Interleukin 13 and Interleukin 4 Receptor- α Polymorphisms in Rhinitis and Asthma

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Key Words

Asthma • Genetics • *IL13* • *IL4R* • Rhinitis • Interaction

Abstract

Background: Asthma and rhinitis may represent two manifestations of the same airway disease. Genetic research can increase our understanding of their common or distinct pathogenesis. *IL13* and *IL4R* polymorphisms are associated with asthma and show gene-gene interaction in asthma. Their role in rhinitis has not been extensively studied. **Methods:** Association of *IL13* and *IL4R* polymorphisms in relation to rhinitis, asthma, serum IgE and skin test response was studied in: (1) 188 trios ascertained through a proband with rhinitis who were clinically not affected by asthma; (2) 407 trios with an asthmatic proband, and (3) 118 asthma cases and 102 unrelated healthy controls using family-based association testing, logistic regression, and analysis of variance as appropriate. Gene-gene interaction was evaluated using logistic regression analysis. **Results:** *IL13* C–1111T (rs1800925) was significantly associated with rhinitis and atopic phenotypes in rhinitis trios that were not affected by clinical asthma. *IL13* Arg130Gln (rs20541) and G870A (rs1295685) were

consistently associated with asthma and serum IgE in both asthma populations. *IL4R* Glu375Ala (rs1805011) and Ser411Leu (rs1805013) were associated with asthma in the asthma case-control population. Combining risk genotypes of *IL13* Arg130Gln with *IL4R* Glu375Ala, and *IL13* C–1111T with *IL4R* Ser478Pro yielded increased risks for asthma compared to their separate effects. **Conclusion:** *IL13* polymorphisms were associated with asthma and rhinitis without clinical asthma; thus, these polymorphisms may constitute a common etiologic pathway for their development. In addition, the study replicates a previously reported interaction of *IL13* and *IL4R* polymorphisms in asthma.

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Introduction

The prevalence of atopic diseases including rhinitis and asthma has been rising worldwide during the last decades. Epidemiological surveys show that rhinitis occurs in 60–85% of patients with allergic and nonallergic asthma [1, 2] and constitutes a risk factor for the development of asthma [3]. This has led to the concept that rhinitis and

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asthma may constitute two manifestations of one airway disease characterized by inflammation of both the upper and lower airways. However, only 10–32% of patients with rhinitis develop clinical asthma [2, 4], which challenges this concept.

Rhinitis and asthma are both complex genetic diseases. Twin and family studies suggest a considerable genetic contribution, with an estimated heritability of 60–70% for both rhinitis and asthma. Genes important for both diseases as well as disease-specific genes, next to environmental factors, likely play a role in their susceptibility [5, 6].

Two genes that are among the most extensively studied in relation to asthma are interleukin 13 (*IL13*) and the interleukin 4 receptor α -chain (*IL4R*). *IL13*, a cytokine typically produced during T-helper type 2 cell responses, promotes allergic inflammation by signaling through the *IL4R* inducing isotype class switch from IgG to IgE production in B cells. Polymorphisms in genes encoding *IL13* and *IL4R* may therefore constitute a common etiologic pathway in the allergic inflammation observed in asthma and rhinitis.

To determine whether polymorphisms in *IL13* and *IL4R* contribute to the development of rhinitis, we studied *IL13* and *IL4R* single-nucleotide polymorphisms (SNPs) in Dutch rhinitis patients who were clinically not affected by asthma and compared our findings with two additional independent Dutch asthma populations. In addition, we set out a replication study of our previously reported [7] interaction of *IL13* and *IL4R* SNPs in asthma.

Patients and Methods

Study Populations

Three Dutch Caucasian populations were selected: (1) rhinitis trios, ascertained through 226 patients affected by rhinitis; (2) asthma trios, ascertained through 407 asthma patients, and (3) a case-control population with asthma cases ($n = 118$) and healthy controls ($n = 102$). Rhinitis and asthma patients were recruited through media appeals and among outpatients in the Departments of Allergology and Pulmonology of the University Medical Center Groningen and local hospitals. The healthy controls were recruited among new employees in an animal laboratory. The local medical ethics committees approved this study, and all participants provided written informed consent.

Ascertainment

Rhinitis Trios. Rhinitis patients were selected based on a positive answer to the following questions: 'Do you have hay fever?' and 'Do you have a runny or stuffed nose when you are near animals (e.g. dogs, cats, horses), near feathers (e.g. in pillows), or in a dusty part of the house, or when you are near trees, grasses and

flowers?' and a self-reported absence of a history of asthma or use of asthma medication. Patients were excluded when affected by nasal polyps, occupational rhinitis or concomitant diseases that could prevent the phenotypic analysis. Following clinical characterization, 38 patients (17%) were excluded from further analyses because they had asthma or probable asthma as defined by our asthma algorithm [8]. In this algorithm, airway hyperresponsiveness (AHR), respiratory symptoms, smoking, airway obstruction and bronchodilator response are used to establish an asthma diagnosis. Exclusion was based on AHR combined with: (1) reversible airway obstruction ($\geq 9\%$ from baseline; $n = 4$); or (2) reporting of ≥ 2 airway symptoms suggestive of asthma (i.e. nocturnal symptoms, dyspnea, cough, wheeze and/or asthma attacks; $n = 26$). The remaining patients were excluded because they reported ≥ 2 airway symptoms suggestive of asthma ($n = 2$), had reversible airway obstruction combined with dyspnea ($n = 1$), or information was incomplete ($n = 5$).

Asthma Trios. Asthma trios were selected if the patient reported doctor-diagnosed asthma. The diagnosis of asthma was objectively confirmed based on symptoms and clinical tests according to the asthma algorithm [8] (see above).

Asthma Case-Control Population. Individuals in the case-control population participated in studies on the comparison of methacholine and adenosine monophosphate bronchial provocation [9]. Asthma cases were included if they met the following criteria: clinical diagnosis of asthma, AHR, at least one positive skin prick test out of 17 common aeroallergens and reversibility to β_2 -agonist $\geq 9\%$ of predicted FEV₁ [9]. Controls participated in a study population of young adults who were mostly asymptomatic [10]. Individuals with AHR who reported ≥ 1 airway symptom suggestive of asthma (wheeze, cough, dyspnea and/or asthma attacks) were excluded.

Clinical Evaluation

Before clinical characterization, all participants were asked to stop inhaled corticosteroids at least 14 days before testing, inhaled long-acting β -agonists and antihistamines 48 h before testing, and inhaled short-acting β -agonists and anticholinergics 8 h before testing. Therapy with oral corticosteroids was continued in 7 patients from the asthma trios.

Rhinitis and Asthma Trios. Rhinitis and asthma trios were characterized using standardized protocols described previously [11]. In brief, a modified version of the British Medical Council questionnaire was applied [12]. AHR to histamine was assessed using a 30-second inhalation protocol as described by De Vries et al. [13]. A subject was considered hyperresponsive if the provocative concentration of histamine producing a 20% fall in forced expiratory volume in 1 s (PC₂₀) was ≤ 32 mg/ml. All patients underwent intracutaneous skin testing with 12 common aeroallergens (mixed grasses, tree pollen, mixed weeds, house dust mite, dog, cat, feather mix, guinea pig, *Aspergillus fumigatus*, *Alternaria alternata*, *Cladosporium herbarum*, *Penicillium notatum*). An individual was regarded skin prick test positive if the mean of the maximum and perpendicular wheal diameter was ≥ 5 mm in reaction to one or more allergens, provided the control was negative.

Asthma Case-Control Population. In asthma cases, AHR to methacholine was tested with a 2-min tidal breathing method [9] and considered positive if the PC₂₀ methacholine was < 8 mg/ml. Healthy controls were challenged with a breath-actuated dosimeter using a modification of a standardized protocol [10]. AHR for

Table 1. Characteristics of the rhinitis and asthma trios, asthma patients, and healthy controls

	Rhinitis trios proband	Asthma trios proband	Asthma case-control	
			patients	controls
Total number	188 ¹	407	118	102
Mean age $\pm$ SD, years	32.8 $\pm$ 8.4	34.3 $\pm$ 8.8	31.8 $\pm$ 8.3	26.9 $\pm$ 5.4
Male, %	45.2	36.9	65.8	44.1
Mean FEV ₁ % predicted $\pm$ SD				
pre-bronchodilator	103.5 $\pm$ 11.4	87.4 $\pm$ 16.4	78.1 $\pm$ 18.6	104.6 $\pm$ 12.9
AHR, % positive	22.4 ²	91.0 ²	100.0 ³	4.0 ⁴
Serum IgE				
Geometric mean, kU/l	114	109	237	46
Interquartile range, kU/l	42–266	38–305	114–568	19–122
≥ 1 Positive skin test, %	96.2	82.9	100	NA

NA = Not available.

¹ Individuals that had asthma according to the asthma algorithm were excluded. ² PC₂₀ histamine (% <32 mg/ml). ³ PC₂₀ methacholine (% <8 mg/ml). ⁴ PD₂₀ methacholine (% <10 μ mol).

these individuals was defined as a cumulative dose of inhaled methacholine causing a 20% fall in FEV₁ $\leq$ 10 μ mol [10]. Serum IgE levels in all three populations were measured by means of an enzyme-linked fluorescent assay (Mini Vidas; Biomérieux Inc., Marcy l'Etoile, France).

Molecular Methods

DNA was isolated from lymphocytes using standard procedures (Puregene kit, Gentra Inc. Minneapolis, Minn., USA). We evaluated three SNPs in *IL13*, C–1111T (rs1800925), Arg130Gln (rs20541), G870A (rs1295685) and five in *IL4R*, Ile50Val (rs1805010), Glu375Ala (rs1805011), Ser411Leu (rs1805013), Ser478Pro (rs1805015) and Gln551Arg (rs1801275).

The *IL13* polymorphisms were haplotype tagging SNPs selected from the publicly available database of the International HapMap Project [14]. For *IL4R*, we chose to include SNPs most likely to have a functional impact, based on previous results [7]. Genotyping was performed using the MassARRAY SNP genotyping system (Sequenom Inc., San Diego, Calif., USA).

Statistical Methods

Genotype data of control individuals were analyzed for deviations from Hardy-Weinberg equilibrium using χ^2 statistics (1 degree of freedom).

The phenotypes allergic rhinitis, asthma, AHR, total serum IgE levels and skin prick test response were tested for association with *IL13* and *IL4R* genotypes. Total serum IgE was tested as a quantitative variable, after logarithmic transformation to approximate a normal distribution.

In the trios, allele or haplotype transmission distortion from the parents to affected children was analyzed using the family-based association test and its haplotype option (<http://biostat.harvard.edu>). In the case-control group, association testing was performed using logistic regression and analysis of variance when appropriate. We corrected for the number of tested SNPs ($n = 3$ and 5 for *IL13* and *IL4R*, respectively) and phenotypes ($n = 2$ for

rhinitis trios and case-controls, and $n = 3$ for asthma trios), by using the Benjamini and Hochberg Step-up False Discovery Rate correction [15]. $p < 0.05$ was considered statistically significant.

Haplotype frequencies in the case-control population were estimated by the expectation-maximization algorithm and used to estimate the separate haplotype frequencies among affected and unaffected individuals. The difference in these frequency distributions was evaluated by a log-likelihood ratio test (in-house software).

Howard et al. [7] previously reported interaction between *IL13* C–1111T and *IL4R* S478P with susceptibility to asthma in our Dutch family study. We similarly tested for gene-gene interaction in asthma cases and controls using logistic regression analyses of combined genotypes. Gene-gene interaction could not be investigated in the trio populations because the study design required transmission disequilibrium testing to combinations of risk genotypes and the numbers of transmitted combined genotypes were considered too small to give reliable estimates.

Results

A total of 188 rhinitis trios, 407 asthma trios, 118 asthma cases, and 102 healthy controls were evaluated and their clinical characteristics are presented in table 1. A total of 165 rhinitis trios and 295 asthma trios were classical trios, consisting of the proband and both parents.

Genotyping

The SNPs were in Hardy-Weinberg equilibrium in all three populations. Allele frequencies in each study population are reported in table 2. SNPs Arg130Gln and G870A in *IL13* were in almost complete linkage disequi-

Table 2. SNPs genotyped in *IL4R* and *IL13* and their minor allele frequencies in studied populations

Gene name/ SNP	rs No.	Alleles ¹	Rhinitis pro- bands	Asthma pro- bands	Asth- ma cases	Healthy controls
<i>IL13</i>						
C-1111T ²	rs1800925	C/T	0.23	0.22	0.23	0.17
Arg130Gln	rs20541	G/A	0.20	0.23	0.28	0.18
G870A ²	rs1295685	G/A	0.20	0.23	0.27	0.18
<i>IL4R</i>						
Ile50Val	rs1805010	A/G	0.45	0.51	0.46	0.46
Glu375Ala	rs1805011	A/C	0.13	0.12	0.08	0.17
Ser411Leu	rs1805013	C/T	0.05	0.05	0.07	0.03
Ser478Pro	rs1805015	T/C	0.19	0.17	0.16	0.19
Gln551Arg	rs1801275	A/G	0.22	0.22	0.24	0.27

¹ Major alleles first. ² No amino acid change.

librium (LD) and both SNPs showed moderate LD ($r^2 = 0.20-0.33$) with C-1111T in all three populations. LD between Ile50Val and other *IL4R* SNPs was low in all populations. The remaining SNPs within *IL4R* were in strong LD in all populations, except for the SNPs Glu375Ala and Ser411Leu, which did not show LD in the asthma case-control study (fig. 1).

Association Analyses

Rhinitis Trios. *IL13* C-1111T was significantly associated with allergic rhinitis and serum IgE ($p = 0.04$ for both phenotypes, table 3). The T allele was transmitted more frequently than expected. Furthermore, we found a borderline association of *IL4R* Ile50Val with rhinitis ($p = 0.06$). The 50Ile allele was transmitted more frequently than expected. Family-based haplotype association analyses showed results consistent with SNP analyses. The *IL13* haplotype -1111T/130Arg/870G (haplotype frequency 8%) was significantly associated with rhinitis and serum IgE ($p = 0.03$ for both phenotypes). The *IL4R* haplotype with the wild-type alleles for *IL4R* SNPs (50Ile/375Glu/411Ser/478Ser/551Gln, haplotype frequency 41%) was positively associated with rhinitis ($p = 0.04$).

Asthma Trios. Consistent association of *IL13* SNPs Arg130Gln and G870A was found with asthma, AHR, skin test response, and total serum IgE ($p < 0.05$; table 3). None of the *IL4R* SNPs showed significant association with asthma, AHR, skin test response or total serum IgE. Analyses of *IL13* haplotypes supported the results of SNP analyses. The haplotype with both minor alleles of

the associated SNPs Arg130Gln and G870A (-1111C/130Gln/870A, frequency 7%) was positively associated with asthma, AHR, skin test response and total serum IgE ($p = 0.02, 0.03, 0.02$ and 0.08 , respectively). Haplotypes of *IL4R* SNPs were not significantly associated with asthma or any atopy phenotype.

A subgroup analysis of allergic (i.e. skin test positive) individuals within the asthma and rhinitis trios showed comparable results. Interestingly, *IL4R* Glu375Ala and Ser478Pro wild-type alleles were associated with serum IgE in the allergic asthma trios.

Asthma Case-Control Population. Consistent with the asthma trios, the case-control population showed association of *IL13* SNPs Arg130Gln and G870A with asthma and total serum IgE ($p < 0.05$; table 4). Genotypes with 1 or 2 copies of the 130Gln and 870A alleles were significantly more prevalent in asthmatics than in controls, and were associated with elevated serum IgE levels. The *IL4R* SNPs Glu375Ala and Ser411Leu were also associated with the presence of asthma ($p = 0.02$). The homozygous 375Glu (wild-type) genotype was more prevalent in asthma cases, and thus carriers of the minor alleles showed decreased risks for asthma (table 3). The *IL4R* haplotype with the wild-type allele 411Ser and minor alleles for the remaining *IL4R* SNPs (50Val/375Ala/411Ser/551Arg/478Pro) had a protective effect since the haplotype was significantly less prevalent in asthma cases (3.8%) than in controls (9.4%, $p = 0.03$). Adjustment for age and gender by using regression analyses did not substantially alter the magnitude ($<10\%$ change), the direction or the significance of the association results (data not shown).

Gene-Gene Interaction – Asthma Case-Control Population

The genotypes *IL13* -1111CT/TT and *IL4R* 478SerSer, previously reported to show gene-gene interaction for asthma in our Dutch family study [7], were not significantly associated with asthma separately, yet their combination showed a trend to association with an increased risk of asthma compared to combined low-risk genotypes -1111CC and 478SerPro/ProPro (odds ratio: 2.3; 95% confidence interval: 1.0–5.5, $p = 0.06$; fig. 2). In combination, the genotypes that separately showed the strongest evidence for an association with asthma in the case-control population, i.e. *IL13* 130Gln carriers and *IL4R* 375Glu homozygotes, conferred an increased risk of asthma compared to combined low-risk genotypes 130ArgArg and 375AlaAla/GluAla (odds ratio: 4.6; 95% confidence interval: 1.8–11.9, $p = 0.001$; fig. 2).

Fig. 1. Position of and LD (D' and r^2) between SNPs analyzed in *IL4R* (a) and *IL13* (b). D' and r^2 between the SNPs within each gene were calculated in each population separately using the expectation-maximization algorithm after first deriving as much phase as possible in the trio populations (in-house software).

Discussion

We present the associations of polymorphisms in *IL13* and *IL4R* with rhinitis and asthma in a Dutch population. This is the first study that selected a group of rhinitis patients who were not affected by clinical asthma according to extensive clinical evaluation. In these patients, *IL13* polymorphisms were associated and *IL4R* polymorphisms showed a trend for association with rhinitis and

total serum IgE. Additionally, *IL13* polymorphisms were also associated with asthma, skin test response, and total serum IgE in two independent asthma populations that were clinically evaluated in a similar standard way. Furthermore, our data confirm an additive influence of *IL13* and *IL4R* variants on the susceptibility for asthma, as previously shown by Howard et al. [7].

This study is distinct because the rhinitis patients were subjected to an extensive clinical evaluation and asthma

Fig. 2. Combinations of genotypes of *IL4R* and *IL13* increase the risk of developing asthma. The upper panel shows genotype effects (odds ratios and 95% confidence intervals by logistic regression) of SNPs. The lower panel shows effects of combinations of genotypes from *IL4R* and *IL13* SNPs.

Table 3. Association of *IL4R* and *IL13* polymorphisms with rhinitis, asthma and atopy phenotypes, and in the rhinitis and asthma trio populations by using family-based association testing

SNPs	Allele	Rhinitis			log IgE in rhinitis trios			Asthma			Skin test response in asthma trios			log IgE in asthma trios		
		n ¹	Z score	p	n ¹	Z score	p	n ¹	Z score	p	n ¹	Z score	p	n ¹	Z score	p
<i>IL13</i>																
C-1111T	T	83	2.07	0.04 (0.24 ²)	83	2.06	0.04 (0.24 ²)	144	0.38	0.71	134	0.55	0.59	161	1.31	0.19
Arg130Gln	Gln	69	0.22	0.82	69	0.48	0.63	130	1.98	0.05 (0.08 ²)	121	2.29	0.02 (0.06 ²)	151	2.11	0.04 (0.07 ²)
G870A	A	80	0.52	0.60	80	0.86	0.39	113	2.23	0.03 (0.07 ²)	107	2.71	0.007 (0.06 ²)	133	2.66	0.008 (0.04 ²)
<i>IL4R</i>																
Ile50Val	Val	125	-1.87	0.06	125	-1.28	0.20	172	0.065	0.95	164	0.72	0.47	200	0.01	0.99
Glu375Ala	Ala	64	0.24	0.80	64	-0.33	0.74	89	-0.69	0.49	85	-0.60	0.55	101	-0.70	0.49
Ser411Leu	Leu	27	0.00	1.00	27	-0.14	0.89	43	0.91	0.37	39	0.00	1.00	48	0.94	0.35
Ser478Pro	Pro	81	0.41	0.68	81	-0.94	0.93	120	0.34	0.74	115	0.60	0.55	137	0.41	0.68
Gln551Arg	Arg	93	-0.19	0.85	93	-0.70	0.48	143	0.76	0.45	133	0.40	0.69	160	0.54	0.59

Significant p values (p < 0.05) are printed in bold type.

¹ Number of informative families.

² p value after False Discovery Rate correction; family-based association testing was performed with an additive model and a biallelic test.

was objectively excluded. To explicitly study the relation of *IL13* and *IL4R* SNPs in rhinitis separately from asthma, we had to exclude 38 (17%) of the 226 patients initially recruited for a self-reported history of rhinitis without asthma. They actually had asthma according to an asthma algorithm that is used to objectively define asthma for genetic research purposes [7, 8, 16]. Our results thus indicate that, due to the high coexistence of asthma and rhinitis, previous genetic studies on rhinitis may have been confounded by the presence of asthma. Notwithstanding this, other studies did report similar findings showing

associations of *IL13* and *IL4R* polymorphisms with cedar pollinosis [17], nonatopic rhinitis [18], self-reported medical diagnosis of hay fever [19], and allergic rhinitis [20, 21]. These results are strengthened by our findings.

This study on an independent Dutch asthma population shows a similar genetic interaction between the polymorphisms C-1111T in *IL13* and Ser478Pro in *IL4R* and the development of asthma in Caucasian subjects previously shown by Howard et al. [7]. Several studies have addressed a possible genetic interaction between SNPs of the *IL13* and *IL4R* gene, yet with different asthma and al-

Table 4. Association of *IL4R* and *IL13* polymorphisms with asthma, and serum IgE in the asthma case-control study

Gene SNP and genotype ¹	Asthma				log IgE	
	patients ²	controls ²	odds ratio ³	p	mean	p ¹
<i>IL13</i>						
C-1111T						
CC	67	65	1.0 (reference)		1.97	
CT/TT	43/5	23/4	1.7 (1.0–3.1)	0.07	2.13/2.24	0.10
Arg130Gln						
ArgArg	57	62	1.0 (reference)		1.95	
GlnArg/GlnGln	51/6	24/3	2.3 (1.3–4.1)	0.005 (0.03⁴)	2.15/2.37	0.02 (0.04⁴)
G870A						
GG	57	62	1.0 (reference)		1.95	
GA/AA	52/5	25/3	2.2 (1.2–3.9)	0.007 (0.02⁴)	2.13	0.03 (0.05⁴)
<i>IL4R</i>						
Ile50Val						
IleIle	31	18	1.0 (reference)		2.23	
IleVal/ValVal	58/22	22/14	0.8 (0.4–1.6)	0.48	2.10/2.14	0.29
Glu375Ala						
GluGlu	97	64	1.0 (reference)		2.07	
GluAla/AlaAla	19/0	24/3	0.5 (0.2–0.9)	0.02 (0.2⁴)	1.91/1.99	0.18
Ser411Leu						
SerSer	100	88	1.0 (reference)		2.03	
SerLeu/LeuLeu	16/0	5/0	2.8 (1.0–8.0)	0.05	2.03/–	1.00
Ser478Pro						
SerSer	82	63	1.0 (reference)		2.06	
SerPro/ProPro	25/5	25/5	0.8 (0.4–1.4)	0.39	1.91/2.08	0.23
Gln551Arg						
GlnGln	67	51	1.0 (reference)		2.12	
GlnArg/ArgArg	38/8	30/8	0.9 (0.5–1.6)	0.78	1.90/2.05	0.05

Skin tests were not available. Significant associations ($p < 0.05$) are printed in bold type.

¹ The associations with asthma best fitted a dominant genetic model, and thus individuals with one or two copies of the minor alleles were shown in comparison to individuals carrying no copy.

² Numbers do not always add up to 118 patients and 102 controls due to missing genotype data.

³ 95% confidence interval is shown in parentheses.

⁴ p value after False Discovery Rate correction.

lergic phenotypes and variable polymorphisms [22–25], or with other genes [26, 27]. The odds ratio for asthma in the current study was greater with polymorphisms Arg-130Gln and Glu375Ala than with the polymorphisms C-1111T and Ser478Pro, suggesting an additive effect. There was no indication for a multiplicative interaction on a logarithmic scale. However, we interpret this finding with caution since the power to distinguish such an effect is low due to the sample size of our study, resulting in wide confidence intervals as shown in figure 2. However, we think that considering the small sample size, it is even more remarkable to find consistent results with high odds ratios in the same direction as in previous studies. Only one study has addressed the biological effect of combined

polymorphisms in *IL13* and *IL4R* [28] and described a synergistic effect of recombinant *IL13* Arg130Gln and *IL4R* Q551R on IL13-induced gene transcription. However, this study does not explain the mechanism underlying this synergism. In order to fully understand the role of variation in these genes in the development of disease, the next challenge will be to elucidate the functionality of these combined SNPs.

Since we have tested three *IL13* polymorphisms and five *IL4R* polymorphisms with two phenotypes in the rhinitis and asthma case-control population and three phenotypes in the asthma trio population, one might argue that some of our results reflect type I statistical errors. After correction for multiple testing using False

Discovery Rate [15], the associations of *IL13* SNPs with asthma and serum IgE in the case-control population remained significant (*p* values ranging from 0.02 to 0.05). By evaluating whether *IL13* and *IL4R* polymorphisms contribute to rhinitis as well as to asthma, we aimed to test a specific biologically plausible hypothesis and to evaluate the role of these polymorphisms in the Dutch population, and found results in line with other publications. Therefore, we think these are true findings and chose to present our results without correction for multiple comparisons.

The genetic contribution of *IL13* and *IL4R* polymorphisms to the development of both rhinitis and asthma suggests that these polymorphisms influence the development of allergic sensitization and allergic inflammation in both the upper and the lower airways. *IL4R* Glu375Ala and Ser478Pro were associated with serum IgE in the allergic asthma trios, indicating that these SNPs may represent susceptibility alleles for allergy with asthma patients.

We found an association of rhinitis and asthma with variation in the same genes, but the SNPs and haplotypes were different in rhinitis and asthma. SNPs may have different biological effects depending on cell type or biological milieu. Functional studies have shown that *IL13* -1111C/T [29] and Arg130Gln [30, 31] and *IL4R* Gln551Arg [32, 33], and Ile50Val [34] have functional consequences such as changes in transcription rates, enhanced activity or signaling through the protein, or changes in serum protein levels in diverse cell types.

Some effects are suggested to depend on the biological context, e.g. the -1111T allele is associated with increased *IL13* transcription only in a Th2-differentiated cell, not in T-naïve cells [29]. Therefore we speculate that development of rhinitis and asthma is induced by variation in *IL13*, but the SNP or haplotype combinations may determine whether an individual develops rhinitis, asthma, or both in conjunction with environmental and cellular conditions.

In conclusion, our study strongly suggests that *IL13* polymorphisms influence the development of rhinitis as well as asthma and their associated allergic phenotypes IgE and skin prick test response. This supports the hypothesis that rhinitis and asthma may be tissue-specific regional manifestations of one disease and shows that *IL13* polymorphisms constitute a common etiologic pathway for the development of these conditions.

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