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The common G-allele of interleukin-18 single-nucleotide polymorphism is a genetic risk factor for atopic asthma. The SAPALDIA Cohort Study

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Summary

Background IL-18 is a pleiotrophic cytokine involved in both, T-helper type 1 (Th1) and Th2 differentiation. Recently genetic variants in the IL-18 gene have been associated with increased risk of atopy and asthma.

Objective To examine the relationship of a genetic, haplotype-tagging promotor variant – 137G/C in the IL-18 gene with atopic asthma in a large, well-characterized and population-based study of adults.

Methods Prospective cohort study design was used to collect interview and biological measurement data at two examination time-points 11 years apart. Multivariate logistic regression analysis was used to assess the association of genotype with asthma and atopy.

Results The G-allele of the IL-18 promotor variant (– 137G/C) was associated with a markedly increased risk for the prevalence of physician-diagnosed asthma with concomitant skin reactivity to common allergens. Stratification of the asthma cases by skin reactivity to common allergens revealed an exclusive association of IL-18 – 137 G-allele with an increased prevalence of atopic asthma (adjusted odds ratio (OR): 3.63; 95% confidence interval: (1.64–8.02) for GC or GG carriers vs. CC carriers), and no according association with asthma and concomitant negative skin reactivity (adjusted OR: 1.13; 0.66–1.94). The interaction between IL-18 – 137G/C genotype and positive skin prick test was statistically significant ($P = 0.029$). None of 74 incident asthma cases with atopy at baseline exhibited the CC genotype.

Conclusion Our results strongly suggest that this variant of the IL-18 gene is an important genetic determinant involved in the development of atopic asthma.

Keywords asthma, atopy, cohort analysis, general population, IL-18, single-nucleotide polymorphism

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Introduction

Experimental evidence points to the involvement of IL-18 in the aetiology of atopic disease. IL-18 has been suggested to be an important determinant of both the T-helper type 1 (Th1) and Th2 differentiation [1]. IL-18, initially termed IFN- γ inducing factor, drives the Th1 response, in synergy with IL-12, and thus leads via IFN- γ -mediated pathways to cytokine type I production [2]. Recently it has been shown that IL-18 can also influence Th2 response by enhancing IL-4 and histamine [3], IL-13 [4] and IgE production [5] in synergy with other Th2-stimulating factors. This pleiotrophic role of IL-18 is further strengthened by its broad expression pattern in various cells of the immune, nervous and endocrine system, but also osteoblastic stromal cells, epidermal keratinocytes, intestinal and airway epithelial cells [2].

In addition to the experimental evidence, the involvement of IL-18 in atopic disease is further supported by clinical and epidemiological observations of increased circulating IL-18 levels in the serum of patients during acute phases of asthma [6], but also constitutively in atopic patients with hayfever [7], atopic dermatitis [8, 9] and asthma [9–11] compared with non-atopic healthy subjects. However contrasting reports failing to show an association of high circulating levels of IL-18 with asthma [12] or atopic dermatitis [6] do also exist.

Common polymorphisms have been identified in the promotor region and coding sequence of the IL-18 gene [13–15]. Common haplotypes have recently been identified to affect the transcription of the gene [13]. One of its haplotype-tagging single-nucleotide polymorphism (SNP) at position –137G/C of the IL-18 promotor region has been associated with differential expression levels of IL-18 mediating distinctive INF- γ inducing potential [13].

Given previous work suggesting associations of IL-18 SNPs with various atopic phenotypes [11, 14, 15], we hypothesized *a priori* that the –137G/C polymorphism in the IL-18 gene might modify specifically the relative risk for development of atopic asthma. We therefore investigated this relationship in the large, well-characterized and population-based Swiss cohort on Air Pollution And Lung Disease In Adults (SAPALDIA cohort).

Materials and methods

Study population

The study population investigated was a multi-centre SAPALDIA cohort representative of the Swiss population. The cohort has been previously described in detail [16, 17]. Study participants are predominantly of European-Caucasian ethnicity and Swiss nationality. They were recruited from the inhabitant registries of eight Swiss communities representing the German, French and Italian

language regions. Information and material collected at baseline (1991) and follow-up examination (2002) included anthropometric and lifestyle information, lung function, skin prick test (SPT) (at baseline only) as well as blood samples for DNA extraction (at follow-up only) and for total and specific IgE measures (at baseline only) [16, 17]. Ethical approval for the study was given by the central ethics committee of the Swiss Academy of Medical Sciences and the Cantonal Ethics Committees for each of the eight examination areas. The SAPALDIA cohort examined 9561 subjects at baseline, of which 8047 (84%) agreed to participate fully or partly at the follow-up survey. The current study was restricted to subjects at follow-up who provided a blood sample and who agreed to genetic testing ($n = 6057$). Missing information in at least one of the covariates included in the model ($n = 301$) and failure of the genotyping assay ($n = 22$) reduced the final sample size to 5734 subjects (71% of participants at follow-up). Participants at baseline were less likely to be of intermediate age or to be of Swiss nationality, but were more likely to report asthma or wheezing and to be former smokers when compared with non-participants at baseline [16]. Comparison of participants vs. non-participants at follow-up revealed that non-participants were on average younger and more likely to have been smokers at baseline examination [17]. Unlike participation at baseline asthmatics were less likely to participate at follow-up.

Case definition

Asthma status was assessed by participants reporting of physician-diagnosed asthma at baseline and follow-up examination (affirmative answers to the following two questions 'Have you ever had asthma?' and 'Was this confirmed by a doctor?'). We created the following asthma case categories: (i) asthma at baseline ($n = 348$, of whom $n = 98$ subjects reported asthma at baseline only), (ii) asthma at either baseline or follow-up (ever asthma; $n = 530$), (iii) incident asthma ($n = 182$ subjects reporting asthma at follow-up, but not at baseline). While asthma phenotype was ascertained by questionnaire data for this study, results on bronchial hyper-responsiveness measured at baseline and follow-up (for details see [17]) confirmed that participants reporting physician-diagnosed asthma were significantly more likely to respond positive to the methacholine test (Tables 1 and 2). Reversibility of airflow obstruction was not assessed in this study.

Atopic asthma was defined as asthma in subjects who had a positive SPT at baseline. Subjects reporting physician-diagnosed asthma and having a negative skin prick at baseline were defined as non-atopic asthma cases. The SPT assessed allergic sensitization to cat, dust mite, timothy grass, *Parietaria*, birch, moulds (*Cladosporium* and *Alternaria*) and was considered positive for a weal

Table 1. Characteristics of the study population, the SAPALDIA cohort study

N	Age ($\pm$ SD) (y)	Female (%)	Asthma family history (%)	Childhood respiratory infection	Baseline BHR* (%)	Baseline FEV ₁ ($\pm$ SD) % predicted	Follow-up FEV ₁ ($\pm$ SD) % predicted	Baseline atopy test			Follow-up allergy reports		
								SPT+%	IgE ($\pm$ SD) (kU/L)	Phadiatop +%	Pets (%)	Dust mite (%)	Pollen (%)
All	5734	41.2 (11.4)	50.2	20.4	7.9	15.9	100.1 (13.6)	22.8	98.2 (269.5)	29.2	9.1	23.0	28.7
Non-asthma	5204	41.3 (11.4)	50.2	19.2	7.2	13.4	100.9 (13.2)	20.0	90.3 (264.8)	26.1	6.6	21.2	25.8
Asthma reported													
Ever asthma	530	40.2 (11.6)	50.8	32.6	14.9	42.6	93.0 (15.7)	50.6	175.8 (301.3)	59.4	33.8	40.2	56.8
Baseline	348	41.2 (11.3)	51.2	33.6	19.0	47.6	92.2 (15.8)	55.8	183.7 (312.3)	62.5	38.5	40.8	58.1
New asthma reports at follow-up	182	38.3 (11.8)	50.0	30.8	7.1	33.3 [†]	94.7 (15.3)	40.7	160.1 (278.4)	53.0	24.7	39.0	54.4

*BHR was investigated at baseline in subjects eligible for methacholine challenge ($n = 4532$); [†]BHR prevalence among new asthma reports at follow-up refers to BHR test at follow-up in eligible subjects ($n = 3548$).

SAPALDIA, Swiss cohort on Air Pollution And Lung Disease In Adults; FEV₁, forced expiratory volume in 1 s.

Table 2. Comparison of characteristics in skin prick negative vs. positive participants, the SAPALDIA cohort study

N	Age ($\pm$ SD) (y)	Female	Asthma family history (%)	Childhood respiratory infection	Baseline BHR* (%)	Baseline FEV ₁ ($\pm$ SD) % predicted	Follow-up FEV ₁ ($\pm$ SD) % predicted	Baseline atopic test			Follow-up allergy reports		
								SPT+%	IgE ($\pm$ SD)	Phadiatop +%	Pets %	Dust mite (%)	Pollen (%)
Skin prick (-)													
All	4425	42.0 (11.2)	51.7	18.7	7.9	13.2	100.5 (13.6)	—	58.0 (135.1)	12.4	4.5	18.9	18.7
Non-asthma	4163	42.0 (11.2)	51.3	17.8	7.5	12.0	100.9 (13.2)	—	56.2 (131.5)	11.8	3.7	18.1	17.5
Asthma reported													
Ever asthma	262	42.6 (10.9)	58.0	33.6	14.9	33.9	93.3 (16.7)	—	86.6 (179.6)	22.5	16.8	31.7	37.8
Baseline	154	44.4 (10.0)	58.4	37.0	19.5	33.3	92.6 (17.0)	—	59.2 (87.5)	18.8	19.5	29.9	34.4
New asthma reports	108	40.1 (11.6)	57.4	28.7	8.3	34.6 [†]	94.1 (13.4)	—	127.4 (257.9)	28.0	13.0	34.2	42.6
Skin prick (+)													
All	1309	38.5 (11.7)	45.4	26.1	7.9	24.9	99.1 (13.6)	+	238.2 (486.6)	86.4	24.6	36.8	62.3
Non-asthma	1041	38.7 (11.7)	45.8	24.6	6.2	18.7	100.7 (14.7)	+	231.0 (512.9)	83.9	18.0	33.7	59.0
Asthma reported													
Ever asthma	268	37.9 (11.8)	45.7	31.7	14.9	50.3	92.8 (14.7)	+	266.5 (366.4)	95.7	50.4	48.5	75.4
Baseline	194	38.7 (11.7)	45.4	30.1	18.6	57.5	91.8 (14.9)	+	286.8 (385.7)	97.3	53.6	49.5	76.8
New asthma reports	74	35.7 (11.7)	39.2	33.8	5.4	31.6 [†]	90.9 (18.3)	+	210.4 (303.4)	90.9	41.9	46.0	71.6

*BHR was investigated at baseline in subjects eligible for methacholine challenge ($n = 4532$); [†]prevalence among new asthma reports at follow-up refers to BHR test at follow-up in eligible subjects ($n = 3548$).

SAPALDIA, Swiss cohort on Air Pollution And Lung Disease In Adults; FEV₁, forced expiratory volume in 1 s.

diameter > 3 mm [16, 18]. Additional baseline atopy markers were measurements using an *in vitro* fluoro-enzyme immunoassay (CAP FEIA system, Pharmacia, Uppsala, Sweden) for circulating serum levels of total IgE ($n = 5351$) and for Phadiatop test for specific allergen sensitization ($n = 5379$) [18, 19]. At follow-up interview information about the presence of allergic symptoms was collected. Three sets of questions were asked to distinguish allergy to pets, pollen and dust. The exact wording of the questions was: 'When you are near animals, such as cats, dogs or horses, do you ever: start to cough? Start to wheeze? Get a feeling of tightness in your chest? Start to feel short of breath? Get a runny or a stuffy nose or start to sneeze? Get itchy or watering eyes?' Identical questions were asked for allergy to dust ('When you are in a dusty part of the house or near pillows or duvets...') and to pollen ('When you are near trees, grass or flowers, or when there is a lot of pollen about...'). Allergy to pets, dust, or pollen was defined as at least one symptom report.

Control definition

Ever asthma cases were compared with subjects without asthma at baseline and follow-up. Baseline asthma cases were cross-sectionally compared with subjects without asthma at baseline, irrespective of their asthma status at follow-up.

Genotyping

The IL-18 -137G/C (dbSNP124; refSNP ID: rs187238) was genotyped using liquid-handling assisted setup and a fluorescent 5'-nuclease real-time PCR (TaqMan, Applied Biosystems, Rothrent, Switzerland) assay on an ABI Prism 7000 sequence detection system (ABI, Rotkreuz, Switzerland). DNA samples of all participants who gave informed consent were genotyped. SNP-specific minor groove binder (MGB) probes and primers used were as follows: MGB probes 5'-AGAAAA[G/C]ATTTTCATGAA AATAGT-3', forward 5'-CACAGAGCCCCAACTTTTACG-3', reverse 5'-AAAAGAAGTACTCGTATCCTCTGCC-3'. A random sample of > 5% of all DNA samples was re-genotyped and verified. Reproducibility of genotype attribution was extremely high (> 99.5%). We tested Hardy-Weinberg equilibrium (HWE) using Arlequin Version 2.000 [20] to test for agreement with random mating and to evaluate population stratification.

Statistical analysis

One-way analyses of variance and χ^2 tests were used to analyse the unadjusted associations of various characteristics and IL-18 genotype with asthma and atopy status. Multiple logistic regression analysis was conducted to

adjust for study centre and known asthma risk factors as potential confounders of the IL-18 genotype/asthma association. Covariates included in the model were study centre (to adjust for language region), sex, age and body mass index at baseline, family history of asthma, smoking status at baseline and follow-up, history of early childhood respiratory infection before the age of 5 years, reported allergy to pets, dust and pollen at follow-up (for models with asthma at follow-up only), skin reactivity to common airborne allergens at baseline. The modification of the IL-18 genotype/asthma association by atopy at baseline was assessed by including an interaction term between IL-18 genotype (dichotomized as GG/GC vs. CC genotype) and SPT (positive vs. negative) in the logistic regression models. Although the statistical tests performed for this publication focused on our *a priori* hypothesis and various associated sensitivity analyses, we also present *P*-values adjusted for multiple testing ($n = 8$) by using the conservative Bonferroni's correction [21]. Statistical analysis was performed using STATA Version 8.1 SE (Stata Corporation, College Station, TX, USA). Two-sided *P*-values of < 0.05 and < 0.10 were considered as statistically significant for main effects and interactions, respectively.

Results

The characteristics of the participants and the distribution of asthma and atopy are presented in Table 1. Our study included 530 physician-diagnosed asthma cases reported at either baseline or follow-up examination and 5204 non-asthmatic controls. In this Swiss population sample aged 18–60 years at baseline 50% of asthmatics and non-asthmatics, respectively, were of female sex. A family history of asthma was common (20%) and positively associated with the presence of asthma among participants (19% in non-asthmatics vs. 33% in asthmatics, $P < 0.001$). Childhood respiratory infections before the age of 5 years were significantly associated with physician-diagnosed asthma later in life (7% in controls vs. 15% in asthmatics, $P < 0.001$). Percent predicted forced expiratory volume in 1 s (FEV₁) was smaller among asthmatics than controls at baseline ($P < 0.001$) and at follow-up ($P < 0.001$). Asthmatics were more likely to exhibit airway hyper-responsiveness than non-asthmatics. Twenty percent of non-asthmatics and 51% of asthmatics exhibited allergen-specific skin reactivity at baseline ($P < 0.001$).

We further analysed the distribution of the above-mentioned characteristics stratified by SPT results (Table 2). Irrespective of case-control status, subjects with a positive SPT at baseline were younger on average ($P < 0.001$ for all subjects), were more likely to be of male gender ($P < 0.001$ for all subjects), and were more likely to

Table 3. Interleukin-18 – 137 G/C-allele and genotype frequency stratified by asthma diagnosis and result of skin prick test, the SAPALDIA cohort study

	Genotype IL-18 – 137 G/C (%)				<i>P</i> -value*	Frequency of	
	<i>N</i>	GG (%)	GC (%)	CC (%)		G-allele	C-allele
All	5734	54.1	38.7	7.2		0.734	0.265
Non-asthma	5204	53.9	38.7	7.5		0.732	0.268
Asthma reported							
Ever asthma	530	55.9	39.4	4.7	0.066	0.756	0.244
Baseline	348	56.8	38.2	4.9	0.188	0.760	0.240
New reports	182	53.9	41.8	4.4	0.263	0.747	0.253
Ever asthma cases stratified by skin prick test at baseline							
Non-atopic asthma	262	53.4	40.1	6.5	0.805	0.735	0.265
Atopic asthma	268	58.2	38.8	3.0	0.012	0.776	0.224
Atopy markers measured [†]							
Skin prick test (<i>n</i> = 5734)							
Negative	4425	54.2	38.6	7.3		0.734	0.265
Positive	1309	53.7	39.9	7.0	0.839	0.734	0.266
Phadiatop (<i>n</i> = 5379)							
Negative	3807	54.5	38.5	7.0		0.737	0.263
Positive	1572	53.4	38.9	7.6	0.632	0.729	0.271
Total IgE (<i>n</i> = 5351) (kU/L)							
< 100	4193	54.1	38.8	7.1		0.736	0.264
> 100	1158	54.2	38.3	7.6	0.787	0.733	0.267

* χ^2 -test for the comparison of the genotype distributions between asthmatics vs. non-asthma controls; [†]Stratification by asthma status did not change association of atopy markers with genotype.

SAPALDIA, Swiss cohort on Air Pollution And Lung Disease In Adults.

have additional indicators of atopy present (elevated circulating IgE serum levels ($P < 0.001$); positive Phadiatop ($P < 0.001$); allergic symptoms to pets ($P < 0.001$); allergic symptoms to dust mite ($P < 0.001$); allergic symptoms to pollen ($P < 0.001$)). The presence of allergen-specific skin reactivity at baseline was positively associated with a family history of asthma among controls ($P < 0.001$), but not asthma cases ($P = 0.65$).

Unadjusted IL-18 allele and genotype frequencies, stratified by asthma and atopy status are presented in Table 3. The allele frequency of the rare C-allele of IL-18 – 137 was 0.265 in the Swiss general population. C-allele frequency and CC genotype were slightly less prevalent among asthma cases when compared with controls. Irrespective of asthma case definition, the difference did not reach statistical significance, but CC genotype frequency was lowest among subjects with incident asthma (4.4% vs. 7.5% in non-asthma controls). Upon stratification of ever asthma by SPT result, we observed a statistically significant inverse association of the CC with atopic asthma (3.0% vs. 7.5%, $P = 0.012$), but not with non-atopic asthma (6.5% vs. 7.5%, $P = 0.805$).

We furthermore assessed the association of this IL-18 SNP with atopy itself by comparing genotype distribution across available baseline markers for atopy. Contrary to its association with atopic asthma, the IL-18 SNP was not associated with atopy, a result that was not altered if the analyses were restricted to controls.

Logistic regression analysis adjusting for study centre indicated an increased risk for physician-diagnosed asthma among subjects carrying at least one G-allele (Table 4), irrespective of case definition. Subjects homozygous for the G-allele had 1.7-fold (1.11–2.59) increased likelihood to report asthma at either baseline or follow-up than CC genotypes. The observed risk associations persisted upon adjustment of additional potential confounders.

The association between IL-18 genotype and asthma was strongly modified by atopy at baseline (P interaction = 0.035). GG genotype was associated with atopic asthma (OR: 3.18; 1.50–6.73), but not with non-atopic asthma (OR: 1.14; 0.68–1.92). Adjustment for additional covariates further strengthened the association with atopic asthma (OR: 3.63; (1.64–8.02) and P for interaction = 0.029). None of the 74 subjects with incident asthma and a positive SPT at baseline exhibited the CC genotype (data not shown).

Discussion

We found a functionally relevant haplotype-tagging SNP in the IL-18 gene to be associated with the prevalence and most likely with the incidence of atopic, physician-diagnosed asthma.

Sequence variation in the IL-18 gene has been described previously [13, 14] and haplotype analysis [13, 15] on a limited number of SNPs revealed functionally

Table 4. Association of interleukin-18 – 137 G/C SNP with physician-diagnosed asthma, the SAPALDIA cohort study

	N Case/control	OR*	95% CI	P-value	P-value [§]	OR [†]	95% CI	P-value	P-value [§]
Ever asthma									
CC	25/388	–	–	1		–		1	
GC	209/2012	1.68	1.10, 2.58	0.019	–	1.73	1.10, 2.73	0.017	–
GG	296/2804	1.70	1.11, 2.59	0.015	–	1.80	1.16, 2.83	0.009	–
Baseline physician-diagnosed asthma									
CC	17/396	–	–	1		–		1	
GC	133/2088	1.54	0.92, 2.59	0.100	–	1.50	0.88, 2.56	0.135	–
GG	198/2902	1.64	0.99, 2.73	0.055	–	1.66	0.98, 2.79	0.058	–
Ever asthma cases stratified by skin prick test at baseline									
Non-atopic asthma									
CC	17/305	–	–	1		–		1	
GC	105/1601	1.22	0.72, 2.07	0.463	–	1.26	0.73, 2.18	0.404	–
GG	140/2257	1.14	0.68, 1.92	0.618	–	1.13	0.66, 1.94	0.647	–
Atopic asthma									
CC	8/83	–	–	1		–		1	
GC	104/411	2.86	1.34, 6.12	0.007	0.056	2.84	1.27, 6.33	0.011	–
GG	156/547	3.18	1.50, 6.73	0.003	0.024	3.63	1.64, 8.02	0.001	0.008
P-interaction [‡]				0.035				0.029	

*Adjustment study centre; [†]Additional adjustments for ever asthma and asthma diagnosis at follow-up: area, sex, age, BMI at baseline, family history of asthma, respiratory infection in early childhood, smoking status at baseline as well as follow-up, reported allergy at follow-up to pets, dust mite, pollen. For asthma diagnosis at baseline: area, sex, age, BMI at baseline, family history of asthma, respiratory infection in early childhood, smoking status at baseline; [‡]Interaction term for genotype (GG/GC vs. CC) with skin prick test at baseline (positive vs. negative) [§]Bonferroni's corrected P-value.

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distinct haplotypes associated with different levels of IL-18 gene transcription activity and varying correlation with circulating INF- γ production. The SNP IL-18 – 137G/C tagged two haplotypes that differed by IL-18 transcriptional activity. The common G-allele conferred a higher IL-18 gene expression *in vitro* after PMA/ionomycin stimulation. The C substitution at position – 137 leads to a change in a transcription factor binding site and lowers transcriptional activity of IL-18 and is no longer correlated with the IFN- γ levels [13].

Our results are thus consistent with experimental, clinical and epidemiological evidence suggesting that high IL-18 activity is associated with aetiology and progression of asthma. IL-18 is expressed in airway epithelium of healthy controls and asthmatics [22] and increased IL-18 levels have been repeatedly found in asthmatics and allergic patients. Recent association studies of genetic IL-18 variants have provided evidence for association with atopic disease [11, 14, 15, 23], as well as chronic inflammatory disease [24], however, negative association have also been reported with asthma [25] and atopy [11]. IL-18 is in fact a promising candidate asthma gene. The gene is located at 11q22 and thus in a candidate region identified by genome-wide linkage studies [26–28]. Whether the SNP at position – 137 in the IL-18 gene is itself causative or merely in strong linkage with the causal locus cannot be judged from our study.

The restriction of our finding to atopic asthma deserves some consideration. The distinction of atopic vs. non-

atopic asthma is often implied in the literature. Classification and distinction of atopic from non-atopic asthma have been proposed [29], but the differentiation of two asthma phenotypes in asthma association studies is rarely given. While genome linkage analyses suggest a certain degree of overlap in the aetiology of both, asthma and atopy [30], the distinction of atopic vs. non-atopic asthma is not straight forward [31], given that atopy itself is not clearly definable. Atopic markers, although highly correlated with each other, do not overlap completely, and none of them is consistently associated with clinical symptoms [31]. To differentiate atopic from non-atopic asthma we chose the skin reactivity to common allergens as atopy marker. It is established that asthma is associated with skin test reactivity to allergens and high serum IgE levels [32]. Total IgE measurement and Phadiatop's test had been conducted on a reduced number of SAPALDIA participants. When we conducted the logistic regression analysis by using total IgE levels (> 100 kU/L as cut-off or Phadiatop's test) as stratification criteria, we observed qualitatively comparable results. The distinctive power of SPT is further supported by our observation, that asthmatics with negative skin reactivity to allergens exhibited normal total IgE levels and low prevalence of sensitization to common allergens.

Major strengths of our population-representative study are its large sample size, its detailed characterization of the participants, as well as the availability of biological material for DNA and blood analysis. The availability of

various atopy markers allowed for detailed evaluation of the degree of overlap between different atopy markers.

Limitations of the study include the lack of biological atopy markers at follow-up, given that it is unclear how stable atopy status remains throughout adult life. This may have caused some misclassification between atopic and non-atopic asthma. However, an indication of the high degree of persistency of the atopy status can be obtained from the follow-up interview response to questions relating to allergic symptoms to common allergen as dust mite, pets and pollen. The distribution of allergic symptoms 11 years after the SPT showed a markedly higher prevalence among skin reactive subjects. A potential reporting bias might be reflected by the fact that 98 participants no longer reported physician-diagnosed asthma at follow-up, despite the fact that they did so at baseline. But only 2% of these subjects still reported current asthma symptoms compared with 30.2% of the subjects with new reports of physician-diagnosed asthma (data not shown). Exclusion of these subjects from the association analysis did not substantially modify previous results.

There is a theoretical concern for selection bias arisen from the association of age, smoking and Swiss nationality with participation at baseline and follow-up. We examined if IL-18 genotype is related to Swiss nationality, age or smoking behaviour among our participants for this current study, and no associations were found. Given that these factors are not confounders of the IL-18 genotype/asthma association in our study, the unequal distribution of these factors between participants and non-participants should not impact on the validity of our study findings. It is furthermore unlikely that IL-18 genotype was a determinant of participation rate among asthmatics. Another concern of our association study may be population stratification as the Swiss population consists of multiple language groups. Deviation of HWE was not observed within the three language groups presented in our study (French, German, Italian). Neither language group nor Swiss nationality did modify the observed associations between IL-18 genotype and asthma. Given the low power of HWE [33], genotype data from additional unlinked markers elsewhere in the genome is ideally available to test for stratification [34, 35]. Limited funding prohibited this control of population stratification in our study. But given the known genetic homogeneity of Caucasian Western-Central European populations [36–38] we do not expect that population stratification in this Swiss cohort invalidated the observed associations.

In conclusion we showed for the first time that a polymorphism in IL-18 is associated with atopic, but not with non-atopic asthma. Future studies need to confirm this results by investigating the association in different populations using various criteria for differentiating between non-asthmatics, atopic and non-atopic asthmatics. Genetic studies must follow-up on this finding by a more

comprehensive assessment of genetic variation in and adjacent to the IL-18 gene.

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