European Journal of Human Genetics (2002) 10, 82–85

www.nature.com/ejhg

SHORT REPORT

Relation between tumour necrosis factor polymorphism TNFα-308 and risk of asthma

John S Witte*,[1], Lyle J Palmer[1,2], Richard D O'Connor[3,4], Penelope J Hopkins[5] and Jeff M Hall[5]

[1]*Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio, USA;* [2]*Channing Laboratory, Harvard Medical School, 181 Longwood Avenue, Boston, Massachusetts, USA;* [3]*Sharp Rees-Stealy Medical Group, San Diego, California, USA;* [4]*Department of Pediatrics, University of California at San Diego, California, USA;* [5]*PPGx, Inc. La Jolla, California, USA*

Tumour necrosis factor (TNF) alpha affects immune response and airway inflammation, which are characteristics of asthma. Genetic factors may impact TNFα levels, and several polymorphisms in the TNF gene cluster on chromosome 6p21 have been associated with TNFα production and potential increased risk of asthma. The present paper evaluates the relation between two single nucleotide polymorphisms (SNPs) in the TNF gene cluster and asthma risk. The SNPs investigated here are guanine (G) to adenosine (A) substitutions in the TNFα and lymphotoxin alpha (LTα) genes. The TNFα SNP is at position -308 in the promoter region (TNFα-308), while the LTα SNP is in the first intron *Nco*I recognition sequence (LTα-*Nco*I). (For both SNPs the G allele is denoted as 1, and the A allele 2.) We determined TNFα-308 and LTα-*Nco*I genotypes in 511 individuals: 236 asthma cases and 275 non-asthmatic controls. Data were analysed by logistic regression of asthma status on the genotypes and potential confounders. TNFα-308*2 was positively associated with asthma, and this relation was strengthened when restricting cases to individuals reporting acute asthma: the adjusted odds ratio (OR) comparing carriers of one or two TNFα-308*2 alleles *versus* none was 1.86 (95% confidence interval (CI)=1.03–3.34, *P*=0.04). Further restricting the subjects to those with a family history of asthma, and those of European-American ancestry strengthened the association even more: adjusted OR=3.16 (95% CI=1.04–9.66; *P*=0.04). LTα-*Nco*I*1 was weakly associated with asthma, and analysis of both genes suggests that only the TNFα-308*2 allele increases risk of asthma.
European Journal of Human Genetics (2002) **10,** 82–85. DOI: 10.1038/sj/ejhg/5200746

Keywords: asthma; candidate gene; case-control study; genetics; tumour necrosis factor alpha; single nucleotide polymorphism

Introduction

Asthma is a complex disease with several intermediate phenotypes, notably atopy and chronic airway inflammation.[1] A potential risk factor for asthma is tumour necrosis factor (TNF) alpha, a pleiotropic cytokine involved with immune inflammatory responses.[2,3] High levels of TNFα have been observed in the bronchoalveolar lavage fluid, serum, and bronchial submucosa of asthmatics.[4,5] In addition, following an allergen challenge, the secretion of TNFα is higher among atopic than non-atopic individuals.[6–8] Therefore, TNFα appears to be implicated in multiple characteristics of asthma: airway inflammation, increased bronchial hyper-responsiveness, and atopy.[2,8–12]

Genetic factors may affect TNFα levels, and the TNF locus is within the Class III region of the human major histocompatibility complex (MHC) on chromosome 6p21.[13] This locus includes two closely linked genes that encode the cytokines TNFα and lymphotoxin alpha (LTα) (also known as TNFβ). A

*Correspondence: JS Witte, Department of Epidemiology and Biostatistics, Case Western Reserve University, W-G72, 2109 Adelbert Road, Cleveland, Ohio 44106-4945, USA Tel: 216 368 6839; Fax: 216 368 3970; E-mail: witte@darwin.cwru.edu
Received 13 July 2001; revised 9 October 2001; accepted 16 October 2001

TNFα promoter SNP (guanine (G, allele '1') to adenosine (A, allele '2') at position -308, TNFα-308) and a LTα-*Nco*I SNP (A to G in the first intron) have been positively associated with clinical symptoms of asthma and self-reported asthma.[14–20] These SNPs have also been shown to influence the rate of transcription and protein production of TNFα and LTα, respectively.[21–23] However, other studies have observed equivocal or inverse associations between the TNFα-308*2 and LTα-*Nco*I*1 alleles and asthma.[19,24–26] In light of these conflicting results, we have undertaken a case-control study of 511 individuals to further investigate the potential association between these alleles and asthma, and whether this relation is stronger among individuals with acute asthma and/or a family history of asthma.

Materials and methods

All subjects were recruited from an outpatient clinic at the Sharp Rees-Stealy Medical Group in San Diego, California, USA. Individuals diagnosed by a physician with asthma (coded as ICD-9 493) were included as cases in the present study. Almost all diagnoses of asthma were confirmed by bronchodilatory response to albuterol. Controls were those individuals confirmed as non-asthmatic, with no previous history of asthma, hayfever, allergic rhinitis, or eczema. A total of 236 cases and 275 controls were recruited into the study. Blood was taken by venipuncture from these subjects, and a questionnaire asked about family history of asthma, basic demographic information, and for the cases, how often they had acute asthma attacks. The latter question was used to distinguish cases with sufficiently severe symptoms to be considered an attack (not just a sense of shortness of breath). Institutional Review Board (IRB) review and approval was obtained for the study protocol from Western IRB (Olympia, Washington, USA), and all study subjects gave informed consent.

DNA was extracted from whole blood using a kit from Gentra Systems, Inc. (Minnesota, USA). SNP genotypes were determined using the TaqMan assay.[27] Samples were assayed in triplicate in a Robbins 96-well plate. The primers for TNFα-308 and LTα-*Nco*I were derived from previously published studies.[24,25] Specifically, TNFα-308 detection was carried out by PCR with the following oligonucleotide primers: 5′-AAACAGACCACAGACCTGGTCC-3′ and 5′-CCATCCTCCC-TGCTCCGATTCCG-3′. LTα-*Nco*I detection was carried out by PCR with the following oligonucleotide primers: 5′-CCGTG-CTTCGTGCTTTGGACTA-3′ and 5′-AGAGCTGGTGGGGA-CATGTCTG-3′. Fragments were amplified in PCR reactions containing 20 ng genomic DNA, 900 nM forward unlabelled inner primer, 900 nM reverse unlabelled inner primer, 200 nM FAM labelled probe, 200 nM TET labelled probe and 1× Perkin-Elmer TaqMan Reagent Mix #43C4447. PCR reactions were preincubated at 50°C for 2 min, then 95°C for 10 min. Two-step thermocycling was performed for 45 cycles: denaturation at 95°C for 30 s and annealing at 64°C for 30 s. Upon completion of thermocycling, the fluorescence was read on an ABI 7700 Sequence Detector using the allelic discrimination software. FAM to TET ratios for each sample DNA, normalised against the TAMRA signal, indicated the genotype of each patient and was further confirmed by similar signals from known control DNAs. The TNF-308 and LTα-*Nco*I assays did not amplify for three and eight subjects, respectively. These individuals were excluded from the analyses of the corresponding SNP.

Basic descriptive statistics, stratified by asthma status, were calculated for demographic factors considered potential effect modifiers or confounders (eg, age, ethnicity). To estimate the relative risks of asthma for carriers of the TNF-308*2 or LTα-*Nco*I*1 variants, odds ratios (ORs) were calculated using logistic regression. The genotypes with one or two variants were combined to reflect a dominant model (eg, for TNF-308, 2,2 and 1,2 combined *vs* 1,1). We also evaluated the relation of asthma risk and TNF-308 – LTα-*Nco*I haplotypes (estimated with the EM algorithm), and combinations of their genotypes. We statistically adjusted for or stratified on the following potential confounders in the logistic regression models: age at diagnosis, sex, ethnicity, physical activity, and family history of asthma. (Adjusting for body mass index and smoking had no appreciable statistical impact on our results, and so these covariates are not included in our final models.) An investigation of the need for interaction or polynomial terms resulted in our inclusion of an age × sex interaction term in the final model. All statistical analyses were undertaken with SAS software (SAS Institute Inc., Cary, North Carolina, 2000).

Results

The average age of study subjects included here was 42 years (standard deviation=14 years). In comparison with controls, cases were more likely to be European-American and to be less physically active (Table 1). Furthermore, cases had much stronger family histories of asthma than controls: over 60% of cases reported that one or more of their first-degree relatives had asthma, while only 25% of controls reported such a family history ($P<0.001$).

Cases were more likely than controls to carry one or two copies of the TNFα-308*2 allele: 30% of the cases, *vs* 22% of the controls, had one or more copies of TNFα-308*2 (P=0.03) (Table 2). Logistic regression analyses indicated that having one or two copies of the TNFα-308*2 allele appeared to increase the risk of asthma (Table 2). The magnitude of this association was increased when restricting the cases to those with acute asthma (ie, one or more attacks per month) (adjusted OR=1.86; 95% CI=1.03 – 3.34; P=0.04). Further restricting the subjects to those with a family history of asthma (ie, ⩾first-degree relative), and those of European-American ancestry increased the association even more: adjusted OR=3.16 (95% CI=1.04 – 9.66; P=0.04).

A substantially weaker relation was observed for LTα-*Nco*I: 57% of the cases *vs* 53% of the controls, had one or more copies of LTα-*Nco*I*1 (P=0.26), and possessing one or two copies of the LTα-*Nco*I*1 allele was marginally associated with an increased risk of asthma (adjusted OR=1.41; 95% CI=0.95 – 2.10; P=0.09) (Table 2).

An analysis of TNFα-308 and LTα-*Nco*I haplotypes gave results similar to those observed for TNFα-308 alone. In particular, comparing the TNFα-308 – LTα-*Nco*I haplotype *2 – *1 *vs* the haplotype *1 – *2 gave an OR=1.54 (95% CI=1.05 – 2.24; P=0.02). Furthermore, genotype analyses that compared subjects with at least one copy of TNFα-308*2 or one copy of LTα-*Nco*I*1 to those without any of these alleles gave even further attenuated results (adjusted OR=1.30; 95% CI=0.88 – 1.92; P=0.20).

Discussion

We observed a statistically significant positive association between carrying one or two copies of the TNFα-308*2 allele and asthma risk. The magnitude of this association was increased when looking at subjects with more acute asthma, a family history, and European-American ethnicity. The weak relation between LTα-*Nco*I*1 and asthma may simply reflect the strong linkage disequilibrium between this allele and TNFα-308*2 ($P<0.001$ here), which are only 2781 bp apart.[21] This would also help explain why the haplotype analysis did not strengthen the association beyond that observed for TNFα-308*2 alone.

The finding for TNFα-308*2 is biologically plausible because TNFα is a potent pro-inflammatory cytokine involved in the airway's inflammation and atopic response in asthma,[2,3,7,8] and TNFα-308*2 has been shown to be associated with higher levels of TNFα production.[22] Furthermore, previous genetic epidemiologic studies support our results.[15–18] Other genetic epidemiologic studies, however, have been equivocal with regard to the relation between TNFα-308*2 and asthma.[19,24–26] These conflicting results may reflect differing: study designs; sample sizes; environmental interactions; or even molecular/statistical analyses. Nonetheless, the results observed here help to further establish that TNFα-308 (or a nearby gene) is causally related to asthma. (The TNFα-308 alleles could simply be a marker for one or more functional polymorphisms in the MHC that affect asthma susceptibility.) Furthermore, this relation may be strongest among individuals with acute asthma and a family history of this phenotype.

The present study was more ethnically diverse than previous reports, and this diversity differed between cases and controls. Nevertheless, we addressed this issue in our analyses by first adjusting for ethnicity, and then restricting subjects to those with European-American ancestry. The observation that family history is a strong risk factor for disease may in part reflect the potential over-reporting of family history among cases and under-reporting among controls. In an attempt to make the self-report of family history as accurate as possible, we asked study subjects whether anyone in their immediate family currently or previously had asthma, or had ever been prescribed an asthma medicine.

In conclusion, our observations suggest that TNFα-308*2 – or a neighbouring polymorphism – is involved in the pathogenesis of asthma, and this relation is strengthened among cases with acute asthma, a family history, and

Table 1 Characteristics of study population

Variable	*Cases* (n = *236*)	*Controls* (n = *275*)	P *value*†
Mean age (SD)*	43.1 (14.4)	41.3 (13.9)	0.16
Ethnicity (%)			
European-American	71.6	61.8	
Hispanic-American	15.7	21.7	
African-American	7.2	8.4	
Asian-American	5.5	5.1	0.07
Family History of Asthma (%)			
No first-degree relatives	39.0	73.4	
1 first-degree relative	27.1	17.1	
⩾2 first-degree relatives	33.9	9.5	<0.001
Physical activity‡ (%)			
Less	30.5	20.4	
Same	41.1	43.6	
More	28.4	36.0	0.02

*For means, standard deviations given in parenthesis. †*P*-values from *t*-test comparing means or chi-square test comparing counts (ie, for covariates given in percentages). ‡Self-reported: in comparison with others of the same age and sex.

Table 2 Case-control comparisons of TNF-308 and LTα-*Nco*I genotypes

TNF-308 Genotype	*LTα-*NcoI *Genotype*	*Cases,* n *(%)*	*Controls,* n *(%)*	*OR (95% CI)*[a,b]	*OR (95% CI)*[a,b,c]	*OR (95% CI)*[a,b,d]
*1/*1		164 (70%)	212 (78%)	1.0 (referent)	1.0 (referent)	1.0 (referent)
*1/*2		67 (29%)	55 (20%)			
*2/*2		4 (2%)	6 (2%)			
Any *2		71 (30%)	61 (22%)	1.58 (1.02 – 2.44)	1.86 (1.03 – 3.34)	1.72 (1.02 – 2.89)
	*2/*2	98 (43%)	128 (47%)	1.0 (referent)	1.0 (referent)	1.0 (referent)
	*2/*1	101 (43%)	117 (43%)			
	*1/*1	31 (13%)	28 (10%)			
	Any *1	132 (57%)	145 (53%)	1.41 (0.95 – 2.10)	1.40 (0.81 – 2.42)	1.46 (0.91 – 2.34)

[a]Odds ratios (OR) and 95% confidence intervals (CI) from dominant logistic regression model. [b]Adjusted for age sex, age, sex × age interaction, ethnicity, physical activity, and family history of asthma. [c]Cases restricted to those with acute asthma. [d]Restricted to European-Americans.

European-American ancestry. Additional genetic epidemiologic studies that focus on individuals from these sub-groups, and directly measure TNFα levels in conjunction with TNF genotypes will help to further clarify the potentially important role of TNF polymorphism in asthma pathogenesis.

References

1 Sandford A, Weir T, Pare P: The genetics of asthma. *Am J Respir Crit Care Med* 1996; **153**: 1749 – 1765.
2 Shah A, Church MK, Holgate ST: Tumour necrosis factor alpha: a potential mediator of asthma. *Clin Exp Allergy* 1995; **25**: 1038 – 1044.
3 Barbara J, Van Ostade X, Lopez A: Tumour necrosis factor-alpha (TNF-α): The good, the bad and potentially very effective. *Immunol Cell Biol* 1996; **74**: 434 – 443.
4 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.
5 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.
6 Ying S, Robinson DS, Varney V, *et al*: TNF alpha mRNA expression in allergic inflammation. *Clin Exp Allergy* 1991; **21**: 745 – 750.
7 Gosset P, Tsicopoulos A, Wallaert B, *et al*: Increased secretion of tumor necrosis factor alpha and interleukin-6 by alveolar macrophages consecutive to the development of the late asthmatic reaction. *J Allergy Clin Immunol* 1991; **88**: 561 – 571.
8 Hallsworth MP, Soh CP, Lane SJ, Arm JP, Lee TH: Selective enhancement of GM-CSF, TNF-alpha, IL-1 beta and IL-8 production by monocytes and macrophages of asthmatic subjects. *Eur Respir J* 1994; **7**: 1096 – 1102.
9 Klebanoff SJ, Vadas MA, Harlan JM, *et al*: Stimulation of neutrophils by tumor necrosis factor. *J Immunol* 1986; **136**: 4220 – 4225.
10 Kips JC, Tavernier JH, Joos GF, Peleman RA, Pauwels RA: The potential role of tumour necrosis factor alpha in asthma. *Clin Exp Allergy* 1993; **23**: 247 – 250.
11 Koizumi A, Hashimoto S, Kobayashi T, Imai K, Yachi A, Horie T: Elevation of serum soluble vascular cell adhesion molecule-1 (sVCAM-1) levels in bronchial asthma. *Clin Exp Immunol* 1995; **101**: 468 – 473.
12 Hakonarson H, Herrick DJ, Serrano PG, Grunstein MM: Mechanism of cytokine-induced modulation of beta-adrenoceptor responsiveness in airway smooth muscle. *J Clin Invest* 1996; **97**: 2593 – 2600.
13 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 USA* 1987; **84**: 8535 – 8539.
14 Campbell D, Britton J, Pavord I, *et al*: LTa NcoI polymorphism at the TNF locus correlates with clinical symptoms of asthma. *Eur J Resp Dis* 1995; **8**: 552s.
15 Moffatt M, Cookson W: Tumour necrosis factor haplotypes and asthma. *Hum Mol Genet* 1997; **6**: 551 – 554.
16 Chagani T, Pare PD, Zhu S, *et al*: Prevalence of tumor necrosis factor-alpha and angiotensin converting enzyme polymorphisms in mild/moderate and fatal/near-fatal asthma. *Am J Respir Crit Care Med* 1999; **160**: 278 – 282.
17 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.
18 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.
19 Malerba G, Trabetti E, Patuzzo C, *et al*: Candidate genes and a genome-wide search in Italian families with atopic asthmatic children. *Clin Exp Allergy* 1999; **29** (Suppl 4): 27 – 30.
20 Winchester EC, Millwood IY, Rand L, Penny MA, Kessling AM: Association of the TNF-alpha-308 (G→A) polymorphism with self-reported history of childhood asthma. *Hum Genet* 2000; **107**: 591 – 596.
21 Messer G, Spengler U, Jung MC, *et al*: Polymorphic structure of the tumor necrosis factor (TNF) locus: an NcoI polymorphism in the first intron of the human TNF-beta gene correlates with a variant amino acid in position 26 and a reduced level of TNF-beta production. *J Exp Med* 1991; **173**: 209 – 219.
22 Wilson AG, di Giovine FS, Blakemore AI, Duff GW: Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum Mol Genet* 1992; **1**: 353.
23 Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW: Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997; **94**: 3195 – 3199.
24 Albuquerque R, Hayden C, Palmer L, *et al*: Association of polymorphisms within the tumour necrosis factor (TNF) genes and childhood asthma. *Clin Exp Allergy* 1998; **28**: 578 – 584.
25 Trabetti E, Patuzzo C, Malerba G, *et al*: Association of a lymphotoxin alpha gene polymorphism and atopy in Italian families. *J Med Genet* 1999; **36**: 323 – 325.
26 Louis R, Leyder E, Malaise M, Bartsch P, Louis E: Lack of association between adult asthma and the tumour necrosis factor alpha-308 polymorphism gene. *Eur Respir J* 2000; **16**: 604 – 608.
27 Heid CA, Stevens J, Livak KJ, Williams PM: Real time quantitative PCR. *Genome Res* 1996; **6**: 986 – 994.