

Association of TGF- β_1 , CD14, IL-4, IL-4R and ADAM33 gene polymorphisms with asthma severity in children and adolescents

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Abstract

Objective: To verify the association of transforming growth factor- β_1 (TGF- β_1) (C-509T and T869C), CD14 (C-159T), IL-4 (C-590T), IL-4R (ILe50Val) and ADAM33 (S_2) gene polymorphisms with asthma severity in a sample of patients with mild, moderate and severe persistent atopic asthma.

Methods: A clinical, laboratory, prospective study was performed in patients with persistent atopic asthma, compared to a control group at Hospital Universitário da Universidade Estadual de Campinas between 2006 and 2007. Analysis of the TGF- β_1 T869C gene polymorphism was performed using the technique polymerase chain reaction (PCR) + amplification refractory mutation system (ARMS). TGF- β_1 C-509T, CD14 C-159T, IL-4 C-590T, IL-4Ra ILe50Val, and ADAM33 S2 gene polymorphisms were detected by PCR and restriction enzyme.

Results: This study included 88 patients with persistent atopic asthma (27 mild, 23 moderate and 38 severe) and 202 healthy blood donors. As to T869C polymorphism (TGF- β_1), there was an association between the CC genotype and patients with severe asthma. There was no association in polymorphisms C-509T (TGF- β_1), C-590T (IL-4) and S_2 (ADAM33). When distribution of C-159T polymorphism genotype frequency (CD14) in severe asthma was compared with the control group, there was a significant result with the TT genotype. There was significant association of the Val/Val genotype (IL-4R) with mild asthma.

Conclusion: Our results indicate that T869C (TGF- β_1), C-159T (CD14) and Val/Val (IL-4R) polymorphisms might be involved in modulation of asthma severity.

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Introduction

Asthma is the most common chronic disease in childhood and adolescence. It is caused by genetic and environmental factors, and many genes have been identified in its pathogenesis.¹

Some studies, also including twins,² have shown that a number of genes and their polymorphisms influence immune

and pulmonary development and response to environmental factors, contributing to asthma occurrence and/or severity. The transforming growth factor- β_1 (TGF- β_1) is located on chromosome 19q13.^{3,4}

Studies on the association of TGF- β_1 C-509T and T869C gene polymorphisms with risk of asthma are controversial; some found a positive association,⁵⁻⁸ whereas in others such

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association is negative,^{9,10} with high levels of TGF- β_1 , plasma immunoglobulin E (IgE) and higher risk of asthma.

TGF- β_1 protein is a multifunctional cytokine that is increased in the bronchial lavage fluid of asthmatics when compared with that of nonasthmatic individuals.^{11,12} It is important in growth, transformation, tissue repair, fibrosis and modulation of immune inflammatory responses.¹³ Its function in asthma is not completely known yet.

The CD14 gene is located on chromosome 5q. The C-159T polymorphism has been associated with changes in CD14 and IgE levels in many populations of different ethnicities.^{14,15}

CD14 is a multifunctional receptor expressed in the surface of monocytes, macrophages and neutrophils or serum soluble.¹⁶ It is the main receptor of lipopolysaccharides (LPS) or inhaled endotoxins, which are potent inducers of pulmonary inflammation and may activate the immune system and cause Th1 differentiation and/or Th2 suppression.¹⁷

It has been proposed that altered CD14 expressions, more increased in asthmatics after LPS inhalation,¹⁸ can change the balance of Th1-Th2 cells, influencing IgE levels and inflammation in allergic diseases such as asthma.¹⁴

Thus, changes in CD14 expression seem to be important, especially in allergic asthma, and such expression is regulated, at least partially, by the gene.

The interleukin 4 (IL-4) gene, located on chromosome 5q31, has also been associated with atopy. IL-4 is the main cytokine responsible for change in B lymphocyte from immunoglobulin M (IgM) to IgE.¹⁹

Nucleotide replacement (C-T) in position -590 of the IL-4 gene promoting region is present in approximately 27% of Caucasians. The IL4-590T allele was associated with increased expression of *in vitro* gene and with higher levels of *in vivo* IgE.²⁰ The IL4-590T was associated with asthma, rhinitis and atopy in a study including children at risk for allergic diseases.¹⁹ That allele has also been associated with low values of forced expiratory volume in 1 second (FEV₁) in a Caucasian population with asthma.²¹ These data suggest that the C-590T polymorphism could influence asthma severity.

The IL-4 receptor gene (IL-4R) is located on chromosome 16 p12.1-p11.2, a region associated with increase in IgE responses. It plays a major role in allergic inflammation through its function of signaling IL-4 and IL-13.²²

Many single nucleotide polymorphisms (SNP), identified in the coding region of that gene, were associated with asthma and atopy.^{23,24} The Ile50Val polymorphism has been extensively studied and associated with IgE levels and atopic asthma²³; however, not all associations were replicated in other populations.^{25,26}

The disintegrin and metalloprotease 33 gene (ADAM33) is located on chromosome 20p13, and 37 SNP were initially identified.²⁷ Since the first report of association between

ADAM33 polymorphisms and asthma in two Caucasian populations in the United Kingdom and the USA, a large number of studies has been published, showing different results. Various associations between different asthma phenotypes, as well as bronchial hyperresponsiveness (BHR) and different SNP in the gene, have been demonstrated.²⁸⁻³⁰

ADAM33 was the first gene identified by positional cloning to be published as a possible candidate for the development of asthma and BHR.²⁷ The ADAM33 protein is expressed in smooth muscle cells of bronchia and pulmonary fibroblasts, playing a major role in airway remodeling.³¹

Asthma has been described as a heterogeneous group of syndromes, but few advances have occurred to distinguish the varied clinical phenotypes from the genetic-molecular perspective.

This study aimed at verifying the association of TGF- β_1 (C-509T and T869C), CD14 (C-159T), IL-4 (C-590T), IL-4R (Ile50Val) and ADAM33 (S_2) gene polymorphisms with asthma severity.

Methods

A clinical, laboratory and prospective study was carried out at the departments of genetics and pediatrics (sector of pediatric pulmonology) at Faculdade de Ciências Médicas da Universidade Estadual de Campinas (UNICAMP) in 2006-2007.

This study was evaluated and approved by the ethics committee of that institution (protocol 172/2006), and all patients signed an informed consent term before starting the study.

Children and adolescents of both genders and with persistent atopic asthma (PAA) (mild, moderate and severe) were included.

The patients were diagnosed according to degree of asthma severity based on the criteria of the Global Initiative for Asthma³² (GINA), and all had spirometric evidence of response to use of beta-agonist drugs, positive reactivity in skin tests of immediate hypersensitivity to inhaling antigens and increase in IgE serum levels. The DNA was extracted from peripheral blood cells, according to the traditional method of phenol/chloroform.³³

Methods, primer sequence and restriction enzymes, as well as size of fragments generated by TGF- β_1 , CD14, IL-4, IL-4R and ADAM33 gene polymorphisms,³⁴⁻³⁷ are described in Table 1.

Statistical analysis

Analyses of the associations between asthmatic patients and the control group were performed by Pearson's chi-square test, and in some analyses, due to the value of cases, genotypes were aggregated and Fisher's exact test was used. Odds ratio (OR) was calculated by Fisher's exact test. Difference between groups was statistically significant when the test

Table 1 - Description of methods, primer sequence, restriction enzymes and size of fragments generated by TGF- β_1 , CD14, IL-4, IL-4R and ADAM33 gene polymorphisms

Polymorphisms	Method	Primers	Amplified fragment (pb)	Restriction enzymes and fragments (pb)	Reference
TGF- β_1 gene (T869C)	PCR-ARMS	General primer (sense): 5'-tccgtgggatactgagacac-3'	241		34
		C primer (antisense): 5'-gcagcggtagcagcagcg-3'			
		T primer (antisense): 5'-agcagcggtagcagcagca-3'			
(C-509T)	PCR	Internal control primer 1: 5'-gcctccaaccattccctta-3'	429		34
		Internal control primer 2: 5'-tcacggattctgtgtgttc-3'			
CD14 gene (C-159T)	PCR + RE	5'-ccgcttctgtcctttctagg-3'			6
		5'-aaagcgggtgatccagatg-3'	406	Eco 81I (SauI), 223 and 183	
IL-4 gene (C-590T)	PCR + RE	5'-gtccaacagatgaggttcac-3'			35
		5'-gcctctgacagttttatgtaatc-3'	497	AvaII, 144 and 353	
IL-4R gene (Ile50Val)	PCR + RE	5'-taaacttgggagaacatggt-3'			36
		5'-tggggaaagatagagtaata-3'	195	AvaII, 177 and 19	
ADAM33 gene (S_2)	PCR + RE	5'-ggcaggtgtgaggagcatcc-3'			36
		5'-gcctccgtgttctcagga-3'	273	RsaI, 254 and 19	
ADAM33 gene (S_2)	PCR + RE	5'-cgagaccatgacaccttct-3'			37
		5'-gcagcagggtcccttct-3'	148	Hinp I, 63 and 85	

ARMS = amplification refractory mutation system; PCR = polymerase chain reaction; RE = restriction enzyme.

value was $p < 0.05$. Epi-Info, version 1.1.2 was the statistical software used.

Results

A total of 88 asthmatic children and adolescents were assessed: 53% girls, 7-18 years, mean age of 10.3 ± 2.79 years and ethnic group comprised of 92% Caucasians, 7% mulattos and 1% Negroids. The control group was composed of 202 individuals (mean age 34 ± 11.3 years), blood donors, 79.2% Caucasians, 20.3% Negroids and 0.5% of Eastern ethnicity.

Among patients with PAA, there were 27 mild, 23 moderate and 38 severe (Table 2).

As to the T869C polymorphism (TGF- β_1), the sample of asthmatic patients was in Hardy-Weinberg equilibrium (HWE)

($\chi^2_{(2)} = 2.83$; $p = 0.24$), which did not occur with the control sample ($\chi^2_{(2)} = 10.58$; $p = 0.005$). There was no difference in genotype distribution between asthmatics and the control group ($\chi^2_{(2)} = 0.67$; $p = 0.71$), as well as between groups of variable severity. However, comparison of each severity group with the control group showed significant difference in relation to severe asthma and the control group ($\chi^2_{(2)} = 9.70$; $p = 0.007$). Such difference was due to an accumulation of individuals with CC genotype in severe asthmatics in relation to the control group.

Therefore, the CC genotype could be a severity factor for asthma ($\chi^2 = 7.80$; $p = 0.005$; OR = 2.99, 1.25-7.09).

For the TGF- β_1 C-509T gene polymorphism, the sample of asthmatic patients was in HWE ($\chi^2_{(2)} = 0$; $p = 1$), as well as

Table 2 - Genotype distribution of polymorphisms: T869C, C-509T (TGF- β_1), C-159T (CD14), Ile50Val (IL-4Ra), C-590T (IL-4) and S_2 (ADAM33) in asthmatic patients and controls

Genotypes	Mild asthma (n = 27)	Moderate asthma (n = 23)	Severe asthma (n = 38)	Control (n = 202)
TGF- β_1 (T869C)				
TT	4 (14.8%)	4 (17.4%)	10 (26.3%)	43 (21.3%)
TC	21 (77.7%)	18 (78.2%)	16 (42.1%)	132 (65.3%)
CC	2 (7.4%)	1 (4.3%)	12 (31.6%)	27 (13.4%)
Total	27	23	38	202
TGF- β_1 (C-509T)				
CC	12 (44.4%)	6 (26%)	14 (36.9%)	58 (28.7%)
CT	13 (48.1%)	12 (52.2%)	17 (44.7%)	112 (55.4%)
TT	2 (7.4%)	5 (21.7%)	7 (30.4%)	32 (15.8%)
Total	27	23	38	202
CD14 (C-159T)				
TT	4 (14.8%)	4 (17.4%)	12 (31.6%)	8 (4%)
TC	16 (59.2%)	11 (47.8%)	14 (36.8%)	131 (64.8%)
CC	7 (26%)	8 (34.8%)	12 (31.6%)	63 (31.2%)
Total	27	23	38	202
IL-4Ra (Ile50Val)				
Val/Val	9 (33.3%)	5 (21.7%)	2 (5.2%)	39 (19.3%)
Val/Ile	14 (51.9%)	13 (56.5%)	25 (65.8%)	96 (47.5%)
Ile/Ile	4 (14.8%)	5 (21.7%)	11 (29%)	67 (33.2%)
Total	27	23	38	202
IL-4 (C-590T)				
CC	9 (33.3%)	10 (43.5%)	19 (50%)	67 (33.1%)
CT	13 (48.1%)	11 (47.8%)	17 (44.7%)	108 (53.5%)
TT	5 (18.5%)	2 (8.7%)	2 (5.3%)	27 (13.4%)
Total	27	23	38	202
ADAM33 (S_2)				
CC	5 (18.5%)	1 (4.3%)	5 (13.2%)	11 (5.4%)
CG	9 (33.3%)	12 (52.1%)	17 (44.7%)	136 (67.3%)
GG	13 (48.1%)	10 (43.5%)	16 (42.1%)	55 (27.2%)
Total	27	23	38	202

the control sample ($\chi^2_{(2)} = 1.79$; $p = 0.41$). The CT and TT genotypes were grouped when showing value < 5 for statistical analysis. There were no significant differences as to asthma presence and severity.

As to the C-159T polymorphism (CD14), the sample of asthmatic patients was in HWE ($\chi^2_{(2)} = 0.21$; $p = 0.90$), which did not occur with the control sample ($\chi^2_{(2)} = 18.72$; $p = 0.00008$). Genotype distribution according to severity group did not show statistical difference.

When genotype distribution of severe asthma was compared with the control group, there was a significant result

with the accumulation of TT genotype in relation to severe asthma ($\chi^2_{(2)} = 44.02$; $p = 0.000000$). It was verified that the TT genotype represented a risk factor for severe asthma ($\chi^2 = 31.94$; $p = 0.000$; OR = 11.19, 3.74-34.24).

As to the Ile50Val polymorphism (IL-4R), the sample of patients ($\chi^2_{(2)} = 1.47$; $p = 0.47$) and controls ($\chi^2_{(2)} = 0.11$; $p = 0.94$) was in HWE. When genotype distribution was performed according to asthma severity, in comparison between mild and severe asthma, there was significant statistical difference with increase of patients with the Val/Val genotype in

mild asthmatics. The Val/Val genotype represented a protection factor for asthma severity ($\chi^2_{(2)} = 8.85$; $p = 0.004$; OR = 9.0, 1.54-90.89).

As to the C-590T polymorphism (IL-4), the sample of patients ($\chi^2_{(2)} = 0.06$; $p = 0.96$) and controls ($\chi^2_{(2)} = 1.27$; $p = 0.53$) was in HWE, and there were no significant differences as to asthma presence and severity.

As to the S-2 polymorphism (ADAM33), the sample of asthmatic patients was in HWE ($\chi^2_{(2)} = 0.11$; $p = 0.94$), which did not occur with the control sample ($\chi^2_{(2)} = 19.50$; $p = 0.00058$). When genotype distribution was compared between severity groups, there were no changes.

Discussion

This is the first Brazilian study verifying the association of TGF- β_1 (C-509T and T869C), CD14 (C-159T), IL-4 (C-590T), IL-4R (ILe50Val) and ADAM33 (S_2) gene polymorphisms with asthma severity.

Asthma can be said to be a complex genetic disease in which there are multiple genetic effects interacting with the environment to modify susceptibility and severity of this disease.³⁸

In some polymorphisms, the control sample was not in HWE. It is believed that this is due to the fact that, in the Law, an ideal population is recommended, and our control sample was comprised of blood donors, among whom only healthy individuals are selected. With regard to the polymorphisms under investigation, since they interfere with mechanisms related to inflammation by using blood donors, individuals who have unfavorable genotypes will be excluded. That results in use of a more homogeneous and antagonistic population in phenotypical terms in relation to the asthmatic population and, therefore, small changes could be better identified. Anyhow, more value was given to results found between severity groups.

Pulleyn et al.⁵ found an association between the TGF- β_1 C-509T gene polymorphism and asthma severity, and there was significant difference in frequency of C-509T genotypes when the mild and severe group and controls were compared. There was a high level of homozygotes for the -509T allele in the severe group. Almost twice more severe asthmatics (1.8 times) had the TT genotype, when compared to asthmatics in the mild group, and such difference increased to 5.6 times when compared to controls. A significant difference was also present when only the mild and severe groups were compared. These results suggest that this allele is more associated with severity than with asthma or atopy onset, in contrast with studies in which polymorphisms have been compared between asthmatics and controls, as in IL-13.³⁹

There is evidence that TGF- β_1 C-509T and T869C gene polymorphisms are associated with gene transcriptional activity. The C-509T polymorphism, in homozygosis, was associated with low levels of circulating TGF- β_1 concentration, when

compared with other genotypes, in white women and in the Indian population.³ On the other hand, the CC genotype of T869C polymorphism was related to a high concentration of TGF- β_1 in Japanese individuals,⁴⁰ but not in white individuals.⁴¹ In another study, Li et al.⁸ verified that the C-509T and T869C polymorphisms increased risk of asthma and atopy in Mexican children.

It has been suggested that increase in TGF- β_1 expression should be harmful to the airways of asthmatic individuals, stimulating their remodeling. About that paradigm, genetic variations associated with high levels of TGF- β_1 , such as the T allele of C-509T and the C allele of T869C, should be associated with high prevalence or increase in asthma severity.⁷

The TGF- β_1 T869C polymorphism, in our sample, showed an association between CC genotype and patients with severe asthma. There was no significant difference between the total group of asthmatics and the healthy control group regarding CC genotype. However, Mak et al.,⁷ studying 250 Chinese patients with asthma, showed that individuals with CC genotype were more susceptible to asthma. As to the C-509T polymorphism, there was no association in our study.

The CD14 C-159T gene polymorphism has been associated with increased *in vitro* CD14 expression and in the serum of children,¹⁴ with altered IgE serum levels and positive allergic tests in different populations.^{14,15} Kedda et al.³⁵ confirmed associations between C-159T and atopy, but did not find association between this polymorphism and asthma or asthma severity in an Australian population.

It is possible that the C-159T polymorphism has an influence related to age in the development of atopy. Such polymorphism has been associated with increased expression of CD14 in the serum of children, and there are no differences in CD14 levels in the serum of adult blood donors with different genotypes for CD14.³⁵ In addition, a longitudinal study including white individuals from Australia aged between 8-25 years showed that those with the CC genotype were probably the same that had earlier atopy, suggesting that the influence of the -159C polymorphism in atopy could be age specific.⁴² There is evidence that atopy and asthma are close entities and relates to a positive association between IgE serum level, positive allergic tests, hyperresponsiveness and development of asthma.⁴³ Therefore, it is expected that alleles associated with atopy have a higher prevalence in asthmatics than in the general population.¹⁵ There are no publications showing association between the C-159T polymorphism and asthma severity, although it has been suggested that this polymorphism changes severity of air flow obstruction in asthmatic individuals.³⁵

In the present study, when genotype distribution of the C-159T polymorphism in severe asthma was compared with the control group, there was a significant result with the TT genotype in relation to severe asthma. It was verified that the TT genotype represented a risk factor for severe asthma.

In the IL-4 C-590T gene polymorphism, its variant is related to increased gene transcription. Incidence of such polymorphism in a study including an American population was 40%, and the T allele was associated with increased IgE production, positive allergic tests and asthma.²⁰ Such association was not found in Australian, British⁴⁴ and Italian individuals.⁴⁵ Studies including Japanese individuals confirmed the associations of that polymorphism with asthma²⁵ and atopic dermatitis.²⁰ That polymorphism was also related to atopy²⁰ and decreased pulmonary function.⁴⁴ Kamali-Sarvestani et al.,⁴⁶ when analyzing the C-590T polymorphism in Iranian asthmatics, verified that such polymorphism was associated with asthma and could modulate its severity.

IL-4 performs its activities through ligation with its receptor (IL-4R). In the IL-4Ra Ile50Val gene polymorphism, the variant Ile50 was associated with atopic asthma and had no association with nonatopic asthma in Japanese individuals. Conversely, Noguchi et al.²⁵ verified that the Ile50Val polymorphism does not play a role in genetic predisposition of atopy or asthma etiology in another Japanese population. Other studies did not find association between this polymorphism and asthma in other ethnic groups.^{26,47,48} Zhang et al.,⁴⁹ when investigating an association between the Ile50Val polymorphism and asthma and high IgE levels in three Asian populations from China, Malaysia and India, verified that in China the prevalence of the Ile50/Ile50 genotype was significantly lower than in Malaysia, and that genotype is related to increased IgE only in Malaysia. That study showed that the association between that polymorphism and asthma differs in those ethnic groups. Howard et al.⁵⁰ analyzed IL-4R gene polymorphisms in families in Germany and did not find evidence that the Ile50Val is the main responsible for susceptibility to allergy, suggesting that other IL-4R polymorphisms are associated with asthma and atopy.

As to the IL-4 C-590T gene polymorphism, there was no association in our study. As to the IL-4R Ile50Val gene polymorphism, it was verified that the Val/Val genotype represented a protection factor for asthma severity.

Hirota et al.⁵¹ found a significant association with asthma susceptibility in a Japanese population for three SNP (S₂, T₁, T₂). These results are consistent with another study in a Caucasian population in the USA.⁵²

Jongepier et al.²⁸ investigated genetic and environmental factors that could have contributed for such accelerated decline in 200 patients with chronic asthma, who were annually studied for 25 years. Those authors showed that the polymorphism variant of S₂ allele was significantly associated with decline in FEV₁ values and concluded that this ADAM33 variant was not only important in asthma development, but also in disease progression and increase in airway remodeling.

Simpson et al.⁵³ have recently verified that ADAM33 polymorphisms could influence pulmonary function in childhood

and concluded that, in early life, it is genetically modified and may increase risk of asthma. In this study, there was no association regarding ADAM33 and allergy.

In the present study, when genotype distribution of the S₂ polymorphism was compared between severity groups, there were no changes.

It is likely that different results can be found in other populations or race groups. Since ethnic and race differences are common in polymorphic systems, variant alleles or interacting gene groups induce expression of clinical phenotype in different populations.

Therefore, in our study, the TGF- β ₁ T869C, CD14 C-159T, and IL-4R Ile50Val gene polymorphisms can be modulators of asthma severity. Although our study has included several genes and their polymorphisms and has showed that distinct genotypes can have different clinical expressions, other studies including larger populations may clarify the actual role of genetic polymorphisms in varied degrees of asthma severity.

References

- Sandford A, Weir T, Paré P. [The genetics of asthma](#). *Am Resp Crit Care Med*. 1996;153:1749-65.
- Sarafino EP, Goldfeder J. [Genetic factors in the presence, severity, and triggers of asthma](#). *Arch Dis Child*. 1995;73:112-6.
- Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, et al. [Genetic control of the circulating concentration of transforming growth factor type \$\beta\$ 1](#). *Hum Mol Genet*. 1999;8:93-7.
- Watanabe Y, Kinoshita A, Yamada T, Ohta T, Kishino T, Matsumoto N, et al. [A catalog of 106 single-nucleotide polymorphisms \(SNPs\) and 11 other types of variations in genes for transforming growth factor- \$\beta\$ 1 \(TGF- \$\beta\$ 1\) and its signaling pathway](#). *J Hum Genet*. 2002;47:478-83.
- Pulleyn LJ, Newton R, Adcock IM, Barnes PJ. [TGF- \$\beta\$ 1 allele association with asthma severity](#). *Hum Genet*. 2001;109:623-7.
- Silverman ES, Palmer LJ, Subramaniam V, Hallock A, Mathew S, Vallone J, et al. [Transforming growth factor- \$\beta\$ 1 promoter polymorphism C-590T is associated with asthma](#). *Am J Respir Crit Care Med*. 2004;169:214-9.
- Mak JC, Leung HC, Ho SP, Law BK, Ho AS, Lam WK, et al. [Analysis of TGF-beta \(1\) gene polymorphisms in Hong Kong Chinese patients with asthma](#). *J Allergy Clin Immunol*. 2006;117:92-6.
- Li H, Romieu I, Wu H, Sienra-Monge JJ, Ramírez-Aguilar M, del Rio-Navarro BE. [Genetic polymorphisms in transforming growth factor beta-1 \(TGFB1\) and childhood asthma and atopy](#). *Hum Genet*. 2007;121:529-38.
- Buckova D, Izakovicová HL, Benes P, Znojil V, Vácha J. [TGF-beta1 gene polymorphisms](#). *Allergy*. 2001;56:1236-7.
- Heinzmann A, Bauer E, Ganter K, Kurz T, Deichmann KA. [Polymorphisms of the TGF-beta1 gene are not associated with bronchial asthma in Caucasian children](#). *Pediatr Allergy Immunol*. 2005;16:310-4.

11. Minshall EM, Leung DY, Martin RJ, Song YL, Cameron L, Ernst P, et al. [Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma](#). *Am J Respir Cell Mol Biol*. 1997;17:326-33.
12. Redington AE, Madden J, Frew AJ, Djukanovic R, Roche WR, Holgate ST, et al. [Transforming growth factor-beta1 in asthma. Measurement in bronchoalveolar lavage fluid](#). *Am J Respir Crit Care Med*. 1997;156:642-7.
13. Blobe GC, Schiemann WP, Lodish HF. [Role of transforming growth factor beta in human disease](#). *N Engl J Med*. 2000;342:1350-8.
14. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. [A polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E](#). *Am J Respir Cell Mol Biol*. 1999;20:976-83.
15. Koppelman GH, Reijmerink NE, Colin Stine O, Howard TD, Whittaker PA, Meyers DA, et al. [Association of a promoter polymorphism of the CD14 gene and atopy](#). *Am J Respir Crit Care Med*. 2001;163:965-9.
16. Ulevitch RJ, Tobias PS. [Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin](#). *Annu Rev Immunol*. 1995;13:437-57.
17. Macatonia SE, Hosken NA, Litton M, Vieira P, Hsieh CS, Culpepper JA, et al. [Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+T cells](#). *J Immunol*. 1995;154:5071-9.
18. Alexis N, Eldridge M, Reed W, Bromberg P, Peden DB. [CD14-dependent airway neutrophil response to inhaled LPS: role of atopy](#). *J Allergy Clin Immunol*. 2001;107:31-5.
19. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, et al. [Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations](#). *Science*. 1994;264:1152-6.
20. Rosenwasser LJ, Klemm DJ, Dresback JK, Inamura H, Mascali JJ, Klinnert M, et al. [Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy](#). *Clin Exp Allergy*. 1995;25 Suppl 2:74-8.
21. Burchard EG, Silverman EK, Rosenwasser LJ, Borish L, Yandava C, Pillari A, et al. [Association between a sequence variant in the IL4 gene promoter and FEV \(1\) in asthma](#). *Am J Respir Crit Care Med*. 1999;160:912-22.
22. Daniels SE, Bhattacharya S, James A, Leaves NI, Young A, Hill MR, et al. [A genome-wide search for quantitative trait loci underlying asthma](#). *Nature*. 1996;383:247-50.
23. Mitsuyasu H, Izuwara K, Mao XQ, Gao PS, Arinobu Y, Enomoto T, et al. [Ile50Val variant of IL4R alpha upregulates IgE synthesis and associates with atopic asthma](#). *Nat Genet*. 1998;19:119-20.
24. Kruse S, Japha T, Tedner M, Sparholt SH, Forster J, Kuehr J, et al. [The polymorphisms S503P and Q576R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction](#). *Immunology*. 1999;96:365-71.
25. Noguchi E, Shibasaki M, Arinami T, Takeda K, Yokouchi Y, Kobayashi K, et al. [No association between atopy/asthma and the IL50Val polymorphism of IL-4 receptor](#). *Am J Respir Crit Care Med*. 1999;160:342-5.
26. Migliaccio C, Patuzzo C, Malerba G, Trabetti E, Galavotti R, Pescolliderung L, et al. [No linkage or association of five polymorphisms in the interleukin-4 receptor alpha gene with atopic asthma in Italian families](#). *Eur J Immunogenet*. 2003;30:349-53.
27. Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, et al. [Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness](#). *Nature*. 2002;418:426-30.
28. Jongepier H, Boezen HM, Dijkstra A, Howard TD, Vonk JM, Koppelman GH, et al. [Polymorphisms of the ADAM33 gene are associated with accelerated lung function decline in asthma](#). *Clin Exp Allergy*. 2004;34:757-60.
29. Raby BA, Silverman EK, Kwiatkowski DJ, Lange C, Lazarus R, Weiss ST. [ADAM33 polymorphisms and phenotype associations in childhood asthma](#). *J Allergy Clin Immunol*. 2004;113:1071-8.
30. Werner M, Herbon N, Gohlke H, Altmüller J, Knapp M, Heinrich J, et al. [Asthma is associated with single-nucleotide polymorphisms in ADAM33](#). *Clin Exp Allergy*. 2004;34:26-31.
31. Postma DS, Howard T. [ADAM33 gene: confirming a gene without linkage](#). *Clin Exp Allergy*. 2004;34:1-3.
32. GINA Workshop Report. Global strategy for asthma Management and Prevention. [updated 2002 April; cited 2003 Oct 18]. Scientific information and recommendations for asthma programs. NIH Publication N° 02-3659. <http://www.ginasthma.com>.
33. Woodhead JL, Fallon R, Figuered H, Longdale J, Malcon ADB. Alternative methodology of gene diagnosis. In: Davies KE, editor. *Human Genetic diseases - a practical approach*. Oxford: IRL Press; 1986. p. 51-64.
34. Perrey C, Turner SJ, Pravica V, Howell WM, Hutchinson IV. [ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta, and TGF-beta 1 gene polymorphisms](#). *Transpl Immunol*. 1999;7:127-8.
35. Kedda MA, Lose F, Duffy D, Bell E, Thompson PJ, Upham J. [The CD14 C-159T polymorphism is not associated with asthma or asthma severity in an Australian adult population](#). *Thorax*. 2005;60:211-4.
36. Hoebee B, Rietveld E, Bont L, Oosten M, Hodemaekers HM, Nagelkerke NJ, et al. [Association of severe respiratory syncytial virus bronchiolitis with interleukin-4 and interleukin-4 receptor alpha polymorphisms](#). *J Infect Dis*. 2003;187:2-11.
37. van Diemen CC, Postma DS, Vonk JM, Bruinenberg M, Schouten JP, Boezen HM. [A disintegrin and metalloprotease 33 polymorphisms and lung function decline in the general population](#). *Am J Respir Crit Care Med*. 2005;172:329-33.
38. Holloway JW, Beghe B, Holgate ST. [The genetic basis of atopic asthma](#). *Clin Exp Allergy*. 1999;29:1023-32.
39. Heinzmann A, Mao XQ, Akaiwa M, Kreomer RT, Gao PS, Ohshima K, et al. [Genetic variants of IL-13 signalling and human asthma and atopy](#). *Hum Mol Genet*. 2000;9:549-59.
40. Yamada Y, Miyauchi A, Goto J, Takagi Y, Okuizumi H, Kanematsu M, et al. [Association of a polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women](#). *J Bone Miner Res*. 1998;13:1569-76.
41. Perrey C, Pravica V, Sinnott PJ, Hutchinson IV. [Genotyping for polymorphisms in interferon-gamma, interleukin10, transforming growth factor-beta1 and tumor necrosis factor-alpha genes: a technical report](#). *Transpl Immunol*. 1998;6:193-7.
42. O'Donnell AR, Toelle BG, Marks GB, Hayden CM, Laing IA, Peat JK, et al. [Age-specific relationship between CD14 and atopy in a cohort assessed from age 8 to 25 years](#). *Am J Respir Crit Care Med*. 2004;169:615-22.
43. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. [Association of asthma with serum IgE levels and skin-test reactivity to allergens](#). *N Engl J Med*. 1989;320:271-7.

44. Walley AJ, Cookson WO. [Investigation of an interleukin-4 promoter polymorphism for associations with asthma and atopy.](#) *J Med Genet.* 1996;33:689-92.
45. Rigoli L, Di Bella C, Procopio V, Barberio G, Barberi I, Caminiti L, et al. [Molecular analysis of sequence variants in the Fcepsilon receptor I beta gene and IL-4 gene promoter in Italian atopic families.](#) *Allergy.* 2004;59:213-8.
46. Kamali-Sarvestani E, Ghayomi MA, Nekoei A. [Association of TNF-alpha -308 G/A and IL-4 -589 C/T gene promoter polymorphisms with asthma susceptibility in the south of Iran.](#) *J Investig Allergol Clin Immunol.* 2007;17:361-6.
47. Zhang H, Zhang Q, Wang L, Chen H, Li Y, Cui T, et al. [Association of IL4R gene polymorphisms with asthma in Chinese populations.](#) *Hum Mutat.* 2007;28:1046.
48. Liu X, Beaty TH, Deindl P, Huang SK, Lau S, Sommerfeld C, et al. [Associations between specific serum IgE response and 6 variants within the genes IL4, IL13, and IL4RA in German children: the German Multicenter Atopy Study.](#) *J Allergy Clin Immunol.* 2004;113:489-95.
49. Zhang W, Zhang X, Qiu D, Sandford A, Tan WC. [IL-4 receptor genetic polymorphisms and asthma in Asian populations.](#) *Respir Med.* 2007;101:186-90.
50. Howard TD, Koppelman GH, Xu J, Zheng SL, Postma DS, Meyers DA, et al. [Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma.](#) *Am J Hum Genet.* 2002;70:230-6.
51. Hirota T, Hasegawa K, Obara K, Matsuda A, Akahoshi M, Nakashima K. [Association between ADAM33 polymorphisms and adult asthma in the Japanese population.](#) *Clin Exp Allergy.* 2006;36:884-91.
52. Howard TD, Postma DS, Jongepier H, Moore WC, Koppelman GH, Zheng SL, et al. [Association of a disintegrin and metalloprotease 33 \(ADAM33\) gene with asthma in ethnically diverse populations.](#) *J Allergy Clin Immunol.* 2003;112:717-22.
53. Simpson A, Maniatis N, Jury F, Cakelbread JA, Lowe LA, Holgate ST, et al. [Polymorphisms in a disintegrin and metalloprotease 33 \(ADAM33\) predict impaired early-life lung function.](#) *Am J Resp Crit Care Med.* 2005;172:55-60.

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