

ORIGINAL PAPER

Interaction between variants in the interleukin-4 receptor α and interleukin-9 receptor genes in childhood wheezing: evidence from a birth cohort studyE. Melén^{*†‡}, S. Umerkajeff^{*}, F. Nyberg^{*§}, M. Zucchelli[†], A. Lindstedt^{||}, H. Gullstén^{||**}, M. Wickman^{*††}, G. Pershagen^{*} and J. Kere^{†||}

^{*}Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [†]Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden, [‡]Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden, [§]AstraZeneca R&D Mölndal, Mölndal, Sweden, [†]Department of Biosciences, Karolinska Institutet, Stockholm, Sweden, ^{||}Clinical Research Centre, Karolinska University Hospital, Huddinge, Sweden, ^{**}Department of Medical Genetics, University of Helsinki, Helsinki, Finland and ^{††}Department of Occupational and Environmental Health, Stockholm County Council, Stockholm, Sweden

Clinical and
Experimental
Allergy

Summary

Background Several polymorphisms in the IL-4 receptor α (*IL4RA*) gene have been associated with asthma and atopy, but with variable success in different populations. Immunologic studies suggest that *IL4RA* may interact with other cytokines and receptors, and gene–gene interactions have also been observed with respect to asthma. Such interactions have been proposed to explain partly the difficulties in replicating association studies.

Methods Using the prospective birth cohort BAMSE, we examined eight single nucleotide polymorphisms (SNPs) and corresponding haplotypes in the *IL4RA* gene in relation to wheezing and sensitization up to age 4. We also evaluated potential interaction effects (departure from a multiplicative interaction model) between the *IL4RA* SNPs and four SNPs in the IL-9 receptor (*IL9R*) gene previously associated with childhood wheezing.

Results We found no main effect of the *IL4RA* SNPs alone and only weak associations to wheezing and sensitization when haplotypes were considered. Gene–gene interactions between several *IL4RA* and *IL9R* SNPs with regard to wheezing were observed ($P = 0.009$), especially between *IL4RA* Q576R (rs1801275) and *IL9R* rs731476 ($P = 0.005$). An interaction was also seen between *IL4RA* and *IL9R* haplotypes.

Conclusion Variants in the *IL4RA* gene alone may not exert any major influence on susceptibility to asthma-related diseases in childhood, but in combination with other genes, such as *IL9R*, *IL4RA* may be an important gene for disease susceptibility.

Keywords asthma, genetics, IgE, IL-4, IL-9, interaction

Submitted 28 February 2006; revised 26 June 2006; accepted 16 August 2006

Correspondence:

E. Melén, MD, Department of Occupational and Environmental Health, Norrbacka, Karolinska University Hospital, SE-171 76 Stockholm, Sweden.
E-mail: erik.melen@ki.se

Introduction

A number of polymorphisms have been identified in the IL-4 receptor α (*IL4RA*) gene, several of which have been investigated in relation to asthma and atopy [1]. Although conflicting results exist regarding the effect of a particular *IL4RA* variant on asthma or allergy, *IL4RA* is considered to be an important gene for asthma susceptibility based on a number of positive findings across studies and populations. Three single nucleotide polymorphisms (SNPs) have proved to be particularly interesting based on the functional effects on signalling pathways and pathogenesis of allergic diseases: I50V, S503P and Q576R. For example, the combination of *IL4RA* P503 and R576 has been shown

to influence the signal transduction pathways and to lower the total IgE concentrations [2].

There is evidence from both immunologic and genetic studies that *IL4RA* may interact with other cytokines and receptors. A recent study on gene–environment effects in the first year of life showed that the I50V polymorphism may interact with day-care attendance and the number of viral infections with regard to lipopolysaccharide (LPS)-induced IFN- γ production [3]. Further, IFN- γ has been shown to increase the mRNA expression and protein production of *IL4RA*, which is highly relevant for the airway inflammatory response due to viral infections [4].

Gene–gene interactions, the effect of one locus being altered by effects at another locus, have been observed

between e.g. *IL4RA* and *IL4*, as well as *IL4RA* and *IL13*, with respect to asthma and total serum IgE levels [5–8], and between *IL4RA* and *IL10* with respect to respiratory syncytial virus (RSV) bronchiolitis [9]. A joint effect of *IL4RA* and *IL1A* variants on the risk of atopy has also been suggested [10]. Such gene–gene interactions have been suggested to account partly for the difficulties in replicating association results in complex diseases, which may also be true for *IL4RA* [11, 12].

Crosstalk between different cytokines is an important feature of the immune response, where one cytokine can stimulate expression of the other and *vice versa*. IL9, for instance, has been shown to induce expression of several cytokines, among them IL4 [13]. On the other hand, expression of IL9 in T cells is stimulated provided that both IL4 and IL10 are present [14]. IL9 has also been reported to potentiate the capacity of IL4 to induce IgE production by normal human B lymphocytes [15]. The differentiation of naive T₀-helper cells to TH2 cells, producing e.g. IL4, IL5, IL9 and IL13, is considered to be central in the pathogenesis of allergic disease. Both IL4 and IL9 are important mediators in allergic inflammation and asthma (see reviews [16, 17]), and their receptors (*IL4RA* and *IL9R*) use both common and specific signalling pathways [18].

Using the prospective birth cohort BAMSE, we first examined eight SNPs and the corresponding haplotypes in the *IL4RA* gene in relation to wheezing and sensitization to common inhalant and food allergens. Second, we evaluated potential interaction effects between the studied *IL4RA* SNPs and four SNPs in the *IL9R* gene, which have previously been associated with childhood wheezing in the BAMSE population [19].

Methods

Study design

The BAMSE study is based on a well-characterized, representative cohort of children followed prospectively from birth. It has previously been presented in detail [20, 21]. In brief, between 1994 and 1996, 4089 newborn infants were recruited and data on parental allergic diseases and residential characteristics were requested in questionnaires filled out by the parents when the child was 2 months old (median age). This study base comprised 75% of all eligible children born in pre-defined areas of both urban and suburban districts of Stockholm. Sixteen per cent of the children have one or two parents born outside Scandinavia. When the children were 1, 2 and 4 years old, new questionnaires were mailed to all parents, this time with the main focus on the children's symptoms related to wheezing and other allergic diseases. Questions related to wheezing followed the International Study of Asthma and Allergies in Childhood (ISAAC) question-

naire, slightly modified ('Has your child ever/after the age of 1/after the age of two had a problem with wheezing?' and 'If your child has had symptoms of wheezing, on how many occasions has the child had these symptoms the last 12 months?') [22]. At approximately 4 years of age (mean 4.3), blood samples were drawn from 2614 children (64%) and analysed for serum IgE antibodies to inhalant and food allergens (Phadiatop[®]; a mixture of cat, dog, horse, birch, timothy, mugwort, *Dermatophagoides pteronyssinus* and *Cladosporium* allergens and fx5[®]; a mixture of milk, egg white, soy bean, peanut, fish and wheat allergens; Phadia AB, Uppsala, Sweden). A positive test result was defined as an IgE antibody level ≥ 0.35 kU_A/L. For the genetic analyses, 2298 of these blood samples were available after exclusion of 69 samples because of too little blood, 81 samples due to lack of questionnaire data and 166 samples because parental consent to genetic analyses was not obtained.

Definitions of cases and controls

In a case-cohort sampling design, a sequential random sample of 709 children (357 girls, 352 boys) from the 'genetics cohort' ($n = 2298$) was selected and those who fulfilled any wheezing criterion up to the age of 4 were identified as cases ($n = 167$), while the remainders were used as random controls (282 girls, 260 boys). In addition, all the other 375 children in the 'genetics cohort' fulfilling the wheezing criteria were included, resulting in a total of 542 wheezing cases (214 girls, 328 boys). The following criteria were used for the selection and classification of wheezing children: (1) recurrent transient wheezing (defined as ≥ 3 episodes of wheezing between 3 months and 2 years of age, but no episode in the last 12 months at 4 years, $n = 173$), (2) early onset persistent wheezing (≥ 1 episode of wheezing between 3 months and 2 years of age and ≥ 1 episode in the last 12 months at 4 years, $n = 230$) and (3) late-onset wheezing (no episode of wheezing between 3 months and 2 years of age, but ≥ 1 episode in the last 12 months at 4 years, $n = 139$). Atopy-associated wheezing was defined as any type of wheezing in combination with sensitization to inhalant and/or food allergens. Detailed characteristics of these cases have been presented elsewhere [19, 23]. The randomly sampled subcohort of 709 children also makes unbiased within-subcohort analysis of other outcomes possible, such as sensitization to either inhalant or food allergens ($n = 195$, 27.5%). Owing to occasional failure in DNA extraction ($n = 29$) and genotyping, the number of individuals presented in the tables is somewhat lower than stated above.

Single-nucleotide polymorphisms and genotyping

DNA was extracted from peripheral blood leucocytes using a standard non-enzymatic method or the PUREGENE KIT

(Gentra Systems, Minneapolis, MN, USA). For selection of SNPs in the *IL4RA* gene, the literature was carefully evaluated in order to include all functional and informative SNPs previously associated with asthma or allergy. Besides, a number of additional, SNPs (some less validated) were selected from the National Center for Biotechnology Information (NCBI) database to cover the gene as well as possible. The design of the project was, however, performed before the publication of the HapMap data and the SNP tagging approach was not established, although we tried to take tagging SNPs into consideration. Initially, we selected 29 potential SNPs in the *IL4RA* gene and these were analysed in 35 unrelated individuals. A total of 10 markers were found to be monomorphic, two had a success below 85% and one were out of Hardy-Weinberg equilibrium (HWE, $P < 0.01$). Of the remaining 16 SNPs, all-coding (four SNPs) and four non-coding SNPs with reliable assays and different locations in the gene were selected for complete analyses. All eight *IL4RA* SNPs were in HWE (all BAMSE samples), and the overall genotyping success rate was $>90\%$ for each SNP (no systematic difference in success rate between cases and controls was seen). Twenty-five individuals (nine cases, 16 controls) were totally excluded due to complete genotyping failure. Selection and quality assessments of the four *IL9R* SNPs included in this study have been presented elsewhere [19]. Briefly, out of 26 potential *IL9R* SNPs tested, eight were found to be polymorphic and were further analysed. However, two of these SNPs had low minor allele frequencies ($< 5\%$), or had low information content in preliminary haplotype tagging analyses and were therefore excluded, and two SNPs were out of HWE, leaving four intronic SNPs for analyses in all cases and controls.

Primers for multiplexing PCR and extension reactions were designed by the SpectroDesigner software (Sequenom GmbH, San Diego, CA, USA). PCR and extension reactions were performed according to standard protocols, and detailed information is available on request (erik.mellen@ki.se). The SNP analysis was performed by MALDI-TOF mass spectrometry (matrix-assisted laser desorption/ionisation-time of flight; Sequenom GmbH), and the genotyping results were also manually double checked.

Statistical analyses

Allele frequencies and success rates were calculated according to standard procedures, and deviations of observed genotype frequencies from the HWE were identified by the χ^2 -test. Block-wise inheritance of the eight markers was checked by estimating correlation coefficient, r^2 , using Haploview 3.2 [24]. The frequency of haplotypes was estimated in case and control subjects combined using the expectation maximization algorithm [25], implemented in the Haploblocks programme (M. Zucchelli and J. Kere, unpublished data). P -values for

differences in allele, genotype and haplotype frequencies between cases and controls were obtained using the χ^2 test. Odds ratios (OR) and 95% confidence intervals (CI) for comparison of haplotype frequency in wheezing cases vs. controls and sensitized vs. non-sensitized children were calculated using standard procedures. A multiple logistic regression model was used to test for gene-gene interaction between *IL4RA* and *IL9R* SNPs by adding an interaction term between the genotypes of interest (analyses performed with STATA, Statistical Software, Version 8.0, Collage Station, TX, USA). A likelihood-ratio test (LRT) between the models with and without interaction terms was then performed to test the null hypothesis of no genotype-genotype interaction. Thus, the obtained P -value estimates a departure from a multiplicative interaction model on the OR scale and indicates whether the effect (OR) of one genotype is altered by effects of another genotype. In order to evaluate all possible interaction effects between the genotypes, each SNP was, if possible, coded 0 (wild-type homozygous), 1 (heterozygous) and 2 (rare homozygous). Such log-additive coding has been suggested when the mode of inheritance is unknown [26]. Rs1805015, rs731478 and rs1973880 were, however, coded 0 (wild-type homozygous) and 1 (heterozygous and rare homozygous) due to few rare homozygous individuals. Interaction between haplotypes in *IL4RA* and *IL9R* was also estimated using LRTs between the models with and without interaction terms. The regression model was adjusted for a number of potential confounders (sex, ethnicity, socio-economic status, maternal smoking during pregnancy or at enrollment, mother's age at enrollment, pet ownership, breastfeeding, number of siblings and residential building characteristics). As no confounding effect on the associations of interest was seen by these variables alone or together, the results are presented unadjusted. Each analysis was also performed in girls and boys separately, and to test for evidence of effect modification by sex, an interaction term between genotype and sex was added to the logistic model. A permutation test was used to estimate the overall significance (empirical P -value) for the whole haplotype set, that is, to test whether the observed distribution of haplotypes could be expected by chance [27]. Thus, the haplotypes were treated as fixed, while the phenotypes (case or control) of the haplotypes (chromosomes) were randomized. The proportion of 10 000 such iterations (randomized χ^2 -tests), where a stronger association was found than in the actual data, provided the empirical P -value of the observation. A similar permutation approach was used to obtain an empirical P -value for the overall significance (global test) of any genotype-genotype interaction between the *IL4RA* and *IL9R* SNPs of interest (by estimation of the proportion of such permutations where the χ^2 -test statistics for an interaction effect exceeded the χ^2 test statistics for interaction in the actual data). Using this approach, one global

hypothesis of no genotype–genotype interactions was tested rather than multiple hypotheses at the same time.

Power calculations showed that using 200 cases and 400 controls with an estimated allele frequency of 10% in the controls, and a relative risk of 2.0, the power would be 89% ($P < 0.05$). The corresponding power for a relative risk of 1.5 and allele frequency of 20% in the controls was estimated to be 74%. Thus, for estimation of the genetic effects from single markers and the most common haplotypes, the study power for persistent wheezers was relatively good ($n = 230$), but lower for the late-onset wheezing group ($n = 139$). For this reason, joint analyses with all wheezing cases were also performed.

Results

SNP localizations, minor allele frequencies (MAF) and P -values for allelic association with any type of wheezing up to 4 years are presented in Table 1. Overall, significant allelic association between the *IL4RA* SNPs and the outcomes tested was not observed, $P > 0.05$ for all but one comparison [rs1805011 (E375A) and recurrent transient wheezing, $P < 0.05$]. Association was also tested with a dominant model (rare homozygous together with heterozygous vs wild-type homozygous), which revealed a significant association between rs1805010 (I50V) and sensitization ($P < 0.05$) whereas all other tests were non-significant. Estimation of the linkage disequilibrium (LD) between the SNPs showed high r^2 -values for SNPs 1–4

and SNPs 5–7, respectively, indicating that there are two separate blocks within this region of the gene (Fig. 1). This LD pattern was confirmed by HapMap data for the same region using the CEPH samples (Utah residents with ancestry from northern and western Europe) [28]. An indirect comparison of our eight SNPs and the 22 SNPs genotyped in the HapMap project spanning 48 kb (CEPH samples) showed that our SNPs largely represent the diversity of the gene in Europeans and both the 5' and 3' ends were covered. Haplotype analyses of SNPs 1–4 suggested weak protective associations with wheezing up to 4 years (haplotype CGAA) and sensitization at 4 years (haplotype TAGG), but the empirical P -value was non-significant in both analyses (Table 2). Haplotype associations between SNPs 5–7, including the P503-R576 haplotype, and the current outcomes also showed no significant results. If all seven SNPs within Blocks 1 and 2 (SNPs 1–7) were considered together, one haplotype (CGGGATA) with an estimated frequency of 5.5% was overrepresented in sensitized compared with non-sensitized children ($P < 0.05$); otherwise no additional information was gained from this analysis compared with haplotype associations with SNP 1–4. Regarding sex-specific effects, no significant difference of haplotype (or genotype) effects on wheezing or sensitization was seen between boys and girls.

As the main effects of *IL4RA* were weak in this population, we also evaluated potential gene–gene interactions between *IL4RA* and *IL9R* variants, according to an interaction model recently described by Marchini et al. [11].

Table 1. Association between *IL4RA* polymorphisms and wheezing up to 4 years*

SNP [†]	SNP localization	MAF cases (n = 521)	MAF controls (n = 509)	P-value [‡]
rs1110470 [C/T]	5' UTR	0.50	0.47	0.19
rs1805010 [A/G]	I50V/I75V	0.43	0.46	0.14
rs2074572 [G/A]	Intronic	0.34	0.37	0.22
rs2072130 [G/A]	Intronic	0.34	0.37	0.16
rs1805011 [A/C]	E375A/E400A	0.13	0.12	0.29
rs1805015 [T/C]	S478P/S503P	0.17	0.16	0.55
rs1801275 [A/G]	Q551R/Q576R	0.23	0.22	0.82
rs1049631 [A/G]	3' UTR (upstream poly A)	0.44	0.46	0.28

*Includes all types of wheezing up to the age of 4 years.

[†]Nucleotides indicated in brackets (major/minor allele), which correspond to the coded amino acids when relevant. Two different positions are given for the coded amino acids, as both designations occur in the literature.

[‡] P -values for differences in allele frequencies between cases and controls using the χ^2 test.

MAF, minor allele frequency; A, alanine; E, glutamate; I, isoleucine; P, proline; Q, glutamine; R, Arginine; S, serine; V, valine; *IL4RA*, *IL-4* receptor α .

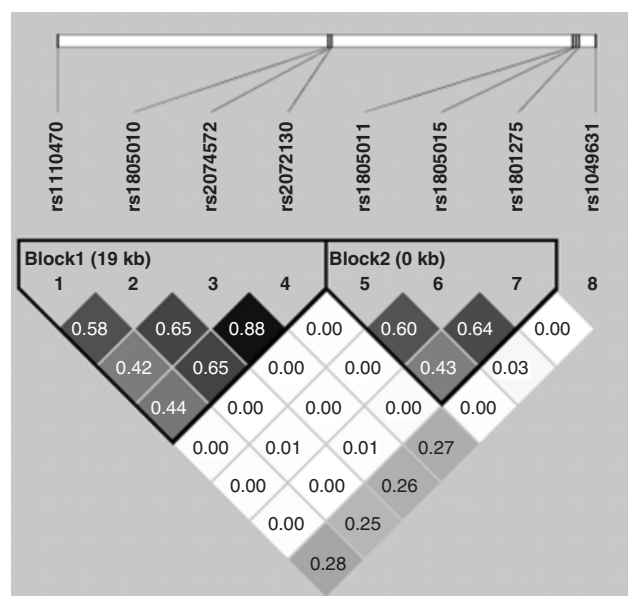


Fig. 1. *IL-4* receptor α single nucleotide polymorphisms (SNPs) according to location in the gene (contig accession NT 010393); see also Table 1. The numbers in each box correspond to the pair-wise correlation coefficient r^2 between respective SNPs. The block structure was estimated using Haploview (Solid spine of linkage disequilibrium) [24].

Table 2. Haplotype frequencies of *IL4RA* variants (SNP 1–4) in wheezing cases and controls and sensitized and non-sensitized children

Haplotype	Frequency Cases	Frequency controls	OR (95% CI)	Empirical <i>P</i> -value*
Wheezing up to 4 years				
TAGG	0.47	0.45	1.07 (0.91–1.26)	0.43
CGAA	0.31	0.35	0.80 (0.67–0.96)	
CAGG	0.10	0.08	1.23 (0.93–1.64)	
CGGG	0.07	0.08	0.91 (0.66–1.24)	
TGAA	0.01	0.01	1.72 (0.75–3.94)	
Sensitization at 4 years				
TAGG	0.41	0.47	0.80 (0.63–1.00)	0.29
CGAA	0.34	0.34	1.00 (0.79–1.28)	
CAGG	0.10	0.08	1.24 (0.84–1.84)	
CGGG	0.09	0.08	1.25 (0.84–1.88)	
CGGA	0.02	0.01	2.04 (0.80–5.26)	

*Empirical *P*-value was estimated by randomized χ^2 tests (10 000 permutations) for difference in haplotype distribution (the whole haplotype set) in cases vs. controls.

CI, confidence interval; OR, odds ratio; *IL4RA*, IL-4 receptor α ; SNP, single nucleotide polymorphism.

Table 3. *P*-values for genotype interactions between *IL9R* and *IL4RA* SNPs using any wheezing as outcome

<i>IL4RA</i> SNPs	<i>IL9R</i> SNPs			
	Rs731477	rs731476	rs731478	rs1973880
rs1805010 (I50V)	0.39	0.20	0.28	0.011
rs1805011 (E375A)	0.39*	0.12	0.41	0.28
rs1805015 (S503P)	0.030	0.045	0.11	0.034
rs1801275 (Q576R)	0.07	0.005	0.43	0.25

*rs1805011 was coded AC, CC vs. AA when combined with rs731477.

Each SNP was coded 0 (wild-type homozygous), 1 (heterozygous) and 2 (rare homozygous), except for rs1805015, rs731478 and rs1973880, which were coded 0 (wild-type homozygous) and 1 (heterozygous and rare homozygous) due to few rare homozygous individuals.

P-values for departure from a multiplicative interaction model were obtained by likelihood-ratio tests between the models with and without interaction terms.

P = 0.009 for a global test of interaction between *IL4RA* and *IL9R* SNPs after 10 000 permutations.

IL4RA, IL-4 receptor α ; SNP, single nucleotide polymorphism; *IL9R*, IL-9 receptor.

Evidence of interaction (*P* < 0.05) was suggested between several of the *IL4RA* and *IL9R* SNPs, especially between *IL4RA* Q576R and *IL9R* rs731476 (*P*-value for interaction 0.005, Table 3) with regard to wheezing up to 4 years, but not to sensitization. These findings were further supported by a significant global test for interaction between the SNPs in the two genes (*P* = 0.009). Figure 2 shows how the

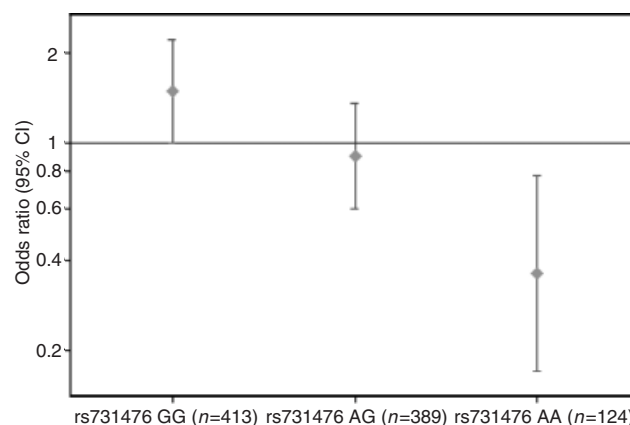


Fig. 2. Effect of IL-4 receptor α (*IL4RA*) Q576R genotypes (QR/RR vs. QQ) on wheezing, in relation to IL-9 receptor (*IL9R*) single nucleotide polymorphisms rs731476 genotypes. Main effect of *IL4RA* Q576R (rs1801275) on wheezing: odds ratio (OR) = 0.99 (0.77–1.28) when comparing QR/RR vs. QQ. Main effect of *IL9R* rs731476 on wheezing: OR = 0.78 (0.59–1.03), AG vs. GG and OR = 0.59 (0.39–0.88), AA vs. GG. *P*-value for interaction: 0.005.

Table 4. Number of wheezing cases and controls for each combination of *IL4RA* rs1801275 (Q576R) and *IL9R* rs731476 genotypes

<i>IL4RA</i> : rs1801275 (Q576R)	<i>IL9R</i> : rs731476		
	GG	AG	AA
QQ (controls)	120	120	34
QR/RR (controls)	63	76	37
QQ (cases)	129	123	38
QR/RR (cases)	101	70	15
Total	413	389	124

The figures represent the number of children included in the interaction analysis (*P* = 0.005) presented in Fig. 2.

IL4RA, IL-4 receptor α ; *IL9R*, IL-9 receptor.

effect of *IL4RA* Q576R genotypes (coded as RR/QR vs. QQ due to limited number of RR individuals as presented in Table 4) on wheezing is dependent on the *IL9R* rs731476 genotype. Interaction analyses were not performed separately in the wheezing subgroups (transient, late onset and persistent wheezing), as the power for each group was not satisfactory. Although, we did not see any effect of the *IL4RA* P503–R576 haplotype in our data, we also tested whether this haplotype and the protective *IL9R* haplotype GAGC [19] could have a joint effect on wheezing or sensitization. In the presence of the *IL9R* GAGC haplotype, *IL4RA* P503–R576 showed a protective effect on wheezing (OR = 0.58, 95% CI 0.35–0.96), whereas no effect was seen in the absence of *IL9R* GAGC (*P*-value for interaction = 0.013, Fig. 3). No interaction was seen between this haplotype (or any of the *IL4RA* haplotypes) and *IL9R* GAGC with regard to sensitization, or for the combination

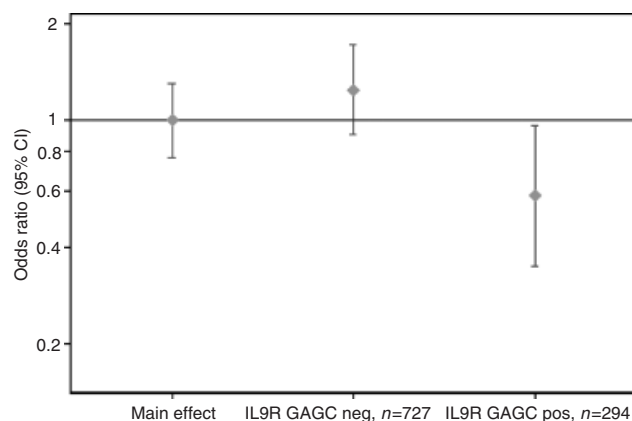


Fig. 3. Effect of IL-4 receptor α 503P-576R haplotype on wheezing, in relation to IL-9 receptor (*IL9R*) haplotype GAGC (neg, not carrier of the GAGC haplotype, pos, carrier of 1 or 2 GAGC haplotypes). *P*-value for interaction: 0.013.

of *IL4RA* SNPs 1-4 haplotypes and *IL9R* GAGC with regard to wheezing.

Discussion

These data suggest that the *IL4RA* and *IL9R* genes interact in influencing the susceptibility to develop wheezing in children. Significant interaction effects could be seen on both genotype and haplotype levels, which strengthen the findings.

The *IL4RA* SNP Q576R showed the strongest interaction with *IL9R* (rs731476) and Q576R has previously been associated with asthma and atopy in other studies (with R576 as the risk allele) [29, 30]. However, two recent studies demonstrate conflicting results with regard to atopic asthma in children, R576 being reported both as a non-risk and a risk allele [6, 31]. Regarding functional effects, Q576R has been shown to affect the signal transduction alone [29, 32], or in combination with other *IL4RA* SNPs such as I75V and S503P [2, 33].

Wheezing in pre-school children is well-known to be triggered by lower respiratory tract infections rather than IgE-mediated mechanisms (see review [34]). The BAMSE children are by study design only up to 4 years of age, and the majority of the children with the wheezing definitions reflect non-atopic wheezing (71%) [19]. Polymorphisms in the *IL4RA* gene have been associated with susceptibility to various childhood infections, for example Q576R and RSV [35], and I50V and the number of viral infections with regard to LPS-induced IFN- γ production [3]. The *IL9R* SNP of particular interest, rs731476, is located in the intronic region between exons 1 and 2, and has no proven functional effects. Intronic SNPs may, however, affect splicing and mRNA stability. It appears plausible that the *IL9R* SNP acts jointly with Q576R in terms of signal transduction and the immunological response that affects children's susceptibility of wheezing. We do not believe

that there is any direct interaction between the *IL4RA* and *IL9R* genes, but that the interaction rather mirrors the combined effect of the protein products encoded by the different genetic variants.

The associations on the genotype level from previous studies on Q576R do not give a clear picture in terms of risk and non-risk alleles. In this study, we show that the effect of the R allele on wheezing in children may be dependent on variants in an *IL9R* intronic SNP (rs731476), and both increased risk (if rs731476 = GG) and decreased risk (if rs731476 = AA) were observed. Given the substantial difference in allele frequencies observed between populations [28], the phenomenon of epistasis, the effect of one particular locus being altered by effects at another locus, in this case *IL4RA* and *IL9R* polymorphisms, may in part explain discrepant results from different studies [11]. Lack of replication of a single locus has been suggested to be explained by the fact that epistasis effects are more important than the main effect of a particular locus [12, 36]. Suppose that a population has a high frequency of homozygous GG for the *IL9R* SNP rs731476, then *IL4RA* R576 will be considered a risk allele for wheezing, whereas R576 will be considered as a non-risk allele in a population with a high frequency of homozygous AA for rs731476.

Interaction (or epistasis) effects between genes can be assessed by several methods and under different assumptions (e.g. on additive or multiplicative scales, and considering various effect measures) [37]. Estimation of interaction by assessing departure from multiplicative effects is currently in quite common use [7, 36-39]. Failure in this study to demonstrate *IL4RA*-*IL9R* interaction with regard to sensitization, despite the observed interaction for wheezing, may have several explanations. First, the BAMSE genetic study was primarily designed to evaluate the role of genetic factors for wheezing up to the age of 4 rather than sensitization, even if the case-cohort sampling design allows for such analyses. The power to detect gene-gene interactions is, however, low when the number of cases is below 200, which is the case for sensitization, although interaction-based analyses can be more powerful than single-locus approaches when the main effects are weak [11]. Second, despite the important role of the IL4 and IL9 pathways in the pathophysiology of sensitization and allergy, the studied *IL4RA* and *IL9R* SNPs may not have any particular joint influence on sensitization. Third, our analyses assumed a multiplicative mode of interaction and not for example an additive mode. Fourth, even the significant observed interaction between the *IL4RA* and *IL9R* genes for wheezing may be due to chance and may not reflect a true biological interaction effect. However, as interaction effects were observed between several SNPs and further supported by a significant global test of interaction after 10 000 permutations, we believe that this explanation is less likely.

The overall main effect of the *IL4RA* SNPs and haplotypes on wheezing and sensitization in this population was not conclusive in any direction. Similar results have been presented from three German studies on children with asthma or atopy, [7, 40, 41]. Furthermore, the major outcome in this study was wheezing up to the age of 4, which does not necessarily share the characteristics of established asthma or atopic asthma that most other studies have used as an outcome [1]. We have previously shown that the effects of *IL9R* haplotypes were predominantly seen in boys, although allelic associations could be demonstrated when boys and girls were analysed together [19]. In this study, we did not see any effect modification by sex with regard to associations between *IL4RA* variants and wheezing or sensitization. Estimation of the interaction between *IL4RA* and *IL9R* polymorphisms was not performed in boys and girls separately, as the power for further subdivision of the population was not satisfactory.

In summary, we have evaluated the effects of *IL4RA* SNPs and haplotypes on childhood wheezing and sensitization. No main effect of the *IL4RA* variants on any of the outcomes tested could be demonstrated. However, our data suggest that there is an interaction between the effects of *IL4RA* and *IL9R* genes in the development of wheezing in children. This finding supports the notion of a gene-gene interaction as a common phenomenon in complex diseases such as asthma and allergy, where the effect of *IL4RA* variants has been shown to interact with several other genes in the immune system [5–10].

Acknowledgements

We wish to thank all children and parents in the BAMSE cohort, all nurses at the Health Care Centres and the BAMSE staff, especially Stina Gustavsson and Eva Hallner, at the Department of Occupational and Environmental Health, Stockholm County Council.

Sources of research support: Stockholm County Council, The Swedish Asthma and Allergy Association, The Swedish Foundation for Health Care Science and Allergy Research, The Swedish Society of Medicine, Konsul Th.C. Bergh's Foundation, The Swedish Heart and Lung Foundation and The Swedish Environmental Protection Agency. Fredrik Nyberg is employed by AstraZeneca and AstraZeneca also supports his part-time adjunct position as Lecturer in Molecular Epidemiology at Karolinska Institutet. AstraZeneca has not contributed any direct financing to this study.

References

- 1 Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun* 2006; 1–6.
- 2 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.
- 3 Hoffjan S, Nicolae D, Ostrovskaya I *et al.* Gene-environment interaction effects on the development of immune responses in the 1st year of life. *Am J Hum Genet* 2005; 76:696–704.
- 4 Yamamoto S, Kobayashi I, Tsuji K *et al.* Upregulation of interleukin-4 receptor by interferon-gamma: enhanced interleukin-4-induced eotaxin-3 production in airway epithelium. *Am J Respir Cell Mol Biol* 2004; 31:456–62.
- 5 Howard TD, Koppelman GH, Xu J *et al.* Gene-gene interaction in asthma: *IL4RA* and *IL13* in a Dutch population with asthma. *Am J Hum Genet* 2002; 70:230–6.
- 6 Lee SG, Kim BS, Kim JH *et al.* Gene-gene interaction between interleukin-4 and interleukin-4 receptor alpha in Korean children with asthma. *Clin Exp Allergy* 2004; 34:1202–8.
- 7 Liu X, Beaty TH, Deindl P *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.
- 8 Kabesch M, Schedel M, Carr D *et al.* *IL-4/IL-13* pathway genetics strongly influence serum IgE levels and childhood asthma. *J Allergy Clin Immunol* 2006; 117:269–74.
- 9 Hoebee B, Bont L, Rietveld E *et al.* Influence of promoter variants of interleukin-10, interleukin-9, and tumor necrosis factor-alpha genes on respiratory syncytial virus bronchiolitis. *J Infect Dis* 2004; 189:239–47.
- 10 Adjers K, Pessi T, Karjalainen J, Huhtala H, Hurme M. Epistatic effect of *IL1A* and *IL4RA* genes on the risk of atopy. *J Allergy Clin Immunol* 2004; 113:445–7.
- 11 Marchini J, Donnelly P, Cardon LR. Genome-wide strategies for detecting multiple loci that influence complex diseases. *Nat Genet* 2005; 37:413–7.
- 12 Moore JH. The ubiquitous nature of epistasis in determining susceptibility to common human diseases. *Hum Hered* 2003; 56:73–82.
- 13 Wiener Z, Falus A, Toth S. *IL-9* increases the expression of several cytokines in activated mast cells, while the *IL-9*-induced *IL-9* production is inhibited in mast cells of histamine-free transgenic mice. *Cytokine* 2004; 26:122–30.
- 14 Houssiau FA, Schandene L, Stevens M *et al.* A cascade of cytokines is responsible for *IL-9* expression in human T cells. Involvement of *IL-2*, *IL-4*, and *IL-10*. *J Immunol* 1995; 154:2624–30.
- 15 Dugas B, Renaud JC, Pene J *et al.* Interleukin-9 potentiates the interleukin-4-induced immunoglobulin (IgG, IgM and IgE) production by normal human B lymphocytes. *Eur J Immunol* 1993; 23:1687–92.
- 16 Chatila TA. Interleukin-4 receptor signaling pathways in asthma pathogenesis. *Trends Mol Med* 2004; 10:493–9.
- 17 Soussi-Gounni A, Kontolemos M, Hamid Q. Role of *IL-9* in the pathophysiology of allergic diseases. *J Allergy Clin Immunol* 2001; 107:575–82.
- 18 Knoops L, Renaud JC. *IL-9* and its receptor: from signal transduction to tumorigenesis. *Growth Factors* 2004; 22:207–15.
- 19 Melen E, Gullsten H, Zucchelli M *et al.* Sex specific protective effects of interleukin-9 receptor haplotypes on childhood wheezing and sensitisation. *J Med Genet* 2004; 41:e123.
- 20 Melen E, Bruce S, Doekes G *et al.* Haplotypes of G protein-coupled receptor 154 are associated with childhood allergy and asthma. *Am J Respir Crit Care Med* 2005; 171:1089–95.

- 21 Wickman M, Kull I, Pershagen G, Nordvall SL. The BAMSE project: presentation of a prospective longitudinal birth cohort study. *Pediatr Allergy Immunol* 2002; 13 (Suppl. 15):11–3.
- 22 Asher MI, Keil U, Anderson HR *et al*. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; 8:483–91.
- 23 Melen E, Kere J, Pershagen G, Svartengren M, Wickman M. Influence of male sex and parental allergic disease on childhood wheezing: role of interactions. *Clin Exp Allergy* 2004; 34:839–44.
- 24 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21:263–5.
- 25 Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol Biol Evol* 1995; 12:921–7.
- 26 Schaid DJ. General score tests for associations of genetic markers with disease using cases and their parents. *Genet Epidemiol* 1996; 13:423–49.
- 27 Laitinen T, Kauppi P, Ignatius J *et al*. Genetic control of serum IgE levels and asthma: linkage and linkage disequilibrium studies in an isolated population. *Hum Mol Genet* 1997; 6:2069–76.
- 28 Altshuler D, Brooks LD, Chakravarti A, Collins FS, Daly MJ, Donnelly P. A haplotype map of the human genome. *Nature* 2005; 437:1299–320.
- 29 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.
- 30 Rosa-Rosa L, Zimmermann N, Bernstein JA, Rothenberg ME, Khurana Hershey GK. The R576 IL-4 receptor alpha allele correlates with asthma severity. *J Allergy Clin Immunol* 1999; 104:1008–14.
- 31 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.
- 32 Kruse S, Braun S, Deichmann KA. Distinct signal transduction processes by IL-4 and IL-13 and influences from the Q551R variant of the human IL-4 receptor alpha chain. *Respir Res* 2002; 3:24.
- 33 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.
- 34 Custovic A, Murray C, Simpson A. Allergy and infection: understanding their relationship. *Allergy* 2005; 60 (Suppl. 79): 10–3.
- 35 Hoebee B, Rietveld E, Bont L *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.
- 36 Moore JH, Williams SM. New strategies for identifying gene-gene interactions in hypertension. *Ann Med* 2002; 34:88–95.
- 37 Cordell HJ. Epistasis: what it means, what it doesn't mean, and statistical methods to detect it in humans. *Hum Mol Genet* 2002; 11:2463–8.
- 38 Cordell HJ, Clayton DG. A unified stepwise regression procedure for evaluating the relative effects of polymorphisms within a gene using case/control or family data: application to HLA in type 1 diabetes. *Am J Hum Genet* 2002; 70:124–41.
- 39 Millstein J CD, Gilliland FD, Gauderman WJ. A testing framework for identifying susceptibility genes in the presence of epistasis. *Am J Hum Genet* 2006; 78:15–27.
- 40 Wjst M, Kruse S, Illig T, Deichmann K. Asthma and IL-4 receptor alpha gene variants. *Eur J Immunogenet* 2002; 29:263–8.
- 41 Woitsch B, Carr D, Stachel D *et al*. A comprehensive analysis of interleukin-4 receptor polymorphisms and their association with atopy and IgE regulation in childhood. *Int Arch Allergy Immunol* 2004; 135:319–24.