



Association of STR polymorphisms in *CMA1* and *IL-4* with asthma and atopy: The SAPALDIA Cohort

Martin Hersberger^{a,*}, Gian-Andri Thun^b, Medea Imboden^b, Anita Brandstätter^c, Vanessa Waechter^a, Monika Summerer^c, Peter Schmid-Grendelmeier^d, Andreas Bircher^e, Lucia Rohrer^f, Wolfgang Berger^g, Erich W. Russi^h, Thierry Rochatⁱ, Florian Kronenberg^c, Nicole Probst-Hensch^b

^a Division of Clinical Chemistry and Biochemistry, University Children's Hospital Zurich and Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland

^b Swiss Tropical and Public Health Institute, Basel, Switzerland, and University of Basel, Basel, Switzerland

^c Division of Genetic Epidemiology, Department of Medical Genetics, Molecular and Clinical Pharmacology, Innsbruck Medical University, Innsbruck, Austria

^d Allergy Unit, Department of Dermatology, University Hospital Zurich, Zurich, Switzerland

^e Division of Allergy, University Hospital Basel, Basel, Switzerland

^f Institute of Clinical Chemistry, University Hospital Zurich, Zurich, Switzerland

^g Institute of Medical Genetics, University of Zurich, Zurich, Switzerland

^h Pulmonary Division, University Hospital Zurich, Zurich, Switzerland

ⁱ Pulmonary Division, University Hospital of Geneva, Geneva, Switzerland

ARTICLE INFO

Article history:

Received 20 April 2010

Accepted 16 August 2010

Available online 22 August 2010

Keywords:

CMA1

UGB

CC10

CC16

SCGB1A1

TNF- α

IL-4

Genetics

Haplotype

Polymorphism

Short tandem repeat

STR

Asthma

Bronchial hyperresponsiveness

Atopy

ABSTRACT

Asthma is a chronic pulmonary disorder that is characterized by airway inflammation and bronchial hyper-reactivity. Several genetic loci have been associated with asthma, and some of these associations have been replicated in independent studies. However, larger population-based replication studies for the association of short tandem repeat (STR) polymorphisms with asthma are limited. In this study, we investigated the association of STR polymorphisms in genes encoding mast cell chymase (*CMA1*), uteroglobin (*UGB*), tumor necrosis factor- α (*TNF- α*) and interleukin-4 (*IL-4*) with asthma and atopic phenotypes in the large population-based Swiss Cohort Study SAPALDIA. Our results show that the STR polymorphism in the *CMA1* gene is associated with asthma and that this association is even stronger with atopic asthma. Similarly, we observed a weak association of the *IL-4* 2-allele with asthma that tended to be stronger for atopic asthma than for nonatopic asthma. This minor *IL-4* 2-allele was also associated with higher IgE levels, with a higher risk for a positive skin prick test and with a trend for a higher risk for bronchial hyperresponsiveness. These results support previous findings suggesting a role for *CMA1* and *IL-4* in atopic asthma and for *IL-4* in atopy in general.

© 2010 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.

1. Introduction

Asthma is a pulmonary disorder which is characterized by airway inflammation presenting with reversible airway obstruction and bronchial hyperreactivity [1]. Asthma is a complex disease, with different clinical phenotypes that include as major characteristics a variable degree of airflow obstruction, bronchial hyperresponsiveness, and airway inflammation. Both environmental and genetic factors contribute to the inception and the development of asthma [2]. Genetic factors contributing to asthma were identified in linkage studies followed by fine mapping, in case-control studies investigating an association of candidate genes involved in inflam-

mation and tissue regeneration with asthma [3], as well as in large genome-wide association studies [4–6]. Although the association of single nucleotide polymorphisms of several genes with asthma have been reinvestigated in replication studies, larger population-based replication studies for the association of STR polymorphisms with asthma are usually lacking [3].

The STR polymorphisms are multiallelic markers and are powerful to detect a linkage disequilibrium of a certain genetic region with an inherited trait [7,8]. Such STR polymorphisms have been useful in genome wide family based linkage studies to identify the genetic cause for a number of monogenic diseases [9–12]. However, there is also indication that some of the STR polymorphisms have functional consequences influencing transcription [13–15], protein abundance and protein function [16–18]. For example, the

* Corresponding author.

E-mail address: martin.hersberger@kispi.uzh.ch (M. Hersberger).

minor alleles of the STR polymorphism in the promoter of *ALOX5* encode different numbers of binding sites for the transcription factor SP1 and were shown to result in reduced transcription of the *ALOX5* gene [14,15]. In addition, STR alleles with a higher number of CAG repeats, which translate into longer glutamine stretches in the androgen receptor or in the huntingtin protein, are associated with X-linked spino-bulbar muscular atrophy and Huntington's disease, respectively [16,17]. Combining the results of associations of single nucleotide and STR polymorphisms in a gene with a certain phenotype, may well support the search for the functional variation through better haplotype prediction in polygenic diseases.

We identified four STR polymorphisms of interest in the literature, three of which show an association with asthma and one of which leads to functional consequences and is located in a gene involved in the pathogenesis of asthma. A STR polymorphism in the gene encoding the mast cell chymase (*CMA1*) has been associated with asthma in two small case-control studies [19,20], whereas a single-nucleotide polymorphism in the promoter of *CMA1* did not show an association with asthma [21]. *CMA1* is a protease involved in inflammatory mechanisms that acts in concert with histamine. *CMA1* is released from activated mast cells in asthmatic airways and has profound effects on inflammation, airway smooth muscle cell functioning, and airway remodeling [22]. Injection of purified *CMA1* into the skin of guinea pigs provokes an increase in microvascular leakage and induces a marked neutrophilia and eosinophilia [23,24].

Two polymorphic regions in the uteroglobin gene (*UGB*, also known as CC10, CC16, or SCGB1A1) have been associated with asthma in smaller case-control studies. The first polymorphic region is a STR located in the first intron of the *UGB* gene, which showed an association with asthma [25]. The second region is a single-nucleotide polymorphism (SNP; A38G) in *UGB*, which was found to be associated with asthma in four studies [26–29], whereas no association was detected in two other populations [25,30]. *UGB* is expressed primarily in the respiratory tract and is secreted by nonciliated bronchiolar secretory (Clara) cells, known to play important immunomodulatory and anti-inflammatory roles in airway inflammation. Reduced *UGB* levels have been reported in serum and airways of asthmatic patients [31,32], which increased during acute asthma episodes [33].

Single nucleotide polymorphisms in the genes coding for tumor necrosis factor (TNF)- α and interleukin (IL)-4 have been associated with asthma in several studies [3] including those by our group [34,35]. In contrast, there are only limited data available on the association of STRs in these genes with asthma. No study has investigated the association of a STR in the locus of the *TNF- α* gene with asthma, although there is indication that the TNF α gene in the *TNF- α* gene alters TNF- α secretion of leukocytes [36], and only one study reported an association of the *IL-4* STR with asthma in children [37]. Both of these cytokines play a central role in asthma and atopy, with TNF- α levels linked to several pathologic features, such as hyperresponsiveness in asthma, and IL-4 identified as a master switch for B-cells to synthesize IgE [2].

To investigate the association of the STR polymorphisms in the *CMA1*, the *UGB*, *TNF- α* , and *IL-4* genes previously associated with asthma and atopic disease, we analyzed these STRs in the large population-based Swiss Cohort Study SAPALDIA (Swiss Study on Air Pollution and Health in Adults).

2. Subjects and methods

2.1. Study population

The SAPALDIA study population has been described before [38,39]. It consists of a random population sample of adults aged 18–60 years at baseline from eight communities in Switzerland. A total of 9651 persons participated in the first assessment in 1991. At the follow-up

examination in 2002, 8047 participants were reassessed with at least a short screening questionnaire. At the follow-up examination, 6058 participants provided questionnaire data, provided a blood sample, and consented to genetic analysis. Included in this study are 5749 participants whose DNA was successfully genotyped for at least one of the STR polymorphisms and who had no missing information on asthma at the baseline examination. Written informed consent was obtained from all study participants. The study was approved by the Swiss Academy of Medical Sciences and the regional ethics committees.

2.2. Definition of asthma, atopy and bronchial hyperresponsiveness

Participants underwent a detailed, computer-assisted interview comprising questions about smoking behavior, exposure to environmental tobacco smoke, workplace exposure to dust and fumes, co-morbidities including asthma, medication use and socioeconomic factors. Physician-diagnosed asthma was defined by affirmative answers to “Have you ever had asthma?” and “Was this confirmed by a doctor?” Current asthmatic patients, comprising a subset of those whose asthma was diagnosed by a physician, were classified by an affirmative answer to at least one of the following two questions: “Have you had an attack of asthma in the last 12 months?” and “Are you currently taking any medicines including inhalers, aerosols, or tablets for asthma?” Asthmatics were further subdivided into atopic asthmatic patients with positive allergen-specific IgE test results or with positive skin prick test results and into nonatopic asthmatic patients without a positive test result for either of these two tests.

A skin prick test conducted at baseline and performed with Phazet assessed allergic sensitization to cat, dust mite, timothy grass, *Parietaria*, birch, and molds (*Cladosporium* and *Alternaria*). It was considered positive for a wheal diameter >3 mm [38,40].

Total serum IgE was measured at baseline using the Pharmacia CAP FEIA system. The presence of specific serum IgE antibodies to a mixture of relevant respiratory allergens (pollen, house dust mites, molds, and animal epithelia) was assessed by the Phadiatop test (Phadia Uppsala, Sweden). Positive reaction was defined as proposed by the manufacturer. All serum testing for IgE was done in the Allergy Laboratory of the Department of Dermatology, University Hospital Zurich in 1993. Blood samples of two volunteers (both consistently Phadiatop positive) were used as quality control [40,41].

Bronchial hyperresponsiveness (BHR) was assessed at baseline by methacholine challenge (Provocoline, Roche, Nutley, NJ). Increasing concentrations of methacholine (0.39, 1.56, 6.25, and 25.0 mg/ml solutions in a phosphate buffer without phenol) were administered through an aerosol dosimeter [42]. The presence of BHR was defined as a fall of $\geq 20\%$ in forced expiratory volume in 1 second (FEV₁) up to a cumulative dose of 2 mg (8.37 μ mol).

Never smokers were defined as persons who at the time of the baseline interview had smoked less than 20 packs of cigarettes or 360 g of tobacco during their lifetime. Former smokers reported quitting at least 1 month before the interview.

Body mass index (BMI) at baseline was divided into four categories: underweight (BMI <20 kg/m²), normal weight (BMI 20–25 kg/m²), overweight (BMI ≥ 25 –30 kg/m²), and obese (BMI ≥ 30 kg/m²).

2.3. Genetic analysis

DNA extraction from ethylenediaminetetraacetic acid (EDTA)-buffered whole blood has been previously described [38]. A total of 5902 DNA samples were subjected to STR-multiplex genotyping. Before amplification, the DNA concentration was measured with a NanoDrop spectrophotometer (PEQLAB Biotechnologies, Erlangen, Germany), and the samples were normalized to contain 20 ng/ μ l

DNA with a TECAN Freedom EVO liquid handling platform (TECAN, Maennedorf, Switzerland). Two negative controls were assayed on each 96-well plate.

Seven STRs were genotyped in one multiplex polymerase chain reaction (PCR). Primers were designed with VisualOMP (DNA Software, Ann Arbor, MI). For amplification, 5 μ l of Qiagen Multiplex PCR Mastermix (Qiagen, Hilden, Germany), 2 μ l of water, and 1 μ l of the primer master mix were dispensed (Microsynth, Balgach, Switzerland). Primer sequences and final concentrations are listed in the Supplementary Table 1. Next, 2 μ l of normalized DNA was added. The amplification reaction was conducted on a thermal cycler (Bio-Rad DNA Engine) with the following settings: 95°C for 15 minutes and 30 reaction cycles of 94°C for 30 seconds, 56.5°C for 90 seconds, and 72°C for 60 seconds, followed by a final extension at 60°C for 30 minutes. The PCR products were 20 times diluted with Hi-Di formamide, and then 1 μ l of the diluted PCR-product was mixed with 8.8 μ l of Hi-Di formamide and 0.2 μ l of GeneScan-500 LIZ size standard (all Applied Biosystems, Foster City, CA). Samples were denatured at 95°C for 5 minutes, and the electrophoresis was run on an ABI 3130xl Genetic Analyzer using POP-7 polymer (both AB).

Results were analyzed using GeneMapper version 4.0 (AB). For each STR, 5 homozygous samples were subjected to Sanger sequencing to calibrate the allele calling algorithm in GeneMapper.

A fraction of samples (5%, corresponding to 4 DNA samples per 96-well plate) was genotyped twice for quality assurance. The discordance rate was 0. The call rates for the seven STRs were 97.5% (*UGB*), 98.1% (*CMA1*), 98.5% (*cPLA2 α*), 98.5% (*IL-4*), 98.4% (*iNOS-M3*), 97.4% (*iNOS-M1*), and 97.8% (*TNFD*). All investigated STRs were in Hardy–Weinberg equilibrium.

The STR investigated in *UGB* is located between Alu repeat elements in intron 1 of the gene [43]. The STR in *CMA1* is located 254 bp downstream of the gene (accession number BV210164) [20]. The STR in *IL-4* is located in intron 3 (VE6523) of the gene [37] and the STR of the *TNFD* locus is located 8 kb downstream of the *TNF- α* gene in intron 3 of the *LST-1* gene [44].

2.4. Statistical analysis

Baseline characteristics of study participants included in this article and allele distributions between asthma patients and controls were compared by χ^2 test. The cross-sectional associations of STR genotypes with physician-diagnosed or current asthma at baseline were assessed using multivariable unconditional logistic regression. All regression models included study area, gender, and age at baseline as covariates. Additional adjustment for atopy, smoking status or smoking intensity, early childhood infection, family history of asthma and exposure to gas, dust, or fumes did not materially alter the results presented and was therefore omitted.

Interaction between genotypes and markers of atopy was tested by integrating multiplicative terms in the regression models.

All statistical analyses were performed using Stata version 9.2 (Stata Corporation, College Station, TX). Significance levels for two-sided tests of main effects were chosen at $\alpha = 0.05$, and at $\alpha = 0.10$ for tests of effect modification [45].

3. Results

The characteristics of the study population overall, stratified according to the presence of asthma, are shown in Table 1. Participants with asthma were more likely to have a family history of asthma and to have experienced respiratory infections during childhood than controls. Participants with asthma were also more likely to be ex-smokers but were less likely to be current smokers at the time of investigation. Asthmatics had a lower FEV₁, had more bronchial hyperresponsiveness, and showed more often aspects of atopy, such as positive skin prick test results, antibodies to common inhalant allergens in the Phadiatop, and higher levels of total IgE, than did controls.

The allele distribution between participants with and without asthma for the STR polymorphisms in the *CMA1*, *UGB*, *TNF- α* , and *IL-4* genes are shown in the Supplementary Table 2. They were comparable to the previously published frequencies for all the STRs analyzed [20,25,37,46,47] and were not different between participants with and without asthma for the *UGB*, *TNF- α* , and *IL-4* genes. However, the overall allele frequencies for the *CMA1* alleles ($p = 0.04$) differed between subjects with and without current asthma (Fig. 1) with the largest difference observed for the 39-allele between groups. Logistic regression analysis using a co-dominant model revealed that the 39-allele of the *CMA1* STR was associated with both, current asthma (odds ratio [OR] = 1.72, 95% confidence interval [CI] = 1.09–2.72; Table 2) and physician-diagnosed asthma (OR = 1.39, 95% CI = 1.03–1.88; Table 3). Stratification of the case groups into atopic and nonatopic asthma patients showed that homozygous carriers of the *CMA1* 39-allele had an increased risk for atopic but not for nonatopic asthma (Tables 2 and 3). We detected an increased risk for homozygous carriers of the *CMA1* 39-allele for current asthma with a positive skin prick test (OR = 3.37, 95% CI = 1.66–6.84; Table 2) and with a positive Phadiatop test result (OR = 3.12, 95% CI 1.68–5.79), whereas homozygosity for the *CMA1* 39-allele did not modulate the risk in current asthma patients with a negative skin prick test (OR = 0.94, 95% CI = 0.47–1.88) or with a negative Phadiatop test result (OR = 0.53, 95% CI = 0.22–1.32). The interaction was strongest between the positive Phadiatop test result and the *CMA1* 39-allele (Table 2) (p interaction = 0.009), and less so between the positive skin prick test and the *CMA1* 39-allele (p interaction = 0.06). Similar results were ob-

Table 1
Baseline characteristics of the SAPALDIA study population

Characteristic	All ^a (n = 5749)	No current asthma (n = 5595)	Current asthma (n = 154)	p Value
Age, y, mean $\pm$ SD	41.24 $\pm$ 11.40 (n = 5749)	41.24 $\pm$ 11.40 (n = 5595)	41.18 $\pm$ 11.32 (n = 154)	0.94
Female, %	50.27 (n = 5749)	50.19 (n = 5595)	53.25 (n = 154)	0.45
Asthma family history, %	20.18 (n = 5698)	19.68 (n = 5545)	38.56 (n = 153)	<0.001
Childhood respiratory infection, %	8.07 (n = 5748)	7.81 (n = 5594)	17.53 (n = 154)	<0.001
Never smoker, %	47.18 (n = 5742)	46.96 (n = 5588)	55.19 (n = 154)	0.04
Ex-smoker, %	23.01 (n = 5742)	22.82 (n = 5588)	29.87 (n = 154)	0.04
Current smoker, %	29.82 (n = 5742)	30.23 (n = 5588)	14.94 (n = 154)	<0.001
BHR, %	15.82 (n = 4457)	14.87 (n = 4370)	63.22 (n = 87)	<0.001
Baseline FEV ₁ , mean $\pm$ SD	3.58 $\pm$ 0.84 (n = 5450)	3.59 $\pm$ 0.83 (n = 5302)	3.16 $\pm$ 0.97 (n = 148)	<0.001
SPT + (%)	22.71 (n = 5545)	21.88 (n = 5403)	54.23 (n = 142)	<0.001
IgE, kU/l, mean $\pm$ SD ^b	3.39 $\pm$ 1.52 (n = 5328)	3.36 $\pm$ 1.51 (n = 5185)	4.51 $\pm$ 1.59 (n = 143)	<0.001
Phadiatop+, %	28.84 (n = 5329)	27.84 (n = 5186)	65.03 (n = 143)	<0.001

BHR, bronchial hyperresponsiveness; FEV₁, forced expiratory volume in 1 second; SPT, skin prick test.

^aSubjects with at least one successfully genotyped short tandem repeat (STR) and complete information on both asthma definitions.

^bMeasured IgE-levels were log-transformed.

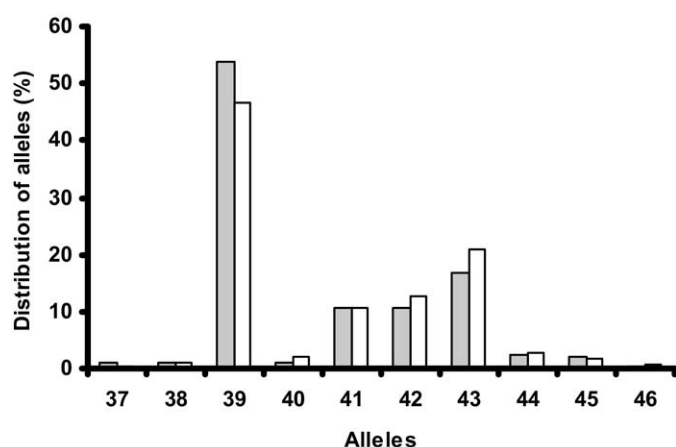


Fig. 1. *CMA1* allele distribution in 5749 study participants with (grey columns, $n = 154$) and without (white columns, $n = 5595$) current asthma of the population-based SAPALDIA Study.

served with physician diagnosed asthma after stratification (Table 3).

Previous studies also reported associations of individual alleles of the STR polymorphisms in the *UGB*, *TNF- α* , and *IL-4* genes with asthma or other inflammatory diseases. We therefore investigated the association of these specific alleles with asthma. No association was observed in our sample for the *UGB* allele 4–8 or the *TNF- α* 23-allele with either physician diagnosed or current asthma, while the *IL-4* 2-allele showed a trend for an increased risk in both asthma subgroups (Supplementary Table 3a–3c). Using a recessive model, we detected a weak association of the *IL-4* homozygous 2/2 genotype with current asthma (Table 4), and stratification according to the atopy status indicated that the *IL-4* homozygous 2/2 genotype would increase the risk for atopic (ORs = 1.73–2.25; Table 4 and Supplementary Table 4) but not for nonatopic asthma (ORs = 0.33–1.46). The latter result was only significant in physician-diagnosed asthma with a positive Phadiatop (Supplementary Table 4), two

Table 2
Association of *CMA1* 39-allele with current asthma^a

	Cases/controls (n)	OR	95% CI	p Value
Asthma	All			
Non-39/non-39	34/1617	Ref		
39/non-39	72/2714	1.25	0.83/1.90	0.29
39/39	45/1237	1.72	1.09/2.72	0.02
				$r^2 = 0.017$
Asthma with negative skin prick test	Nonatopic asthma			
Non-39/non-39	19/1195	Ref		
39/non-39	31/2064	0.91	0.51/1.63	0.76
39/39	15/942	0.94	0.47/1.88	0.86
Asthma with negative Phadiatop				
Non-39/non-39	15/1056	Ref		
39/non-39	27/1824	0.96	0.51/1.83	0.91
39/39	7/842	0.53	0.22/1.32	0.18
Asthma with positive skin prick test	Atopic asthma			
Non-39/non-39	12/365	Ref		
39/non-39	36/548	2.07	1.06/4.05	0.03
39/39	27/263	3.37	1.66/6.84	0.001
Asthma with positive Phadiatop				
Non-39/non-39	16/437	Ref		
39/non-39	41/691	1.67	0.92/3.02	0.09
39/39	34/309	3.12	1.68/5.79	<0.001
Interaction (skin prick test and <i>CMA1</i> -39)				0.06
Interaction (Phadiatop and <i>CMA1</i> -39)				0.009

^aAdjusted for age, gender, and area.

Table 3
Association of *CMA1* 39-allele with physician diagnosed asthma^a

	Cases/controls (n)	OR	95% CI	p Value
Asthma	All			
Non-39/non-39	87/1564	Ref		
39/non-39	177/2609	1.21	0.92/1.57	0.17
39/39	93/1189	1.39	1.03/1.88	0.03
				$r^2 = 0.010$
Asthma with negative skin prick test	Nonatopic asthma			
Non-39/non-39	33/1181	Ref		
39/non-39	79/2016	1.36	0.90/2.07	0.14
39/39	35/922	1.31	0.80/2.13	0.28
Asthma with negative Phadiatop				
Non-39/non-39	29/1042	Ref		
39/non-39	69/1782	1.31	0.84/2.05	0.23
39/39	24/825	0.98	0.56/1.71	0.95
Asthma with positive skin prick test	Atopic asthma			
Non-39/non-39	48/329	Ref		
39/non-39	92/492	1.26	0.86/1.84	0.23
39/39	55/235	1.60	1.05/2.46	0.03
Asthma with positive Phadiatop				
Non-39/non-39	52/401	Ref		
39/non-39	101/631	1.23	0.86/1.76	0.26
39/39	64/279	1.77	1.19/2.65	0.005
Interaction (skin prick test and <i>CMA1</i> -39)				0.62
Interaction (Phadiatop and <i>CMA1</i> -39)				0.08

^aAdjusted for age, gender, and area.

terms that also showed a weak interaction (p for interaction = 0.08; Supplementary Table 4).

To investigate whether the *CMA1* 39-allele, *UGB* 4–8-allele, *TNF- α* 23-allele, or *IL-4* 2-allele is associated with atopic phenotypes, we analyzed the association of these candidate alleles with bronchial hyperresponsiveness, IgE levels, positive skin prick tests and Phadiatop results. The homozygous genotype of the minor *IL-4* 2-allele was associated with higher IgE levels compared with all other *IL-4* genotypes (Table 5). Stratification of the IgE levels according to asthma case-status showed that the association of *IL-4* 2-allele with higher IgE levels was seen mainly in controls and less so in asthma patients. The homozygous genotype of *IL-4* 2-allele was also associated with a higher risk for a positive skin prick test

Table 4
Association of *IL-4* 2-allele with current asthma^a

	Cases/controls (n)	OR	95% CI	p Value
Asthma	All			
Non-2/non-2	146/5439	Ref		
2/2	8/144	2.13	1.02/4.45	0.04
				$r^2 = 0.017$
Asthma with negative skin prick test	Nonatopic asthma			
Non-2/non-2	63/4113	Ref		
2/2	2/97	1.46	0.35/6.09	0.61
Asthma with negative Phadiatop				
Non-2/non-2	49/3640	Ref		
2/2	1/93	0.84	0.11/6.23	0.87
Asthma with positive skin prick test	Atopic asthma			
Non-2/non-2	72/1139	Ref		
2/2	5/42	1.84	0.70/4.87	0.22
Asthma with positive Phadiatop				
Non-2/non-2	87/1398	Ref		
2/2	6/43	2.25	0.92/5.50	0.08
Interaction (skin Prick test and <i>IL-4</i> -2)				0.37
Interaction (Phadiatop and <i>IL-4</i> -2)				0.77

^aAdjusted for age, gender, and area.

Table 5
Association of *IL-4* 2-allele with IgE levels

	Mean log total IgE (kU/l)	95% CI	p Value
All ^a			
Non-2/non-2 (5174)	3.39	3.35/3.43	0.02
2/2 [142]	3.68	3.44/3.93	
No physician diagnosed asthma			
Non-2/non-2 (4844)	3.33	3.29/3.37	0.03
2/2 [129]	3.62	3.37/3.88	
Physician diagnosed asthma			
Non-2/non-2 (330)	4.28	4.11/4.46	0.54
2/2 [13]	4.55	3.71/5.40	
No current asthma			
Non-2/non-2 (5038)	3.36	3.31/3.40	0.02
2/2 [135]	3.65	3.40/3.90	
Current asthma			
Non-2/non-2 (136)	4.65	4.35/4.95	0.81
2/2 [7]	4.80	3.62/5.98	

^aAdjusted for age, gender, area, and asthma at baseline.

and with a trend for a higher risk for bronchial hyperresponsiveness, further supporting a role for this STR polymorphism in atopic disease (Table 6).

4. Discussion

Here we report our investigation of the association of the STR polymorphisms in the *CMA1*, *UGB*, *TNF- α* , and *IL-4* genes involved in inflammation with the risk for asthma and atopy in the SAPALDIA cohort. All of these STRs have previously been associated with asthma, asthma-related phenotypes or other inflammatory diseases like nephropathy. The main findings of this large population-based cohort study are that the STR in the *CMA1* is associated with atopic but not with nonatopic asthma and that the STR in the *IL-4* gene is associated with total serum IgE levels, measures of atopy, and weakly with asthma itself.

The association of the *CMA1* STR with asthma confirms the findings of a previous study in an Indian sample and the limitation of the association to atopic asthma patients expands these findings. Similar to the Indian sample, we observed a difference in allele distribution between asthma patients and controls. In both studies 39-allele carriers were overrepresented in asthma patients, whereas 43-allele carriers were underrepresented. A significant association with asthma was observed in SAPALDIA for the 39-allele, whereas in the Indian study the 43-allele was significantly inversely associated with asthma [20]. Stratification of the SAPALDIA sample further revealed that the *CMA1* 39-allele increases the risk for atopic asthma although it was not found to be associated with nonatopic asthma. In line with our findings, the Indian sample consisted of atopic asthma patients only, supporting the restriction of association of the *CMA1* 39-allele with atopic asthma. This restriction would argue for a role of the mast cell chymase during atopic responses of the mast cells, which seems to be independent of a modulation of IgE levels (data not shown). Indeed, the chymase is released from activated mast cells together with histamine through degranulation following IgE activation [22]. Chymase activates PAR-1 and augments neutrophil and eosinophil recruitment [48] and probably plays a role in fibrosis [48]. However, whether this STR in the *CMA1* gene is functional or in linkage disequilibrium with another causal variant in this genetic region responsible for the asthma risk increase is currently not known.

The STR in intron 3 of the *IL-4* gene showed a weak association with asthma, but was associated with IgE levels and measures of atopy in our study. *IL-4* plays a central role in IgE regulation by triggering isotype switching from IgM to IgE and IgG4 and enhances the expression the low-affinity receptor for IgE and MHC class II molecules. Furthermore, *IL-4* is necessary for the promotion of its

own production and thus is critical for the induction and maintenance of allergy [37]. In this line, we find that the minor 2-allele of the *IL-4* STR polymorphism is associated with higher IgE levels and an increased risk for a positive skin prick test result. Several studies have reported an association of different *IL-4* polymorphisms with IgE levels, and some but not all studies have shown an association with asthma and other atopic diseases [3,49]. In our study, the *IL-4* STR was associated only with current asthma in a recessive model; however, after stratification for atopic asthma we observed also an association with physician-diagnosed atopic asthma. Hence, it may well be that the minor allele of the *IL-4* STR represents a risk factor for atopic asthma. Intriguingly, this STR is highly linked with the C-589T promoter polymorphism in the *IL-4* gene (Levin's δ = 0.81 [37]) which has previously been associated with several asthma phenotypes [37]. Similar to our observation for the *IL-4* STR, the *IL-4* C-589T promoter polymorphism was associated with atopic asthma but not with nonatopic asthma in a recent meta-analysis when a recessive model was applied [50]. Such a congruency in the phenotypic expression between the two highly linked polymorphisms would argue that one of the polymorphic loci would be functional or that both are in linkage with another functional variant. There is indication that the *IL-4* T-589 allele is associated with higher binding of transcription factors [51] and increased transcription compared with the C-589 allele [52]; however, whether this leads to an increased transcription *in vivo* is currently not known.

In contrast to the linkage of the *IL-4* STR to a potentially causative polymorphism, there is no indication for functionality or linkage with a causative polymorphism for the *UGB* or the *CMA1* STRs. Any observed association of such a nonfunctional STR with a phenotype or disease will not infer causality but will associate a certain genetic locus with a phenotype or disease. Therefore, the observed association of the *CMA1* STR with atopic asthma most likely only represents a proxy for causative variants within *CMA1* or within neighboring genes.

The lack of association of the *UGB* STRs with asthma and atopy in our large population-based study conflicts with a previous publication investigating this STR in atopic asthma. There are several possible explanations for this discrepancy often observed in genetic association studies for polygenic diseases. The genetic and environmental differences between populations may mask an effect of a STR in a second population through altered gene–gene and environment–gene interactions. In this line, the *UGB* STR has been associated with atopic asthma in Indians [25], whereas we could not detect such an association in Swiss asthma patients. By contrast, the limited power of a study to detect an association of a STR could also result in lack of repetition of a previously found association. In this line, the Indian study was larger. Similarly, the limited power of our study could have resulted in lack of association of the *TNF- α* STR with asthma phenotypes, however, the studies showing an association of this STR with nephropathy were smaller [46,47].

Table 6
Association of *IL-4* 2-allele with atopy and bronchial hyperresponsiveness*

	Cases/controls (n)	OR	95% CI	p Value
Positive skin prick test				
Non-2/non-2	1211/4176	Ref		
2/2	47/99	1.51	1.05/2.18	0.03
Positive Phadiatop				
Non-2/non-2	1485/3689	Ref		
2/2	49/94	1.20	0.84/1.73	0.31
Positive bronchial hyperresponsiveness				
Non-2/non-2	676/3658	Ref		
2/2	27/87	1.53	0.96/2.44	0.08

*Adjusted for age, gender, area, and asthma at baseline.

Furthermore, it seems that linkage of the *TNF- α* STR with the asthma-associated -308 *TNF- α* polymorphism [35] is limited (Le-wontins $D' = 0.5\text{--}0.6$ [53]).

This study has both strengths and limitations. In contrast to the previous case-control studies investigating the STRs in the *CMA1* and the *IL-4* gene, the SAPALDIA study is a large population-based study with detailed health information, which allows the differentiation between atopic and nonatopic asthma. The high-quality data on genetic variants and the large size of the study also allows credible estimates of the effect of a certain genotype on phenotypic measures of atopy, like IgE levels. Limitations of our study include a medium-sized number of asthma patients compared with the large number of controls, limiting the power for the assessment of interactions.

In summary, we show in the large population-based SAPALDIA study, that the STR polymorphism in the *CMA1* gene is associated with atopic asthma and that the STR polymorphism in *IL-4* is weakly associated with asthma, IgE levels, and atopy. These results support previous findings suggesting a role for *CMA1* and *IL-4* in atopic asthma and of *IL-4* in atopy in general, and they corroborate that polymorphisms in these genes might modulate the individual risk for atopy and asthma.

Acknowledgments

We are indebted to the study participants, the persons providing technical and administrative support, and the medical teams and field workers at the local centers. We are particularly grateful to the SAPALDIA participants and their continued participation. We also very much appreciate the technical assistance of Margot Haun and Gertraud Erhart. This work has been supported by the Georg und Berta Schwyzer-Wyniker Stiftung. Recent support for the SAPALDIA project has been provided by a major grant from the Swiss National Foundation for Scientific Research (grant number 3347CO-108796/1, from April 1, 2006, to March 31, 2009) as part of the national program for cohort studies, by a 2008 grant from the Geneva Lung League (Ligue Pulmonaire Genevoise), by a 2008 grant from the Zurich Lung League (Lungen Liga Zurich). Over the last years (i.e., from 1991 to 2006), the SAPALDIA project has been supported by numerous grants from the Swiss National Foundation for Scientific Research (e.g., grant numbers 4026-28099, 3247BO-104283, 3247BO-104288, 3247BO-104284, 32-65896.01, 32-59302.99, 32-52720.97, 32-4253.94); special contributions from governmental agencies from Switzerland: Canton's government of Aargau, Basel-Stadt, Basel-Land, Geneva, Luzern, Valais, Ticino, and Zurich, Federal Office for Forest, Environment and Landscape, Federal Office of Public Health, Federal Office of Roads and Transport; and private, non-profit organizations: Swiss Lung League, The Canton's Lung League of Basel Stadt/Basel Landschaft, Geneva, Ticino, and Zurich. Genotyping was done within the "Sequencing and Genotyping Core Facility" of the Innsbruck Medical University, a facility supported by the IFTZ of the University.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.humimm.2010.08.008.

References

- [1] Cohn L, Elias JA, Chupp GL. Asthma: Mechanisms of disease persistence and progression. *Annu Rev Immunol* 2004;22:789–815.
- [2] Busse WW, Lemanske RF Jr. Asthma. *N Engl J Med* 2001;344:350–62.
- [3] Weiss ST, Raby BA, Rogers A. Asthma genetics and genomics 2009. *Curr Opin Genet Dev* 2009;19:279–82.
- [4] Moffatt MF, Kabisch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 2007;448:470–3.
- [5] Himes BE, Hunninghake GM, Baurley JW, Rafaels NM, Sleiman P, Strachan DP, et al. Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. *Am J Hum Genet* 2009;84:581–93.
- [6] Repapi E, Sayers I, Wain LV, Burton PR, Johnson T, Obeidat M, et al. Genome-wide association study identifies five loci associated with lung function. *Nat Genet* 2010;42:36–44.
- [7] Ott J, Rabinowitz D. The effect of marker heterozygosity on the power to detect linkage disequilibrium. *Genetics* 1997;147:927–30.
- [8] Chapman NH, Wijman EM. Genome screens using linkage disequilibrium tests: Optimal marker characteristics and feasibility. *Am J Hum Genet* 1998;63:1872–85.
- [9] Petukhin K, Fischer SG, Pirastu M, Tanzi RE, Chernov I, Devoto M, et al. Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. *Nat Genet* 1993;5:338–43.
- [10] Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, et al. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat Genet* 1999;22:352–55.
- [11] Votruba M, Moore AT, Bhattacharya SS. Demonstration of a founder effect and fine mapping of dominant optic atrophy locus on 3q28-qter by linkage disequilibrium method: A study of 38 British Isles pedigrees. *Hum Genet* 1998;102:79–86.
- [12] Pandolfo M, Sirugo G, Antonelli A, Weitnauer L, Ferretti L, Leone M, et al. Friedreich ataxia in Italian families: Genetic homogeneity and linkage disequilibrium with the marker loci D9S5 and D9S15. *Am J Hum Genet* 1990;47:228–35.
- [13] Warpeha KM, Xu W, Liu L, Charles IG, Patterson CC, Ah-Fat F, et al. Genotyping and functional analysis of a polymorphic (CCCTT) (n) repeat of NOS2A in diabetic retinopathy. *FASEB J* 1999;13:1825–32.
- [14] Silverman ES, Du J, De Sanctis GT, Radmark O, Samuelsson B, Drazen JM, Collins T. Egr-1 and Sp1 interact functionally with the 5-lipoxygenase promoter and its naturally occurring mutants. *Am J Respir Cell Mol Biol* 1998;19:316–23.
- [15] In KH, Asano K, Beier D, Grobholz J, Finn PW, Silverman EK, et al. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. *J Clin Invest* 1997;99:1130–7.
- [16] Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington disease chromosomes. *Cell* 1993;72:971–83.
- [17] Zitzmann M, Nieschlag E. The CAG repeat polymorphism within the androgen receptor gene and maleness. *Int J Androl* 2003;26:76–83.
- [18] Hersberger M, Muntwyler J, Funke H, Marti-Jaun J, Schulte H, Assmann G, et al. The CAG repeat polymorphism in the androgen receptor Gene is associated with HDL-cholesterol but not with coronary atherosclerosis or myocardial infarction. *Clin Chem* 2005;51:1110–5.
- [19] Hossny EM, Amr NH, Elsayed SB, Nasr RA, Ibraheem EM. Association of polymorphisms in the mast cell chymase gene promoter region (–1903 g/A) and (TG)n(GA)m repeat downstream of the gene with bronchial asthma in children. *J Investig Allergol Clin Immunol* 18:376–81.
- [20] Sharma S, Rajan UM, Kumar A, Soni A, Ghosh B. A novel (TG)n(GA)m repeat polymorphism 254 bp downstream of the mast cell chymase (CMA1) gene is associated with atopic asthma and total serum IgE levels. *J Hum Genet* 2005;50:276–82.
- [21] Iwanaga T, McEuen A, Walls AF, Clough JB, Keith TP, Rorke S, et al. Polymorphism of the mast cell chymase gene (CMA1) promoter region: Lack of association with asthma but association with serum total immunoglobulin E levels in adult atopic dermatitis. *Clin Exp Allergy* 2004;34:1037–42.
- [22] Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* 2006;117:1277–84.
- [23] He S, Walls AF. Human mast cell chymase induces the accumulation of neutrophils, eosinophils and other inflammatory cells in vivo. *Br J Pharmacol* 1998;125:1491–500.
- [24] He S, Walls AF. The induction of a prolonged increase in microvascular permeability by human mast cell chymase. *Eur J Pharmacol* 1998;352:91–8.
- [25] Sharma S, Ghosh B. Association of an intragenic microsatellite marker in the CC16 gene with asthma in the Indian population. *J Hum Genet* 2004;49:677–83.
- [26] Choi M, Zhang Z, Ten Kate LP, Collee JM, Gerritsen J, Mukherjee AB. Human uteroglobin gene polymorphisms and genetic susceptibility to asthma. *Ann NY Acad Sci* 2000;923:303–6.
- [27] Laing IA, de Klerk NH, Turner SW, Judge PK, Hayden CM, Landau LI, et al. Cross-sectional and longitudinal association of the secretoglobin 1A1 gene A38G polymorphism with asthma phenotype in the Perth Basin Infant Asthma Follow-up cohort. *Clin Exp Allergy* 2009;39:62–71.
- [28] Laing IA, Goldblatt J, Eber E, Hayden CM, Rye PJ, Gibson NA, et al. A polymorphism of the CC16 gene is associated with an increased risk of asthma. *J Med Genet* 1998;35:463–7.
- [29] Saadat M, Saadat I, Saboori Z, Emad A. Combination of CC16, GSTM1, and GSTT1 genetic polymorphisms is associated with asthma. *J Allergy Clin Immunol* 2004;113:996–8.
- [30] Gao PS, Mao XQ, Kawai M, Enomoto T, Sasaki S, Tanabe O, et al. Negative association between asthma and variants of CC16(CC10) on chromosome 11q13 in British and Japanese populations. *Hum Genet* 1998;103:57–84.
- [31] Shijubo N, Itoh Y, Yamaguchi T, Sugaya F, Hirasawa M, Yamada T, et al. Serum levels of Clara cell 10-kDa protein are decreased in patients with asthma. *Lung* 1999;177:45–52.
- [32] Van Vyve T, Chanez P, Bernard A, Bousquet J, Godard P, Lauwerijs R, Sibille Y. Protein content in bronchoalveolar lavage fluid of patients with asthma and control subjects. *J Allergy Clin Immunol* 1995;95:60–8.

- [33] Martin AC, Laing IA, Khoo SK, Zhang G, Rueter K, Teoh L, et al. Acute asthma in children: Relationships among CD14 and CC16 genotypes, plasma levels, and severity. *Am J Respir Crit Care Med* 2006;173:617–22.
- [34] Castro-Giner F, Kogevinas M, Imboden M, de Cid R, Jarvis D, Machler M, et al. Joint effect of obesity and TNF α variability on asthma: Two international cohort studies. *Eur Respir J* 2009;33:1003–9.
- [35] Castro-Giner F, Kogevinas M, Machler M, de Cid R, Van Steen K, Imboden M, et al. TNF α –308G > A in two international population-based cohorts and risk of asthma. *Eur Respir J* 2008;32:350–61.
- [36] Turner DM, Grant SC, Lamb WR, Brenchley PE, Dyer PA, Sinnott PJ, Hutchinson IV. A genetic marker of high TNF-alpha production in heart transplant recipients. *Transplantation* 1995;60:1113–7.
- [37] Kabesch M, Tzotcheva I, Carr D, Hoffer C, Weiland SK, Fritzsche C, et al. A complete screening of the IL4 gene: Novel polymorphisms and their association with asthma and IgE in childhood. *J Allergy Clin Immunol* 2003;112:893–8.
- [38] Ackermann-Lieblich U, Kuna-Dibbert B, Probst-Hensch NM, Schindler C, Felber DD, Stutz EZ, et al. Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991–2003: Methods and characterization of participants. *Soz Präventivmed* 2005;50:245–63.
- [39] Martin BW, Ackermann-Lieblich U, Leuenberger P, Kunzli N, Stutz EZ, Keller R, et al. SAPALDIA: Methods and participation in the cross-sectional part of the Swiss Study on Air Pollution and Lung Diseases in Adults. *Soz Präventivmed* 1997;42:67–84.
- [40] Wuthrich B, Schindler C, Medici TC, Zellweger JP, Leuenberger P. IgE levels, atopy markers and hay fever in relation to age, gender and smoking status in a normal adult Swiss population. SAPALDIA (Swiss Study on Air Pollution and Lung Diseases in Adults) Team. *Int Arch Allergy Immunol* 1996;111:396–402.
- [41] Wuthrich B, Schindler C, Leuenberger P, Ackermann-Lieblich U. Prevalence of atopy and pollinosis in the adult population of Switzerland (SAPALDIA study). Swiss Study on Air Pollution and Lung Diseases in Adults. *Int Arch Allergy Immunol* 1995;106:149–56.
- [42] Leuenberger P, Schindler C, Schwartz J, Ackermann-Lieblich U, Tara D, Perruchoud AP, et al. Occupational exposure to inhalative irritants and methacholine responsiveness. *Scand J Work Environ Health* 2000;26:146–52.
- [43] Hay JG, Danel C, Chu CS, Crystal RG. Human CC10 gene expression in airway epithelium and subchromosomal locus suggest linkage to airway disease. *Am J Physiol* 1995;268:L565–75.
- [44] Spink CF, Keen LJ, Middleton PG, Bidwell JL. Discrimination of suballeles present at the TNF α microsatellite locus using induced heteroduplex analysis. *Genes Immun* 2004;5:76–9.
- [45] Fleiss JL. Analysis of data from multiclinic trials. *Control Clin Trials* 1986;7:267–75.
- [46] Tuglular S, Berthoux P, Berthoux F. Polymorphisms of the tumour necrosis factor alpha gene at position -308 and TNF α microsatellite in primary IgA nephropathy. *Nephrol Dial Transplant* 2003;18:724–31.
- [47] Thibaudin D, Thibaudin L, Berthoux P, Mariat C, Filippis JP, Laurent B, et al. TNFA2 and d2 alleles of the tumor necrosis factor alpha gene polymorphism are associated with onset/occurrence of idiopathic membranous nephropathy. *Kidney Int* 2007;71:431–7.
- [48] Reed CE, Kita H. The role of protease activation of inflammation in allergic respiratory diseases. *J Allergy Clin Immunol* 2004;114:997–1008.
- [49] Chien YH, Hwu WL, Chiang BL. The genetics of atopic dermatitis. *Clin Rev Allergy Immunol* 2007;33:178–90.
- [50] Li Y, Guo B, Zhang L, Han J, Wu B, Xiong H. Association between C-589T polymorphisms of interleukin-4 gene promoter and asthma: A meta-analysis. *Respir Med* 2008;102:984–92.
- [51] Rosenwasser LJ, Borish L. Genetics of atopy and asthma: The rationale behind promoter-based candidate gene studies (IL-4 and IL-10). *Am J Respir Crit Care Med* 1997;156:S152–5.
- [52] Akkad DA, Arning L, Ibrahim SM, Epplen JT. Gender specifically associated promoter polymorphism in multiple sclerosis affects interleukin 4 expression levels. *Genes Immun* 2007;8:703–6.
- [53] Spink CF, Keen LJ, Mensah FK, Law GR, Bidwell JL, Morgan GJ. Association between non-Hodgkin lymphoma and haplotypes in the TNF region. *Br J Haematol* 2006;133:293–300.

Online material:

Table 1 (online): Primer sequences and concentrations for PCR amplification. Forward primers were labeled with FAM, YYE (Yakima Yellow), and AT550		
STR	Sequence	Final concentration
CMA1	5'-FAM-TGCACATAGTGACCTGATTC-3'	200 nM
	5'-GTGTCTTTTCTTAAGCTGGAAAT-3'	200 nM
iNOS M1	5'-FAM-AGCCTACAACCTGCATTCGTC-3'	600 nM
	5'-TTGCTTGAGGCCAGGAGTTC-3'	600 nM
UGB	5'-YYE-AAGCTCTGTGTTGAAGGGAA-3'	400 nM
	5'-TGGGCAACAGAGCGAGATTC-3'	400 nM
iNOS M3	5'-AT550-GGGAAAGAATGGACAATTTCA-3'	200 nM
	5'-TTGGCCAAAACAACTGAAA-3'	200 nM
IL-4	5'-AT550-CAGGACAAAGACTGTACAAT-3'	200 nM
	5'-AGTCATATCCCAAATCTGTTC-3'	200 nM
cPLA2 α	5'-YYE-ATGCAAAAATGCTCTAGGTT-3'	200 nM
	5'-ATGTAAATTAATCAGTCCTAAAT-3'	200 nM
TNFd	5'-FAM-GGACTCTGTCTCCAAAGAAA-3'	200 nM
	5'-TTGGTGCCTCTCTTGTCTCT-3'	200 nM

Table 2 (online): Allele distribution between cases and controls		
CMA1 alleles	current asthma (n, %)	No current asthma (n, %)
34	1, 0.33	4, 0.04
35	0	2, 0.02
36	0	4, 0.04
37	2, 0.66	49, 0.44
38	4, 1.32	121, 1.09
39	162, 53.64	5188, 46.59
40	3, 0.99	248, 2.23
41	32, 10.60	1194, 10.72
42	32, 10.60	1413, 12.69
43	52, 17.22	2320, 20.83
44	8, 2.65	327, 2.94
45	5, 1.66	173, 1.55
46	1, 0.33	84, 0.75
47	0	3, 0.03
52	0	1, 0.01
57	0	1, 0.01
58	0	1, 0.01
59	0	3, 0.03

UGB alleles	Current asthma (n, %)	No current asthma (n, %)
2-8	0	2, 0.02
3-7	2, 0.66	32, 0.29
3-8	45, 14.90	1660, 15.01
3-9	105, 34.77	4038, 36.52
3-10	44, 14.57	1809, 16.22
3-11	4, 1.32	106, 0.96
3-13	0	8, 0.07
4-8	99, 32.78	3377, 30.54
4-9	3, 0.99	35, 0.32
4-10	0	3, 0.03

TNF-α alleles	Current asthma (n, %)	No current asthma (n, %)
19	0	4, 0.04
20	0	68, 0.61
21	25, 8.22	870, 7.85
22	7, 2.30	231, 2.08
23	152, 50.00	5634, 50.81
24	101, 33.22	3228, 29.11

25	14, 4.61	877, 7.91
26	5, 1.64	166, 1.50
27	0	5, 0.05
28	0	3, 0.03
29	0	1, 0.01
30	0	1, 0.01

IL-4 alleles	Current asthma (n, %)	No current asthma (n, %)
IL4 2	41, 13.31	1584, 14.19
IL4 3	267, 86.69	9579, 85.79
IL4 4	0	3, 0.03

Table 3a (online): Association of IL4 2-allele with asthma*				
	Cases/controls (n)	OR	95% CI	P-value
<u>Physician diagnosed asthma</u>				
Non-2/non-2	269/3995	ref		
2/non-2	78/1243	0.93	0.72 / 1.21	0.58
2/2	14/138	1.52	0.86 / 2.68	0.15
<u>Current asthma</u>				
Non-2/non-2	121/4143	ref		
2/non-2	25/1296	0.66	0.43 / 1.02	0.06
2/2	8/144	1.96	0.94 / 4.10	0.07

*adjusted for age, sex and area.

Table 3b (online): Association of TNF-α 23 allele with asthma*				
	Cases/controls (n)	OR	95% CI	P-value
<u>Physician diagnosed asthma</u>				
Non-23/non-23	86/1327	ref		
23/non-23	181/2599	1.08	0.83 / 1.41	0.58
23/23	90/1413	0.97	0.72 / 1.32	0.87
<u>Current asthma</u>				
Non-23/non-23	36/1377	ref		
23/non-23	80/2700	1.13	0.76 / 1.69	0.54
23/23	36/1467	0.93	0.58 / 1.48	0.75

*adjusted for age, sex and area.

Table 3c (online): Association of UGB allele 4-8 with asthma*				
	Cases/controls (n)	OR	95% CI	P-value
<u>Physician diagnosed asthma</u>				
Non-4-8/non-4-8	173/2605	ref		
4-8/non-4-8	149/2177	1.03	0.82 / 1.29	0.80
4-8/4-8	33/542	0.93	0.63 / 1.36	0.70
<u>Current asthma</u>				
Non-4-8/non-4-8	65/2713	ref		
4-8/non-4-8	73/2253	1.36	0.97 / 1.91	0.08
4-8/4-8	13/562	0.98	0.54 / 1.80	0.95

*adjusted for age, sex and area.

Table 4 (online): Association of IL4 2-allele with physician diagnosed asthma*				
	Cases/controls (n)	OR	95% CI	P-value
Asthma				
Non-2/non-2	347/5238	ref		
2/2	14/138	1.55	0.88 / 2.72	0.13
				$r^2=0.009$
Asthma with negative skin prick test	Non-atopic Asthma			
Non-2/non-2	146/4030	ref		
2/2	2/97	0.59	0.14 / 2.44	0.47
Asthma with negative Phadiatop				
Non-2/non-2	123/3566	ref		
2/2	1/93	0.33	0.05 / 2.39	0.27
Asthma with positive skin prick test	Atopic Asthma			
Non-2/non-2	186/1025	ref		
2/2	11/36	1.73	0.86 / 3.48	0.13
Asthma with positive Phadiatop				
Non-2/non-2	207/1278	ref		
2/2	12/37	2.00	1.02 / 3.91	0.04
Interaction (Skin Prick test and <i>IL4</i> 2-allele)				0.17
Interaction (Phadiatop and <i>IL4</i> 2-allele)				0.08

*adjusted for age, sex and area.