

Haplotypes of the interleukin-4 receptor α chain gene associate with susceptibility to and severity of atopic asthma

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Summary

Background Development of asthma is likely to depend on a complex interaction between environmental and genetic factors. Several groups have suggested the gene of the IL-4 receptor α chain (IL4R) as a candidate gene for the development of asthma, although association with single polymorphisms has shown contradicting results.

Objective We chose to analyse IL4R gene haplotypes and assess their possible relevance in susceptibility to asthma and to certain clinical phenotypes.

Methods IL4R gene haplotypes were analysed, based on the three markers C-3223T, Q551R and I50V, using the expectation–maximization algorithm, in 170 atopic asthma patients and 350 controls, all adult Swedish Caucasians.

Results Our data showed significantly higher levels of soluble IL-4R (sIL-4R) in asthma patients compared with controls ($P < 0.0001$). Furthermore, we showed a significant association between the IL4R haplotype containing the alleles T-3223, V50 and R551 (TVR) of the IL4R gene, and susceptibility to atopic asthma, with a frequency of 6.5% in the patients compared with 1% in the controls ($P < 0.0005$). A subgroup of patients with heterozygous or homozygous state for the T-3223, V50 and R551 alleles, also had lower levels of sIL-4R in their circulation compared with patients with homozygous state in the C-3223, I50 and Q551 alleles ($P < 0.05$) and showed less severe asthma according to lung function test ($P < 0.05$). Analysis of single markers showed the T-3223 IL4R allele to associate with lower serum levels of sIL-4 receptor ($P < 0.0001$) and patients carrying the T allele also had more symptoms of active asthma (wheezing, $P < 0.01$; coughing, $P < 0.05$ and breathing difficulties, $P < 0.01$).

Conclusion Our data suggest that asthmatic patients with low levels of sIL-4 receptor may represent a genetically distinct subgroup of atopic asthma. TVR haplotype analyses confirm the importance of IL4R as a candidate gene for susceptibility to asthma. This finding may have implications for the understanding of the pathogenesis of asthma and possibly for the development of more specific therapies.

Keywords adult, asthma, atopy, candidate gene, gene polymorphism, haplotypes, IL4R, soluble IL-4 receptor, susceptibility, Swedish Caucasians

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Introduction

The prevalence of asthma has more than doubled within the last 20–30 years in Sweden and in the middle of the 1990s the incidence was estimated to be about 7% of the population [1]. Factors contributing to the development of atopy and asthma are not well defined, although both genetic predisposition and environmental triggers are known to be of importance [2]. Genome-wide search and linkage studies to find asthma and atopy susceptibility loci, have mapped an area to chromo-

some 16 where the gene for IL-4 receptor α chain is located [3, 4].

The IL-4 receptor ligand, IL-4, is known to be one of the key cytokines in the pathogenesis of asthma through the induction of isotype switching to IgE and differentiation of T-helper type 2 (Th2) lymphocytes. Both IL4 and IL4R gene knockout mice show deficiencies in the production of Th2 lymphocytes [5], while the latter also show reduced IgE responses [6].

Several polymorphisms in the IL4R gene have been associated with phenotypes related to asthma and atopy [7], although these data were not confirmed in all studies. The Q551R polymorphism was first investigated in association with atopy: the R allele was shown to be more frequent in

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atopic patients and associated with an enhanced signalling of the IL-4 receptor [8]. While some studies did not find the Q551R polymorphism to be a susceptibility marker for asthma or atopy [9, 10], another study indicated the Q551 allele to associate with atopy [11].

Another polymorphism, I50V was found to be associated with atopic asthma and increased IgE levels in a Japanese population [12]. However, these findings were not reproduced in other ethnic groups [13].

The promoter polymorphism, C to T at position -3223 in the IL4R gene was shown to influence the levels of the soluble IL-4 receptor (sIL-4R) [14]. Secreted forms of sIL-4 occur naturally in both mice and humans and have been described to act as antagonists to IL-4 [15]. *In vivo* treatment with sIL-4R, in a mouse model, prevented the development of hypersensitivity and airway hyper-responsiveness [16]. However, the C-3223 T polymorphism was never reported in association with asthma/atopic disease.

The aim of our study was to analyse certain IL4R gene haplotypes to assess their possible relevance for the susceptibility to asthma and to certain clinical phenotypes in a selected population of adult Swedish Caucasians.

Materials and methods

Patients and control population

The patient group consisted of 170 Swedish Caucasians, 40% males and 60% females with an average age of 35 years (range 17–63), recruited from the Allergy and Asthma Clinic at Sahlgrenska University Hospital, Trollhättan Hospital and Borås Hospital (all in the western part of Sweden). All patients were sensitized to one or more inhaled allergens and an elevated level of total IgE was considered as one of the selection criteria. The patients also fulfilled at least one of the following inclusion criteria: asthma symptoms, need of bronchodilators (β_2 -stimulants) and lung function test (forced expiratory volume in 1 s (FEV₁) or peak expiratory flow (PEF)) $\leq 90\%$ predicted. For the control group, 350 healthy adult Swedish Caucasian blood donors were recruited from the Blood Bank of Sahlgrenska University Hospital. The control group consisted of 50% males and 50% females with an average age of 45 years (range 20–71 years).

Permission for this study was granted by the Ethics Committee of Göteborg University, and all volunteers gave informed consent.

Genotyping

DNA used for genotyping was extracted from ethylenediaminetetraacetic acid treated blood using the salting out method [17].

Genotypes were determined for the IL4R C-3223T, Q551R, I50V polymorphisms by the PCR and restriction enzyme digestion. PCR primer sequences for the C-3223T polymorphism were: 5'AAACAAGTAAGCGTGTTAG GAT and 5'AGTCAAGTCATCCTTCGTCGGTT, which resulted in a PCR fragment of 727 bp. PCR primer sequences for the I50V polymorphism were: 5'TGGCAAGCAAGAGAGGCAACC CT and 5'CTCACCATGCTCGCTGGGCTTGA which resulted in a PCR fragment of 353 bp. PCR primer sequences

for the Q551R polymorphism were: TGGCCGCACAGGT GGCAGGTAA and TCGGCCCCCACCAGTGGCTACC, which gave a PCR fragment of 464 bp. PCR was carried out in a total volume of 25 μ L containing 30 ng genomic DNA, 2 mM MgCl₂ for the Q551R and I50V polymorphisms and 4 mM MgCl₂ for the C-3223T polymorphism, 0.2 mM dNTP, 0.4 μ M of each primer and 1.25 U AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). Cycling conditions were: 94 °C 12 min, 30 cycles of 94 °C 30 s, 60 °C 30 s, 72 °C 60 s followed by 72 °C 5 min. A conservative recognition site was included in the PCR product to insure efficiency of the cleavage with restriction endonucleases; genotyping was repeated for some of the samples as a quality control. For genotyping of the Q551R polymorphism a different method, allelic discrimination using the LightCycler system (Roche, Mannheim, Germany), was also used as a control with a 100% consistency.

The genotypes for the IL4R C-3223T, Q551R, and I50V polymorphisms were detected by enzyme cleavage of different PCR products. For the C-3223T polymorphism, 4 μ L of the PCR product was used for digestion with 2 U BclI (New England Biolabs Inc., Hertfordshire, UK), in 100 μ g/mL bovine serum albumin, 1 $\times$ NEBuffer 1 and at a final volume of 25 μ L. The TT genotypes resulted in four fragments with the size 55, 63, 114, and 495 bp; the CC genotypes gave three fragments with the size 63, 114, and 550 bp and the TC genotypes produced all five fragments. For the Q551R polymorphism 3 μ L of the PCR product was used for digestion with 1.6 U BsmFI (New England Biolabs Inc.) in 100 μ g/mL BSA, 1 $\times$ NEBuffer 4 and at a final volume of 25 μ L. The genotype QQ (A at position 22876) resulted in two fragments of the size 124 and 340 bp; the genotype RR (G at position 22876) gave three fragments of the size 21, 124, and 319 bp; and the QR genotype resulted in all four fragments. For the I50V polymorphism, 3 μ L of the PCR product was used for digestion with 6 U MspI (New England Biolabs Inc.), in 1 $\times$ NEBuffer 2 and at a final volume of 25 μ L. The genotype VV (G at position 4679) resulted in three fragments with the size 84, 112, and 157 bp; the II genotype (A at position 4679) resulted in two fragments with the size 112 and 241 bp; and the VI genotype gave all four fragments. Digestion with BclI and MspI were performed at 37 °C for 90 min followed by inactivation at 65 °C for 20 min. BsmFI digestion was done in 65 °C for 90 min following inactivation at 80 °C for 20 min. The products were separated on a 4–20% polyacrylamide gel and the genotypes determined by the size of the different digested bands as described earlier [18].

Determination of soluble interleukin 4 receptor and immunoglobulin E

One hundred sixty-eight patients and 198 control individuals were used according to their IL4R Q551R and IL-4R C-3223T genotype for analyses of sIL-4 receptor levels in plasma, using the Quantikine human IL-4sR immunoassay (R&D systems, Abingdon, UK). The samples were analysed in duplicates following the manufacturer's instructions and the mean value was used for statistical analysis. Standard curves were generated with human recombinant sIL-4R provided and the detection limit was 15.6 pg/mL.

UniCAP total IgE assay (Pharmacia & Upjohn, Uppsala, Sweden) was used for the measurements of total IgE in plasma samples from 170 patients and 160 controls following the manufacturer's instructions. Standard curves were generated using the UniCAP total IgE calibrators and the detection limit was 2 kU/L.

Statistical analysis

Genotype and allele frequencies were compared using the χ^2 test or Fisher's exact test. Differences were considered significant at $P < 0.05$. Quantitative data were analysed by ANOVA *post hoc* test (Fisher's PLSD). Logistic and multivariate regression analyses were used to assess effects of single nucleotide polymorphisms and haplotypes for susceptibility to asthma, on levels of sIL-4 receptor and total IgE and on values of lung tests (in patients). All calculations were made using StatView, version 4.5. (SAS Institute Inc., Cary, NC, USA) or MINITAB, version 10.5 (Minitab Inc., State College, PA, USA). Logarithmic transformation was performed on the data for sIL-4R levels, before use in the statistical evaluation.

Haplotype frequencies were estimated for 272 controls and 170 patient samples using the expectation-maximization (EM) algorithm with the help of the Arlequin software 2.000 [19]. In addition, we used PHASE software, version 2.0.2 for confirmation of the haplotype frequencies and for assigning of haplotypes for individuals [20].

Results

Genetic markers of susceptibility

To assess association with susceptibility to atopic asthma, we compared the frequency of genotypes and/or haplotypes for three IL4R gene polymorphisms, C-3223T, Q551R and I50V, in 170 atopic patients and in 330 healthy control individuals (Tables 1 and 2). All analysed genotypes were found to be in Hardy-Weinberg equilibrium.

All eight theoretically possible haplotypes were found in the analysed groups, with three main haplotypes dominating in 76% and 80% of all the chromosomes in the controls and the patients.

We found no association with asthma for any of the markers when analysed alone using any possible model of inheritance (in Table 1 the data are expressed according to a co-dominant model). However, there was a statistically significant difference in haplotype frequencies comparing patients and controls in $8 \times 2 \chi^2$ test ($P < 0.0005$) (Table 2). This was mostly because of a difference in the frequency between the patient and control groups for the TVR and C-3223, V50,R551 (CVR) haplotypes. A separate analysis of the frequencies of the TVR haplotype against all other haplotypes showed a relatively strong difference ($P < 0.0001$).

Soluble interleukin-4 receptor

The plasma levels of sIL-4R were analysed in 168 patients and 198 control individuals.

We found that the patients expressed significantly higher levels of sIL-4R compared with the controls (38.6 ± 0.9 vs. 33.4 ± 0.7 pg/mL, $P < 0.0001$) (Fig. 1). When analysing both

Table 1. Genotype frequencies for three markers in the IL4R gene

SNP and genotype	Frequency (%)		<i>P</i> -value χ^2 test
	Controls	Patients	
IL4R551			
RR	3 (9)	6 (10)	0.1
RQ	30 (86)	35 (60)	
QQ	67 (192)	59 (100)	
IL4R50			
VV	20 (56)	23 (39)	0.7
VI	51 (145)	49 (83)	
II	29 (83)	28 (48)	
IL4R3223			
CC	47 (130)	47 (79)	0.3
CT	47 (129)	43 (73)	
TT	6 (17)	10 (17)	

Absolute number of cases appears in the parentheses.

IL4R, gene of the IL-4 receptor α chain; SNP, single nucleotide polymorphism.

Table 2. Haplotype frequencies for three markers in the IL4R gene

Haplotype	Haplotype frequency (%)		<i>P</i> -value χ^2 test
	Controls	Patients	
Ht1 (C-I-Q)	44 (238)	41 (137)	<0.0005*
Ht2 (T-V-Q)	26 (141)	24 (79)	
Ht3 (C-V-Q)	10 (55)	11 (38)	
Ht4 (C-V-R)	9 (48)	5 (18)	
Ht5 (C-I-R)	8 (43)	11 (37)	
Ht6 (T-I-Q)	2 (11)	1 (3)	
Ht7 (T-V-R)	1 (8)	6.5 (22)	
Ht8 (T-I-R)	0 (0)	0.5 (2)	

*When analysing all eight haplotypes against each other.

Absolute numbers of cases appear in parentheses.

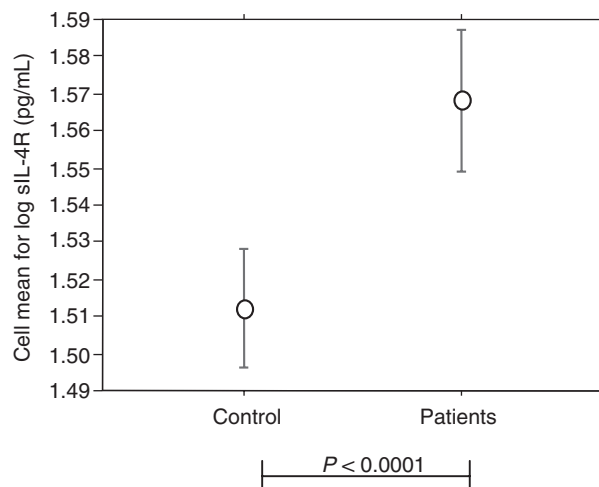


Fig. 1. Levels of soluble IL-4 receptor (sIL-4R) in patients and controls. Mean $\pm$ 95% confidence interval.

the patients and the controls the results showed significantly lower levels of sIL-4R in individuals who carried the IL4R T-3223 allele compared with those homozygous for C-3223 (CT, 33.2 ± 0.7 vs. CC, 39.0 ± 0.9 pg/mL, $P < 0.0001$) (Fig. 2).

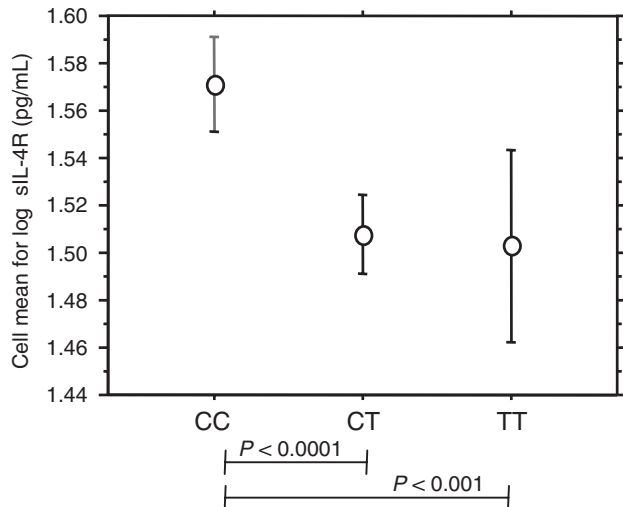


Fig. 2. Gene of the IL-4 receptor α chain, C-3223T polymorphism in association with soluble IL-4 receptor (sIL-4R) levels. Patients and controls taken together, mean $\pm$ 95% confidence interval.

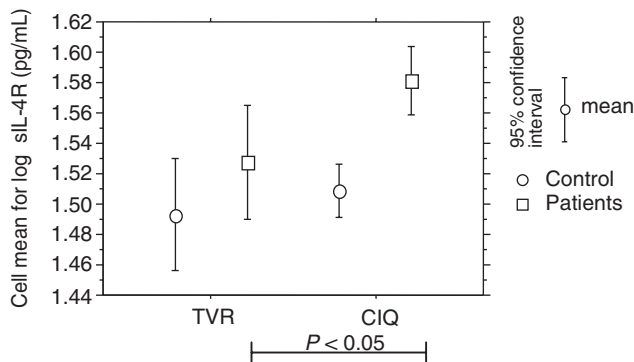


Fig. 3. Soluble IL-4 receptor (sIL-4R) levels in comparison between individuals homozygous or heterozygous in the haplotype containing the alleles T-3223, V50 and R551 (TVR) compared with individuals homozygous in the CIQ alleles, in patients ($\square$) compared with controls (o).

These results were independent of disease, as shown by multiple regression analysis.

A subgroup of the patients homozygous or heterozygous for the TVR alleles expressed significantly lower levels of sIL-4R as compared with the patients homozygous for the C-3223, I50, Q551 (CIQ) alleles (34.8 ± 1.6 vs. 39.8 ± 1.1 pg/mL, $P < 0.05$), although this was still higher than in controls (Fig. 3).

Disease activity in relation to genetic markers

Detailed data on clinical symptoms and laboratory parameters were used for studies of associations with the analysed genetic markers. When comparing the phenotypes of atopic asthma with respect to the analysed genotypes, an association between the IL4R haplotypes and the measurements of percentage of expected %PEF and absolute values of PEF were found (Fig. 4). Patients with the CIQ IL4R haplotype had significantly lower %PEF in comparison with patients with the TVR haplotype ($101 \pm 2\%$ vs. $96.5 \pm 0.9\%$, $P < 0.05$). This effect was also found in multivariate logistic regression analysis with %PEF or absolute PEF values as dependent variable (in the last case gender was included in the

Table 3. Symptoms of asthma activity in association with the C-3223T IL4R genotypes

Symptoms	Genotype frequency (%)		P-value χ^2 test
	CC	TT/TC	
Wheezing			
Yes	21	43	<0.01
No	57	42	
Cough			
Yes	28	48	<0.05
No	48	39	
Breathing difficulties			
Yes	38	63	<0.01
No	41	25	

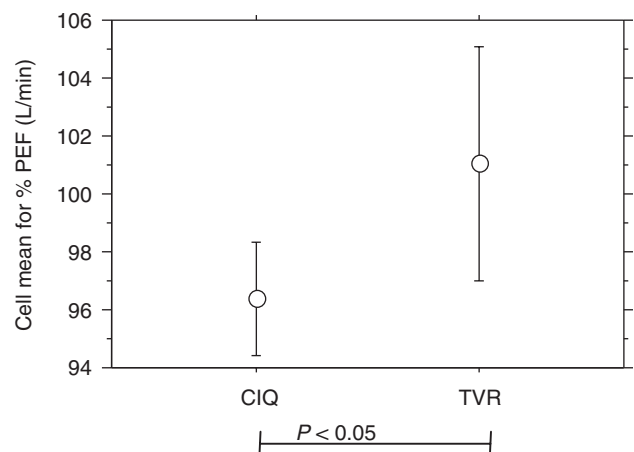


Fig. 4. Percentage of expected peak expiratory flow (%PEF) measurements in patients with asthma in association with haplotype containing the alleles T-3223, V50 and R551 (TVR) compared with CIQ.

model). No influence was found of genetic markers on the FEV₁ and forced vital capacity (FVC) or %FEV₁ and %FVC.

By analysing the separate genetic markers in relation to clinical symptoms, we noted that the C-3223T polymorphism associated with asthma activity. Individuals with the T allele had more active asthma as to wheezing ($P < 0.01$), coughing ($P < 0.05$) and breathing difficulties ($P < 0.01$) (Table 3).

Immunoglobulin E levels

We measured the IgE levels in 170 patients and 160 controls. There were no differences in total IgE levels in individuals with different genotypes analysed as single markers or haplotypes, neither for the patients nor for the controls.

Discussion

Our study demonstrates a previously not shown association between the T-3223 V50 R551 haplotype of the IL4R gene and susceptibility to atopic asthma, while none of the markers

alone was associated with the disease. The fact that some markers are linked and that certain haplotypes may have a stronger impact on disease compared with single markers alone is supported by other studies [21, 22]. A recent investigation demonstrated that the combination of two markers in the IL4R gene, V50 and R551, associated with susceptibility to asthma and resulted in a receptor with an enhanced sensitivity to IL-4, an association that was stronger than when analysing either of the single markers alone [22].

The EM algorithm for haplotype frequency estimation has repeatedly been shown to provide an accurate and robust approach for the analysis of population data with unknown phase [23]. This algorithm is based on the assumption of Hardy–Weinberg equilibrium of each of the markers and uses simulation for estimation of ambiguous haplotype from the known haplotypes, starting with estimation of unambiguous haplotypes and calculation of genotype frequencies for each simulated dataset. The number of permutations and the initial conditions are important values for the development of reliable results. We performed haplotype frequency estimation using the Arlequin software [19]. The frequency data allowed us to predict individual haplotypes and in our dataset the rare haplotype, TVR, could be estimated unambiguously in two control individuals and in six patients. In addition we used the PHASE software to assign haplotypes to individuals when the combination of alleles was ambiguous.

The IL4R promoter polymorphism, C-3223T, has, to our knowledge, not been reported previously in association with asthma, but the T-3223 allele has been shown to correlate with lower levels of sIL-4R [14].

Little is known about the regulation of the expression of sIL-4R. One study found the production of sIL-4R in humans occurs through proteolytic shedding controlled by metalloproteinases [24] and one might speculate that the I50V polymorphism, located on the extracellular side of the cell, may influence this proteolytic shedding. On the other hand, the sIL-4R has also been shown to be produced by alternative splicing mechanisms [25]. The promoter polymorphism C-3223T interferes with four potential transcription sites [14] and might affect gene transcription. However, the role of these polymorphisms in the regulation of sIL-4R production remains unclear.

We found that asthma patients have more circulating sIL-4R compared with controls. A similar association was also shown in a previous study where they found higher levels of sIL-4R when investigating the bronchoalveolar lavage from asthmatic children [26]. In addition in our patients who were homozygous, or heterozygous for the TVR alleles, the lowest levels of sIL-4R and less severe asthma according to lung function tests were shown. There have been studies regarding the Q551R polymorphism where the R allele was found to act as a protecting allele for IgE-mediated disorders [11, 27]. In addition, some reports show an association between multiple IL4R haplotypes and atopy and asthma [21, 28], even suggesting that the underlying cause of these disorders may be influenced by separate genetic variations. Our own data show that the TVR haplotype associate with asthma. The genetics behind asthma is complex and susceptibility and severity markers need not be the same. This makes it important to find the true phenotypes characterizing the

disorder. The role of sIL-4R, whether it contributes to the development of allergic inflammation or is produced as a response to disease, is not clear.

There are two hypotheses concerning the function of sIL-4R. One is that it acts as an antagonist to IL-4, preventing it from binding to the membrane-bound receptor [15] and the second that it protects IL-4 from degradation and thereby increases its half-life [29, 30]. The effect of sIL-4R in atopic asthma has been tested both in mouse models [16] and as a potential drug in humans [31]. With sIL-4R in the role of an antagonist to IL-4, lower sIL-4R levels could explain the more active asthma state that we found in individuals carrying the T-3223 allele.

We paid special attention to atopic asthma in our study. High level of IgE was one of the selection criteria for the patient group. Nevertheless, as it was shown earlier, the absolute IgE level is not a stable criterion for asthma and we also used specific sensitization as an additional criterion for the patient group. Non-atopic asthma patients were excluded from this study. Further genetic analyses corresponding to the IL4R gene are required to determine the possible relevance of sIL-4R in this group of patients.

In conclusion, we found that asthmatic patients express higher levels of sIL-4R compared with controls. In addition, we also defined a genetically distinct subgroup of patients with atopic asthma through determination of the TVR haplotype in the IL4R gene. Patients with homozygous or heterozygous state for all three of these alleles had lower levels of sIL-4R in the circulation and less severe asthma according to lung function test. This finding confirms the importance of IL4R as a candidate gene for susceptibility to asthma. This may have implications for the understanding of the pathogenesis of asthma and possibly for the development of more specific therapy.

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References

- 1 Socialstyrelsen. Statistics Sweden. Stockholm: Epidemiologic Centre, 2000.
- 2 Tattersfield AE, Knox AJ, Britton JR, Hall IP. Asthma. *Lancet* 2002; 360:1313–22.
- 3 Daniels SE, Bhattacharaya S, James A et al. A genome-wide search for quantitative trait loci underlying asthma. *Nature* 1996; 383:247–50.
- 4 Deichmann KA, Heinzmann A, Forster J et al. Linkage and allelic association of atopy and markers flanking the IL-4-receptor gene. *Clin Exp Allergy* 1998; 28:151–5.
- 5 Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluethmann H, Kohler G. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature* 1993; 362:245–8.
- 6 Noben-Trauth N, Shultz LD, Brombacher F, Urban JF Jr, Gu H, Paul WE. An interleukin 4 (IL-4)-independent pathway for CD4+ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. *Proc Natl Acad Sci USA* 1997; 94:10838–43.

- 7 Howard TD, Koppelman GH, Xu J et al. Gene-gene interaction in asthma: IL4R and IL-13 in a Dutch population with asthma. *Am J Hum Genet* 2002; 70:230-6.
- 8 Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. *N Engl J Med* 1997; 337:1720-5.
- 9 Hackstein H, Hofmann H, Bohnert A, Bein G. Definition of human interleukin-4 receptor alpha chain haplotypes and allelic association with atopy markers. *Hum Immunol* 1999; 60: 1119-27.
- 10 Noguchi E, Shibasaki M, Arinami T et al. Lack of association of atopy/asthma and the interleukin-4 receptor alpha gene in Japanese. *Clin Exp Allergy* 1999; 29:228-33.
- 11 Kruse S, Japha T, Tedner M 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.
- 12 Mitsuyasu H, Izuhara K, Mao XQ et al. Ile50Val variant of IL-4R alpha upregulates IgE synthesis and associates with atopic asthma. *Nat Genet* 1998; 19:119-20.
- 13 Tan EC, Lee BW, Chew FT, Shek L, Tay AW, Tay AH. IL-4Ralpha gene Ile50Val polymorphism. *Allergy* 1999; 54: 1005-7.
- 14 Hackstein H, Hecker M, Kruse S et al. A novel polymorphism in the 5' promoter region of the human interleukin-4 receptor alpha-chain gene is associated with decreased soluble interleukin-4 receptor protein levels. *Immunogenetics* 2001; 53:264-9.
- 15 Fanslow WC, Clifford KN, Park LS et al. Regulation of alloreactivity in vivo by IL-4 and the soluble IL-4 receptor. *J Immunol* 1991; 147:535-40.
- 16 Renz H, Bradley K, Enssle K, Loader JE, Larsen GL, Gelfand EW. Prevention of the development of immediate hypersensitivity and airway hyperresponsiveness following *in vivo* treatment with soluble IL-4 receptor. *Int Arch Allergy Immunol* 1996; 109:167-76.
- 17 Aldener-Cannava A, Olerup O. HLA-DPA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) and distribution of DPA1 alleles in Caucasian, African and Oriental populations. *Tissue Antigens* 1996; 48:153-60.
- 18 Padyukov L, Hahn-Zoric M, Blomqvist SR et al. Distribution of human kappa locus IGKV2-29 and IGKV2D-29 alleles in Swedish Caucasians and Hong Kong Chinese. *Immunogenetics* 2001; 53:22-30.
- 19 Schneider S, Roessli D, Excoffier L. A Software for Population Genetic Data Analysis. Switzerland: Genetics and Biometry Laboratory, University of Geneva, 2000.
- 20 Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003; 73:1162-9.
- 21 Ober C, Leavitt SA, Tsalenko A et al. Variation in the interleukin 4-receptor alpha gene confers susceptibility to asthma and atopy in ethnically diverse populations. *Am J Hum Genet* 2000; 66:517-26.
- 22 Risma KA, Wang N, Andrews RP et al. V75R576 IL-4 receptor alpha is associated with allergic asthma and enhanced IL-4 receptor function. *J Immunol* 2002; 169:1604-10.
- 23 Clark VJ, Metheny N, Dean M, Peterson RJ. Statistical estimation and pedigree analysis of CCR2-CCR5 haplotypes. *Hum Genet* 2001; 108:484-93.
- 24 Jung T, Schrader N, Hellwig M, Enssle KH, Neumann C. Soluble human interleukin-4 receptor is produced by activated T cells under the control of metalloproteinases. *Int Arch Allergy Immunol* 1999; 119:23-30.
- 25 Kruse S, Forster J, Kuehr J, Deichmann KA. Characterization of the membrane-bound and a soluble form of human IL-4 receptor alpha produced by alternative splicing. *Int Immunol* 1999; 11:1965-70.
- 26 Fitch PS, Brown V, Schock BC, Ennis M, Shields MD. Interleukin-4 and interleukin-4 soluble receptor alpha levels in bronchoalveolar lavage from children with asthma. *Ann Allergy Asthma Immunol* 2003; 90:429-33.
- 27 Kauppi P, Lindblad-Toh K, Sevon P et al. A second-generation association study of the 5q31 cytokine gene cluster and the interleukin-4 receptor in asthma. *Genomics* 2001; 77:35-42.
- 28 Beghe B, Barton S, Rorke S et al. Polymorphisms in the interleukin-4 and interleukin-4 receptor alpha chain genes confer susceptibility to asthma and atopy in a Caucasian population. *Clin Exp Allergy* 2003; 33:1111-7.
- 29 Sato TA, Widmer MB, Finkelman FD et al. Recombinant soluble murine IL-4 receptor can inhibit or enhance IgE responses in vivo. *J Immunol* 1993; 150:2717-23.
- 30 Jung T, Wagner K, Neumann C, Heusser CH. Enhancement of human IL-4 activity by soluble IL-4 receptors *in vitro*. *Eur J Immunol* 1999; 29:864-71.
- 31 Borish LC, Nelson HS, Corren J et al. Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J Allergy Clin Immunol* 2001; 107:963-70.