

Polymorphisms in the interleukin-4 and interleukin-4 receptor α chain genes confer susceptibility to asthma and atopy in a Caucasian population

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Summary

Background IL-4 by binding to its receptor (IL-4R) is essential for the development of airway inflammation present in asthma, through the induction of IgE synthesis in B cells and differentiation of T cells to a Th2 phenotype.

Objective To investigate the role of four common polymorphisms in the IL-4 (IL4-34CT and IL4-589CT) and IL-4R α chain (IL4RAI50V and IL4RAQ576R) genes in conferring susceptibility to the development of atopy and/or asthma.

Methods Two polymorphisms in the IL-4 gene promoter, IL4-34CT and IL4-589CT, and two polymorphisms in the IL-4R α chain gene, IL4RAI50V and IL4RAQ576R, have been genotyped using PCR-based methods in 341 asthmatic families and in 184 non-asthmatic adults recruited from the south of England.

Results Case-control analysis did not reveal differences in the distribution of the four polymorphisms between asthmatics and controls. However, the transmission disequilibrium test showed that the IL4-589 T allele was preferentially transmitted to asthmatic children ($P = 0.036$) and that the IL4RAQ576 was preferentially transmitted to children with atopic asthma ($P = 0.018$). Haplotype analysis showed a strong association between the IL4-34T/-589T haplotype and asthma *per se* ($P = 0.041$), and a strong association between the IL4RA I50/Q576 haplotype and atopic asthma ($P = 0.006$).

Conclusion Our data suggest that polymorphisms in the IL-4 and IL-4R α chain genes might play a role both conferring susceptibility to and modulating severity of atopy and asthma.

Keywords asthma, bronchial hyperresponsiveness, cytokines, genes, polymorphisms

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Introduction

Asthma is a respiratory disease characterized by recurrent respiratory symptoms, reversible variable airway obstruction, airway inflammation and increased airway responsiveness [1]. Atopy, an immune disorder involving IgE antibody responses to ubiquitous allergens, is one of the most important risk factors for the development of asthma. It is thought that asthma and atopy result from an interaction between genetic and environmental factors [2]. The airway inflammation in asthma is characterized by enhanced T cell secretion of Th2 cytokines in the airways and by a deposition of interstitial collagens in the lamina reticularis beneath the true epithelial basement membrane associated with an increased number of myofibroblasts that leads to airway remodelling [3].

The Th2 cytokine, IL-4, is a pleiotropic cytokine produced by activated T cells, mast cells and basophils which plays a

crucial role in the development of asthma through its role in the induction of IgE synthesis by B cells and differentiation to a Th2 phenotype in T cells [4], and also because IL-4, together with IL-13, acts on epithelial cells and fibroblasts within the airways, increasing matrix deposition and proliferation of smooth muscle [5, 6]. The IL-4 receptor (IL-4R) consists of two subunits: the α chain (IL-4R α), which is a high-affinity IL-4 binding site [7] shared with the IL-13R, and the common γ chain (γ c) shared with several other cytokine receptors that amplifies signalling of the α chain [7]. Binding of IL-4 to its receptor induces activation of several signalling molecules that can translocate to the nucleus and bind to specific DNA sequences in the promoter regions of target genes (e.g. I ϵ , CD23, MHC II) [7].

The gene encoding for IL-4 is located on chromosome 5q(5q31–33) that has been shown to be in linkage with atopy and asthma in several studies [2], while the gene encoding for the IL-4R α chain (IL4RA) is located on chromosome 16p(16p12.1) [8], another region shown to be in linkage with atopy in two different populations [9, 10].

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Two common polymorphisms consisting of a C to T exchange at position – 589 and – 34 from the open reading frame in the promoter of the IL-4 gene (IL4-589CT and IL4-34CT, respectively) [11, 12] have been associated with asthma and/or atopy; however, published data are controversial as many studies have been unable to reproduce these findings [2]. A number of single nucleotide polymorphisms (SNPs) have been identified in the coding region of the IL4RA gene, some of them leading to amino acid changes in the receptor [13–16]. An extracellular variant of IL-4R α chain, consisting of a substitution of valine at amino acid 50 for isoleucine (I50V), has been associated with atopy and atopic asthma [17]. A variant of the intracellular domain of IL-4R α consisting of a substitution of glutamine at position 576 (including signal peptide) for arginine (Q576R) has been associated with hyper-IgE syndrome and severe atopic eczema [14] and with enhanced signalling through the IL-4R α [14, 18]. However, this has not been confirmed in two recent studies [16, 19]. At this stage it is difficult to identify which SNP(s) contribute to the associations seen with atopy, and which merely show linkage disequilibrium with other disease-causing SNPs.

Given the central role of the IL-4/IL-4R α pathway in atopy and asthma, we have investigated the role of four common polymorphisms in the IL4 and IL4RA genes in conferring susceptibility to the development of atopy and/or asthma in a large population of asthmatic families recruited from the south of England.

Methods

Subjects and clinical assessment

Three hundred and forty-one Caucasian families were recruited from the Southampton area with at least two biological siblings (age 5–21 years) with a current physician diagnosis of asthma and taking asthma medication on a regular basis as previously described [20, 21]. One hundred and eighty-four healthy, non-asthmatic adult controls with no personal or family history of asthma were recruited from the same Southampton area through blood donor clinics (Table 1). Ethical approval was obtained from the Southampton and South West Hampshire and the Portsmouth and South East Hampshire Local Research Ethics Committees.

Molecular methods

Genomic DNA was extracted from 10 mL whole blood using a Genomic DNA Maxi-Prep kit (Qiagen, Crawley, UK). Genotyping for the IL4-589CT polymorphism was performed according to a previously published 5' Nuclease Assay (TaqMan) [22]. Genotypes for the IL4-34CT polymorphism were assigned using PCR amplification followed by *MnII* restriction digestion and gel electrophoresis [17]. PCR products were visualized using Microplate Array Diagonal Gel Assay Analysis (MADGE) [22] on 7.5% polyacrylamide gels followed by Vistra green staining.

Genotypes for the I50V polymorphism of the IL4RA gene were assigned using PCR amplification followed by *RsaI* restriction digestion and gel electrophoresis. Primers and PCR conditions used for IL4RI50V genotype analysis were IL4R-I50V-U-5' CGC GCC TCC GTT GTT CTC AGG AT-3' and IL4R-I50V-L-5' TGC GGT TCC TGG AGG TAC GTC-3' and 35 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 30 s and extension at 72 °C for 30 s. PCR products were visualized using 7.5% MADGE and Vistra green staining.

Genotyping for IL4RAQ576R was performed according to a published PCR-SSP protocol [23] with minor modifications. The results of genotyping techniques for the four polymorphisms typed were validated by fluorescent-based direct dideoxy DNA sequencing using BigDye[®] Terminator on an ABI 377 automated sequencer (Perkin-Elmer Applied Biosystem, Warrington, UK).

Generation of phenotypic scores for genotype–phenotype analysis

Generation of phenotypic scores used to describe disease severity, such as bronchial hyper-responsiveness (BHR), symptom score, treatment score, asthma severity score, atopy and atopy severity score, was performed as previously described by Sayers et al. [21].

Case report form (CRF) asthma The CRF asthma phenotype was defined as a positive response to the questionnaire: 'Have you ever had asthma?' and 'Was this confirmed by a doctor?' and 'Have you used any medicines to treat asthma, or any breathing problems, at any time in the last 12 months?'

Table 1. Phenotypic characteristics of study cohort

	Non-asthmatic parents	Asthmatic parents	Sibling 1	Sibling 2	Controls
Age (years)	40.7	40.2	13.0	9.9	42.3
Gender (% male)	51.0	47.1	56.9	53.6	47.0
Asthma (% doctor)	0	100	100	100	0
Eczema (% questionnaire)	25.8	50.8	57.8	62.4	0
Hayfever (% questionnaire)	38.0	69.8	64.2	47.0	8.7
FEV ₁ (% predicted)	103.39	94.12	94.74	95.62	ND
BHR (1/L Slope+30)*1000	26.79	17.22	14.55	12.00	ND
Log IgE (age corrected)	0.49	1.01	1.84	1.93	ND
N	492	189	341	338	184

ND: not determined.

Statistical analysis

Genetic association analysis Association of the IL4 and IL4RA polymorphism with asthma and atopy phenotypes was evaluated using the transmission/disequilibrium test (TDT) [24], case-control analyses and a disease severity analysis (SPSS Program version 10, Chicago, IL, USA). Data were analysed in three separate groups: (1) the first affected (CRF positive) sibling (sib 1); (2) the second affected sibling (CRF positive) (sib 2); and (3) parents with a doctor's diagnosis of asthma. For the TDT, trios of mother, father and first affected sibling with available genotype were analysed using a program written in STATA (version 6) by David Clayton (MRC Biostatistics Unit, Cambridge, UK). Dichotomous variables analysed using TDT were: CRF positive, CRF positive and positive atopy (defined by positive SPT and/or raised specific IgE), CRF positive and raised total IgE (age corrected), CRF and PC20 < 4 and CRF and PC20 ≤ 16. For case-control studies, the genotype and allele frequencies in the asthmatic sib 1, asthmatic sib 2 and asthmatic parent cohorts were compared to the control non-asthmatic population (Table 2). All the groups were tested for Hardy-Weinberg equilibrium. The only dichotomous variable analysed using a χ^2 test was CRF positive. A *P*-value ≤ 0.05 was considered significant.

Within the three asthmatic cohorts, *T*-tests, ANOVA and non-parametric tests were carried out as appropriate on the following variables: total IgE (age corrected and log transformed to improve normality), force expiratory volume in one second (FEV₁%) predicted, BHR (slope transformed to $[1/(L \text{ slope} + 30)] \times 1000$ to improve normality), atopy severity score and asthma severity score. A *P*-value ≤ 0.05 was considered significant. Haplotype frequencies were inferred from parental haplotypes as far as possible for doubly heterozygous parents and siblings. The 3% of doubly heterozygous IL-4 haplotypes were unassigned and classed as missing (43 cases). The 6% of doubly heterozygous parents and siblings for IL4RA were unassigned and classed as missing (90 cases). Haplotype frequencies were inferred from a program written in SPSS (version 10).

Case-control studies were also undertaken using haplotypes. Frequencies of single haplotypes in the asthmatic sib 1, asthmatic sib 2 and asthmatic parent cohorts were compared to frequencies in the control non-asthmatic population.

Frequencies of haplotypes in the control population were estimated using Arlequin (version 2.000), which implements the EM algorithm to estimate maximum likelihood haplotype frequencies [25]. The CRF-positive phenotype was analysed using a χ^2 test, and a *P*-value ≤ 0.05 was considered significant. Interactions between the IL4 and IL4RA polymorphisms were assessed by performing the TDT for receptor haplotypes conditioned on promoter haplotype of siblings, and examining changes in the significance of variables. *P*-values were not adjusted for multiple comparisons for the following reasons: (i) prior evidence from linkage studies and candidate gene studies in other samples suggested that IL4 and IL4RA genes were asthma and atopy susceptibility genes and (ii) that the individual IL4 and IL4RA alleles and haplotypes are not independent of each other.

Results

Genotyping and allele frequencies

Table 1 summarizes the phenotypic characteristics of the study cohorts. Genotype frequencies were determined for each polymorphism (see Table 2). The IL4-34 T allele was found at a frequency of 0.15, and the IL4-589 T allele at a frequency of 0.16. The IL4RAI50, isoleucine allele (A) was found at a frequency of 0.55; the IL4RAR576, glutamine allele (G) was found at a frequency of 0.23. Allele frequencies did not differ significantly from Hardy-Weinberg equilibrium assessed using χ^2 analysis. Haplotype frequencies for the IL4 and IL4RA polymorphisms are summarized in Table 3. The most common haplotypes were IL4-34C/IL4-589C with a frequency of 0.85 for IL-4, and IL4RAI50/IL4AQ576 at a frequency of 0.46 for IL4RA.

Genetic association analyses

(i) **TDT analysis:** The phenotypes CRF asthma, CRF asthma+atopy (SPT pos and/or increased specific IgE), CRF asthma+raised total IgE, CRF asthma+PC20 < 4, CRF asthma+PC20 < 16 were analysed for both IL4 promoter polymorphisms and IL4RA polymorphisms. The data shown in Table 4 demonstrate that the T allele at position - 589 of the IL-4 gene promoter was preferentially

Table 2. Genotype frequencies of study cohort

Polymorphism	Genotype	Non-asthmatic parents (%)	Asthmatic parents (%)	Sibling 1 (%)	Sibling 2 (%)	Controls (%)
IL4-34CT	CC	339 (69.6)	140 (75.3)	223 (67.3)	217 (70)	132 (72.5)
	CT	142 (29.2)	41 (22)	102 (30.8)	88 (28.3)	48 (26.3)
	TT	6 (1.2)	6 (3.2)	6 (1.8)	5 (1.6)	2 (1.09)
IL4-589CT	CC	336 (69.3)	139 (74.3)	220 (66.4)	216 (69.9)	136 (74.3)
	CT	142 (29.3)	42 (22.4)	105 (31.7)	87 (28.1)	45 (24.5)
	TT	7 (1.4)	6 (3.2)	6 (1.8)	6 (1.9)	2 (1.09)
IL4RA-I50V	AA	145 (29.8)	46 (24.7)	108 (32.7)	83 (26.6)	54 (29.5)
	AG	259 (53.2)	101 (54.3)	159 (48.1)	168 (54)	81 (44.2)
	GG	83 (17)	39 (20.9)	63 (19)	60 (19.3)	48 (26.2)
IL4RA-Q576R	AA	291 (60)	114 (60.9)	205 (62.5)	186 (61.7)	113 (61.4)
	AG	168 (34.6)	62 (33.1)	111 (33.8)	101 (33.5)	61 (33.1)
	GG	26 (5.3)	11 (5.9)	12 (3.65)	14 (4.6)	10 (5.4)

transmitted to CRF-positive children ($P = 0.036$, 1.4-fold preferential transmission), while the T allele at position –34 was not preferentially transmitted to the affected children. The IL4RAQ576 allele was significantly associated with CRF positive plus high total IgE phenotype ($P = 0.018$, 1.4-fold preferential transmission). The IL4RAI50 allele was not preferentially transmitted to affected offspring for any variable analysed.

TDT analysis for haplotypes (Table 5) revealed that the haplotype IL4-34T/IL4-589T was preferentially transmitted to the affected asthmatic children ($P = 0.0041$). The IL4-RAI50/IL4AQ576 haplotype was preferentially transmitted to both asthmatic children ($P = 0.021$) and atopic asthmatic children ($P = 0.006$), while IL4RAI50/IL4AR576 haplotype was preferentially under-transmitted to asthmatic children with a high IgE level ($P = 0.024$).

(ii) *Case-control analyses*: Using asthmatic parents ($n = 189$), sibling 1 (341), sibling 2 (338) and non-asthmatic adult control ($n = 184$) populations did not reveal any significant differences in the distribution of IL4-34CT, IL4-589CT and IL4RAI50V and IL4RAQ576R polymorphisms between the subject groups for asthma diagnosis.

(iii) *Disease severity analysis*: Within the second asthmatic sibling group, the disease severity analysis revealed a positive association between both IL4-34CT and IL4-589CT polymorphisms and BHR ($P = 0.002$, mean difference 3.8 (SE 1.2), CC group less BHR than CT and TT groups and $P = 0.002$, mean difference 3.8 (SE 1.2), CC less BHR than CT and TT, respectively). This equates to a change in the average ATS category from category 2 (mild) for the CC group to an average of category 3 (moderate) for the CT/TT group.

Within the first asthmatic sibling group, the disease severity analysis revealed a positive association between the IL4-RAQ576R polymorphism and total serum IgE level ($P = 0.009$, mean difference 0.4 (SE 0.1)). The AA group had both a higher median total IgE (296 kU/L) than the AG and GG groups (200 kU/L) and atopy severity score ($P = 0.023$, mean difference 0.3 (SE 0.1)). The AA group had a more severe atopy (mean atopy severity score 1.4) than the AG and GG groups (mean atopy severity score 1.1).

As an illustration of the clinical relevance of the observed difference in atopy severity score, we characterized the relationship of components of the score to the numbers of skin prick test and specific IgE responses that an individual has. The score ranged between 0 and 4.5, with non-atopic individuals having a mean of 0.02 (SD 0.04) and atopic individuals having a mean of 1.7 (SD 1.0). The highest scores >4 (very severe atopy) were observed in 12 subjects. These individuals had 4–6 positive skin prick tests (out of six) and 6–7 positive specific IgE tests (out of seven). Subjects with a score of 1.4 had, on average, a greater number of positive skin prick tests (1–2) and positive specific IgE tests (4–6) than those with a score of 1.1 (1–2 positive skin prick tests and 3–5 positive specific IgE tests). No differences were found between genotype groups of IL4RAI50V for any of the variables analysed.

(iv) *Gene-gene interaction*: The analysis of possible interactions between IL4 and IL4RA polymorphisms for CRF asthma, CRF asthma+atopy and CRF asthma+high total IgE level did not reveal any significant interactions between the four polymorphisms (data not shown).

Table 3. Haplotype frequencies in study cohort (unrelated parents)

IL4-34/IL4-589	Frequency (%)	IL4RAI50/IL4RA-Q576R	Frequency (%)
IL4-34C/IL4-589C	1116 (85.2)	IL4RAI50/IL4RAQ576	580 (46.6)
IL4-34C/IL4-589T	5 (0.4)	IL4RAI50/IL4RA-R576	111 (8.9)
IL4-34T/IL4-589C	0 (0.0)	IL4RAV50/IL4RA-Q576	408 (32.8)
IL4-34T/IL4-589T	189 (14.4)	IL4RAV50/IL4RA-R576	145 (11.7)
Total	1310	Total	1244

Linkage disequilibrium (D') calculated using Arlequin Version 2.000

IL4-34/IL4-589	$D' = 0.999$	$P < 0.0001$
IL4RAI50/IL4RA-Q576R	$D' = 0.2194$	$P < 0.0001$

Table 4. Variables showing significant TDT tests

Polymorphism	Significant variable	No. of genotyped offspring	Allele	Observed transmissions	Expected transmissions	P-value
IL-4-34CT	CRF asthma	337	C	76	88.5	0.06
			T	101	88.5	
IL-4-589CT	CRF asthma	337	C	75	89	0.036
			T	103	89	
IL4RA-I50V	–					
IL4RA-Q576R	CRF and raised total IgE	302	A	113	96.5	0.018
			G	80	96.5	

Discussion

This study has investigated four polymorphisms in the IL4 and IL4RA genes for association with asthma and atopy in a large population of 341 Caucasian families recruited from the south of England. Using TDT analysis we found that the T allele at position –589 of IL4 was preferentially transmitted to asthmatic children, while the T allele at position –34 was not. However, analysis of the intrafamilial association of haplotypes of IL4 showed a strong association between the IL4-34T/IL4-589T haplotype and asthma. These results suggest that either the combination of the two polymorphisms has a greater effect on IL-4 transcription than the –589T allele alone or, perhaps more likely, identifies a haplotypic background on which another asthma susceptibility

Table 5. Results of the TDT analysis using haplotypes

Polymorphisms	Significant variable	No. of genotyped offspring	Haplotype	Observed	Expected	P-value
IL4-34CT/-589CT	CRF asthma	328	IL4-1,1*	68	81	0.041
			IL4-2,2	94	81	
IL4RA-I50V/Q576R	CRF asthma	326	IL4R α -1,1**	170	150	0.021
			IL4R α -1,2	33	41.5	NS
			IL4R α -2,1	117	126.5	NS
			IL4R α -2,2	53	55	NS
	CRF asthma and atopy 2	282	IL4R α -1,1	149	132	0.037
			IL4R α -1,2	30	35.5	NS
			IL4R α -2,1	104	111	NS
			IL4R α -2,2	43	47.5	NS
	CRF asthma and raised total IgE	291	IL4R α -1,1	152	130	0.006
			IL4R α -1,2	26	35.5	0.024
			IL4R α -2,1	107	113	NS
			IL4R α -2,2	42	48.5	NS

*1 = -34 C and -589 C; 2 = -34 T and -589 T.

**1 = I50 and Q576; 2 = V50 and R576.

polymorphism lies. The lack of association seen in the case-control study between the IL-4 polymorphisms and doctors' diagnosis of asthma may represent either a lack of power (type II error) or be a result of comparing paediatric asthmatics with adult non-asthmatics, although no association was seen in the comparison of the parents with asthma with the controls. Power calculation shows that this analysis only had 35% power to detect an odds ratio of 1.5.

There are a number of other polymorphisms in IL4 and the adjacent gene, IL13, including an amino acid variant of IL13 (R130Q) that has been shown to be associated with total IgE levels, atopy and asthma in several studies. Another variant at position -1055 in the promoter region of IL13 has also been reported to be associated with atopic asthma in a Dutch population and to alter IL-13 production (reviewed in [26]). However, given the relatively low linkage disequilibrium that exists between the IL-4 SNPs and the R130Q polymorphism of IL-13 in this study population (Holloway and Beghé, unpublished observation), it is unlikely that the association observed with the IL-4 promoter polymorphisms is due to linkage disequilibrium with a functional polymorphism of IL-13. Further to this, Noguchi et al. [27] have recently demonstrated in a Japanese population that significant association of haplotypes consisting of IL4 promoter polymorphisms and polymorphisms lying in the intergenic region between the IL4 and IL13 genes show significant association with asthma.

Since analysis within the asthmatic cohorts revealed that both IL-4 promoter polymorphisms were significantly associated with BHR in the second sibling cohort (and not the first sibling cohort), we compared the differences between the two cohorts of children. We found that the only variable showing a significant difference between the two cohorts was in fact BHR, which was higher in the second sibling cohort ($\text{BHR } (1/L \text{ slope} + 30) \times 1000 = 14.5 \text{ vs. } 12; P = 0.004$). Therefore, we hypothesize that the T allele may be associated with the severity of asthma. This is in agreement with data published by Burchard et al. [28] and Sandford et al. [29] showing an association of T allele at locus -589 with the severity of FEV₁ and with the risk of developing life-

threatening asthma. However, the lack of any association with the asthma severity score seen in this study is uninformative due to the small number of severe asthmatics in the cohort, which consists mainly of mild/moderate asthmatic subjects.

No associations were found between the IL4 polymorphisms and any measure of atopy, such as total or specific IgE levels, suggesting that polymorphisms of the IL-4 promoter may be more related to *asthma per se* than to atopy or atopic asthma. This is in contrast with previous reports in which the -589T variant has been associated with asthma and higher IgE levels in American and Japanese populations [11, 30], and it has been associated *in vitro* with enhanced IL4 transcription and higher binding affinity to nuclear transcription factors [11]. To date no studies have examined the effect of IL4-34CT polymorphism or the two polymorphisms in combination on IL4 gene transcription. In agreement with our findings, two recent high resolution studies of the 5q31-33 region identify regions of linkage and haplotypes associated with atopy and high total IgE phenotypes distal to the IL-4-IL13 locus [31, 32].

As IL-4 exerts its biological function through its receptor, the IL-4R α chain gene is also a candidate gene for asthma and atopy. The analysis of two common polymorphisms in the coding region of the IL-4R α chain revealed that the IL4RAQ576 allele showed non-significant preferential transmission to asthmatic children. However, by further restricting the phenotype to CRF-positive plus high total IgE, despite the reduction in sample size, significant association was seen ($P = 0.018$), suggesting that the IL4RAQ576 allele may be influencing susceptibility to atopy or atopic asthma rather than asthma *per se*.

The results of the linkage disequilibrium analysis showed that there was weak but significant ($D = 0.22794, P < 0.0001$) disequilibrium between the polymorphisms at amino acids 50 and 576. This differs from the results previously reported for a Caucasian population sample [33]. This difference may reflect a difference in populations (US vs. UK). When the transmission of IL-4R α haplotypes was examined, it was observed that the haplotype IL4RAI50/IL4AQ576 was

preferentially transmitted to asthmatic children. Again, by restriction of the phenotype to asthma plus high total IgE (reduction in sample size from 326 to 291), the significance of the association was increased ($P = 0.006$) and the IL4RAI50/IL4AR576 haplotype was also significantly associated ($P = 0.024$). Ober et al. [33] have previously reported, in a founder population (Hutterites) as well as in an outbred population, strong association between multiple IL-4R α haplotypes and atopy and asthma. In their study eight amino acid polymorphisms of the IL-4 RA were genotyped; however, the authors concluded given the multiple-associated haplotypes that it was likely that another polymorphism outside the coding region of the gene was responsible for the observed association. Further to this, Hackstein et al. [34] recently identified a promoter polymorphism of IL-4R α at position -3223 that was associated with a low serum level of soluble IL-4R α . Potentially, this could alter the susceptibility to atopy or asthma by regulating IL-4 signalling.

In conclusion, we have identified an association between polymorphisms of the IL-4 and IL-4R α chain and atopy and asthma phenotypes in a large well-defined Caucasian cohort. The results support previous studies indicating the role that polymorphism of these genes may play in both determining susceptibility to and modulating severity of atopy and asthma. However, this study also shows the difficulties of interpreting association seen between single polymorphisms of highly polymorphic genes and disease phenotypes. This highlights the need for comprehensive analysis of extended haplotypes across candidate genes in large well-defined populations, combined with functional studies of gene and protein function, in order to ascertain which polymorphism(s) are truly disease associated and which are in linkage disequilibrium with the 'true' disease-associated variants.

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