Joint effect of obesity and *TNFA* variability on asthma: two international cohort studies

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ABSTRACT: **Obesity is a risk factor for asthma. Adipose tissue expresses pro-inflammatory molecules including tumour necrosis factor (TNF), and levels of TNF are also related to polymorphisms in the TNF-α (*TNFA*) gene. The current authors examined the joint effect of obesity and *TNFA* variability on asthma in adults by combining two population-based studies.**

The European Community Respiratory Health Survey and the Swiss Cohort Study on Air Pollution and Lung and Heart Disease in Adults used comparable protocols, questionnaires and measures of lung function and atopy. DNA samples from 9,167 participants were genotyped for *TNFA -308* and lymphotoxin-α (*LTA*) *+252* gene variants.

Obesity and *TNFA* were associated with asthma when mutually adjusting for their independent effects (odds ratio (OR) for obesity 2.4, 95% confidence interval (CI) 1.7–3.2; OR for *TNFA -308* polymorphism 1.3, 95% CI 1.1–1.6). The association of obesity with asthma was stronger for subjects carrying the *G/A* and *A/A TNFA -308* genotypes compared with the more common *G/G* genotype, particularly among nonatopics (OR for *G/A* and *A/A* genotypes 6.1, 95% CI 2.5–14.4; OR for *G/G* genotype 1.7, 95% CI 0.8–3.3).

The present findings provide, for the first time, evidence for a complex pattern of interaction between obesity, a pro-inflammatory genetic factor and asthma.

KEYWORDS: Asthma, atopy, genetic polymorphism, obesity, tumour necrosis factor-α

There is increasing evidence suggesting that obesity is a risk factor for asthma in children and adults [1–3]. Obesity has been associated with both incident and prevalent asthma as well as with severity of the disease. Results from cohort studies have not, however, been consistent. Experimental studies support this association but the mechanisms linking obesity with asthma are complex and remain uncertain [2, 4–7]. Obesity affects pulmonary physiology and induces changes in immunity or inflammatory status. Adipose tissue expresses a number of pro-inflammatory molecules, including tumour necrosis factor (TNF), in a pattern that could be analogous to the immune function of T-lymphocytes and macrophages [8].

Twin studies suggest that obesity and asthma may be caused by shared genetic (genetic pleiotropy) risk factors [9]. Some genes associated with obesity are located on asthma consensus loci on chromosomic regions 5q, 6p, 11q and 12q [6], and TNF-α

(*TNFA*) is among the few genes that have been associated with both asthma and obesity [4, 5].

TNF is a potent pro-inflammatory cytokine, mainly secreted by macrophages. TNF is involved in the regulation of a wide spectrum of biological processes, including cell proliferation, differentiation, apoptosis, lipid metabolism and coagulation, and has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, cancer and inflammation of asthmatic airways. *TNFA* and lymphotoxin-α (*LTA*; also named *TNFβ*) genes are members of the TNF superfamily. *TNFA* and *LTA* are located within the class III region of the major histocompatibility complex region on chromosome 6p21.3, in a cluster of 13 kb, which also includes lymphotoxin-β, a region previously linked to asthma in different genome scans. Polymorphisms in *TNFA* and *LTA* may affect levels of TNF in the airways. The *TNFA -308 G/A* polymorphism, located in the promoter region of *TNFA*, has been associated with

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increased promoter activity and secretion of TNF [10]. The *LTA +252 A/G* polymorphism, located in the first intron of the *LTA* gene, seems to be associated with a high LTA expression [11]. A recent meta-analysis that included the population reported in the current study has shown that the *TNFA -308 A* and *LTA +252 G* alleles are both positively associated with asthma [12].

In the current study, the evaluation of a joint effect of obesity and genetic variants in the *TNFA* and *LTA* genes on asthma is presented, by examining different proposed pathways associating these biological factors with respect to this disease.

MATERIAL AND METHODS
Study population
The analyses in the current report are based on two studies. The European Community Respiratory Health Survey (ECRHS) is a population-based cohort study conducted in 10 countries [13, 14]. The population-based Swiss Cohort Study on Air Pollution and Lung and Heart Disease in Adults (SAPALDIA) was conducted in eight Swiss communities [15, 16]. Initially, 5,065 participants from ECRHS and 6,071 participants from SAPALDIA with complete interview data and DNA samples for genotyping were included [12]. Some subjects from Basel, Switzerland (n=400), were initially included in both datasets, but remained only in the SAPALDIA cohort during the analyses. Due to differences between the two studies in age at inclusion, the present analysis was restricted to the 9,167 subjects who were aged <50 yrs at inclusion. Subjects in both studies could be considered as being mainly of European-Caucasian origin. Ethical approval was obtained for each centre from the appropriate institutional ethics committee and written consent was obtained from each participant.

Asthma and atopy assessment and obesity evaluation
ECRHS and SAPALDIA used identical questionnaires for assessment of respiratory symptoms and asthma. Asthma status at baseline was evaluated in the early 1990s (ECRHS-I and SAPALDIA-I), while the effects of changes in obesity and asthma were assessed at follow-up for both cohorts. The median length of follow-up was 8.9 yrs. The asthma definitions used in the present analysis were similar to those used in previous publications [12, 17]. All subjects defined as having asthma had responded positively to an initial question ''Have you ever had asthma?'' The main asthma definition of ''current asthma'' was based on further positive responses to either of two questions: ''Have you had an attack of asthma in the last 12 months?'' or ''Are you currently taking any medicines including inhalers, aerosols or tablets for asthma?'' ''Physician-diagnosed asthma'' was defined as a further positive response to the question ''Was this confirmed by a doctor?''

Height and weight were assessed in both studies (table 1). Body mass index (BMI) was calculated as weight divided by squared height in metres. BMI was divided into four categories: underweight (BMI <20 kg·m⁻²), normal weight (BMI 20 to <25 kg·m⁻²), overweight (BMI ≥25 to <30 kg·m⁻²) and obese (BMI ≥30 kg·m⁻²).

Skin prick tests were performed in ECRHS and SAPALDIA (Phazet®; Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden). At baseline in both studies, atopic subjects were defined as having positive tests to at least one common aero-allergen (*Dermatophagoides pteronyssinus*, timothy grass, cat and *Cladosporium herbarum*).

Candidate single nucleotide polymorphism selection and genotyping
On the basis of previous studies and functional data [10–12], two single nucleotide polymorphisms (SNPs) were selected for genetic analysis in the two cohorts, *TNFA -308* (rs1800629) and *LTA +252* (rs909253). In SAPALDIA, these polymorphisms were genotyped by real-time PCR. In ECRHS, SNPs were genotyped using the SNPlex™ platform (Applied Biosystems, Foster City, CA, USA). Genotyping was performed at the Centre for Genomic Regulation in the Barcelona Node of the Centro Nacional de Genotipado in Spain. The agreement in genotyping in subjects from Basel, Switzerland (n=400), who were included in both ECRHS and SAPALDIA and were genotyped by both methods, was 99.8%. Genotype distribution for both alleles was consistent with Hardy–Weinberg equilibrium (HWE) in the control group (p>0.05). A previous analysis of these cohorts [12] found similar effects of *TNFA -308* and *LTA +252* on asthma, possibly due to strong linkage disequilibrium between the loci (Chi-squared=7516.29, disequilibrium

TABLE 1	Characteristics of the population in the European Community Respiratory Health Survey (ECRHS) and the Swiss Cohort Study on Air Pollution and Lung and Heart Disease in Adults (SAPALDIA) studies

	Controls			Cases of current asthma		
	ECRHS	SAPALDIA	p-value	ECRHS	SAPALDIA	p-value
Subjects n	4224	4364		426	132	
Age yrs	34.2±7.2	36.3±8.8	<0.01	33.7±7.1	36.5±8.9	<0.01
Sex						
Male	2036 (48.2)	2197 (50.5)	0.03	172 (40.4)	60 (45.8)	0.32
Female	2188 (51.8)	2149 (49.4)		254 (59.6)	71 (54.1)	
Smoking status						
Never	1796 (42.5)	2021 (46.3)	<0.01	219 (51.7)	65 (49.2)	0.16
Ex	892 (21.1)	947 (21.7)		93 (21.9)	39 (29.5)	
Current	1534 (36.3)	1392 (31.9)		112 (26.4)	28 (21.2)	
BMI kg·m⁻²						
Mean±SD	24.1±3.8	23.8±3.6	<0.01	24.7±4.6	24.5±4.5	0.62
<20	387 (9.9)	688 (15.8)	<0.01	33 (8.3)	16 (12.2)	0.31
20 to <25	2194 (56.2)	2495 (56.7)		218 (54.9)	77 (58.8)	
25 to <30	1072 (27.5)	990 (22.8)		96 (24.2)	25 (19.1)	
≥30	249 (6.4)	203 (4.7)		50 (12.6)	13 (9.9)	
TNFA -308						
G/G	2809 (72.1)	3246 (74.4)	0.04	248 (62.4)	99 (75.0)	0.02
G/A	980 (25.1)	1011 (23.2)		132 (33.2)	31 (23.5)	
A/A	110 (2.3)	103 (2.4)		17 (4.3)	2 (1.5)	
LTA +252						
A/A	1997 (47.5)	2089 (47.9)	0.52	165 (38.8)	67 (51.6)	<0.01
A/G	1750 (41.6)	1832 (42.0)		202 (47.5)	59 (44.7)	
G/G	455 (10.8)	439 (10.1)		58 (13.6)	6 (4.5)	

Data are presented as median±SD or n (%), unless otherwise stated. BMI: body mass index; *TNFA*: tumour necrosis factor-α; *LTA*: lymphotoxin-α.

constant (D')=0.98, p<0.001). Results were also similar for the present analysis evaluating obesity, and results for *LTA +252* are, therefore, not shown.

Statistical analysis

The statistical analyses were performed using logistic regression and SNPassoc (version 1.5–1) R package (version 2.6.1) [18, 19]. Logistic regression models were used, adjusted for country (ECRHS) or study area (SAPALDIA), sex, age and smoking status. SNPs were tested in control samples for deviations from HWE [20]. Genotype distribution for both alleles was consistent with HWE in the control group (p >0.05). D' and Chi-squared p-values for marker independence were estimated to determine linkage disequilibrium between both genetic markers. Population stratification in ECRHS data was assessed using two different methods. First, the analysis of 26 unlinked markers (supplementary table E1) was performed by the genomic control approach [21] used in the earlier analysis [12]. It was found that population stratification had a minimal effect (inflation factor 1.06). Secondly, analysis using EIGENSTRAT software (version 1.01) [22] using the same 26 markers showed no evidence of population stratification (supplementary fig. E1).

RESULTS

The general characteristics of the study population are summarised in table 1. Among controls, significant but slight differences were observed between cohorts for mean age, sex, smoking status and mean BMI. Compared with controls, cases with current asthma were more often females and younger, and reported less smoking.

The multivariate analysis of obesity, *TNFA -308* polymorphism and current asthma (table 2) indicated that both obesity and the *TNFA -308* polymorphism are associated with asthma. The risk estimates for *TNFA -308* and obesity were very similar in the unadjusted models compared with the model adjusting mutually for both risk factors, indicating that the effect of each factor is not dependent on the other. Adjustment by smoking status or further adjustment by atopy did not modify the risk estimates. Similar associations were observed for

physician-diagnosed asthma, while no clear pattern was observed between BMI categories and atopy.

The percentages of underweight and obese subjects were higher for carriers of the *A/A TNFA -308* genotype (18% and 2%, respectively) compared with the more frequent *G/G* genotype (13% and 1%, respectively). These differences were only statistically significant for underweight subjects (p=0.02). The association of *TNFA -308* with underweight was only observed in the ECRHS and not in SAPALDIA but the difference between studies was not statistically significant.

Figure 1 shows risk estimates for obesity and current asthma after stratifying by *TNFA -308* genotypes. The analysis was performed combining *G/A* and *A/A* carriers (dominant genetic model) because analysis for each genotype was not possible due to the low prevalence of *A/A* (3%). The risk tended to be higher for those with the *G/A* and *A/A* genotypes (odds ratio (OR) 3.17, 95% confidence interval (CI) 1.73–5.66) compared with the *G/G* genotype (OR 1.94, 95% CI 1.28–2.85), but the interaction was not statistically significant (p=0.40). This difference in risk was more pronounced among nonatopics for current asthma (OR 6.09, 95% CI 2.49–14.39) and physician-diagnosed asthma (OR 5.62, 95% CI 2.79–11.02), while the *TNFA -308* polymorphisms did not seem to modify the effect of obesity and asthma in atopics (OR 1.67, 95% CI 0.77–3.28, and OR 1.66, 95% CI 0.84–3.15, respectively). The interaction between *TNFA* and obesity in relation to asthma among nonatopics was statistically significant (p=0.05 for current asthma and p=0.03 for physician-diagnosed asthma).

The association of *TNFA -308* with asthma stratified by BMI is shown in table 3 (contrary to figure 1, which showed the ORs for BMI stratified by *TNFA -308*). A positive association of *TNFA -308* polymorphisms with current asthma was observed for all subjects (OR 1.34, 95% CI 1.10–1.62). The highest risks were observed for those underweight (OR 1.94, 95% CI 1.00–3.68) and obese subjects (BMI $\geqslant$30 kg·m^{-2}; OR 1.70, 95% CI 0.89–3.19). The increased risk for *TNFA -308* among obese

TABLE 2	Multivariate model associating current asthma with tumour necrosis factor-α (*TNFA*) -308 polymorphism and obesity	
Variables	**OR (95% CI)**	**Mutually adjusted OR (95% CI)**
BMI kg·m^{-2}		
<20	0.78 (0.56–1.07)	0.76 (0.54–1.06)
20 to <25	1 (ref)	1 (ref)
25 to <30	0.95 (0.75–1.20)	0.91 (0.71–1.15)
$\geqslant$30	2.36 (1.72–3.21)	2.23 (1.60–3.06)
TNFA -308		
GG	1 (ref)	1 (ref)
GA	1.33 (1.08–1.62)	1.41 (1.14–1.73)
AA	1.41 (0.83–2.28)	1.48 (0.85–2.42)

Logistic regression adjusted by country, age, sex, smoking status. OR: odds ratio; CI: confidence interval; BMI: body mass index; ref: reference value.
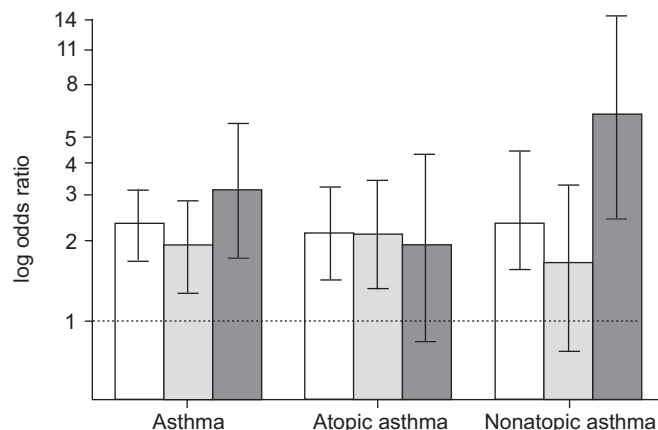

FIGURE 1. Odds ratios (bars) and 95% confidence intervals (lines) for the association of obesity (body mass index (BMI) $\geqslant$30 kg·m^{-2}) and current asthma by atopy and tumour necrosis factor-α (*TNFA*) -308 genotypes. □: all subjects; ▨: G/G; ■: G/A+A/A. The reference group corresponded to subjects with normal weight (BMI 20 to <25 kg·m^{-2}). The logistic regression was adjusted for country, age, sex and smoking status.

TABLE 3 Association of tumour necrosis factor-α (*TNFA*) -308 G/A+A/A genotype and asthma stratified by body mass index (BMI) categories

	All		Atopic asthma		Nonatopic asthma	
	OR (95% CI)[#]	p-value[¶]	OR (95% CI)[#]	p-value[¶]	OR (95% CI)[#]	p-value[¶]
Current asthma	1.34 (1.10–1.62)		1.35 (1.05–1.72)		1.33 (0.93–1.87)	
BMI <20	1.94 (1.00–3.68)	0.24	2.32 (1.02–5.24)	0.23	2.29 (0.68–7.43)	0.21
BMI 20 to <25	1.27 (0.97–1.64)	ref	1.25 (0.90–1.73)	ref	1.05 (0.63–1.70)	ref
BMI 25 to <30	1.52 (0.99–2.30)	0.53	1.64 (0.95–2.78)	0.43	1.17 (0.54–2.38)	0.83
BMI ⩾30	1.70 (0.89–3.19)	0.41	1.05 (0.41–2.55)	0.66	3.15 (1.18–8.54)	0.05
Physician-diagnosed asthma	1.10 (0.93–1.29)		1.18 (0.96–1.46)		0.94 (0.70–1.24)	
BMI <20	1.43 (0.85–2.36)	0.30	1.61 (0.84–3.06)	0.45	1.58 (0.66–3.56)	0.10
BMI 20 to <25	1.04 (0.83–1.09)	ref	1.09 (0.83–1.43)	ref	0.70 (0.45–1.06)	ref
BMI 25 to <30	1.13 (0.78–1.62)	0.80	1.32 (0.80–2.12)	0.58	0.93 (0.52–1.59)	0.46
BMI ⩾30	1.76 (0.99–3.10)	0.10	1.32 (0.57–2.99)	0.78	1.93 (0.85–4.26)	0.03

OR: odds ratio; CI: confidence interval; ref: reference value. [#]: logistic regression adjusted by country, sex, age and smoking status. [¶]: for interaction between *TNF* -308 G/A+A/A and BMI categories; reference categories BMI 20 to <25 kg·m^{-2} and G/G genotype.

subjects were observed in both SAPALDIA (OR 2.42) and ECRHS (OR 1.56), while there was heterogeneity between studies in the results for underweight (test for heterogeneity Q=5.00, p=0.03). Among underweight subjects, the increased risk of current asthma was associated with *TNFA* -308 irrespective of atopic status (table 3), while the increased risk of *TNFA* -308 among obese subjects was only observed for nonatopics, with an OR of 3.15 (95% CI 1.18–8.54) for current asthma and 1.93 (95% CI 0.85–4.26) for physician-diagnosed asthma. The interaction term for *TNFA* -308 and obesity was the same as that shown for figure 1.

DISCUSSION

In the present study, the joint effects of obesity and the *TNFA* gene were evaluated in adults with asthma. The findings suggest that obesity and *TNFA* are associated with asthma in a complex pattern that involves both independent and combined pathways, and that some of these pathways may be modified by sex and atopic status. The combination of two studies increased statistical power but also served to verify the consistency of findings in two populations examined with similar protocols but by different research groups.

The mechanisms through which obesity provokes asthma remain unclear. Common genetic pathways have been proposed as an explanation for the covariation of obesity and asthma [5]. The current study is the first to evaluate both obesity and *TNFA* variants simultaneously in a large population sample. A model showing the possible links between obesity, TNF and asthma is proposed in figure 2.

There is rising evidence that links both obesity and *TNFA* variants to asthma (pathways A and B, fig. 2). Results of several cross-sectional and prospective studies [1, 2, 4, 8], as well as a recent meta-analysis [1], are in favour of obesity being a risk factor for asthma (pathway A, fig. 2). Results from the ECRHS and SAPALDIA studies, particularly the effect of weight gain during the follow-up period on the occurrence of new-onset asthma, support these findings [23]. Obesity may

affect the pulmonary physiology and modify the immunological system, leading to pro-inflammatory processes [4–6, 8]. Some studies have suggested an increased risk for asthma and asthma-related symptoms among underweight subjects [24–27]. The reasons for the impact of underweight on asthma are unclear [24], and further research is needed to elucidate potential pathways. Although this effect was not observed in the current study, an increased risk of *TNFA* -308 was seen among underweight subjects, which may suggest a role of underweight and systemic inflammatory state on asthma.

The *TNFA* -308 polymorphism has been linked with an increased TNF expression [10] and has been previously associated with asthma [12]. The current results indicate that *TNFA* -308 is associated with asthma (pathway B, fig. 2) but not with atopy [12].

Only a weak association was found between *TNFA* -308 and obesity (pathway C, fig. 2), and an association was also found with underweight, although this was observed only in the ECRHS and not in SAPALDIA study. Results from a meta-analysis suggest that *TNFA* -308 is associated with the
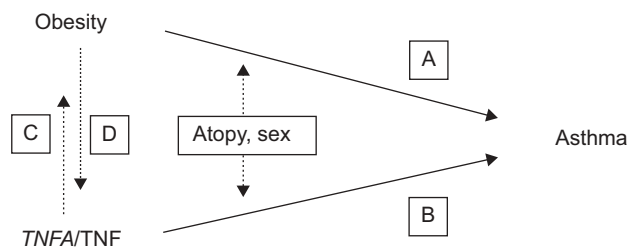
FIGURE 2. Pathways associating obesity, the tumour necrosis factor-α (*TNFA*) gene/tumour necrosis factor (TNF) cytokine and asthma. ——:independent effects of obesity (line A) and *TNFA*/TNF (line B) on asthma, for which there exists strong evidence. ·····: pathways for which epidemiological evidence is less clear; the potential interaction of obesity and *TNFA* (lines C and D) and the potential modification of these effects by atopy and sex.

development of obesity [28]. In contrast, experimental studies suggest that TNF is involved in body weight homeostasis by increasing lipolysis, favouring muscle cell catabolism and stimulating general proteolysis [29].

In a reverse pathway, TNF could be involved in inflammatory changes produced by obesity (pathway D, fig. 2). This pathway could only be examined indirectly by evaluating whether the effect of obesity on asthma was modulated by the *TNFA -308* polymorphism. As hypothesised, the asthma risk associated with obesity was strongest in carriers of the *TNF -308 A* allele. This observation is consistent with the hypothesis that the influence of adipose tissue on TNF signalling is genotype dependent. Adipose tissue expresses several cytokines, including TNF-α [8], that may simulate the immune function of T-lymphocytes and macrophages [4]. Increased levels of TNF in serum have been observed in obese subjects [30, 31], as well as in asthmatics [32]. TNF-mediated inflammation is common in both obesity and asthma and it is plausible that it is upregulated in both conditions [5].

It has been suggested that the effect of obesity on asthma and atopy could be sex dependent, being greater in post-pubertal and adult females than in males [1, 4, 6, 8, 23]. The current results suggest a slightly stronger effect of obesity on physician-diagnosed asthma among females compared with males. The present study also reported an increased risk for atopy in females and a significant interaction between sex and obesity for atopy. However, overall results are inconsistent [1, 4, 7, 8, 14], including those in a recent meta-analysis [33].

The current study found that the effect of obesity was stronger for subjects with nonatopic asthma. Other studies have also reported similar findings [34, 35], or have found an association for asthma but not for other atopic diseases [36, 37]. GILLILAND *et al.* [34] and KRONANDER *et al.* [35] found an increased risk for nonatopic asthma regardless of sex, while they observed an increased risk for atopic asthma only among obese females. Raised levels of high-sensitivity C-reactive protein, an inflammatory marker known to be related to smoking, obesity and cardiovascular disease, were found to be significantly associated with respiratory symptoms and nonallergic asthma but not with allergic asthma [38].

The current analysis presents, for the first time a joint evaluation of obesity and TNF in a large population-based study. Despite the large sample size, the evaluation of asthma and obesity in relation to the minor *TNFA -308* genotype (*A/A*) was based on small numbers. In addition, some of the pathways evaluated related to obesity could have different effects in pre- and post-menopausal females. In current study, most females were pre-menopausal. Other issues that could not be adequately addressed in the present study regard other measures of obesity, such as waist-to-hip ratio, that have been shown to be associated with a high risk of cardiovascular diseases [39]. Finally, the analysis of the impact of obesity on TNF-α signalling would have been more informative had circulating TNF-α levels been measured.

The present study strengthens the existing evidence for an effect of obesity and tumour necrosis factor on asthma, and shows for the first time that obesity interacts with genetic factors in the causation of asthma. In conclusion, the study shows that obesity and tumour necrosis factor are associated with asthma in a complex pattern that involves both independent and combined effects.

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