

Self-Reported Allergic Rhinitis and/or Allergic Conjunctivitis Associate with *IL13* rs20541 Polymorphism in Finnish Adult Asthma Patients

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Keywords

Allergic rhinitis · Allergic conjunctivitis · Asthma ·
Interleukin 13 · Sensitization

Abstract

Background: Our aim was to observe factors associated with *IL13* rs20541 polymorphism and other factors with or without allergic comorbidities such as subject-reported allergic rhinitis (AR) and/or allergic conjunctivitis (AC) symptoms in adult asthmatics. **Methods:** A population-based sample of Finnish adult asthma patients ($n = 1,156$) and matched controls ($n = 1,792$) filled in a questionnaire. Asthma was diagnosed based on a typical history of asthma symptoms and lung function tests. Skin prick tests with 17 aeroallergens and blood tests including analysis of interleukin 13 (IL13) rs20541 (G/A) genotypes were performed for a subsample ($n = 193$). **Results:** The proportion of asthmatics reporting AR was 61.9% and reporting AC was 40.7%. After adjustments, the presence of the *IL13* rs20541A– allele (OR 3.06, 95% CI 1.42–6.58, $p = 0.004$) or multisensitization (adjusted OR 4.59,

95% CI 1.48–14.26, $p = 0.008$) was associated with AR/AC asthma. Nasal polyps and acetylsalicylic acid-exacerbated respiratory disease was also associated with AR/AC asthma. **Conclusions:** Adult AR/AC asthma could putatively be a phenotype, characterized by the presence of atopic and/or eosinophilic factors and a high prevalence of the *IL13* rs20541A– allele. Studies on the mechanisms behind this and in other populations are needed.

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Introduction

The increased prevalence of asthma and allergic diseases is a major public health problem worldwide [1]. Atopy, family history, inhaled irritants, and upper airway inflammation are known risk factors of asthma [2–5].

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Up to 80% of asthma patients have allergic rhinitis (AR) [5]. A case-control study of French adult asthma patients showed that 92.4% of AR patients reported conjunctivitis symptoms [6]. In a Cameroonian community-based survey, 15.9% of adult asthma patients reported allergic conjunctivitis (AC) symptoms, whereas only 5.2% of nonasthmatic controls reported allergic rhinoconjunctivitis (ARC) symptoms [7]. A Finnish population-based study found that allergic rhinoconjunctivitis doubles the risk for incident asthma in adults [8].

Genome-wide association studies have identified several atopy-associating single nucleotide polymorphisms (SNPs) [9]. The T helper cell 2 (Th2) cytokine interleukin 13 (IL13) has been shown to be a central mediator of allergy and certain phenotypes of asthma [10]. Numerous SNPs have been identified in the *IL13* gene, namely rs20541 (+2044 G/A, R130Q G/A), which has been found to associate with allergy and/or asthma phenotypes in several ethnically diverse populations [11]. G2044A is located in exon 4 at position 2044 and causes a switch of the amino acid arginine to glutamine at codon 130 (R130Q) which may affect ligand-receptor interaction [12]. A recent study has found an association between rs20541 polymorphism and positive SPT result [13]. Another study failed to find statistical significance between rs20541 polymorphism and treatment and symptom severity of asthma [14]. Another group also reports an association between the *IL13* AA rs20541 genotype and airway remodeling in Japanese asthmatics [15].

We recently showed that a multisensitization pattern, AR or AC characterize adult-onset asthma [16]. The aim here was to further observe this allergic multimorbidity and its association with the *IL13* rs20541 polymorphism.

Methods

The study was approved by the Ethics committee of Pirkanmaa Hospital District. Written informed consent was obtained from the subjects. Asthma patients ($n = 1,156$) were Finnish population-based subjects (aged over 30 years) with entitlement to special reimbursement for asthma medication from the Social Insurance Institution of Finland (requiring a lung-function test, confirmed physician-diagnosed asthma, and regular use of asthma medication). For each asthma case, 1 or 2 controls ($n = 1,792$) without asthma were selected through a register covering the entire population. Asthma patients and nonasthmatic controls were matched for age, gender, and area of residence by postal code. AR was determined by the question: "Have you ever had hay fever or other rhinitis symptoms that are related to pollens or animals?" AC or atopic dermatitis (AD) were addressed through the question: "Have you ever had allergic eye or allergic skin symptoms?"

Lung function tests, blood tests, and skin prick tests (SPT) were performed in 1997 for a subsample as previously described [16]. One case and its corresponding control were withdrawn due to lacking information of AR/AC, thus the subsample consisted of 193 cases and 261 controls. DNA from whole blood samples and analysis of the *IL13* rs20541 polymorphism was performed according to previously described methods [17, 18]. The GA polymorphism at rs20541 of the *IL13* gene was analyzed using the ABI Prism 7000 sequence detection system for both PCR and allelic discrimination (Applied Biosystems, Foster City, CA, USA). A commercial kit from Applied Biosystems was used (TaqMan[®], Assay on Demand, purchase No. 185118655). The allele distribution of the cytokine gene studied followed Hardy-Weinberg equilibrium.

Statistics were analyzed with SPSS Base 18 Statistical Software Package (SPSS, Chicago, IL, USA). Comparisons were made by nonparametric Fisher's exact, Kruskal-Wallis, and Mann-Whitney U tests and binary logistic regression. Models for binary logistic regression were adjusted by 8 potential confounding factors: age, age of asthma onset, gender, occupation, nasal polyps (NP), acetylsalicylic acid-exacerbated respiratory disease (AERD), parental allergy, and either the *IL13* rs20541 genotype or the number of positive SPT values. Two-tailed p values < 0.05 were considered statistically significant.

Results

A total of 716 (61.9%) asthma patients and 524 (29.2%) control subjects reported AR symptoms, which associated significantly with asthma (OR 3.85, 95% CI 3.19–4.64, $p < 0.001$, by conditional logistic regression). The corresponding percentages of subjects reporting AC were 40.7 and 19.6%, respectively, and reporting AD were 33.6 and 22.8%, respectively. They also associated significantly with asthma (AC: OR 2.73, 95% CI 2.32–3.21, $p < 0.001$; AD: OR 1.7, 95% CI 1.48–2.05, $p < 0.001$). Only the presence of AR and/or AC were chosen for further evaluation due to their higher prevalence compared to AD.

When observing the subsample that underwent clinical tests ($n = 454$), the *IL13* rs20541 GA or AA genotype was not associated with the asthma phenotype ($p = 0.63$, OR 1.10, 95% CI 0.74–1.64). When observing only the asthma subgroup ($n = 193$), several factors associated with AR/AC symptoms, including the *IL13* rs20541 GA or AA genotype, the polysensitization pattern, sensitization to pollen or animal dander allergen(s), 3–17 positive SPT values, female gender, younger age, younger age at asthma onset, office/service work, NP, AERD, and parental allergy (Table 1). The following factors did not associate with AR/AC asthma: the severity of asthma symptoms, duration of asthma symptoms, smoking habits, FEV₁, body mass index, peripheral blood eosinophils, and total serum IgE (S-IgE; Table 1). Furthermore, the

Table 1. Characteristics of asthma patients with and without AR/AC symptoms

	AR ¹ and/or AC ¹		<i>p</i> value
	no (<i>n</i> = 72)	yes (<i>n</i> = 121)	
Gender			
Male	36 (50.0)	33 (27.3)	0.002
Female	36 (50.0)	88 (72.7)	
Age, years	64 (40–79)	56 (31–79)	0.001
Age of onset, years	53 (1–70)	46 (6–69)	0.001
Duration of symptoms, years	6 (1–53)	7 (1–58)	0.56
BMI ¹	26.4 (19.6–39.8)	25.9 (17.8–40.4)	0.39
FEV ₁ , %	88.7 (17–129)	87.8 (23–147)	0.090
B-eos, 10 ⁻⁹ /L	0.16 (0.04–0.50)	0.15 (0.02–0.92)	0.35
S-IgE, IU/mL	37.0 (4–1,461)	54.0 (4–5,900)	0.32
<i>IL13</i> rs20541 genotype			
GG	34 (48.6)	28 (23.7)	0.002
GA	29 (41.4)	65 (55.1)	
AA	7 (10.0)	25 (21.2)	
AERD ¹			
No	64 (94.1)	98 (83.1)	0.040
Yes	4 (5.9)	20 (16.9)	
NP ²			
No	68 (94.4)	101 (83.5)	0.026
Yes	4 (5.6)	20 (16.5)	
Smoking status			
Nonsmoker	34 (47.2)	63 (52.1)	0.31
Ex/current smoker	38 (52.8)	58 (47.9)	
Asthma symptoms			
None/mild	30 (41.7)	46 (38.0)	0.65
Moderate/severe	42 (58.3)	75 (62.0)	
Occupation			
Office/service work	13 (18.1)	35 (28.9)	0.018
Retired	45 (62.5)	50 (41.3)	
Other	14 (19.4)	36 (29.8)	
Parental allergy			
No	65 (92.9)	88 (72.7)	0.001
Yes	5 (7.1)	33 (27.3)	
Sensitized			
No	42 (58.3)	45 (37.2)	0.001
Monosensitized ³	18 (25.0)	25 (20.7)	
Polysensitized ⁴	12 (16.7)	51 (42.1)	
Pollen sensitization			
No	63 (87.5)	71 (58.7)	<0.001
Yes	9 (12.5)	50 (41.3)	
Animal dander sensitization			
No	60 (83.3)	66 (54.5)	<0.001
Yes	12 (16.7)	55 (45.5)	
Mite sensitization			
No	56 (77.8)	91 (75.2)	0.73
Yes	16 (22.2)	30 (24.8)	
Mold sensitization			
No	63 (87.5)	103 (85.1)	0.83
Yes	9 (12.5)	18 (14.9)	
SPT-positive values			
0	42 (58.3)	45 (37.2)	<0.001
1–2	23 (39.1)	25 (20.7)	
3–17	7 (9.7)	51 (42.1)	

Data are presented as *n* (%) or the median (min.–max. range). AR, allergic rhinitis; AC, allergic conjunctivitis; BMI, body mass index; B-eos, peripheral blood eosinophils; S-IgE, total serum IgE; AERD, acetylsalicylic acid-exacerbated respiratory disease; SPT, skin prick test; NP, nasal polyps. *p* values were determined by Fisher's exact test (dichotomous) or by Mann-Whitney U test (continuous).

¹ Subject-reported disease/symptom.

² Subject-reported doctor-diagnosed disease.

³ At least 1 positive SPT value to 1 allergen type (pollen/animal dander/mite/mold).

⁴ Positive SPT values to ≥2 allergen types.

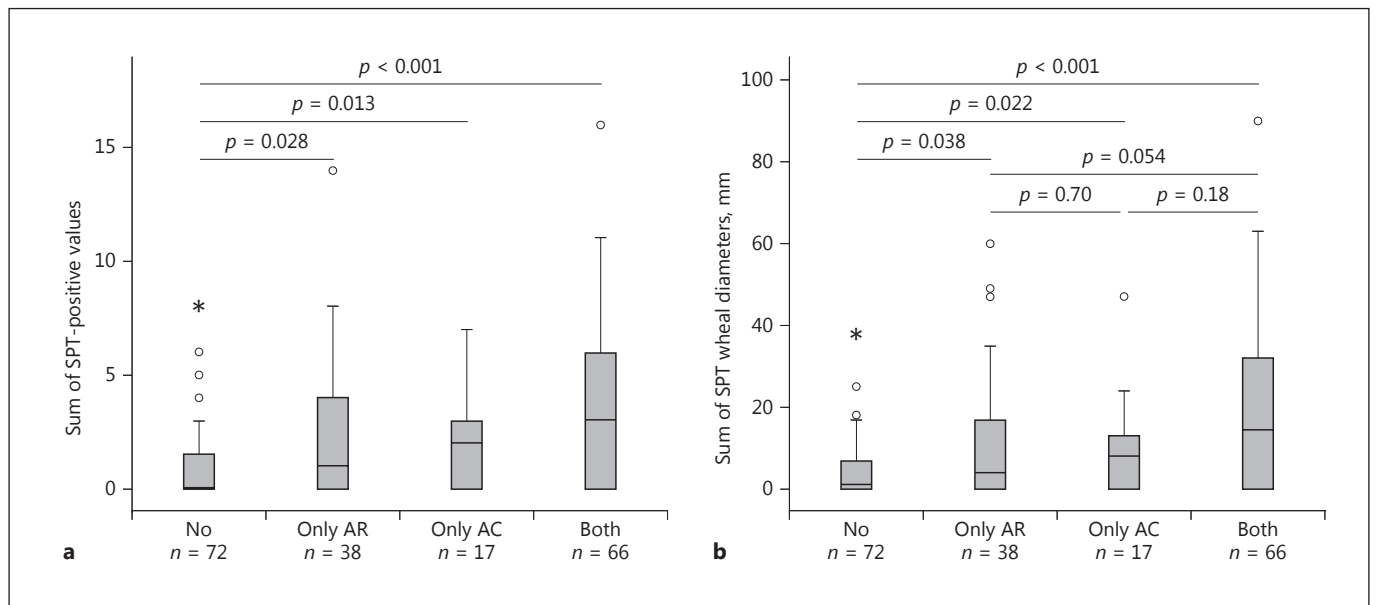


Fig. 1. Median sum of positive SPT values (**a**) and median sum of SPT wheal diameters (**b**) in the asthma subgroups.

following were also not associated: subject-reported use of peroral or inhaled corticosteroids, education or occupational education, childhood spent in the countryside or on a farm, or parental asthma ($p > 0.05$, data not shown).

In logistic regression analyses of the asthma group, the presence of the *IL13* rs20541 GA or AA genotype associated significantly with AR/AC (raw OR 3.04, 95% CI 1.61–5.71, $p = 0.001$; adjusted OR 3.06, 95% CI 1.42–6.58, $p = 0.004$) when the *IL13* rs20541 GG genotype was a reference. Three to seventeen positive SPT values associated significantly with AR/AC (raw OR 5.20, 95% CI 1.90–14.27, $p = 0.001$; adjusted OR 4.59, 95% CI 1.48–14.26, $p = 0.008$) when 0–2 positive SPT values was taken as the reference. Having 1 or more positive SPT values was not significantly associated with AR/AC (OR 1.01, 95% CI 0.50–2.05, $p = 0.97$) when 0 positive SPT values was the reference.

The median sum of positive SPT results and the size of SPT wheal diameters was greater in the asthma subgroups with AR and/or AC symptoms compared to the subgroup without AR/AC (Fig. 1). The highest sum of positive SPT results and largest SPT wheal diameters were detected in the asthma subgroup with both AR and AC symptoms (Fig. 1). S-IgE was not associated with the *IL13* rs20541 GA or AA genotype in asthmatics ($p > 0.05$, data not shown).

Discussion

The current challenge is to prevent atopy in an attempt to reduce the asthma burden [5–8]. Also, a significant proportion of asthmatics fail to respond to therapy [19]. Our finding of a high prevalence of AR/AC symptoms in adult asthmatics is in line with these observations [5–8]. After adjustments, the *IL13* rs20541 GA or AA genotype, or a multisensitization pattern affected the risk of AR/AC in the asthma group. *IL13* rs20541A– allele-carrying patients had a predisposition towards multisensitization and AR/AC symptoms in asthma. The *IL13* rs20541A allele was not related to the whole asthma group, which could indicate that this genotype would be characteristic of a true atopic asthma endotype. We demonstrated a dose-dependent association between sensitization and the presence of AR and/or AC symptoms in asthmatics, strengthening the previous reports that sensitization is not a dichotomous value [16, 20].

NP and AERD were also associated with AR/AC asthma. Hence, adult AR/AC asthma seems to include atopic and eosinophilic characteristics. Further studies to prove this are required.

Female gender was associated with AR/AC asthma, reflecting putatively gender-based differences in asthma pathogenesis, which are perceived as more severe and having a more pronounced effect on activities of daily liv-

ing in women [21]. Parental allergy, young patients, and those with a younger age at onset were further factors associated with AR/AC symptoms, which could indicate the burden of the atopic asthma phenotype in Finland. The association of office/service work with AR/AC symptoms might be in part explained by the fact that these occupations are more frequent in younger age groups, which has more polysensitization and AR/AC symptoms. However, these factors were not independently associated with AR/AC.

S-IgE was not associated with AR/AC asthma or with the *IL13* polymorphism, indicating that S-IgE is affected by several genome-environmental interactions. AR/AC did not associate with the severity of asthma. In accordance with this, variation exists in the severity of the atopic asthma phenotypes, depending on eosinophilia, female gender, and early multiple allergies [20, 22].

Natural variation in the coding region of *IL13* has been suggested to be an important genetic determinant of susceptibility to allergy [11, 23]. A meta-analysis suggests that the *IL13* rs20541 G/A polymorphism is associated with (allergic) asthma [24]. A study group examined the impact of *IL13* rs20541 G→A on the functional properties of IL-13 by directly comparing the activity of wild-type IL-13 and IL-13 R130Q on primary human cells [11]. The group showed that IL-13 R130Q was significantly more active than wild-type IL-13 in inducing STAT6 and CD23 in monocytes and hydrocortisone-dependent IgE switching in B cells, and it was neutralized less effectively. The IL-13 variant was unable to engage T cells, which suggests that increased allergic inflammation in carriers of *IL13* rs20541 A depends on enhanced IL-13-mediated Th2 effector functions rather than increased Th2 differentiation. A recent meta-analysis found that the *IL13*+1923C/T polymorphism was significantly associated with the risk of asthma; however, the authors were not able to study the

effect of modification by atopic status [25]. The *IL13*-RAD50 and *IL4*-KIF3A loci were found to be associated with AR and asthma [9]. However, severe forms of asthma are not all IL-13 endotypes of asthma and only some forms of Th2 asthmatics respond to anti-IL-4 or anti-IL-13 therapy, as was recently demonstrated [26].

To conclude, an adult atopic/eosinophilic asthma phenotype could putatively be characterized by a high prevalence of the *IL13* rs20541A allele, a multisensitization pattern, and AR/AC symptoms. However, this needs to be proven by studies on pathomechanisms and in increased populations. Further studies are still needed on specific biomarkers and risk-conferring genes for each asthma phenotype and possible endotype.

Acknowledgements

The authors thank Sinikka Repo-Koskinen for excellent assistance. The study was supported in part by research grants from the Finnish Medical Society Duodecim, the Finnish Society of Allergy and Immunology, the Jane and Aatos Erkko Foundation, the Finnish Cultural Foundation, the Tampere Tuberculosis Foundation, the Väinö and Laina Kivi Foundation, and the Yrjö Jahns-son Foundation.

Author Contributions

K.Å., A.L., S.T.-S., and J.K., planned the study, performed analyses and wrote the manuscript. M.H. provided the laboratory tests. J.P., H.H., R.R., D.Y.W., and M.J.M. provided expertise in analyses. All authors critically reviewed the manuscript.

Disclosure Statement

The authors declare that they have no conflicts of interest.

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