

Polymorphisms in the *IL18* gene are associated with specific sensitization to common allergens and allergic rhinitis

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Background: Atopy has been linked to chromosome 11q22, a region that harbors the *IL18* gene. IL-18 enhances IL-4/IL-13 production and induces IgE production that is directly associated with the pathogenesis of atopic disorders.

Objective: We sought to investigate whether genetic abnormalities in the regulatory regions of the *IL18* gene predispose, in part, to susceptibility to atopy.

Methods: Among a white population of 105 families, the oldest child was examined with regard to atopic phenotypes and single-nucleotide polymorphisms (SNPs) within the *IL18* gene.

Results: We have identified 5 novel SNPs in the *IL18* gene (–920[t/c], –133[c/g], and –132[a/g] in promoter 2 [upstream of exon 2]; +179[c/a; Ser35Ser] in exon 4; and +486[c/t; Phe137Phe] in exon 6). Three SNPs are located in promoter 2, and one (–133[c/g]; nuclear factor 1 site) was significantly associated with high serum IgE levels ($P = .001$; odds ratio, 3.96) and specific sensitization to common allergens ($P = .005$; OR, 4.12). In addition, previously identified SNPs in exon 1 (+113[t/g] and +127[c/t]) and in promoter 1 (–137[g/c], GATA3 site) of the *IL18* gene were significantly associated with high IgE levels ($P \leq .005$; OR, 3.27–3.90) and specific sensitization ($P = .02$ to $.008$; OR, 3.27–3.83). The SNP +127(g/t) in exon 1 was also a susceptibility locus for seasonal allergic rhinitis ($P = .008$; OR, 3.22).

Conclusion: *IL18* might be responsible for the linkage effects seen in the chromosomal region 11q22, which has been found previously with the phenotype “sensitization to mite allergen.” Thus a suspected direct role of *IL18* in the pathogenesis of atopy has been strengthened by the presence of 8 common SNPs in the promoter regions of *IL18*. (J Allergy Clin Immunol 2003;111:117–22.)

Key words: *IL-18*, IgE, atopy, allergy, sensitization, rhinitis, polymorphisms

Abbreviations used

NF: Nuclear factor
OR: Odds ratio
SAR: Seasonal allergic rhinitis
SNP: Single-nucleotide polymorphism

Atopy is characterized by the presence of specific IgE antibodies to common allergens in individuals with a genetic predisposition who respond with immediate hypersensitivity on exposure to specific allergens. Atopy affects up to 40% of the population in Western societies and underlies the development of allergic diseases, such as asthma, allergic rhinitis, and atopic dermatitis. Although environmental factors play important roles in the development of atopy, there is a strong genetic predisposition in atopic subjects.¹

A number of whole-genome scans have recently been completed to elucidate the genetic basis of this common and complex disease and to provide evidence for linkage of atopy to multiple chromosomal regions, including replicated regions on chromosomes 5q, 6q, 11q, 12q, and 13q.² In addition, a number of single-nucleotide polymorphisms (SNPs) in candidate genes have been described that are nonrandomly overtransmitted to atopic subjects. These SNPs might influence physiologic functions that are known to be relevant disease mechanisms in atopy, such as influencing IgE production or modifying IgE effector function.³

The production of specific IgE is initiated by T_H2 cells that release IL-4 and IL-13 in response to antigen presentation. Binding of IL-4 and IL-13 to the IL-4R α chain recruits signal transducer and activator of transcription 6 into the receptor complex, activates germline transcription from the ϵ heavy-chain gene locus, and, together with signals derived by the B-cell surface molecule CD40, induces isotype switching in B cells.⁴

Recently, interest in the role of IL-18 in atopic disorders has increased.⁵ Binding of IL-18 to its receptor leads to the activation of the IL-1R-associated kinase/TNF receptor-associated factor 6 signal transduction pathways, followed by activation of activator protein 1 through c-Jun N-terminal kinase and the nuclear translocation of nuclear factor κ B (NF- κ B).⁶ Because NF- κ B recognition sites (among others) are located in the promoter of several cytokine genes (eg, *IL4*, *IL13*, and *IFNG*), NF- κ B is suggested to upregulate cytokine gene

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expression. IL-18 also induces the mitogen-activated protein kinase signaling pathway to activate signal transducer and activator of transcription 3, which plays a key role in cell-proliferation events.⁷ In synergy with IL-12, IL-18 stimulates T_H1-mediated immune responses, such as the expression of IFN- γ and the production of IgG2a by B cells.⁸ Paradoxically, IL-18 also enhances T_H2 cytokine production (IL-4 and IL-13) and the IgE production in helminth-infected mice⁹ and in a mouse model of ragweed-induced allergy.¹⁰ IL-18 also regulates IgE production in vivo in the absence of allergen.¹¹ Daily administration of IL-18 induced a dose-dependent increase in serum IgE levels in mice. Furthermore, transgenic mice overexpressing IL-18 spontaneously have high serum levels of IgE and IL-4.¹² Collectively, these results indicate that IL-18 has the potential to initiate T_H2 responses and upregulate IgE production. According to these findings suggesting a functional role for this molecule in atopy, recent studies have demonstrated exaggerated levels of IL-18 in the sera of patients, with exacerbation of asthma, atopic dermatitis, and allergic rhinitis.^{5,13-15} Therefore it might be plausible that genetic variations within the *IL18* gene might influence T_H2 responses and predispose to exaggerated IgE production to specific allergens.

The gene for human IL-18 is located on chromosome 11q22.2-22.3 (Locus ID 3606, National Center for Biotechnology Information, National Institutes of Health) close to the marker D11S927 (distance approximately 2 cM). Importantly, linkage of this marker to the phenotype "sensitization to mite allergen" has been shown recently (108.7 cM, nonparametric linkage 2.52, $P = .006$).¹⁶ The maximum LOD score over disease model statistics amounted to 3.76 at position 130.84 cM on the same chromosome, directly next to the gene for IL-18.¹⁶ Recently, a genome-wide search for atopy susceptibility genes in Dutch families also identified chromosome 11q22 as an important candidate region for susceptibility to atopy.¹⁷ A genome-wide screen in a cross between Biozzi BP2 and BALB/c mice found evidence for linkage of the allergic phenotype in 4 loci syntenically homologous to 5 human regions, including chromosome 11q22.¹⁸ Thus SNPs in the genes of IL-18 might be responsible for the observed linkage between 11q22 and susceptibility to atopy.

In the context of multiple sclerosis, common SNPs in promoter 1 and exon1 of the *IL18* gene have already been described.¹⁹ Our interest was to search for additional common SNPs in the whole *IL18* gene, which is composed of 6 exons and probably 2 promoters, and to investigate their association with atopic phenotypes.

METHODS

Screening population

The screening for SNPs was performed in a separate population of 50 unrelated white individuals recruited randomly without regard for atopic history. These individuals were not included in association analyses.

Atopic population

Individuals were recruited in the Southwestern part of Germany, as described elsewhere.²⁰ An atopic child served as the index patient. The genotyping and association studies were performed in 105 atopic and nonatopic individuals (oldest child of the family; mean age, 17 years) of 260 subjects aged from 4 to 28 years recruited through 2 prior studies. Therefore all individuals analyzed in this study were genetically independent from each other. Among them, 69% (72 individuals) showed specific sensitization to common inhalant allergens, 62% (65 individuals) had total IgE serum concentrations of higher than 100 kU/L, and 24% (25 individuals) had seasonal allergic rhinitis (SAR). Determination of allele frequencies, linkage disequilibrium, and Hardy-Weinberg estimates were performed.

Specific IgE was detected by means of ELISAs against 2 mixtures of grass pollens, mite allergens (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), cat dander, and birch pollens (Magic Lite; Chiron Diagnostics, Fernwald, Germany). The cut-off point for a positive test result was 1.43 ML units.²¹ Detectable IgE to at least one of the 4 allergens or a positive skin prick test response to one of the same allergens were the criteria to identify any specific sensitization. Measurement of total serum IgE was carried out by using an enzyme allergosorbent test (Phadezym; Pharmacia, Uppsala, Sweden).²²

Approval

The collection of serum and DNA material, as well as the experimental protocol, have been approved by the Ethics Commission of the University of Freiburg.

Amplification of genomic DNA by means of PCR

DNA was extracted from peripheral blood leukocytes according to standard protocols and column purified (DNA midi kit; Qiagen, Hilden, Germany). We screened for SNPs in the whole *IL18* gene by using the appropriate primers to obtain 300-bp fragments (Table I). PCR was carried out in a volume of 10 μ L containing 30 ng of DNA, 5 pmol of each primer, 0.06 U of Taq-Polymerase (Pharmacia, Uppsala, Sweden), and 2 mmol of deoxyribonucleoside triphosphate mix, with the buffer recommended by the supplier. After denaturation for 5 minutes at 94°C, 35 cycles were carried out, with each cycle consisting of 45 seconds at 94°C, 90 seconds at the annealing temperature (Table I), and 90 seconds at 72°C. The last synthesis step was extended to 10 minutes.

Single-strand conformation polymorphism analysis

The amplified products were resolved on nondenaturing 10% or 12% polyacrylamide gels containing 10% glycerol at 20°C for 2 hours.²³ The gels were silver stained, as described elsewhere.²⁴ Sequencing was performed for all observed single-strand conformation polymorphism variants.

Sequencing

Sequencing with the dideoxy chain termination method was performed on an automated sequencer (ABI310; Applied Biosystems, Weiterstadt, Germany).

Restriction fragment length polymorphism analysis

PCR for genotyping was performed with mutated primers, introducing a restriction site for restriction fragment length polymorphism analysis of the respective genotype (Table II). All enzymes were from NEB (Frankfurt, Germany). Restriction was performed

TABLE I. Primers for the screening of polymorphisms

	Primer	Annealing temperature
Promoter 1	5'-taacatgttgaacataagcccta-3'; 5'-ggggcagccgcttagcagctag-3'	54°C
Exon 1	5'-ccaaggagtgccgacagcagctct-3'; 5'-gactgctcatcgtagtgatgct-3'	56°C
Promoter 2 (from -2480 to exon 2)	5'-actctttatagattcttctc-3'; 5'-caataatgactaggctgtgagc-3'	55°C
	5'-tggtcagcaatgaagccataa-3'; 5'-cagggtttcaccacgttgccc-3'	
	5'-agaactttgagagccaagt-3'; 5'-tgtataattttggcctccaa-3'	53°C
	5'-atgcctgtaatctcaagcactt-3'; 5'-attagtagtactgtgactctg-3'	
	5'-tgtctcaagatctctgcaata-3'; 5'-atcaaggatacaggattga-3'	50°C
	5'-tgcacaccgtattggaagagg-3'; 5'-cttctcttacaaccagtgagg-3'	
	5'-gacaatgaagtacaaaccttc-3'; 5'-tcagcttatgaatacccatgt-3'	50°C
Exon 2	5'-gttcagtactaggccattatc-3'; 5'-tgttctatggcattagccttac-3'	53°C
Exon 3	5'-aagccaagacagacccttaaac-3'; 5'-gaaggcagattgtagcatgag-3'	53°C
Exon 4	5'-cttctggaacagaagattgtca-3'; 5'-agcacggaacaatgtaagcag-3'	52°C
Exon 5	5'-tgttctgtgcaagaattctc-3'; 5'-gacaagggttggtctgaggat-3'	54°C
Exon 6, 1	5'-agggtcagtactgtgttcaga-3'; 5'-cacaagctagaagatccaac-3'	53°C
Exon 6, 2	5'-gaagtgtccagagacatgata-3'; 5'-ggctactacactcagctaat-3'	54°C

TABLE II. Primer for restriction fragment length polymorphism analysis

Polymorphism	Primer	Annealing temperature	Enzyme
Promoter 1, -656	5'-taacatgttgaacataagcccta-3'; 5'-gaaagtaagcttggggagccgg-3'	54°C	<i>Ngo</i> IV
Promoter 1, -607	5'-tggtgcagaaagtgtaaaaatttaa-3'; 5'-cggataccatcattagaataatat-3'	50°C	<i>Ssp</i> I
Promoter 1, -137	5'-atgcttctaatggactaagga-3'; 5'-gtaatatcactatttcatgaatt-3'	50°C	<i>Eco</i> RI
Exon 1, 113	5'-ccaaggagtgccgacagcagctct-3'; 5'-ggggcagccgcttagcagctag-3'	54°C	<i>Nhe</i> I
Exon 1, 127	5'-atgcttctaatggactaagga-3'; 5'-agactgcagcaggtgtgggcc-3'	56°C	<i>Msc</i> I
Promoter 2, -920	5'-tgtctcaagatctctgcaata-3'; 5'-tacctattttctgtgtgtgca-3'	53°C	<i>Pst</i> I
Promoter 2, -133	5'-gtattcataagctgaaactcccg-3'; 5'-tgttctatggcattagccttac-3'	53°C	<i>Sma</i> I
Promoter 2, -132	5'-gttcagtactaggccattatc-3'; 5'-gatgaacaatctgacattcaattaa-3'	53°C	<i>Ase</i> I

in a volume of 10 μ L containing 5 μ L of the PCR product and the buffer recommended by the supplier for 90 minutes at 37°C. The fragments were resolved on 12% polyacrylamide gels. Two known homozygous individuals for the different alleles and one heterozygous individual (previously confirmed by sequencing) were included in each reaction. The genotyping was performed by 2 investigators blinded to the phenotypes. Restriction analysis was performed in duplicate, and SNPs segregated correctly.

Statistical analysis

Association studies were performed with 105 individuals who were the oldest children of their families. The importance of the genotype as a predictor (presence of the polymorphic allele) for the dichotomous variables of specific sensitization, serum IgE level of higher than 100 kU/L, and SAR was estimated with logistic regression analysis (PROC LOGIST Statistical Analysis System; SAS Institute Inc, Cary, NC).^{25,26} All regression analyses were adjusted for age and sex. Odds ratios (ORs) estimating the relative risks and their 95% CIs are given.

RESULTS

Polymorphisms

Screening for common SNPs in the *IL18* gene was performed by means of single-strand conformation polymorphism analysis. PCR products covered the coding gene, as well as the adjacent splice sites and promoter regions. We identified the following SNPs: -656(t/g), -607(a/c), and -137(g/c) in promoter 1 and +113(t/g) and

+127(c/t) in exon 1 (all described by Giedraitis et al¹⁹). In addition, we localized 3 new common SNPs in the promoter 2 region (-920[t/c], -133[c/g], and -132[a/g]; Fig 1) and 2 silent mutations in exon 4 (+179[c/a], Ser35Ser) and exon 6 (+486[c/t], Phe137Phe; not yet described). No SNPs were found in the other exons and regions of the *IL18* gene. The distance between the 2 promoters amounts to approximately 9 kb.

Allelic frequencies

We determined the frequencies of the alleles that differed from the published genotype (Table III). All variants were in Hardy-Weinberg equilibrium (data not shown).

Linkage disequilibrium

Linkage disequilibrium was tested between all pairs of SNPs in the *IL18* gene. All SNPs were in strong linkage disequilibrium ($P < .005$).

Association studies

All SNPs found in the *IL18* gene, except the 2 silent mutations in exon 4 (+179[c/a]; Ser35Ser) and exon 6 (+486[c/t]; Phe137Phe), were included in the association studies. The SNPs in promoter 1 (-137C), exon 1 (113G and 127T), and promoter 2 (-133G) showed strong associations with atopic phenotypes. These SNPs were significantly associated with high IgE levels ($P \leq .005$; OR,

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147661 acattaaata ataagatcta ctgtgaggac taactctctg taatttcaaa gtagtaaa
147721 glltaaaaca tgttcaaga tctctgcaat aa[ctgtagca caacagaaaa taggtatttc 920(t/c)
147781 tattaatgac agggtaaaa gtaactaa aaactactgt gtttgtttcc tgcaactaat
147841 catggaggga atgctaaatt tcagaggttg gtgaaataac atgtgtattt ttttcccaat
147901 caaggttcaa agatllctca cactgagaac tctatttcca taacaaaatt ctggaagcct
147961 gcaacacgta ttggaagagg ggaggaagg aaagcaaal ggaaggattt aaattttttt
148021 caaatccctg atcccttgat tttacagcaa gattgtattt atgtattact tgtgtttaa
148081 alalagllala atagagactc cagatcaaaa atcaccgcag ctgaggaga aagagggcca
148141 caaatgcca gggccttca gctllctca accclgcctg taccctcaga tgggaagcct
148201 tttttatcat tgtttacact ttatgcattt gcaatgaag tcaaaactc tcaagctc
148261 acccatagga acccactggt tgaagagaa ggaatgaagc agtccctcct aaagggcag
148321 attagatgtg tttatggcct cctcaggiga aactatattt atattgacaa tatatttata
148381 tttctcaagg aatactagaa taatgattca gtccgtactc aggcatttla lctacccttt
148441 ataattattt ttaatgagaa aatgctttct atcttccaaa tatctgatga tttgttagag
NF-1
148501 aacacttaaa catgggtatt cctagctgca accclgagga gllllaliga lglcaagatt -133(c/g), -132(a/g)
148561 gttcatcagt atactaggtg attactgac cactgaactt gaaggttcta taagtagta
148621 gtaaaaggta caatcattgt cctctaacag atgctctttt gctttcatta ggaataaaca
148681 tggctgctga accatgaga gacatttcca tcaactllgt quacatgaaa lllallaaa
148741 alagcattla clllalaggl aggcataatg ccatagaaca aataccaggt tcaataaat
148801 ctattcaatt agaaaagatg ttgtgagggt accclllag lgaclctll
Exon 2

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FIG 1. Part of the human *IL18* gene sequence for promoter 2 (upstream of exon 2; NCBI AC067833). Three novel polymorphisms at positions -920(t/c), -133(c/g), and -132(a/g) are shown. The transcription factor NF-1 binds to the site -133/132, which is indicated. Exon 2 is underlined, and the ATG start codon is marked.

TABLE III. Allelic frequencies of common polymorphisms in the human *IL18* gene

Polymorphism	Allelic frequency (n = 105)
Promoter 1, -656G	35.7%
Promoter 1, -607C	40.3%
Promoter 1, -137C	28.3%
Exon 1, 113G	27.4%
Exon 1, 127T	25.9%
Promoter 2, -920C	10.3%
Promoter 2, -133G	30.3%
Promoter 2, -132G	28.2%

Percentages given represent the allelic frequencies of the polymorphic alleles that differ from the published wild type.

3.27-3.96; Table IV). Regarding specific sensitization to common allergens (grass pollens, birch pollens, house dust mite, and cat dander), SNPs in exon 1 (127T) and promoter 2 (-133G) were significant ($P = .008$ and $.005$, respectively; ORs, 3.83 and 4.12, respectively; Table IV). The SNP 127T in exon 1 was also the strongest predictor for SAR ($P = .008$; OR, 3.22). SNPs -607C in promoter 1, -920C in promoter 2, and -132G in promoter 2 were not associated with atopic phenotypes.

DISCUSSION

We identified 5 new SNPs in the *IL18* gene. Three SNPs are likely to be of functional importance and are located in promoter 2 (upstream of exon 2), and one of them (-133G) was highly significantly associated with high IgE levels and specific sensitization to common allergens in this well-characterized white population. In addition, one SNP in exon 1 (127T) of the *IL18* gene (of

5 previously identified common SNPs¹⁹) was also associated with SAR (OR, 3.22). The most significant association with high IgE levels and specific sensitization (ORs, 3.96 and 4.12; $P = .001$ and $.005$; Table IV) was observed for the newly identified variant -133G in the promoter 2 region.

In our association studies we included only the oldest child of the family to gain a population of genetically independent individuals and to optimize the discrimination between affected and nonaffected individuals. This age group therefore represented the most reliable test population, eliminating the possibility of sensitization being not yet detectable.

Recently, the regulatory elements of both *IL-18* promoters have been investigated by means of 5' serial deletion and site-directed mutation. These studies revealed that an IFN consensus sequence-binding protein binding site is critical for the activity of promoter 1 (upstream of exon 1) and that a PU.1 binding site is essential for promoter 2 (upstream of exon 2, bearing the ATG start codon).^{27,28} Activity of promoter 1 was found to be upregulated in activated macrophages and T cells, whereas activity of promoter 2 was constitutively detectable.²⁹ Possible associations and functional implications have yet to be examined.

The most important polymorphism in promoter 1, -137(g/c), is situated in a GATA3 binding site. GATA3 has been proposed to be responsible for specifically inducing the very broad T_H2 phenotype.^{30,31} The most important polymorphism in promoter 2, -133(c/g), is situated in an NF-1 binding site. NF-1 is supposed to activate the transcription of a number of immune regulatory proteins, such as TNF receptor 2, transforming growth factor $\beta 1$, or *IL-1 β* .^{32,33} Concerning functional interpre-

TABLE IV. Association results with *IL18* polymorphisms

Polymorphisms	Specific sensitization			IgE >100 kU/L			SAR		
	OR	CI	P value	OR	CI	P value	OR	CI	P value
Promoter 1, -656G*	2.97	1.13/7.85	.03	2.37	1.02/5.49	.04	2.16	0.88/5.27	.09
Promoter 1, -607C	1.34	0.50/3.57	.56	1.16	0.49/2.76	.75	0.58	0.24/1.42	.23
Promoter 1, -137C*	3.40	1.23/8.40	.02	3.65	1.54/8.63	.003	2.72	1.16/6.38	.02
Exon 1, 113G*	3.27	1.23/8.68	.02	3.27	1.42/7.53	.005	2.56	1.10/5.95	.03
Exon 1, 127T*	3.83	1.40/10.46	.008	3.90	1.64/9.25	.002	3.22	1.36/7.63	.008
Promoter 2, -920C	0.86	0.29/2.57	.78	0.44	0.17/1.19	.11	0.46	0.15/1.42	.18
Promoter 2, -133G*	4.12	1.54/11.03	.005	3.96	1.69/9.30	.001	2.37	1.02/5.51	.05
Promoter 2, -132G	0.48	0.18/1.31	.15	0.99	0.44/2.24	.97	0.80	0.35/1.83	.60

Calculations for the phenotype-specific sensitization with 72 affected and 33 control patients, for the phenotype total serum IgE value of higher than 100 kU/L with 65 affected and 40 control patients, and for the phenotype SAR with 25 affected and 80 control patients. A *P* value of less than .05 was considered significant.

*Significant associations were seen for -656 in promoter 1, -137 in promoter 1, 113G in exon 1, 127T in exon 1, and -133G in promoter 2.

tations, one should, however, bear in mind that substantial linkage disequilibrium between all variants in the *IL18* gene was found. This might reflect a functional link between presence of a combination of SNPs. Thus gene expression of IL-18 seems to be extremely complex, taking into account the existence of 2 promoters and different protein forms of IL-18.³⁴ Given the biologic function of IL-18, gene-gene interactions could also be relevant, as shown before between polymorphisms in the genes encoding IL-13 and the IL-4R α subunit.³⁵

Previous results detected increased IL-18 concentrations in various atopic diseases, such as atopic dermatitis, allergic rhinitis, and asthma exacerbation.^{5,13-15} Additionally, a strong correlation between IL-18, eosinophils, and allergic inflammation in the human lung could be demonstrated.³⁶ Our results might therefore suggest a link between higher IL-18 levels in subjects with atopic diseases and nonrandom overtransmission of SNPs in the *IL18* gene. Because IL-18 enhances T_H2 cytokine production (IL-4 and IL-13) and upregulates IgE production, SNPs in the *IL18* gene might be relevant in inducing the very broad T_H2 phenotype observed in atopic subjects.

We conclude that IL-18 might be responsible for the linkage effects seen in the chromosomal region 11q22. In earlier work, linkage was mainly found with the phenotype "sensitization to mite allergen."¹⁶ Moreover, Koppelman et al¹⁷ recently also found evidence for linkage of the chromosomal region 11q22 with atopy in a genome-wide search. We extend these observations by identifying polymorphisms in the *IL18* promoter region that are associated with high IgE levels, specific sensitization to common allergens, and SAR. Therefore our finding of 8 common SNPs that are likely to be of functional importance strengthen a suspected direct role of IL-18 in the pathogenesis of atopy.

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REFERENCES

- Meyers DA, Marsh DG. Allergy and asthma. In: King RA, Rotter JJ, Motulsky AG, editors. The genetic basis of common disease. New York: Oxford University Press. p. 1992.
- Ober C, Moffatt MF. Contributing factors to the pathobiology. The genetics of asthma. Clin Chest Med 2000;21:245-61.
- Heinzmann A, Deichmann KA. Genes for atopy and asthma. Curr Opin Allergy Clin Immunol 2001;1:387-92.
- Bacharier LB, Geha RS. Molecular mechanisms of IgE regulation. J Allergy Clin Immunol 2000;105:S547-58.
- El-Mezzein RE, Matsumoto T, Nomiya H, Miike T. Increased secretion of IL-18 in vitro by peripheral blood mononuclear cells of patients with bronchial asthma and atopic dermatitis. Clin Exp Immunol 2001;126:193-8.
- Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol 2001;19:423-74.
- Kalina U, Kauschat D, Koyama N, Nuernberger H, Ballas K, Koschmieder S, et al. IL-18 activates STAT3 in the natural killer cell line 92, augments cytotoxic activity, and mediates IFN-gamma production by the stress kinase p38 and by the extracellular regulated kinases p44erk-1 and p42erk-2. J Immunol 2000;165:1307-13.
- Dinarello CA. IL-18: a Th1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol 1999;103:11-24.
- Yoshimoto T, Tsutsui H, Tominaga K, Hoshino K, Okamura H, Akira S, et al. IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. Proc Natl Acad Sci U S A 1999;96:13962-6.
- Wild JS, Sigounas A, Sur N, Siddiqui MS, Alam R, Kurimoto M, et al. IFN-g-inducing factor (IL-18) increases allergic sensitization, serum IgE, Th2 cytokines, and airway eosinophilia in a mouse model of allergic asthma. J Immunol 2000;164:2701-10.
- Yoshimoto T, Mizutani H, Tsutsui H, Noben-Trauth N, Yamanaka K, Tanaka M, et al. IL-18 induction of IgE: dependence on CD4+ T cells, IL-4 and STAT6. Nat Immunol 2000;1:132-7.
- Hoshino T, Kawase Y, Okamoto M, Yokota K, Yamamura K, et al. Cutting edge: IL-18-transgenic mice: in vivo evidence of a broad role for IL-18 in modulating immune function. J Immunol 2001;166:7014-8.
- Wong CK, Ho CY, Ko FW, Chan CH, Ho AS, Hui DS, et al. Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN-gamma, IL-4, IL-10 and IL-13) in patients with allergic asthma. Clin Exp Immunol 2001;125:177-83.
- Tanaka H, Miyazaki N, Oashi K, Teramoto S, Shiratori M, Hashimoto M, et al. IL-18 might reflect disease activity in mild and moderate asthma exacerbation. J Allergy Clin Immunol 2001;107:331-6.
- Verhaeghe B, Gevaert P, Holtappels G, Lukat KF, Lange B, Van Cauwenberge P, et al. Up-regulation of IL-18 in allergic rhinitis. Allergy 2002;57:825-30.
- Kurz T, Strauch K, Heinzmann A, Braun S, Jung M, Ruschendorf F, et al. A European study on the genetics of mite sensitization. J Allergy Clin Immunol 2000;106:925-32.

17. Koppelman GH, Stine OC, XU J, Howard TD, Zheng SL, Kauffman HF, et al. Genome-wide search for atopy susceptibility genes in Dutch families with asthma. *J Allergy Clin Immunol* 2002;109:498-506.
18. Zhang Y, Lefort J, Kearsy V, Lapa e Silva JR, Cookson WO, Vargaftig BB. A genome-wide screen for asthma-associated quantitative trait loci in a mouse model of allergic asthma. *Hum Mol Genet* 1999;8:601-5.
19. Giedraitis V, He B, Huang WX, Hillert J. Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. *J Neuroimmunol* 2001;112:146-52.
20. 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.
21. Kjellman N-IM, Johansson SJO, Roth A. Serum IgE levels in healthy children quantified by a sandwich technique (PRIST). *Clin Exp Allergy* 1976;6:51-9.
22. Kleine-Tebbe T, Eickholt M, Gätjen M. Comparison between MAGIC LITE- and CAP-system: two automated specific IgE antibody assays. *Clin Exp Allergy* 1992;22:475-84.
23. Orita M, Youichi S, Sekiya S. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 1989;5:156-9.
24. Hiort O, Wodtke A, Struve D. Detection of point mutations in the androgen receptor gene using non-isotopic single strand conformation polymorphism analysis. German Collaborative Intersex Study Group. *Hum Mol Genet* 1994;7:1163-6.
25. Kleinbaum DG, Kupper LL, Muller KE. Applied regression analysis and other multivariable methods. 2nd ed. Boston: PWS-Kent Publishing group; 1987.
26. SAS Institute Inc. SAS/STAT software: changes and enhancement through release 6.12. Cary, NC: SAS Institute Inc; 1996.
27. Kim YM, Kang HS, Paik SG, Pyun KH, Anderson KL, Torbett BE, et al. Roles of IFN consensus sequence binding protein and PU.1 in regulating IL-18 gene expression. *J Immunol* 1999;163:2000-7.
28. Kalina U, Ballas K, Koyama N, Kauschat D, Miething C, Arnemann J, et al. Genomic organization and regulation of the human interleukin-18 gene. *Scand J Immunol* 2000;52:525-30.
29. Tone M, Thompson SA, Tone Y, Fairchild PJ, Waldmann H. Regulation of IL-18 (IFN-gamma-inducing factor) gene expression. *J Immunol* 1997;159:6156-63.
30. Kuo CT, Leiden JM. Transcriptional regulation of T lymphocyte development and function. *Annu Rev Immunol* 1999;17:149-87.
31. Ray A, Cohn L. Th2 cells and GATA-3 in asthma: new insights into the regulation of airway inflammation. *J Clin Invest* 1999;104:985-93.
32. Hurle B, Segade F, Rodriguez R, Ramos S, Lazo PS. The mouse tumor necrosis factor receptor 2 gene: genomic structure and characterization of the two transcripts. *Genomics* 1998;52:79-89.
33. Krohn K, Rozovsky I, Wals P, Teter B, Anderson CP, Finch CE. Glial fibrillary acidic protein transcription responses to transforming growth factor-beta1 and interleukin-1beta are mediated by a nuclear factor-1-like site in the near-upstream promoter. *J Neurochem* 1999;72:1353-61.
34. Seya T, Matsumoto M, Shiratori I, Fukumori Y, Toyoshima K. Protein polymorphism of human IL-18 identified by monoclonal antibodies. *Int J Mol Med* 2001;8:585-90.
35. Howard TD, Koppelman GH, Xu J, Zheng SL, Postma DS, Meyers DA, et al. Gene-gene interaction in asthma: IL-4RA and IL-13 in a Dutch population with asthma. *Am J Hum Genet* 2002;70:230-6.
36. Mattes J, Yang M, Mahalingam S, Kuehr J, Webb DC, Simson L, et al. Intrinsic defect in T cell production of Interleukin (IL)-13 in the absence of both IL-5 and Eotaxin precludes the development of eosinophilia and airways hyperreactivity in experimental asthma. *J Exp Med* 2002;195:1433-44.