in *IL-10*). GLM could not be used because of the numerous low frequencies.

Results

Differences Between Allele, Genotype, and Haplotype Frequencies of Cytokine Gene Polymorphisms in Patients With AD and in Healthy Controls

We investigated SNPs in 13 selected cytokines associated with immune reactions in AD patients and unrelated healthy controls. First, deviation from HWE was tested for the control group (data not shown). Most of the SNPs passed the HWE test. We observed a deviation for IL-1α -889 and IL-10 -1082 (*P*=.0015). The healthy controls—all blood donors—were not selected randomly and were free of immunologic disorders such as asthma, AR, and AD. We therefore propose that this exceptional deviation from HWE could have occurred by chance. There was insufficient justification for excluding these SNPs from the association study and we were able to use this group as a control group for children with severe AD. Comparable results have been found in studies comparing the distribution of cytokine gene SNPs in the Czech population [20] and for IL-1α -889 in the Macedonian population, where few SNPs (IL-1α -889, IL-1ß -511, IL-1ß +3962, and IFNγ UTR5644) were not in HWE [21].

The frequencies of genotypes, alleles, and haplotypes were analyzed and compared between patients and controls.

Allele Frequencies

There were no statistically significant differences between patients and controls in terms of the allele frequencies of the SNPs investigated (data not shown).

Genotype Frequencies

We found statistically significant differences between the patients and controls for the genotype frequencies of *IL-4*, *IL-4R*α, *IL-6*, and *IL-10*. The results of the genotype analysis of these SNPs are shown for the 2 groups in Table 1. The genotype frequencies of the other cytokines are not shown.

The most frequent genotypes in the patient group compared to the control group were IL-4 TT at position -1098 (88.6% in patients vs 74.5% in controls, OR=2.67, *P*=.008) and IL-4 CT at position -590 (34.16% in patients vs 19.6% in controls; OR=2.12, *P*=.013) and IL-6 GG at position -174 (46.2% in patients vs 29.1% in controls, OR=2.09, *P*=.007) and IL-6 GG at position -nt565 (47.3% in patients vs 32.0% in controls, OR=2.67, *P*=.015). These polymorphisms were associated with an increased risk of severe AD.

In contrast, we detected significantly lower frequencies of the genotypes IL-4 GT at position -1098 (11.4% in patients vs 25.5% in controls, OR=0.37, *P*=.008); IL-4 CC at position -590 (61.2% in patients vs 75.49% in controls, OR=0.52, *P*=.019); IL-6 CG at position -174 (35.5% in patients vs 51.5% in controls, OR=0.52, *P*=.013); and IL-6 AG at position -nt565 (34.4% in patients vs 52.4% in controls, OR=0.48, *P*=.006) in patients compared to healthy controls and these were associated with a reduced risk of severe AD.

The genotype IL-10 AG at position -1082 was negatively associated with severe AD (OR=0.42, *P*=.002). Interestingly, IL-10 AA at position -1082 was significantly associated with an increased risk of severe AD (OR=2.02, *P*=.019).

The heterozygous CT genotype of *IL-10* at position -819 was also found to be negatively associated with severe AD (OR=0.44, *P*=.003), while the homozygous CC genotype of *IL-10* at position -819 was significantly associated with an increased risk of severe AD (OR=1.88, *P*=.017).

The frequencies of the AA, AC, and CC genotypes of *IL-10* at position -590 were 8%, 25%, and 55% in patients and 5%, 48%, and 49% in controls, respectively. The heterozygous genotype IL-10 AG at position -590 was significantly associated with a reduced risk of AD (OR=0.45, *P*=.005). On the other hand, the genotype IL-10 CC at position -590 was significantly associated with an increased risk of severe AD (OR=1.8, *P*=.023).

Haplotype Frequencies

For the investigation of functional SNPs, we also determined haplotype frequencies of *IL-2* (-330, +166), *IL-4* (-1098, -590, and -33), *IL-6* (-174 and nt565, *IL-10* (-1082, -819, and -592, *TGF-β1* (codon 10 and 25), and *TNF*-α (-308 and -238) in patients and controls. The relative frequencies are shown in Table 2. Each patient and healthy donor had 2 haplotypes.

Significant differences in haplotype frequencies were detected only for *IL-4* and *TNF*-α. The haplotype IL-4 GC was higher in controls (12.8%) than in patients (5.62%) (OR=0.41, *P*=.01). At the same time, the haplotype TNF– AA was lower in patients (0%) than in controls (3.47%) (*P*=.01). However, 3

Table 1. Genotype Frequencies of Selected Cytokine Gene Polymorphisms in Patients With Atopic Dermatitis (AD) and Healthy Controls

			AD		Controls			
Gene	Loci	Genotype	No.	%	No.	%	OR (95%, CI)	*P* Value[b]
IL-4	-1098	G/G	0	-	0	-	-	NS
		G/T	10	11.36	26	25.49	0.37 (0.17-0.83)	.008[c]
		T/T	78	88.64	76	74.51	2.67 (1.21-5.91)	.008[c]
	-590	C/C	54	61.36	77	75.49	0.52 (0.28-0.96)	.019[c]
		C/T	30	34.09	20	19.61	2.12 (1.10-4.10)	.013[c]
		T/T	4	4.55	5	4.90	0.92 (0.24-3.55)	NS
IL-4Rα	+1902	A/A	52	59.09	55	53.92	1.23 (0.69-2.20)	NS
		A/G	32	36.36	43	42.16	0.78 (0.44-1.41)	NS
		G/G	4	4.55	4	3.92	1.17 (0.28-4.81)	NS
IL-6	-174	C/C	17	18.28	20	19.42	0.93 (0.45-1.90)	NS
		C/G	33	35.48	53	51.46	0.52 (0.29-0.92)	.013[c]
		G/G	43	46.24	30	29.13	2.09 (1.16-3.77)	.007[c]
	nt565	A/A	17	18.28	16	15.53	1.22 (0.58-2.57)	NS
		A/G	32	34.41	54	52.43	0.48 (0.27-0.85)	.006[c]
		G/G	44	47.31	33	32.04	1.19 (0.64-2.22)	.015[c]
IL-10	-1082	A/A	29	32.95	20	19.61	2.02 (1.04-3.90)	.019[c]
		A/G	43	48.86	71	69.61	0.42 (0.23-0.76)	.002[c]
		G/G	16	18.18	11	10.78	1.84 (0.80-4.21)	NS
	-819	C/C	55	62.50	48	47.06	1.88 (1.05-3.35)	.017[c]
		C/T	26	29.55	50	49.02	0.44 (0.24-0.80)	.003[c]
		T/T	7	7.95	4	3.92	2.12 (0.60-7.49)	NS
	-592	A/A	8	9.09	5	4.90	1.94 (0.61-6.16)	NS
		A/C	25	28.41	48	47.06	0.45 (0.24-0.82)	.005[c]
		C/C	55	62.50	49	48.04	1.80 (1.01-3.22)	.023[c]

Abbreviations: NS, nonsignificant; OR, odds ratio.
[a]OR AD-to-controls with 95% CI.
[b]Statistical significance, *P*<.05.
[c]Corrected for multiple testing according to Benjamini and Hochberg method [18].

IL-10 haplotypes (GCC, ACC, and ATA) were detected in both groups, but no differences in frequencies of *IL-10* haplotype distribution were detected between the groups. There were also no significant between-group differences for the other haplotype frequencies of the cytokine gene polymorphisms analyzed.

Clinical Outcome of AD Patients

Our study was designed as a prospective study with early enrolment of AD patients and a turning point at 3 years of age established as a critical period for the potential diversification of clinical outcome. As expected, the severity of AD in our series decreased significantly during childhood. The SCORAD index in the second, third, fourth, and fifth year of life were significantly decreased compared to those in the first year (*P*=.011, *P*<.001, *P*=.003, *P*<.001, respectively, Figure). At the turning point of 3 years, skin involvement was less noticeable but still prevalent in 71 patients (95.5%; SCORAD, 10-55). AD had disappeared completely in just 3 patients. During the study period, increased levels (>0.35 kU/L) of specific IgE against inhaled allergens (grass pollen and/or tree pollen) were detected in 50 children (grass pollen only, 17 patients; tree pollen only, 23 patients; and grass and tree pollen, 10 patients). Fourteen patients developed respiratory allergy, namely hay fever (n=7) and bronchial asthma (n=7); 2 children developed both asthma and AR. The cumulative results are shown in Table 3.

Association Between Polymorphisms in the Receptor Alpha Chain of IL-4 *and* IL-10 *and the Onset of Respiratory Allergy in Children With AD During Childhood*

IL-10 and *IL-4Rα* polymorphisms have been previously highlighted as associated with an asthma phenotype and elevated IgE levels [9]. We therefore focused on polymorphic variants of these cytokine genes. We further characterized the association between selected polymorphisms and the course of allergic disease.

We analyzed the relationship between the polymorphisms in *IL-4Rα* (at 1 position) and *IL-10* (at all 3 tested positions) and the process of allergy manifestation, focusing on the onset of AD and, in particular, the appearance of asthma and/or AR, and changes in total IgE and allergen-specific IgE levels. The results are shown in Table 4.

On investigating the polymorphism in *IL-4Rα* at position

Table 2. Haplotype Frequencies of Selected Cytokine Gene Polymorphisms in Patients With Atopic Dermatitis (AD) and Healthy Controls

		AD		Controls			
Gene	Haplotype	No.	%	No.	%	OR (95% CI)	*P* Value[a]
TGF-ß1	CC	8	4.49	16	7.77	0.56 (0.23-1.34)	NS
	CG	63	35.39	61	29.61	1.30 (0.85-1.99)	NS
	TG	107	60.11	129	62.62	0.90 (0.60-1.36)	NS
TNF-α	AA	0	–	7	3.47	–	.011[b]
	AG	26	14.13	27	13.37	1.07 (0.60-1.91)	NS
	GA	9	4.89	10	4.95	0.99 (0.39-2.49)	NS
	GG	149	80.98	158	78.22	1.19 (0.72-1.95)	NS
IL-2	GG	65	38.24	67	33.17	1.25(0.82-1.91)	NS
	GT	1	0.59	1	0.50	1.19 (0.07-19.16)	NS
	TG	62	36.47	72	35.64	1.04 (0.68-1.59)	NS
	TT	42	24.71	62	30.69	0.74 (0.47-1.17)	NS
IL-4	GC	10	5.62	26	12.75	0.41 (0.19-0.87)	.011[b]
	TC	129	72.47	148	72.55	1.00 (0.64-1.56)	NS
	TT	39	21.91	30	14.71	1.63 (0.96-2.75)	NS
IL-6	CA	66	35.48	85	41.26	0.78 (0.52-1.18)	NS
	CG	1	0.54	8	3.88	0.13 (0.02-1.08)	NS
	GA	0	–	1	0.49	–	NS
	GG	119	63.98	112	54.37	1.49 (0.99-2.24)	NS
IL-10	ACC	65	34.57	65	31.55	1.15(0.75-1.75)	NS
	ATA	43	22.87	54	26.21	0.84 (0.53-1.32)	NS
	GCC	80	42.55	87	42.23	1.01 (0.68-1.51)	NS

Abbreviations: NS, nonsignificant; OR, odds ratio.
[a]Statistical significance, *P*<.05.
[b]Corrected for multiple testing according to Benjamini and Hochberg method [18].

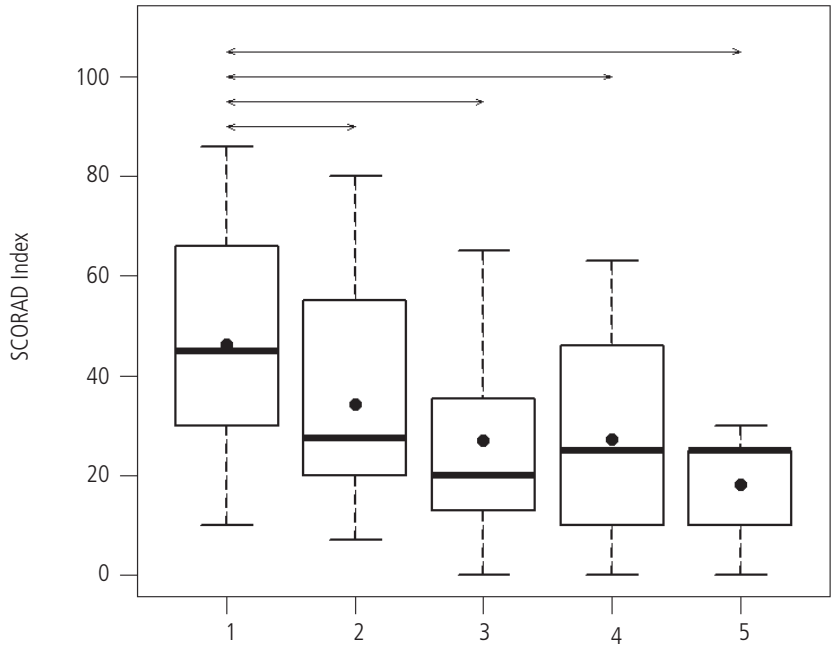

Figure. Association between patient age and SCORAD index. Box plots of SCORAD index for particular age groups (in years)—1: 0.50-1.49, 2: 1.50-2.49, 3: 2.50-3.49, 4: 3.50-4.49, and 5: 4.50-5.49. Full circles: arithmetic averages; bold solid lines: medians; *P* values of <.05 from Mann-Whitney test for independent samples were considered to be significant (*<.05, **<.01, ***<.001). SCORAD indicates SCORing atopic dermatitis.

+1902, we found a significant association ($P<.05$) between the genotypes of *IL-4Rα* and an increased level of tree pollen–specific IgE at the clinical turning point (third-fourth year of life). The AA and AG genotypes were more frequent (50% of all AA genotypes and 47.4% of all AG genotypes) in patients with increased levels of specific IgE than in patients lacking the corresponding specific IgEs (0% for both genotypes). In addition, the GG genotype was characteristic in the patient group (33.3% of all GG genotypes), with a shift from an initially positive specific tree pollen IgE level to a negative value. This particular genotype was not found (0%) in patients who were consistently negative for specific tree pollen IgE or who possessed highly positive specific IgEs at the turning point.

Moreover, the tendency towards the association of *IL-10* SNPs with allergic rhinitis (AR) was found for positions -819C/T and -592C/A ($P=.085$ and $P=.083$, respectively). In the group of patients who developed AR, the AA genotype at position -590 was more frequent than the AC and CC genotypes (60% of all AA genotypes, 4.5% of all AC genotypes, and 10% of all CC genotypes, respectively). On the other hand, in the group of patients without AR, the frequency of the AA genotype was decreased in comparison with that of the AC and CC genotypes (40% of all AA genotypes, 95.5% of all AC genotypes, and 87.5% of all CC genotypes, respectively). We obtained similar results for polymorphisms at the -819 position: the TT genotype was increased in comparison with the CC and CT genotypes (60% of all TT genotypes, 10% of all CC genotypes, and 4.5% of all CT genotypes, respectively) in the group of children with AR, while the frequencies of the CC and CT genotypes were higher than that of the TT genotype in the group of patients without AR (87.5% of all CC genotypes, 95.5% of all CT genotypes, and 40% of all TT genotypes, respectively) (data not shown). No significant differences were detected for any of the other parameters or for any other positions of *IL-10* polymorphisms.

Table 3. Changes in Clinical Status and in Immunoglobulin (Ig) E Levels in Patients (n=74) During Childhood

	Enrolment in Study No. (%)	Withdrawal From Study at 3 Years of Age No. (%)
Atopic dermatitis	74 (100)	71 (95.8)[a]
Asthma bronchiale	2 (2.7)	9 (12.5)[b]
Allergic rhinitis	1 (1.35)	9 (12.5)[b]
Specific IgE against grass pollen	3 (4.05)	27 (36.49)
Specific IgE against tree pollen	6 (8.1)	33 (44.59)[c]

[a]Atopic dermatitis disappeared in 3 patients.
[b]Two children had both asthma and rhinitis at 3 years of age
[c]One patient had positive specific IgE only during infancy (negative at 3 years of age).

Table 4. Association Between *IL-4Rα* and *IL-10* Receptor Gene Polymorphisms and Alteration of Clinical Status of Patients With Atopic Dermatitis

Permution *P* Value[a]	*IL-10*			*IL-4Rα*
	-1082A/G	-819C7T	-592A/C	+1902A/G
Atopic dermatitis	0.771	0.610	0.614	0.644
Asthma bronchiale	0.880	0.467	0.471	0.334
Allergic rhinitis	0.241	0.085 b	0.083b	0.536
Serum level of total IgE	0.970	0.661	0.646	0.289
Serum level of specific IgE against white egg	0.300	0.939	0.937	0.157
Serum level of specific IgE against cow milk	0.694	0.875	0.857	0.516
Serum level of specific IgE against grass pollen	0.506	0.661	0.644	0.664
Serum level of specific IgE against tree pollen	0.821	0.128	0.129	0.036c

Abbreviation: Ig, immunoglobulin.
[a]Statistical significance was set at $P<.05$.
[b]$P<0.1$ (Corrected for multiple testing according to Benjamini and Hochberg method) [18].
[c]$P<.05$.

Discussion

AD is a complex multifactorial disease with a strong genetic predisposition. Several studies have demonstrated a genetic linkage to AD in different populations worldwide [2].

In our association study, we focused on an array of cytokines and receptors thought to play an important role in the regulation of immune response and particularly in the pathogenesis of AD [2,6] and compared the distribution of alleles, genotypes, and haplotypes between AD patients and controls. We described the genotypes of 7 polymorphisms

of *IL-4*, *IL-6*, and *IL-10* significantly associated with AD (Table 1). A significant association was also found for *TNF*-α AA and *IL-4* GC haplotypes and AD (Table 2). Functional polymorphisms of cytokine genes and their receptor genes are among the most investigated inherited factors that might influence the occurrence and course of AD, but the results are contradictory [6]. The importance of cytokine genes in susceptibility to AD has also been highlighted by previously published genetic association studies [6,22]. These studies described the chromosomal regions that have a major importance in susceptibility to AD, including regions that contain the genes for several cytokines located on chromosome 5q31-33, such as *IL-3*, *IL-4*, *IL-5*, and *IL-13*. Associations between polymorphisms in *IL-4*, *IL-6*, and *IL-13* and extrinsic AD [23, 24], asthma [25], AR [13], and elevated levels of IgE [13] have been described. Nonetheless, a German study carried out by Reich et al [2] and an American study by Hoffjan et al [8], did not prove any association between cytokine gene polymorphisms and AD. More specifically, studies of *IL-10* polymorphisms have failed to find an association with AD in Caucasian populations [4,6]. This discrepancy may be due to the group of patients analyzed–very young infants with severe AD—or to population dependence.

In our prospective study, we followed the natural course of AD and the changes in its phenotype in the first 3 years of life. We confirmed the previously reported shift toward respiratory allergy in early infancy [26]. Asthma bronchiale and/or AR appeared in more than 20% of patients, and positivity for specific IgE against grass pollen and tree pollen was detected in 36.5% and 44.6% of the patients, respectively. AD disappeared completely in just 3 patients. We also wanted to identify the genetic background represented by a set of cytokine genes, particularly *IL-4R*α and *IL-10*, which might potentially influence the above-described outcome of an early stage of severe AD. Besides the influence on general atopic status, the genetic background including cytokine gene polymorphisms might also influence an "atopic march", ie, dynamic changes in atopic status that occur in a child's first years of life. Such an association has not yet been studied.

The main goal of our prospective study was therefore to investigate the influence of a genetic inherited background on the outcome of initially severe AD. However, the expected influence of an inherited set of cytokine genes showed a relatively mild effect on final outcome. We found an association between IL-4Rα +1902 A/G and the appearance of tree pollen-specific IgE during the first 3 years of life.

It is well known that IL-4 plays an important role in the regulation of IgE production. The IL-4Rα chain is a common component of both IL-4 and IL-13 receptors and is indispensable for the binding and signal transduction of IL-4 [27]. SNPs in this cytokine receptor have many functional consequences. An association between *IL-4R*α polymorphisms and atopy, particularly asthma, has been described as one of the few proven strong associations between cytokine gene polymorphisms and asthma [25,28] or allergic rhinitis [29,30]. Several studies have detected an association between *IL-4R*α and sensitization to pollen allergens [30], total serum IgE [31,32], and the expression of CD23 [15]. We could not confirm an association between this cytokine receptor gene polymorphism and AD in our case-control study or the clinical manifestation of inhalant allergy in our cohort of patients with AD. Nonetheless, we did observe an association between *IL-4R*α and an increased level of specific IgE against common inhalant allergens.

While the association between *IL-4* and *IL-4R*α and AD has already been studied [15,23,30], this is the first time that a positive association with the outcome of atopic march in children has been proven in a prospective study [6].

Furthermore, we identified a trend towards an association between *IL-10* polymorphisms and severe AD in patients who subsequently developed AR (Table 4). No other associations were observed. IL-10, as a cytokine with various roles in immunoregulatory processes, has attracted much attention in other studies evaluating allergic disorders [10]. Although polymorphisms in the *IL-10* gene have been shown to alter both IL-10 mRNA and protein expression, their relevance to the onset of AD or AR and asthma remains unclear [33,34]. *IL-10* SNPs at -1082, -819, and -592 are strongly linked to ethnicity [35]. Only 3 haplotypes of the promoter region have been described in the Caucasian population (GCC, ACC, and ATA) [36], coinciding with our findings. Several studies have reported an association between *IL-10* polymorphisms and atopy and/or allergic diseases. Associations between the ATA haplotype, the low *IL-10*-producing haplotype, and increased eosinophil counts in patients with asthma have been demonstrated [37]. Some studies have reported an association between the ATA haplotype and severity of asthma [9,38]. An association between allelic frequencies in *IL-10* SNPs at -1082, -819, and -592 and total serum IgE levels among AD patients [11, 39] and asthma [34] has been reported. Moreover, statistical significance has been found between IL-10 -592C>A and eosinophil cell count in asthma patients [40]. Here, we add a trend towards the development of AR in children with severe AD bearing -819C/T and -592A/C polymorphisms in the *IL-10* promoter region. Our results do not reach high statistical significance but they do support an important regulatory role of *IL-10* in allergic disorders.

In conclusion, analysis of the polymorphisms of cytokines and their receptor genes revealed a strong association between *IL-4*, *IL-6*, and *IL-10* and atopic dermatitis. An association between *IL-4* and *TNF*α haplotypes was also found.

Moreover, we have reported, for the first time, that *IL-4R*α +1902 polymorphisms and *IL-10* -819 and -592 polymorphisms are associated with an increased level of tree pollen-specific IgE and a slightly increased risk of AR in children with severe AD. *IL-4R*α and *IL-10* polymorphisms thus represent candidate markers for the prediction of potential onset of inhalant allergy in pediatric patients with AD. Further observations of our cohort would enable a more precise mapping of the associations between genetic background and clinical outcome of severe atopy in early childhood.

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