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TNFA -308G>A in two international population-based cohorts and risk of asthma

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ABSTRACT: Genetic association studies have related the tumour necrosis factor-α gene (*TNFA*) guanine to adenine substitution of nucleotide -308 (-308G>A) polymorphism to increased risk of asthma, but results are inconsistent. The aim of the present study was to test whether two single-nucleotide polymorphisms, of *TNFA* and of the lymphotoxin-α gene (*LTA*), are associated with asthma, bronchial hyperresponsiveness and atopy in adults, by combining the results of two large population-based multicentric studies and conducting a meta-analysis of previously published studies.

The European Community Respiratory Health Survey (ECRHS) and Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA) used comparable protocols, including questionnaires for respiratory symptoms and measures of lung function and atopy. DNA samples from 11,136 participants were genotyped at *TNFA* -308 and *LTA* 252. Logistic regression employing fixed and random effects models and nonparametric techniques were used.

The prevalence of asthma was 6%. The *TNFA* -308G>A polymorphism was associated with increased asthma prevalence and with bronchial hyperresponsiveness. No consistent association was found for atopy. The *LTA* 252A>G polymorphism was not associated with any of the outcomes. A meta-analysis of 17 studies showed an increased asthma risk for the *TNFA* -308 adenine allele.

The tumour necrosis factor-α gene nucleotide -308 polymorphism is associated with a moderately increased risk of asthma and bronchial hyperresponsiveness, but not with atopy. These results are supported by a meta-analysis of previously published studies.

KEYWORDS: Asthma, genetics, lymphotoxin-α, polymorphism, tumour necrosis factor

Asthma is a complex disease with both genetic and environmental components. It is characterised by obstruction of the airways of the lung and is related to atopy and bronchial hyperresponsiveness (BHR). Several chromosome regions and candidate genes have been associated with asthma, although the individual genes identified to date exhibit only modest effects and an unknown pattern of inheritance [1–3].

Tumour necrosis factor (TNF) is a potent pro-inflammatory cytokine involved in the inflammation of asthmatic airways [4]. It is located within the class III region of the major histocompatibility complex (MHC) region on chromosome 6p21.3 [5], which has previously been linked to asthma in various genome screens [1, 3, 6]. The TNF-α gene (*TNFA*) and lymphotoxin-α (LT-α) gene (*LTA*, also called *TNFB*) are members of the TNF superfamily. *TNFA* plays an important role in generating and maintaining inflammatory responses and airway hyperreactivity [7, 8]. TNF-α has been found in increased concentrations in the airways [8] and bronchoalveolar lavage fluid of asthmatic patients [9]. Moreover, the TNF-α secretory response to allergens differs between atopic and nonatopic subjects [10]. *LTA* is located closely upstream of *TNFA*, and both exhibit similar biological activity [11].

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Polymorphisms in the two genes may affect the levels of TNF in the airways. The *TNFA* guanine (G) to adenine (A) substitution of nucleotide -308 (-308G>A) polymorphism, located in the promoter region of *TNFA*, has been associated with increased secretion and promoter activity [12]. The *LTA* 252A>G polymorphism, located in the first intron of *LTA* seems to be associated with high LT-α production [13]. The *TNFA* -308A and *LTA* 252G alleles have been positively associated with asthma in many [14–22] but not all studies [23–27].

The aim of the present study was to evaluate whether or not polymorphisms in *TNFA* and *LTA* were associated with asthma, BHR and atopy in adults in two large population-based European cohorts for which comparable methods had been used, the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA) and the European Community Respiratory Health Survey (ECRHS). The role of TNF in asthma susceptibility was also evaluated across smoking categories. Finally, the consistency of the present results was evaluated through a meta-analysis of all published papers on polymorphisms in the two genes.

MATERIALS AND METHODS

Study population

The present study included 11,136 subjects derived from two different population cohorts. The ECRHS was a population-based multicentric cohort study. In the first phase of the study, taking place in most countries in the early 1990s, a random sample of the population aged 20–44 yrs living in the study areas was contacted and asked to complete a short questionnaire concerning respiratory symptoms [28]. In a second phase, an ~20% random subsample of the study population was contacted together with a complementary symptom subsample. The symptom subsample included all subjects reporting asthma-related respiratory symptoms in the short questionnaire who had not been selected in the random sample [29]. Subjects in most centres were followed-up with a median duration of follow-up of 8.9 yrs from the first phase (ECRHS) to the second phase (ECRHS-II). For the present analysis, the population studied consisted of 5,065 subjects with interview information, from whom DNA had additionally been extracted during ECRHS-II (19 centres from 10 countries).

The second cohort study population was that of the SAPALDIA [30, 31]. SAPALDIA subjects were recruited in 1991 as a random sample of adults aged 18–60 yrs from eight Swiss communities representing different language and climatic regions and varying degrees of urbanisation. The median follow-up time for SAPALDIA was 10.9 yrs. Participants with complete interview data and DNA samples available for genotyping were included (n=6,071).

Subjects included in the present analysis could be considered to be mainly of European-Caucasian origin. Some subjects from Basle in Switzerland (n=400) appeared in both datasets and were included only in the SAPALDIA analysis. Ethical approval was obtained for each centre from the appropriate institutional ethics committee and written consent was obtained from each participant.

Asthma, bronchial hyperresponsiveness and atopy

The ECHRS and SAPALDIA used identical questionnaires for the assessment of respiratory symptoms and asthma. Asthma was evaluated at baseline (phase I of both studies) on the basis of reported asthma symptoms and reported physician-diagnosed asthma. The presence of asthma symptoms was based on a positive response to either of two questions concerning: attack of asthma during the 12 months preceding the interview, or current use of asthma medication. Among subjects reporting an asthma attack, 67% also reported use of asthma medication. Alternative definitions of asthma employed in previous studies were also examined. ''Wheeze without a cold'' was defined as a positive response to the following two consecutive questions (the second asked on the basis of a positive response to the first): ''Have you had wheezing or whistling in your chest at any time in the last 12 months?''; ''Have you had this wheezing or whistling when you did not have a cold?'' Physician-diagnosed asthma was defined as a positive response to the following question: ''Have you ever had asthma and was this confirmed by a doctor?''

Data on BHR were available for a total of 8,043 subjects across the two studies. The SAPALDIA and ECRHS used identical spirometric protocols [28, 30], and consenting participants underwent bronchial challenge with methacholine chloride, administered *via* MEFAR® aerosol dosimeters (Mefar, Bovezzo, Italy) [32]. BHR was defined as a 20% fall in forced expiratory volume in one second (FEV_1) from the highest post-diluent FEV_1 during methacholine challenge with a cumulative dose of 1 mg for both the ECRHS and SAPALDIA [33]. BHR associated with the higher cumulative doses delivered in SAPALDIA was not taken into account. A family history of asthma was defined as a report of asthma of either of the parents.

Skin-prick tests (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) were performed in the ECRHS and SAPALDIA. Subjects atopic at baseline in both studies were defined as yielding positive test results to at least one common inhalant allergen (house dust mites (*Dermatophagoides pteronyssinus*), timothy grass, cat and *Cladosporium herbarum)*.

Candidate single-nucleotide polymorphism selection and genotyping

Two single-nucleotide polymorphisms (SNPs), *TNFA* -308G>A (National Center for Biotechnology Information SNP ID rs1800629) and *LTA* 252A>G (rs909253), were selected on the basis of previous evidence of their correlation with serum levels of TNF-α and LT-α [12, 13] and their association with asthma [14–22]. In the SAPALDIA, the *TNFA* -308G>A and *LTA* 252A>G polymorphisms were genotyped using a liquid-handling-assisted set-up and a fluorescent 5′-nuclease real-time PCR (TaqMan; Applera Europe, Rotkreuz, Switzerland) assay with an ABI Prism 7900 sequence detection system (Applied Biosystems, Rotkreuz, Switzerland). SNP-specific primers were designed for the PCR by Applied Biosystems (Applera Europe). The SNP-specific minor-groove-binder probes and forward and reverse primers used were as follows: *TNFA* -308G>A: 5′-CCCGTCC[C/T]CATGCC-3′, 5′-CCAAAAGAAATGGAGGCAATAGGTT-3′, and 5′-GG-ACCCTGGAGGCTGAAC-3′, respectively; and *LTA* 252A>G: 5′-CTGCCATG[A/G]TTCCT-3′, 5′-CAGTCTCATTGTCTCTGT-CACACAT-3′, and 5′-AGAGAGAGACAGGAAGGGAACAG-3′, respectively. A random sample of 10% of all DNA samples was re-genotyped, and all genotypes were confirmed. The genotype call rate was >99%.

For the ECRHS, genotyping was performed at the Centre for Genomic Regulation of the Spanish National Genotyping Centre (Barcelona, Spain). SNPs were genotyped using the SNPlex™ platform (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions and analysed on an Applied Biosystems 3730/3730*xl* DNA Analyzer (Applied Biosystems, Rotkreuz, Switzerland). Allele-calling was performed by cluster analysis using Genemapper (version 4.0) software (Applied Biosystems, Applera Europe). The genotype call rate was >98%. Genotyping quality was controlled in two ways. First, internal positive and negative controls provided by the manufacturer were included in the reaction plates. Secondly, six duplicate samples of two HapMap [34] reference trios were incorporated into the genotyping process. Both genotype concordance and correct Mendelian inheritance were verified. Genotype concordance was tested using SNPator, a web-based tool for genotyping management and SNP analysis developed by the Spanish National Genotyping Centre [35].

The genotyping across the two laboratories that analysed samples of the two cohorts were compared using subjects from the centre in Basle (n= 400), who had been included in both the ECRHS and SAPALDIA. The agreement in genotyping was 99.8%. In addition, the Basle ECRHS samples had been previously genotyped for the *TNFA* -308 marker using both restriction fragment length polymorphism and allele-specific PCR methods [36]. Only very small differences in genotype distribution were observed between those results and the results reported in the present study (Chi-squared=0.02; degrees of freedom (df)=2; p=0.99).

Meta-analysis

Previous articles on the association of the *TNFA* -308G>A or *LTA* 252A>G polymorphism with asthma were sought on PubMed, and backward searches of articles cited in earlier literature reviews or original papers were conducted. The keywords used in the PubMed search were ''asthma AND gene AND (tumour necrosis factor OR TNF)'' for *TNFA* -308G>A and ''asthma AND gene AND (lymphotoxin OR LTA OR TNFB)'' for *LTA* 252A>G.

In the meta-analysis, all studies that met the following criteria were included: 1) design: either population-based cohort, case–control or cross-sectional study; 2) outcome: asthma defined as physician-diagnosed [18, 20, 23, 26, 27, 37–40] or self-reported [16, 17, 19, 25, 29, 41, 42], regardless of age of onset; 3) ethnicity: information available, or, if not available in the published report, available through contact with the authors [41]; 4) method of genotyping reported; 5) complete genotype information available for subjects; and 6) genotypes in Hardy–Weinberg equilibrium. Results from the SAPALDIA and ECRHS were included in the meta-analysis using asthma symptoms and physician-diagnosed asthma as the main outcomes definition.

Statistical analysis

The statistical analysis was performed using the R genetic package (version 1.2.1) of R statistical software (version 2.4.0) [43]. Exact tests were used to test for Hardy-Weinberg equilibrium in control subjects (subjects without asthma symptoms, physician-diagnosed asthma, atopy or BHR) [44]. The normalised disequilibrium constant *D'* and Chi-squared p-values for marker independence were estimated in order to determine linkage disequilibrium between both genetic markers.

Logistic regression analysis was performed in order to determine the adjusted associations between genotypes and disease using co-dominant and additive models. The odds ratio (OR) and p-values corresponding to the 95% confidence interval (CI) were computed using the generalised linear models procedures (glm) from the R statistical package. A p-value of <0.05 was considered significant. Logistic regression models were adjusted for country (ECRHS) or study area (SAPALDIA), sex, age, body mass index (BMI) and smoking status. Haplotype-specific adjusted associations were also evaluated. Haplotypes were reconstructed and analysed using the haplo.glm function of the R library HaploStats.

The impact of population stratification in the present data was assessed by analysing 23 unlinked SNPs (online supplementary material) using a genomic control approach [45]. These SNPs were genotyped in the ECRHS study and in a subsample of the SAPALDIA. The significance of the additive model was corrected by the inflation factor (λ) derived from genomic control for each of the three main outcomes in both the ECRHS and the pooled analysis.

Multifactor dimensionality reduction (MDR) was used on genetic and nongenetic potential determinants jointly in order to find genotype combinations within which the dichotomous outcome variability was much lower than between combinations [46–48]. It is an extension of the combinatorial partitioning method and can be seen as a data reduction technique in that it reduces the dimensionality of multilocus information to a single dimension. The method is nonparametric, assumes no particular genetic model and generates low false-positive rates [47].

In the meta-analysis, the exact test of Hardy–Weinberg was used to test deviations from Hardy–Weinberg equilibrium only in control groups. ORs were estimated for each study using Fisher's exact test of independence for 2×2 (using Fisher's exact test for count data) tables under the prior hypothesis that the rare allele confers susceptibility to asthma. Meta-analysis was performed using the Mantel–Haenszel method using the fixed effects and random effects model with R library rmeta version 2.14. Publication bias was evaluated by measuring the asymmetry of the funnel plot measuring the intercept from regression of standard normal deviates against precision [49].

RESULTS

The characteristics of participants in the ECRHS and SAPALDIA are presented in table 1. Both populations were comparable with regard to sex, BMI and pulmonary function (FEV_1 and forced vital capacity), but not with regard to mean age due to differences in the inclusion criteria for age. Smoking status also differed slightly between studies, but the smoking prevalence in the SAPALDIA is within the range observed between different centres in the ECRHS.

The prevalence of atopy and asthma and minor allele frequency (MAF) of *TNFA* -308G>A and *LTA* 252A>G are shown by study and country in table 2. Asthmatics (and consequently also atopics) were oversampled in the ECRHS since the subcohort with respiratory symptoms was included. Among the random samples of the ECRHS and SAPALDIA,

TABLE 1 Characteristics of participants in the European Community Respiratory Health Survey (ECRHS) and Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA) at baseline

	Both cohorts	ECRHS		SAPALDIA	
		Symptom sample	Random sample	Age 18–44 yrs	All ages
Subjects n	10736	796	3869	3572	6071
Males %	49	44	48	50	50
Age yrs	38±10	34±7	34±7	33±8	41±11
BMI kg·m^{-2}	23.9±3.8	25.1±4.8	23.9±3.7	23.1±3.4	23.8±3.6
Smoking status %					
Never-smoker	45.5	42.7	43.5	48.0	47.1
Ex-smoker	22.4	18.6	21.7	19.6	23.3
Current smoker	32.1	38.7	34.7	32.5	29.6
Asthma symptoms %	5.6	35.8	3.7	2.9	2.9
Physician-diagnosed asthma %	8.8	38.7	6.7	6.2	6.2
Atopy %	21.5	48.4	24.7	22.9	19.8
FEV$_1$ % pred	106.6±14.6	100.3±16.4	105.8±13.3	107.1±13.4	107.8±14.9
FVC % pred	112.3±14.9	107.4±14.3	109.2±13.1	112.3±13.9	114.9±15.5
BHR %	12.5	37.8	11.7	9.2	9.9

Data are presented as mean±SD unless otherwise indicated. BMI: body mass index; FEV$_1$: forced expiratory volume in one second; FVC: forced vital capacity; BHR: bronchial hyperresponsiveness; % pred: % predicted.

the prevalence of atopy ranged from 15% in Germany (ECRHS) to 42% in Australia (ECRHS). The prevalence of asthma symptoms ranged from 2% in Germany, Estonia, Spain and Belgium to 7% in the UK and Australia. Even higher prevalences were reported for physician-diagnosed asthma.

Geographical differences were also observed for the MAFs of both polymorphisms. The lowest MAFs were observed in France (12% for *TNFA* -308G>A and 27% for *LTA* 252A>G) and the highest in the UK (21% for *TNFA* -308G>A and 41% for *LTA* 252A>G). The two polymorphisms were in strong linkage disequilibrium (Chi-squared=7516.29; D'=0.98; r^2=0.60; $p<2.22\times10^{-16}$). The genotype distribution for both alleles was consistent with the Hardy–Weinberg equilibrium in the control group ($p>0.05$), except for the French ECRHS centre in Grenoble ($p<0.001$). The analyses presented in the current article include data from Grenoble. The results were only minimally modified when all of the analyses were repeated excluding this centre (data not shown). No strong effects of population stratification were detected in the present sample, obtaining λ of ~1 (1.05 in atopy, 1.06 in asthma and 1.30 in BHR).

The associations of *TNFA* -308G>A with atopy, asthma symptoms and BHR adjusted for country (ECRHS) or centre (SAPALDIA), sex, age, BMI and smoking status are summarised in table 3. A significant association was found for asthma symptoms and *TNFA* -308GA heterozygotes (OR=1.38; p=0.001) and for the *TNFA* -308A allele (OR=1.30; p=0.002). An analysis of *TNFA* -308G>A by study showed an increased risk of asthma symptoms in the ECRHS but not in the SAPALDIA (tables 3 and 4; fig. 1). The result of the test for heterogeneity between the two studies was significant (Q-statistic 5.92; df=1; p=0.015). In the ECRHS, a significant risk increase for asthma symptoms was observed for the GA and AA genotypes and the A allele (OR=1.49; $p=6.3\times10^{-5}$). Stratification by country in the ECRHS showed no differences in risk (Q=2.66; df=8; p=0.95; table 4; fig. 1) and an increased risk (OR>1) was observed for all countries. ORs for the random subsample of the ECRHS tended to be lower than those of the asthma-enriched subsample (data not shown). In the SAPALDIA, no difference in effect of *TNFA* -308G>A was observed between Latin- and German-speaking regions (Q=0.44; df=1; p=0.51). Exclusion of SAPALDIA subjects who were aged >45 yrs at baseline (so as to compare with a similar population structure as in the ECRHS) did not affect the risk estimates for asthma. The OR for the A allele in all SAPALDIA subjects was 0.94 (p=0.71), whereas the OR for SAPALDIA subjects aged <45 yrs was 0.89 (p=0.57). The observed associations between *TNFA* -308G>A and asthma symptoms were not modified by either sex or atopy. In both the ECRHS and SAPALDIA, *TNFA* -308G>A was associated with a slight increase in BHR prevalence (A allele OR=1.15; p=0.03), with similar risks found in the two studies (A allele OR=1.13 and 1.18, respectively). No significant association was found for *TNFA* -308G>A with atopy (table 3). The ORs for the random subsample of the ECRHS tended to be lower than those of the asthma-enriched subsample (data not shown).

The strength of the association of *TNFA* -308G>A genotypes was different for distinct asthma-related phenotypes. ORs for the A allele and for distinct phenotypes are shown in table 5. A positive association was found for most, but not all, phenotypes examined, although the differences between ECRHS and SAPALDIA remained with regard to phenotypes based on reported asthma symptoms.

Results for *LTA* 252A>G are summarised in table 6. Overall, ORs tended to be lower than for *TNFA* -308G>A. A significantly increased risk of asthma symptoms was observed for the heterozygous GA genotype (OR=1.21; p=0.05), which

TABLE 2 Symptom prevalence at baseline and minor allele frequency (MAF) by study site

Study	Country	Sample size n	Atopy %	Asthma symptoms %	Physician-diagnosed asthma %	BHR %	MAF[#]	
							TNFA -308G>A	*LTA* 252A>G
ECRHS	All	4665 (3869)	29 (25)	9 (4)	12 (7)	16 (12)	0.16	0.32
	Norway	436 (436)	23 (23)	3 (3)	8 (8)	8 (8)	0.19	0.36
	Sweden	643 (457)	41 (30)	2 (6)	21 (8)	19 (8)	0.15	0.37
	UK	358 (289)	39 (34)	18 (7)	20 (8)	23 (17)	0.21	0.41
	Australia	341 (248)	49 (42)	21 (7)	26 (13)	28 (21)	0.14	0.28
	Estonia[¶]	228 (175)	22 (18)	4 (2)	5 (3)		0.15	0.29
	Germany	439 (439)	15 (15)	2 (2)	4 (4)	14 (14)	0.15	0.33
	Belgium	506 (455)	27 (24)	5 (2)	7 (5)	13 (9)	0.18	0.32
	France	549 (522)	28 (28)	7 (5)	13 (11)	14 (12)	0.12	0.27
	Spain	1165 (848)	22 (19)	6 (2)	9 (4)	13 (1)	0.15	0.29
SAPALDIA	All	6071	19.8	2.9	9.9	6.2	0.139	0.305
	German[+]	3308	19.7	2.9	9.8	6.6	0.137	0.307
	Latin[§]	2763	19.9	2.8	10	5.7	0.141	0.303

Data shown in parentheses represent the prevalences in the European Community Respiratory Health Survey (ECRHS) random sample. SAPALDIA: Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults. BHR: bronchial hyperresponsiveness; *TNFA*: tumour necrosis factor-α gene; -308G>A: guanine to adenine substitution of nucleotide -308; *LTA*: lymphotoxin-α gene. [#]: in control subjects without asthma symptoms, physician-diagnosed asthma, cough, phlegm, current wheeze or atopy; [¶]: atopy measured using specific immunoglobulin E (no skin-prick test available) and BHR not measured; [+]: German-speaking centres: Basle, Aarau, Davos, and Wald; [§]: Italian- or French-speaking centres: Lugano, Payerne, Geneva and Montana.

was primarily driven by an association of the G allele with asthma in the ECRHS sample (OR=1.22; p=0.01).

Haplotype analysis

The 252G/-308A haplotype was positively associated with asthma symptoms (OR=1.29; p=0.003) compared to the most common haplotype, 252A/-308G. The prevalence of the 252A/-308A haplotype was <0.01 and could, therefore, not be tested for an association with asthma. No significant overall results were found for the 252G/-308G haplotype (online supplementary material).

Smoking effect

An association of *TNFA* -308G>A (GA and AA genotypes compared to GG genotype) with asthma was significant among never-smokers (OR=1.33; p=0.03) and ex-smokers (OR=1.64; p=0.01), whereas a nonsignificant excess risk was found for current smokers (OR=1.20). This differential effect was only observed in the ECRHS and not in the SAPALDIA. In the ECRHS, the effect of *TNFA* -308G>A was significant among never-smokers (OR=1.50; p=0.01) and ex-smokers (OR=2.36; p=0.0009). However, the p-value for interaction was nonsignificant when examining never- *versus* ever-smokers (p=0.52) or smoking status in three categories (p=0.23).

Multifactor dimensionality reduction

In the nonparametric MDR analysis, multiple genetic loci and environmental exposures associated with asthma were detected simultaneously in the absence of a main effect. An increasing number of interactions was examined, starting from including singular effects up to three-way interactions, and the best fit of each combination of variables was tested through permutation tests using 5,000 replications. Among the seven variables examined, significant results were found for a model including *LTA* 252A>G, smoking and region (with 5,000 permutations, permutation-based p-value of <0.001). The best fit was for a model with two variables included, smoking and region (permutation-based p-value of <0.001).

Meta-analysis

The meta-analysis of *TNFA* -308G>A was performed including the country-specific results from ECRHS countries and the two region-specific results from the SAPALDIA as individual observations along with the 16 published studies listed in table 4 and figure 1. The funnel plot for publication bias was not asymmetric (p>0.10), suggesting a lack of publication bias in the present meta-analysis. The ECRHS and SAPALDIA results were based on asthma symptoms. In total, 4,341 cases of asthma and 13,459 controls were examined (table 4; fig. 1). The combined OR for asthma using a fixed-effects model was 1.32 (p<0.001) and using a random-effects model 1.35 (p=0.001). Similarly to a previous meta-analysis [37], significant heterogeneity was observed between the Caucasian populations (Q=56.57; df=17; p<0.001). Excluding the study of ALBUQUERQUE *et al.* [26], which had an MAF of 31% that was different from all other studies, and the study of SHIN *et al.* [27], with a different MAF to other Asian population studies (17%), the heterogeneity diminished considerably (Q=9.64; df=15; p=0.84). The combined OR in Caucasian populations after the exclusion of the study of ALBUQUERQUE *et al.* [26] was 1.24 (p<0.0001) for the fixed-effects model and 1.29 (p=0.001) for the random-effects model. Heterogeneity was nonsignificant in Asian populations, although the ORs for a study in Korea [27] were markedly different from those of all other studies. The same risk was observed when physician-diagnosed was included instead of asthma symptoms in the meta-analysis,

TABLE 3 Adjusted association of tumour necrosis factor-α gene nucleotide -308 genotype with atopy, asthma symptoms and bronchial hyperresponsiveness (BHR)

	GG		GA			AA			A allele			Corrected[#]
	Case/C	OR	Case/C	OR (95% CI)	p-value	Case/C	OR (95% CI)	p-value	Case/C	OR (95% CI)	p-value	p_{add}
Atopy												
All	1679/5516	1.0	567/1821	0.99 (0.89–1.11)	0.89	54/191	0.82 (0.60–1.13)	0.22	675/2203	0.96 (0.88–1.06)	0.44	0.52	0.53
ECRHS	801/2028	1.0	317/715	1.09 (0.93–1.29)	0.28	24/90	0.59 (0.37–0.95)	0.03	365/895	0.97 (0.85–1.12)	0.69	0.04	0.04
SAPALDIA	878/3488	1.0	250/1106	0.91 (0.78–1.06)	0.23	30/101	1.11 (0.72–1.70)	0.63	310/1308	0.95 (0.83–1.09)	0.48	0.40	
Asthma symptoms												
All	378/7195	1.0	175/2343	1.38 (1.13–1.68)	0.001	19/243	1.39 (0.83–2.32)	0.21	213/2829	1.30 (1.11–1.53)	0.002	0.005	0.006
ECRHS	248/2809	1.0	132/980	1.59 (1.25–2.02)	0.0002	17/110	1.84 (1.04–3.26)	0.04	166/1200	1.49 (1.22–1.81)	6.3×10^{-5}	0.0003	0.0004
SAPALDIA	130/4386	1.0	43/1363	1.03 (0.72–1.46)	0.89	2/133	0.51 (0.13–2.10)	0.35	47/1629	0.94 (0.69–1.29)	0.71	0.57	
BHR												
All	686/5145	1.0	278/1655	1.22 (1.05–1.42)	0.01	27/178	1.08 (0.71–1.65)	0.71	332/2011	1.15 (1.02–1.31)	0.03	0.04	0.08
ECRHS	363/1975	1.0	146/688	1.12 (0.90–1.39)	0.31	18/73	1.32 (0.77–2.27)	0.31	182/834	1.13 (0.95–1.35)	0.18	0.41	0.43
SAPALDIA	323/3170	1.0	132/967	1.32 (1.06–1.64)	0.01	9/105	0.83 (0.42–1.67)	0.61	150/1177	1.18 (0.98–1.41)	0.09	0.04	

Models were adjusted for age, sex, body mass index, country (European Community Respiratory Health Survey (ECRHS)) or study centre (Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA)), and smoking status. G: guanine; A: adenine; C: control; OR: odds ratio; CI: confidence interval; p_{add}: p-value for additive model. [#]: corrected for genomic control.

with an OR of 1.27 ($p<0.001$). A meta-analysis of 10 previous studies on *LTA* 252A>G and asthma [14, 17, 19, 20, 25–27, 38, 40, 41, 42] was also performed, including the data from the present study. The meta-analysis examined a total of 3,120 cases and 12,026 controls, with the combined OR being 1.01 (95% CI 0.99–1.16; $p=0.06$) for the fixed-effects model and 1.08 (95% CI 0.95–1.23; $p=0.22$) for the random-effects model.

DISCUSSION

A genetic association study on the *TNFA* -308G>A and *LTA* 252A>G polymorphisms with asthma and related phenotypes was performed in two large European prospective cohorts. This is the largest association study of *TNFA* -308G>A/*LTA* 252A>G in subjects well phenotyped for atopy and respiratory symptoms using validated questionnaires and measures of lung function, BHR and atopy. A moderate but significant association of the *TNFA* -308GA genotype and the *TNFA* -308A allele with increased asthma risk was found. The risks for BHR were also increased but to a lesser extent, whereas no consistent associations were found for atopy. The present results were verified using alternative nonparametric analyses and a meta-analysis of all published studies. Weaker associations were found for *LTA*. The effects of *TNFA* can be attributed to linkage disequilibrium with other asthma genes, to a main effect of *TNFA* or to an interaction and modification of the effect by environmental exposures.

The two polymorphisms examined are located in the MHC class III region, near human leukocyte antigen (HLA)-B [5]. The *TNFA* -308A allele is in strong linkage disequilibrium with the HLA-A1, -B8 and -DR3 alleles [12], which are also associated with higher levels of *TNFA* -308G>A [50]. Although some studies suggest that the *LTA* 252A>G/*TNFA* -308G>A association is independent of MHC class II alleles [21, 42], MOFFAT *et al.* [14] found that two haplotypes containing the *TNFA* -308A allele (*LTA* 252G/*TNFA* -308A/HLA-DRB1*3 and *LTA* 252G/*TNFA* -308A/HLA-DRB1*2) were more strongly associated with asthma and BHR than other haplotypes containing only *LTA* 252A>G/*TNFA* -308G>A polymorphisms. Identification of the individual effects of *LTA* 252A>G and *TNFA* -308G>A is difficult due to the linkage disequilibrium. The present results indicate that *TNFA* -308G>A is associated with several asthma-related phenotypes, whereas less consistent results were observed for the main effects of *LTA* 252A>G. Haplotype analysis showed that only the model with the *TNFA* -308A allele was associated with asthma, and that this association was equivalent to the model with *TNFA* -308G>A alone. This evidence suggests that the associations observed for *LTA* 252A>G in asthma and BHR may be due to its linkage with *TNFA* -308G>A.

TNF-α is a potent pro-inflammatory cytokine found in high concentrations in bronchoalveolar fluid from asthmatics [8, 9, 51]. *TNFA* nucleotide -308 is located in the promoter region of *TNFA,* and *in vitro* studies have reported increased *TNFA* transcription associated with the *TNFA* -308G>A variant [12, 52]. The A allele has been associated with increased expression [12] and secretion of TNF-α [52, 53], although this association is not uniformly supported [54, 55]. No association of the *TNFA* A allele with atopy was observed, whereas it was observed for asthma symptoms and BHR. Furthermore no modification of the association of *TNFA* -308G>A and asthma by atopic status

TABLE 4 Tumour necrosis factor-α gene guanine to adenine (A) substitution of nucleotide -308 polymorphism and asthma symptoms in the studies used for the meta-analysis and study weighting

First author [ref.]	Year	Population	Case/control A allele n	Weight %
Present study				
ECRHS	2007	Norway	6/135	2.10
		Sweden	54/140	5.07
		Estonia	5/62	1.57
		UK	33/116	4.15
		Australia	24/77	3.94
		Germany	3/121	1.20
		Belgium	9/144	2.78
		France	14/118	3.66
		Spain	18/287	3.91
SAPALDIA	2007	Switzerland	47/1629	5.44
Present overall			213/2829	33.82
Previous studies				
Louis [23]	2000	European Caucasian	31/31	3.70
Buckova [25]	2002	Czech	51/40	4.54
Winchester [16]	2000	British and Irish	13/150	2.69
Bilolikar [38]	2005	British	61/43	3.52
Munthe-Kaas [22]	2007	Norway	130/213	5.59
Albuquerque [26]	1998	Australian Caucasian	58/25	3.44
Moffatt [42]	1997	Australian	52/113	4.79
El Bahlawan [41]	2003	African American from USA	9/53	2.30
Witte [40]	2002	USA	75/67	5.03
Wang [20]	2004	Taiwanese	18/53	3.69
Sandford [17]	2004	Chinese	26/16	3.29
Shin [27]	2004	Korean	54/41	4.52
Aoki [37]	2006	Japanese	21/12	2.77
Hong [39]	2007	Korean	92/14	2.77
Winchester [16]	2000	South Asian	2/39	0.49
Gupta [18]	2005	Indian	42/34	4.13
Sharma [19]	2006	North India	66/37	4.53
Sharma [19]	2006	West India	55/36	4.39
Previous overall			856/1017	66.18
Overall		Total	1069/3846	100

ECRHS: European Community Respiratory Health Survey; SAPALDIA: Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults.

was observed. One study [20] reported that *TNFA* -308G>A was a risk factor for atopic asthma but not for nonatopic asthma, although a number of other studies did not find this interaction [23, 27, 42].

In the present study, the pattern of the interaction between TNF polymorphisms and tobacco smoke was not clear. TNF-α is central to acute cigarette-smoke-induced inflammation and the resulting connective tissue breakdown. Oxidative stress involved in inflammation is partly regulated by cytokines such as TNF-α. *TNFA* -308G>A effects on the inflammatory response to oxidative stress have been suggested in other studies in relation to exposure to ozone, occupational endotoxin and environmental tobacco smoke, but the results have been inconsistent. Interaction of the *LTA* 252A>G and *TNFA* -308G>A polymorphisms with environmental factors has been shown in several studies in relation to smoking, ozone and other environmental or occupational exposures, although the results have not been consistent [56-59].

The present study had low power for the evaluation of differences between countries and regions due to the low MAF of the *TNFA* -308A allele. No effect of population stratification was observed in the present sample, although the number of markers tested might be insufficient for the detection of lower stratification between European populations [60]. The association analyses of both markers revealed differences in risk between the ECRHS and SAPALDIA cohorts for asthma symptoms, whereas more consistent results were obtained for BHR and atopy. The study protocols of both studies were similar, and phenotypes were defined using the same questions. Nevertheless, differences across the two cohorts tended to relate primarily to asthma phenotypes, defined by questionnaire, rather than to phenotypes derived from functional or

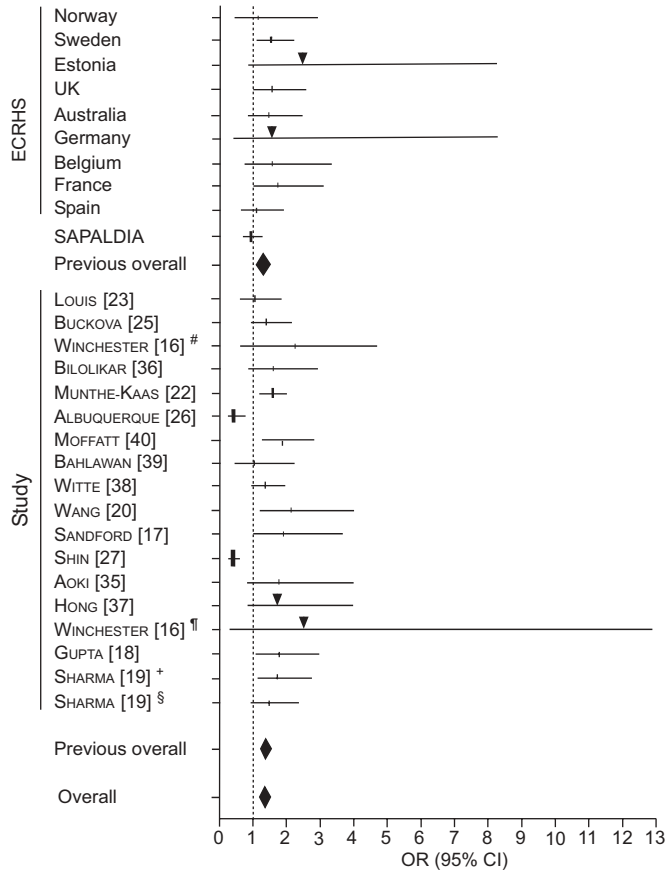

FIGURE 1. Meta-analysis of tumour necrosis factor-α gene guanine to adenine (A) substitution of nucleotide -308 polymorphism and asthma symptoms, including the European Community Respiratory Health Survey (ECRHS) and Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA). Data are presented as A allele odds ratio (OR; ■ (size reflects weighting)) for asthma symptoms and 95% confidence interval (CI; horizontal bars). The centres of the diamonds indicate the combined mean effect of the studies and their extremities the 95% CI; ……: line of no effect. The arrowheads indicate the positions of ORs of low weight. #: British and Irish; ¶: South Asian; +: North India; §: West India.

biological tests. However, a study assessing the internal consistency of respiratory symptoms suggests that international comparisons are not affected by errors due to cross-cultural variations in the reporting of symptoms [61]. The major difference between the two studies was the inclusion in the ECRHS of a subsample with subjects that reported respiratory symptoms in the initial screening questionnaire. This subsample represented >60% of the asthma cases in the ECRHS and led to differences in symptom prevalences compared to the SAPALDIA. The magnitude of asthma risk in relation to *TNFA* genotypes in the ECRHS after exclusion of the asthma-enriched sample tended to be to weaker, although still positive, indicating that inclusion of the asthma-enriched sample could not explain the differences in risk between the two studies. The slightly stronger association observed in the oversampled asthmatics from the ECRHS might, possibly, reflect an underlying stronger effect of the *TNFA* -308G>A genotype in more severe forms of asthma and in asthma persistence as well as progression. There is, however, only very limited evidence supporting such an association [15, 62]. Other potential differences, such as errors in genotyping, could be disregarded. Given the above, the most likely explanation for the absence of an association with self-reported asthma phenotypes in the SAPALDIA is the lack of statistical precision due to a much lower number of cases compared to the ECHRS. The meta-analysis of all published studies reinforces the present results of a positive association between the *TNFA* -308G>A genotype and asthma prevalence, although some geographical variability could be observed. An inverse association was observed in only two studies [26, 27]. However, the combined estimate confirms the association of *TNFA* -308G>A with an increased risk of asthma in European and Asian populations. Population stratification is a concern in large genetic association studies with heterogeneous population [60]. The subjects in the present analysis were almost entirely of European ancestry. However, even within the present study population, differences in allelic frequency were observed between countries, with higher MAFs obtained in the UK. The two previous studies that found an inverse association with asthma risk [26, 27] were outliers with regard to *TNFA*

TABLE 5 Adjusted association of tumour necrosis factor-α gene -308 adenine allele with various asthma-related phenotypes and their combinations in the two cohorts

	Both cohorts		ECRHS		SAPALDIA	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Atopy	0.97 (0.88–1.07)	0.51	0.98 (0.86–1.13)	0.82	0.96 (0.84–1.09)	0.50
Asthma symptoms	1.30 (1.11–1.53)	0.001	1.49 (1.23–1.81)	5.6×10^{-5}	0.94 (0.69–1.29)	0.71
BHR	1.16 (1.02–1.32)	0.03	1.14 (0.95–1.36)	0.16	1.18 (0.98–1.41)	0.09
Physician-diagnosed asthma	1.13 (0.98–1.29)	0.09	1.37 (1.15–1.63)	0.0004	0.84 (0.67–1.05)	0.12
Asthma symptoms and atopy	1.25 (1.02–1.54)	0.03	1.41 (1.12–1.78)	0.003	0.78 (0.47–1.29)	0.33
Asthma symptoms and BHR	1.25 (0.98–1.61)	0.07	1.30 (0.98–1.73)	0.068	1.05 (0.62–1.77)	0.86
Wheeze without a cold	0.97 (0.85–1.09)	0.58	1.01 (0.86–1.18)	0.92	0.90 (0.72–1.11)	0.31

Models are adjusted by age, sex, body mass index, country (European Community Respiratory Health Survey (ECRHS)) or study centre (Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA)) and smoking status. OR: odds ratio; CI: confidence interval; BHR: bronchial hyperresponsiveness.

TABLE 6 Adjusted association of lymphotoxin-α gene nucleotide 252 genotype with atopy, asthma symptoms and bronchial hyperresponsiveness (BHR)

	AA		AG			GG			G allele				Corrected[#]
	Case/C	OR	Case/C	OR (95% CI)	p-value	Case/C	OR (95% CI)	p-value	Case/C	OR (95% CI)	p-value	padd	padd
Atopy													
All	1005/3812	1	1011/3268	1.00 (0.91–1.11)	0.92	269/764	1.09 (0.93–1.28)	0.28	1549/4796	1.03 (0.96–1.11)	0.40	0.52	0.53
ECRHS	545/1445	1	538/1271	1.07 (0.92–1.23)	0.39	148/332	1.09 (0.87–1.36)	0.47	834/1935	1.05 (0.95–1.16)	0.35	0.51	0.52
SAPALDIA	460/2367	1	473/1997	0.95 (0.83–1.09)	0.49	121/432	1.10 (0.88–1.38)	0.41	715/2861	1.01 (0.92–1.12)	0.82	0.45	
Asthma symptoms													
All	255/4837	1	280/4231	1.21 (1.00–1.45)	0.05	65/1017	1.04 (0.77–1.41)	0.78	410/6265	1.08 (0.95–1.23)	0.26	0.44	0.13
ECRHS	165/1997	1	202/1750	1.36 (1.08–1.72)	0.01	58/455	1.40 (1.00–1.96)	0.05	318/2660	1.22 (1.05–1.43)	0.01	0.02	0.02
SAPALDIA	90/2840	1	78/2481	0.98 (0.72–1.34)	0.89	7/562	0.40 (0.18–0.86)	0.02	92/3605	0.80 (0.63–1.02)	0.08	0.02	
BHR													
All	481/3431	1	425/3047	0.97 (0.84–1.12)	0.70	121/732	1.10 (0.88–1.37)	0.39	667/4511	1.02 (0.93–1.13)	0.65	0.60	0.64
ECRHS	271/1374	1	215/1269	0.82 (0.67–1.00)	0.05	76/325	1.09 (0.82–1.46)	0.54	367/1919	0.97 (0.85–1.12)	0.70	0.08	0.13
SAPALDIA	210/2057	1	210/1778	1.16 (0.94–1.42)	0.16	45/407	1.09 (0.77–1.53)	0.63	300/2592	1.08 (0.94–1.25)	0.28	0.39	

Models were adjusted for age, sex, body mass index, country (European Community Respiratory Health Survey (ECRHS)) or study centre (Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA)), and smoking status. A: adenine; G: guanine; C: control; OR: odds ratio; CI: confidence interval; padd: p-value for additive model. #: corrected for genomic control.

-308A allele frequency. In the present meta-analysis, large variation in the allelic frequency of the *TNFA* -308A allele was observed between populations, with lower frequencies in those from Korea, Japan, China and Taiwan. One limitation of the present meta-analysis is that the selection criteria were broad, leading to the inclusion of different age groups and definitions of asthma (*e.g.* paediatric asthma, adult asthma and atopic asthma), and the biological mechanisms involved in each of these asthma-related phenotypes might be different. This is particularly important given the differences in risk observed for different asthma phenotypes in the present study. Since there are limited published data on BHR, it was not possible to perform a meaningful meta-analysis for this end-point.

Conclusions

In the present large international population-based prospective study, the tumour necrosis factor-α gene guanine to adenine substitution of nucleotide -308 polymorphism was associated with a moderately increased risk of asthma and bronchial hyperresponsiveness, whereas no association was found for atopy. These results were supported by a meta-analysis of the published evidence.

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Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults

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REFERENCES

1 Cookson W. The alliance of genes and environment in asthma and allergy. *Nature* 1999; 402: B5–B11.
2 Weiss ST, Raby BA. Asthma genetics 2003. *Hum Mol Genet* 2004; 13: Suppl. 1, R83–R89.
3 Contopoulos-Ioannidis DG, Kouri IN, Ioannidis JP. Genetic predisposition to asthma and atopy. *Respiration* 2007; 74: 8–12.
4 Shah A, Church MK, Holgate ST. Tumour necrosis factor alpha: a potential mediator of asthma. *Clin Exp Allergy* 1995; 25: 1038–1044.
5 Carroll MC, Katzman P, Alicot EM, *et al.* Linkage map of the human major histocompatibility complex including the tumor necrosis factor genes. *Proc Natl Acad Sci U S A* 1987; 84: 8535–8539.
6 Wjst M, Fischer G, Immervoll T, *et al.* A genome-wide search for linkage to asthma. *Genomics* 1999; 58: 1–8.
7 Choi IW, Sun K, Kim YS, *et al.* TNF-α induces the late-phase airway hyperresponsiveness and airway inflammation through cytosolic phospholipase A_2 activation. *J Allergy Clin Immunol* 2005; 116: 537–543.
8 Bradding P, Roberts JA, Britten KM, *et al.* Interleukin-4, -5, and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994; 10: 471–480.
9 Broide DH, Lotz M, Cuomo AJ, Coburn DA, Federman EC, Wasserman SI. Cytokines in symptomatic asthma airways. *J Allergy Clin Immunol* 1992; 89: 958–967.
10 Ying S, Robinson DS, Varney V, *et al.* TNFα mRNA expression in allergic inflammation. *Clin Exp Allergy* 1991; 21: 745–750.
11 Nedwin GE, Naylor SL, Sakaguchi AY, *et al.* Human lymphotoxin and tumor necrosis factor genes: structure,

homology and chromosomal localization. *Nucleic Acids Res* 1985; 13: 6361–6373.
12 Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. *ProcNatl Acad Sci USA* 1997; 94: 3195–3199.
13 Messer G, Spengler U, Jung MC, *et al.* Polymorphic structure of the tumor necrosis factor (TNF) locus: an *Nco*I polymorphism in the first intron of the human TNF-β gene correlates with a variant amino acid in position 26 and a reduced level of TNF-β production. *J Exp Med* 1991; 173: 209–219.
14 Moffatt MF, James A, Ryan G, Musk AW, Cookson WO. Extended tumour necrosis factor/HLA-DR haplotypes and asthma in an Australian population sample. *Thorax* 1999; 54: 757–761.
15 Chagani T, Pare PD, Zhu S, *et al.* Prevalence of tumor necrosis factor-α and angiotensin converting enzyme polymorphisms in mild/moderate and fatal/near-fatal asthma. *Am J Respir Crit Care Med* 1999; 160: 278–282.
16 Winchester EC, Millwood IY, Rand L, Penny MA, Kessling AM. Association of the TNF-α-308 (G>A) polymorphism with self-reported history of childhood asthma. *Hum Genet* 2000; 107: 591–596.
17 Sandford AJ, Chan HW, Wong GW, Lai CK, Chan-Yeung M. Candidate genetic polymorphisms for asthma in Chinese schoolchildren from Hong Kong. *Int J Tuberc Lung Dis* 2004; 8: 519–527.
18 Gupta V, Sarin BC, Changotra H, Sehajpal PK. Association of G-308A TNF-α polymorphism with bronchial asthma in a North Indian population. *J Asthma* 2005; 42: 839–841.
19 Sharma S, Sharma A, Kumar S, Sharma SK, Ghosh B. Association of TNF haplotypes with asthma, serum IgE levels, and correlation with serum TNF-α levels. *Am J Respir Cell Mol Biol* 2006; 35: 488–495.
20 Wang TN, Chen WY, Wang TH, Chen CJ, Huang LY, Ko YC. Gene–gene synergistic effect on atopic asthma: tumour necrosis factor-α-308 and lymphotoxin-α-*Nco*I in Taiwan's children. *Clin Exp Allergy* 2004; 34: 184–188.
21 Li Kam Wa TC, Mansur AH, Britton J, *et al.* Association between -308 tumour necrosis factor promoter polymorphism and bronchial hyperreactivity in asthma. *Clin Exp Allergy* 1999; 29: 1204–1208.
22 Munthe-Kaas MC, Carlsen KL, Carlsen KH, *et al.* HLA Dr-Dq haplotypes and the TNFA-308 polymorphism: associations with asthma and allergy. *Allergy* 2007; 62: 991–998.
23 Louis R, Leyder E, Malaise M, Bartsch P, Louis E. Lack of association between adult asthma and the tumour necrosis factor α-308 polymorphism gene. *Eur Respir J* 2000; 16: 604–608.
24 Zhu S, Chan-Yeung M, Becker AB, *et al.* Polymorphisms of the IL-4, TNF-α, and Fcε RIβ genes and the risk of allergic disorders in at-risk infants. *Am J Respir Crit Care Med* 2000; 161: 1655–1659.
25 Buckova D, Holla LI, Vasku A, Znojil V, Vacha J. Lack of association between atopic asthma and the tumor necrosis factor α-308 gene polymorphism in a Czech population. *J Investig Allergol Clin Immunol* 2002; 12: 192–197.
26 Albuquerque RV, Hayden CM, Palmer LJ, *et al.* Association of polymorphisms within the tumour necrosis factor (TNF) genes and childhood asthma. *Clin Exp Allergy* 1998; 28: 578–584.
27 Shin HD, Park BL, Kim LH, *et al.* Association of tumor necrosis factor polymorphisms with asthma and serum total IgE. *Hum Mol Genet* 2004; 13: 397–403.
28 Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994; 7: 954–960.
29 Jarvis D, Chinn S, Potts J, Burney P. Association of body mass index with respiratory symptoms and atopy: results from the European Community Respiratory Health Survey. *Clin Exp Allergy* 2002; 32: 831–837.
30 Martin BW, Ackermann-Liebrich U, Leuenberger P, *et al.* SAPALDIA: methods and participation in the cross-sectional part of the Swiss Study on Air Pollution and Lung Diseases in Adults. *Soz Praventivmed* 1997; 42: 67–84.
31 Ackermann-Liebrich U, Kuna-Dibbert B, Probst-Hensch NM, *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 Praventivmed* 2005; 50: 245–263.
32 Schwartz J, Schindler C, Zemp E, *et al.* Predictors of methacholine responsiveness in a general population. *Chest* 2002; 122: 812–820.
33 Chinn S, Jarvis D, Burney P. Relation of bronchial responsiveness to body mass index in the ECRHS. *Thorax* 2002; 57: 1028–1033.
34 Anon, International HapMap Project. www.hapmap.org/index.html.en.
35 Genomic Diversity and Population Genomics Node of the Spanish Institute of Bioinformatics. SNPator. www.snpator.org/public/new_login/index.php Date last accessed: August 2007.
36 Imboden M, Nicod L, Nieters A, *et al.* The common G-allele of interleukin-18 single-nucleotide polymorphism is a genetic risk factor for atopic asthma. The SAPALDIA Cohort Study. *Clin Exp Allergy* 2006; 36: 211–218.
37 Aoki T, Hirota T, Tamari M, *et al.* An association between asthma and TNF-308G/A polymorphism: meta-analysis. *J Hum Genet* 2006; 51: 677–685.
38 Bilolikar H, Nam AR, Rosenthal M, Davies JC, Henderson DC, Balfour-Lynn IM. Tumour necrosis factor gene polymorphisms and childhood wheezing. *Eur Respir J* 2005; 26: 637–646.
39 Hong SJ, Kim HB, Kang MJ, *et al.* TNF-α (-308 G/A) and CD14 (-159T/C) polymorphisms in the bronchial responsiveness of Korean children with asthma. *J Allergy Clin Immunol* 2007; 119: 398–404.
40 Witte JS, Palmer LJ, O'Connor RD, Hopkins PJ, Hall JM. Relation between tumour necrosis factor polymorphism TNFα-308 and risk of asthma. *Eur J Hum Genet* 2002; 10: 82–85.
41 El Bahlawan L, Christensen M, Binaei S, Murphy C, Zhang Q, Quasney M. Lack of association between the tumor necrosis factor-α regulatory region genetic polymorphisms associated with elevated tumor necrosis factor-α levels and children with asthma. *Chest* 2003; 123: 374S–375S.
42 Moffatt MF, Cookson WO. Tumour necrosis factor haplotypes and asthma. *Hum Mol Genet* 1997; 6: 551–554.

43 R: a language and environment for statistical computing. Vienna, R Foundation for Statistical Computing. www.r-project.org

44 Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy–Weinberg equilibrium. *Am J Hum Genet* 2005; 76: 887–893.

45 Clayton DG, Walker NM, Smyth DJ, *et al.* Population structure, differential bias and genomic control in a large-scale, case-control association study. *Nat Genet* 2005; 37: 1243–1246.

46 Ritchie MD, Hahn LW, Roodi N, *et al.* Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. *Am J Hum Genet* 2001; 69: 138–147.

47 Ritchie MD, Hahn LW, Moore JH. Power of multifactor dimensionality reduction for detecting gene–gene interactions in the presence of genotyping error, missing data, phenocopy, and genetic heterogeneity. *Genet Epidemiol* 2003; 24: 150–157.

48 Hahn LW, Ritchie MD, Moore JH. Multifactor dimensionality reduction software for detecting gene–gene and gene–environment interactions. *Bioinformatics* 2003; 19: 376–382.

49 Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; 315: 629–634.

50 Jacob CO, Fronek Z, Lewis GD, Koo M, Hansen JA, McDevitt HO. Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor α: relevance to genetic predisposition to systemic lupus erythematosus. *Proc Natl Acad Sci USA* 1990; 87: 1233–1237.

51 Virchow JC Jr, Walker C, Hafner D, *et al.* T cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. *Am J Respir Crit Care Med* 1995; 151: 960–968.

52 Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-α promoter polymorphism effects transcription. *Mol Immunol* 1997; 34: 391–399.

53 Bouma G, Crusius JB, Oudkerk PM, *et al.* Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. *Scand J Immunol* 1996; 43: 456–463.

54 Stuber F, Udalova IA, Book M, *et al.* -308 tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. *J Inflamm* 1995; 46: 42–50.

55 Brinkman BM, Zuijdeest D, Kaijzel EL, Breedveld FC, Verweij CL. Relevance of the tumor necrosis factor α (TNF α) -308 promoter polymorphism in TNF α gene regulation. *J Inflamm* 1995; 46: 32–41.

56 Yang IA, Holz O, Jorres RA, *et al.* Association of tumor necrosis factor-α polymorphisms and ozone-induced change in lung function. *Am J Respir Crit Care Med* 2005; 171: 171–176.

57 Li YF, Gauderman WJ, Avol E, Dubeau L, Gilliland FD. Associations of tumor necrosis factor G-308A with childhood asthma and wheezing. *Am J Respir Crit Care Med* 2006; 173: 970–976.

58 Castro-Giner F, Kauffmann F, de Cid R, Kogevinas M. Gene–environment interactions in asthma. *Occup Environ Med* 2006; 63: 776–786.

59 Wu H, Romieu I, Sienra-Monge JJ, *et al.* Parental smoking modifies the relation between genetic variation in tumor necrosis factor-α (*TNF*) and childhood asthma. *Environ Health Perspect* 2007; 115: 616–622.

60 Marchini J, Cardon LR, Phillips MS, Donnelly P. The effects of human population structure on large genetic association studies. *Nat Genet* 2004; 36: 512–517.

61 Sunyer J, Basagana X, Burney P, Anto JM. International assessment of the internal consistency of respiratory symptoms. *Am J Respir Crit Care Med* 2000; 162: 930–935.

62 Kips JC. Cytokines in asthma. *Eur Respir J* 2001; 18: Suppl. 34, 24s–33s.