An association between asthma and *TNF*-308G/A polymorphism: meta-analysis

Takeshi Aoki · Tomomitsu Hirota · Mayumi Tamari ·
Kunio Ichikawa · Kazunori Takeda · Tadao Arinami ·
Masanao Shibasaki · Emiko Noguchi

Received: 24 January 2006 / Accepted: 30 April 2006 / Published online: 25 July 2006
© The Japan Society of Human Genetics and Springer-Verlag 2006

Abstract Tumor necrosis factor (TNF) is a potent inflammatory cytokine that contributes to airway inflammation in asthma. Previous studies have reported that a G-to-A transition at position –308 (–308G/A, also referred to as *TNF*- α -308*1 and 308*2 respectively), is associated with asthma, but other studies have shown conflicting results. To investigate a possible association between the *TNF*-308G/A polymorphism and asthma, we performed transmission disequilibrium tests and a case–control study (family samples: 495 members in 165 Japanese trio families with one asthmatic child and both parents; case–control samples: 461 Japanese asthmatic children and 465 healthy controls). To increase the sample size and power, we performed a meta-analysis of all available

relevant studies, including 2,477 asthmatics and 3,217 controls. We did not find a significant association between the *TNF*-308G/A polymorphism and childhood atopic asthma in two independent Japanese populations ($P>0.05$); however, meta-analysis revealed that the *TNF*-308G/A polymorphism was statistically significantly associated with asthma. The combined odds ratio with a fixed effects model and with a random effects for *TNF*-308A was 1.46 (95% confidence interval [CI], 1.27–1.68, $P=0.0000001$) and 1.46 (95% CI, 1.20–1.77, $P=0.00014$) respectively. Our data further support the importance of the *TNF* region in the development of asthma.

Keywords *TNF* · Polymorphism · TDT · Case–control · Asthma · Meta-analysis

T. Aoki · K. Ichikawa
Department of Pediatrics, Tsukuba Medical Center
Hospital, Tsukuba, Japan

T. Hirota · M. Tamari
Laboratory of Genetics of Allergic Diseases,
RIKEN SNP Research Center,
Yokohama, Japan

K. Takeda
Institute of Disability Sciences,
Tsukuba, Japan

T. Arinami · E. Noguchi (✉)
Department of Medical Genetics, Graduate School of
Comprehensive Human Sciences, University of Tsukuba,
1-1-1 Tennoudai, Tsukuba 305-8577, Japan
e-mail: enoguchi@md.tsukuba.ac.jp

M. Shibasaki
Department of Pediatrics, Tsukuba College of Technology,
Tsukuba, Japan

Introduction

Asthma is the most common chronic disorder in children, and exacerbation of asthma is a major cause of childhood morbidity and hospitalization. The prevalence of childhood asthma in Japan is 5.1% among infants, 6.4% among children, and 3.2% among adults, and 1.146 million patients received ongoing medical care for asthma in 1996 (Makino et al. 2005). Asthma is a complex and chronic disease in which allergen-induced inflammatory processes in the airways contribute to the development of symptoms, such as wheezing, cough, dyspnea, and breathlessness. Histopathologic studies of patients with asthma revealed infiltration of the airway wall by inflammatory cells such as T cells, eosinophils, and mast cells, and structural changes, i.e., thickening of

all components of the airway wall, in asthmatic airways (Saetta and Turato 2001).

Asthma is believed to be a complex disorder involving genetic and environmental factors, and several asthma susceptibility loci have been identified through genome-wide screening (Daniels et al. 1996; Ober et al. 1998; Wjst et al. 1999; Dizier et al. 2000; Yokouchi et al. 2000; Laitinen et al. 2001). Tumor necrosis factor (TNF) gene is located on chromosome 6p21 within the major histocompatibility complex class III region (Carroll et al. 1987), which has been linked to asthma by several genome-wide linkage studies (Daniels et al. 1996; Wjst et al. 1999; Yokouchi et al. 2000; Nicolae et al. 2005). TNF is a potent pro-inflammatory cytokine and is released during allergic responses by both macrophages and mast cells (Penhica et al. 1984; Thomas et al. 1996; Thomas 2001). TNF blockade inhibited late-phase airway hyperresponsiveness and airway eosinophilia in a mouse model of asthma (Choi et al. 2005), and anti-TNF therapy provided clinical improvement in patients with severe asthma (Howarth et al. 2005). These accumulated data support the idea that TNF plays a major role in the pathogenesis of asthma.

One *TNF* polymorphism, a G-to-A transition at position –308 (–308G/A, also referred to as *TNF-α*-308*1 and 308*2), has been investigated extensively for its association with asthma and asthma-associated phenotypes. Some studies have supported an association with asthma (Moffatt and Cookson 1997; Albuquerque et al. 1998; Chagani et al. 1999; Li Kam Wa et al. 1999; Winchester et al. 2000; Witte et al. 2002; Shin et al. 2004; Wang et al. 2004), whereas other studies have not (Louis et al. 2000; Zhu et al. 2000; Buckova et al. 2002). Moffatt and Cookson (1997) first reported that –308G/A was significantly associated with asthma in an Australian population (Moffatt and Cookson 1997). The –308G/A polymorphism was also reported to be associated with bronchial hyperreactivity (Li Kam Wa et al. 1999). Chagani suggested that the –308 G/A polymorphism may be a risk factor for asthma, but that it does not increase the risk of a fatal asthma attack (Chagani et al. 1999). To date, there have been no studies examining the association between the –308G/A polymorphism of *TNF* and asthma in the Japanese.

The goal of the present study was to investigate whether the –308G/A polymorphism is associated with asthma. We performed association studies in two independent Japanese populations and a meta-analysis of all available studies, and examined a possible association between asthma susceptibility and the –308G/A polymorphism.

Materials and methods

Study populations

Family study

The probands were mite-sensitive asthmatic children who visited the Pediatric Allergy Clinic of the University Hospital of Tsukuba (Japan). A full verbal and written explanation of the study was given to all family members interviewed, and 165 trio families (father, mother, and one affected child, 495 members), including 47 families used for our genome-wide screening (Yokouchi et al. 2000) gave informed consent and participated in the study.

Each family member was questioned regarding allergic symptoms and underwent a physical examination performed by a participating pediatrician. Asthma was diagnosed in participants according to the criteria of the National Institutes of Health, USA (NIH 1997). Patients had to satisfy the two following criteria:

1. Two or more episodes of wheezing and shortness of breath during the previous 12 months
2. Reversibility of the wheezing and dyspnea, either spontaneously or by bronchodilator treatment

Methacholine challenge testing was not done because of the young age of the asthma patients; however, differential diagnosis of asthma in the affected children was made by participating physicians or pediatricians who had treated the children for more than 2 years, and they confirmed each diagnosis of asthma. Because wheezing is often associated with viral respiratory infection in young children (Martinez et al. 1995), we excluded children younger than 3 years of age. The young adult patients included in this study had suffered from chronic asthma since childhood. Total serum IgE levels and IgE levels specific for *Dermatophagoides farinae* were determined with the Pharmacia CAP System (Uppsala, Sweden). This study was approved by the Ethics Committee of the University of Tsukuba.

Case-control study

All case-control participants were Japanese and gave written informed consent to participate in the study in accord with the rules of the Process Committee of the SNP Research Center, the Institute of Physical and Chemical Research (RIKEN), Japan.

The participants were 461 children with asthma (mean age 10.5 years), and 465 healthy individuals

(mean age 44 years) who had neither respiratory symptoms nor a history of asthma-related disease. The children with asthma were patients at Osaka Prefectural Habikino Hospital or National Sagamihara Hospital. The 465 healthy control subjects were recruited through physicians' interviews about whether they had been diagnosed with asthma and/or atopy. All patients with asthma were diagnosed according to the criteria described above. Clinical characteristics of the patients and control subjects are given in Table 1.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes. Polymerase chain reaction (PCR) of the –308G/A polymorphism of *TNF* was carried out with primers 5'-AGGCAATAGGTTTTGAGGGCCAT-3' and 5'-TCCTCCCTGCTCCGATTCCG-3'. Amplification conditions were 94°C for 10 min followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and a final extension of 72°C for 7 min. PCR products were digested with *NcoI* endonuclease at 37°C for 16 h. Digested PCR fragments were subjected to agarose gel electrophoresis and visualized by ethidium bromide staining and ultraviolet transillumination. Expected product sizes for the G allele were 87 and 20 bp, and the expected product size for the A allele was 107 bp.

Statistical methods

Genotype distributions were compared with those expected for samples from populations in the Hardy–

Weinberg equilibrium with an exact test (Wigginton et al. 2005). Transmission disequilibrium testing (TDT) was performed with the ASPEX package (<http://www.aspex.sourceforge.net/>). The significance of differences in the allele and genotype frequencies between the case and control groups was determined by the Chi-squared test. $P < 0.05$ was considered significant. The statistical power of TDT for detecting a polymorphism that directly affected risk was calculated according to the method proposed by Risch and Merikangas (1996). Statistical power for the case–control study was calculated as described previously (Schlesselman 1974).

Meta-analysis

MEDLINE and PubMed database searches were performed to identify studies related to asthma and the –308G/A polymorphism of *TNF*. For computer-based searches, we used a combination of key words related to asthma and *TNF*. Keywords used for the database search were as follows: *TNF*, tumor necrosis factor, polymorphism, variants, and asthma. Articles of any language were considered. To be eligible for inclusion in our study, studies had to meet the following criteria:

1. Design, case–control trial
2. Participants, asthmatic
3. Methods, genotype of –308G/A polymorphisms
4. Results, genotype data

We included our present data in the meta-analysis. To be included in the meta-analysis, asthma could be either physician-diagnosed or self-reported. The following studies were excluded:

1. Studies with incomplete genotype and phenotype data
2. Studies analyzing intermediate phenotypes such as bronchial hyperresponsiveness or wheezing
3. Studies of occupational asthma
4. Family-based association studies

Meta-analysis was performed by the Mantel–Haenszel approach, assuming a fixed effects model, and by the DerSimonian and Laird method, assuming a random effects model, with the Rmeta package (<http://www.cran.r-project.org/bin/windows/base>). We used allele frequency data from each eligible study to examine the association of asthma with –308G/A. Heterogeneity among studies was tested with

Table 1 The clinical details of the study populations

Parameter	Statistic
Families	165
Number of affected children	165
Mean age (years $\pm$ SD)	10.1 $\pm$ 5.8
Male:female	1.57:1
Log(total IgE; mean IU/ml $\pm$ SD)	2.72 $\pm$ 0.60
Number of parents	330
Mean age (years $\pm$ SD)	40.3 $\pm$ 7.3
Male:female	1:1
Log (total IgE) (mean IU/ml $\pm$ SD)	1.97 $\pm$ 0.65
Case–control study	
Number of affected children	461
Mean age (years, range)	10.5 $\pm$ 4.5
Male:female ratio	1.56:1
Log(total IgE) (mean IU/ml $\pm$ SD)	2.59 $\pm$ 0.67
Number of controls	465
Mean age (years $\pm$ SD)	44 $\pm$ 13
Male:female ratio	2.47:1
Log(total IgE) (mean IU/ml $\pm$ SD)	–

Values < 0.34 and > 100 for Df RAST IgE were expressed as 0, and 100 respectively in this study

Chi-squared statistics obtained by adding the weighted squares of the deviations of each estimate from the pooled estimate. Publication bias was examined by plotting a funnel of the reported effect assessed with the odds ratio against the standard error of the natural logarithm of the odds ratio ($P>0.10$; Egger et al. 1997). We estimated the odds ratios by comparing asthmatic participants with control subjects in the same study and calculated odds ratios under the prior hypothesis that individuals who had the –308A allele were more susceptible to asthma. To guard against the possibility that any one study contributed disproportionately to the summary odds ratios, we conducted a sensitivity analysis by recalculating the meta-analysis after omitting one study at a time (Azzam and Mathews 2003). The heterogeneity test was performed for each analysis to determine whether there was evidence of heterogeneity.

Results

TDT in asthmatic families

In the present study, we genotyped two independent asthmatic samples, family, and case–control samples. In the family study, frequencies of the –308GG, GA, and AA genotypes in the parents were 0.96, 0.04, and 0 respectively, and the distribution did not deviate from the expected Hardy–Weinberg equilibrium ($P=1.0$). Preferential transmission of the –308G or –308A allele was not observed by TDT (5 transmitted vs. 7 non-transmitted for –308A, $P=0.78$).

Case–control study

The results of the case–control study are shown in Table 2. The genotype distribution among control subjects did not deviate from the expected Hardy–Weinberg equilibrium ($P=0.07$ for case–control and $P=0.78$ for TDT families). Case and control subjects

among families who underwent TDT were identified, and an artificially constructed case population consisting of transmitted parental alleles to the affected child and a control population of nontransmitted parental alleles, as described by Kirov et al. (1999). No significant differences in the genotype and allele distributions were observed between the case and control groups ($P>0.05$).

Statistical power of the TDT and of the case–control samples was 0.54 and 0.74 respectively, at the alpha level of 0.05 if the relative risk of asthma in those persons carrying a putative risk allele was 1.8 compared with that in persons without the allele.

Meta-analysis

The meta-analysis included a total of 2,477 asthmatic and 3,217 control participants (Fig. 1; Moffatt and Cookson 1997; Albuquerque et al. 1998; Louis et al. 2000; Winchester et al. 2000; Buckova et al. 2002; Witte et al. 2002; El Bahlawan et al. 2003; Sandford et al. 2004; Shin et al. 2004; Wang et al. 2004; Bilollikar et al. 2005; Gupta et al. 2005). Several studies were not included because allele frequency data were not available (Chagani et al. 1999; Li Kam Wa et al. 1999; Trabetti et al. 1999; Lin et al. 2002; Di Somma et al. 2003; Li et al. 2006) or because of an incomplete phenotype (probable asthma; Zhu et al. 2000). We also excluded the family-based association study (Randolph et al. 2005) and a study of occupational asthma (Beghe et al. 2004) from our meta-analysis. The list of articles retrieved, and characteristics of the studies included are shown in Table 3. The genotype distribution among control subjects in each study did not deviate from the expected Hardy–Weinberg equilibrium ($P>0.05$). No publication bias was observed by funnel plot of the reported effects of –308G/A ($P=0.77$). Overall, the combined odds ratio with a fixed effects model for asthma was 1.29 (95% CI, 1.13–1.48, $P=0.00012$), and that with a random effects model was 1.31 (95% CI, 0.99–1.74, $P=0.06$). The result of a

Table 2 Genotype distributions and odds ratio of the TNF-308G/A polymorphism in childhood asthma and controls

	<i>n</i>	Genotype count (frequency)			<i>P</i> value	Allele count (frequency)		<i>P</i> value
		GG	GA	AA		G	A	
Case–control samples								
Asthma	461	441 (0.96)	19 (0.04)	1 (0.00)	0.23	901 (0.98)	21 (0.02)	0.11
Control	465	454 (0.98)	10 (0.02)	1 (0.00)		918 (0.99)	12 (0.01)	
TDT samples								
Case (transmitted allele)	165	160 (0.97)	5 (0.03)	0 (0.00)	0.84	325 (0.98)	5 (0.02)	0.56
Control (non-transmitted allele)	165	158 (0.96)	7 (0.04)	0 (0.00)		323 (0.98)	7 (0.02)	

TDT transmission disequilibrium testing

Fig. 1 Odds ratios (with 95% confidence intervals) of asthma with TNF-308G/A polymorphisms

OR = Odds ratio

Fixed-effects model using Mantel-Haenszel methods, random-effects model using the DerSimonian and Laird method.

heterogeneity test was statistically significant ($Q=55.94$, $df=14$, $P<0.00001$) and was considered evidence of heterogeneity. Therefore, sensitivity analysis was performed by repeated calculation of the combined odds ratio estimate after omission of one study at a time.

The result of sensitivity analysis is shown in Table 4. The result of the heterogeneity test was no longer significant after omission of the study by Shin et al. (2004), indicating that their study contributed disproportionately to the summary odds ratio, suggesting the

Table 3 Characteristics of the studies for the meta-analysis

Population	Case	Control	HWE in controls <i>P</i> value	Reference
British	Physician-diagnosed pediatric asthma from clinic	Subjects without asthma from local schools	0.38	Bilollikar et al. (2005)
Indian	Physician-diagnosed asthma	Healthy subjects without any allergic diseases	1	Gupta et al. (2005)
Korean	Asthmatics recruited from research centers	General populations and spouses without asthma	1	Shin et al. (2004)
Chinese	Pediatric asthma based on ISAAC criteria	Non-asthmatic school children	1	Sandford et al. (2004)
Taiwanese	Pediatric asthma recruited from hospital	Children without asthma based on ISAAC criteria	1	Wang et al. (2004)
Not specified	Pediatric asthma with exacerbation	Healthy controls	0.51	El Bahlawan et al. (2003)
American	Physician-diagnosed asthma from clinic	Subjects without any atopic diseases	0.89	Witte et al. (2002)
Czech	Adult patients based on ATS criteria	General population without asthma	0.47	Buckova et al. (2002)
European Caucasian	Physician-diagnosed asthma from clinic	Hospital workers or blood donors without asthma	1	Louis et al. (2000)
British/Irish	Diagnosed by questionnaire	General population without asthma	0.24	Winchester et al. (2000)
South Asian			0.14	
Australian Caucasian	Physician-diagnosed pediatric asthma from clinic	Children without any atopic diseases (general population)	0.34	Albuquerque et al. (1998)
Australian	Diagnosed by questionnaire	General population without asthma	0.45	Moffatt and Cookson (1997)

HWE Hardy–Weinberg equilibrium, ISAAC International Study of Asthma and Allergies in Childhood, ATS American Thoracic Society

existence of heterogeneity. The combined odds ratio with the fixed effects model and with the random effects model after removal of that study was 1.46 (95% CI, 1.27–1.68, $P=0.0000001$) and 1.46 (95% CI, 1.20–1.77, $P=0.00014$) respectively.

Meta-analysis of six Caucasian populations revealed significant evidence of heterogeneity ($Q=15.47$, $df=5$, $P=0.0085$). After omission of the study by Albuquerque et al. (1998), the homogeneity test was not significant ($P=0.36$). The combined odds ratio with the fixed effects model and random effects model for asthma in the Caucasian populations was 1.57 (95% CI, 1.27–1.92, $P=0.000022$), and 1.58 (95% CI, 1.27–1.96, $P=0.000033$) respectively.

Meta-analysis of seven Asian populations also showed significant evidence of heterogeneity ($Q=36.14$, $df=6$, $P<0.00001$). After omission of the study by Shin et al. (2004), the homogeneity test result was not significant ($P=0.60$). The combined odds ratio with the fixed effects model and random effects model in the

Asian population was 1.71 (95% CI, 1.29–2.25, $P=0.0002$) and 1.70 (95% CI, 1.29–2.25, $P=0.00019$) respectively.

Discussion

In the present study, we did not detect a significant association between the –308G/A polymorphism of *TNF* and childhood asthma in two independent Japanese populations. However, meta-analysis revealed that the polymorphism was significantly associated with asthma, supporting involvement of the –308G/A polymorphism in the development of asthma.

Conflicting results are often obtained in studies of the genetics of complex traits; some studies may show an association between a polymorphism and a disease, whereas others fail to replicate the result. Discrepancies may be due to differences in the populations studied, difficulty in defining the phenotype, or lack of

Table 4 Odds ratios (OR) and sensitivity analysis of meta-analysis for the *TNF*-308G/A polymorphism and asthma

Study number	Reference	OR	95% CI	Summary OR with study removed	95% CI	<i>P</i> value	<i>P</i> (test of heterogeneity)
All				Fixed 1.29 Random 1.31	1.13–1.48 0.99–1.74	0.0001 0.0610	< 0.000001
1	Present study (case-control samples)	1.78	0.87–3.65	Fixed 1.28 Random 1.29	1.12–1.47 0.96–1.73	0.0003 0.0960	< 0.000001
2	Present study (TDT samples)	0.71	0.22–2.26	Fixed 1.31 Random 1.34	1.14–1.49 1.00–1.80	<0.0001 0.0463	< 0.000001
3	Bilolikar(2005)	1.61	1.07–2.40	Fixed 1.26 Random 1.29	1.09–1.45 0.95–1.76	0.0012 0.1057	< 0.000001
4	Gupta (2005)	1.79	1.11–2.89	Fixed 1.26 Random 1.28	1.10–1.45 0.95–1.73	0.0010 0.1097	< 0.000001
5	Shin(2004)	0.39	0.25–0.59	Fixed 1.46 Random 1.46	1.27–1.68 1.20–1.77	<0.0001 0.0001	0.052
6	Sandford (2004)	1.92	1.00–3.69	Fixed 1.27 Random 1.28	1.11–1.46 0.95–1.72	0.0005 0.1051	< 0.000001
7	Wang (2004)	2.15	1.23–3.76	Fixed 1.25 Random 1.26	1.09–1.44 0.94–1.70	0.0013 0.1194	< 0.000001
8	El Bahlawan (2003)	1.04	0.49–2.20	Fixed 1.30 Random 1.33	1.14–1.49 0.99–1.79	0.0001 0.0594	< 0.000001
9	Witte (2002)	1.36	0.95–1.94	Fixed 1.28 Random 1.31	1.11–1.48 0.95–1.80	0.0005 0.0944	< 0.000001
10	Buckova (2002)	1.40	0.90–2.19	Fixed 1.28 Random 1.31	1.12–1.48 0.96–1.78	0.0004 0.0905	< 0.000001
11	Louis (2000)	1.04	0.60–1.79	Fixed 1.31 Random 1.34	1.14–1.51 0.99–1.81	<0.0001 0.0605	< 0.000001
12	Winchester(British/Irish) (2000)	2.19	1.10–4.34	Fixed 1.27 Random 1.27	1.11–1.46 0.95–1.70	0.0005 0.1128	< 0.000001
13	Winchester (South Asian) (2000)	2.62	0.55–12.38	Fixed 1.29 Random 1.29	1.13–1.47 0.97–1.72	0.0002 0.0845	< 0.000001
14	Albuquerque(1998)	0.62	0.37–1.03	Fixed 1.37 Random 1.39	1.19–1.57 1.05–1.84	<0.0001 0.0220	0.00001
15	Moffatt (1997)	1.90	1.29–2.78	Fixed 1.23 Random 1.27	1.07–1.42 0.94–1.72	0.0037 0.1201	< 0.000001

Fixed fixed effects model (Mantel–Haenszel methods), *Random* random effects model (DerSimonian and Laird method). 95% CI and *P* values for the fixed and random effects models with the study removed

statistical power. Asthma is a complex disorder in which multiple genes and environmental factors are involved, and each genetic factor contributes modestly or weakly to the development of asthma. In the present study, the frequency of the –308A allele in the Asian populations was low, and the power to detect an association was not so high, especially in TDT. Therefore, we performed a meta-analysis to increase the power of the analysis for asthma and the –308G/A polymorphism and found an association between the –308G/A polymorphism and asthma susceptibility. To see the effect of the –308G/A polymorphism in specific ethnicities, we performed separate meta-analysis of Caucasian and Asian populations. The studies by Albuquerque et al. (1998) in Caucasian populations and Shin et al. (2004) in Asian populations contributed to heterogeneity in each ethnic group. After omission of these studies from our meta-analysis, results were consistent between the two ethnic groups, indicating that the –308A allele is associated with an increased risk of asthma.

Heterogeneity is considered when a given collection of studies is more heterogeneous than would be expected on the basis of sampling variance alone. This suggests that each of the studies measures an effect that is different from that in the other studies and that the variance comes from different study designs or populations in addition to the usual sampling variance. Sensitivity analysis revealed significant heterogeneity in the present meta-analysis ($P < 0.00001$), and the study by Shin et al. (2004) contributed to the heterogeneity (Table 4). After exclusion of that study, the test of heterogeneity for –308A was not significant ($P = 0.052$), and the summary odds ratio changed from 1.29 to 1.46. There are a number of reasons for the heterogeneity, such as differences in patients and control subjects and ethnicity, or differences in methodology. However, as shown in Table 3, the definition of case and control subjects in the study by Shin et al. (2004) did not differ from that in other studies, and differences in ethnicity or genotyping methods is unlikely to have caused the discrepancy in the present analysis.

It has been reported that the estimate of a genetic effect in the first report to describe an association is upwardly biased (winner's curse phenomenon; Lohmueller et al. 2003). When the initial study showing an association of *TNF* with asthma (Moffatt and Cookson 1997) was excluded from our meta-analysis, the combined odds ratio for the –308A allele was reduced from 1.46 to 1.41, but the association with asthma was still statistically significant ($P = 0.0000088$ for the fixed effects model and $P = 0.001$ for the random

effects model). This indicates that the initial positive study did not substantially influence the results of the meta-analysis of the association of asthma with the –308G/A polymorphism.

In our previous study, the –1037C/T (–857C/T) polymorphism of *TNF*, –1211T/–1043C/–1037C (–1031T/–863C/–857C) haplotype of *TNF*, and the *LTA*/*TNF* haplotypes were associated with asthma (Noguchi et al. 2002; Migita et al. 2005). The present meta-analysis data showed that asthma is associated with the –308G/A polymorphism of *TNF*, further supporting the importance of the *TNF* region in the development of asthma. Screening of the –308G/A polymorphism of *TNF* may be beneficial in identifying individuals susceptible to asthma and for personalized health risk assessments.

References

- Albuquerque RV, Hayden CM, Palmer LJ, Laing IA, Rye PJ, Gibson NA, Burton PR, Goldblatt J, Lesouef PN (1998) Association of polymorphisms within the tumour necrosis factor (TNF) genes and childhood asthma. *Clin Exp Allergy* 28:578–584
- Azzam A, Mathews CA (2003) Meta-analysis of the association between the catecholamine-*O*-methyl-transferase gene and obsessive-compulsive disorder. *Am J Med Genet B Neuropsychiatr Genet* 123:64–69
- Beghe B, Padoan M, Moss CT, Barton SJ, Holloway JW, Holgate ST, Howell WM, Mapp CE (2004) Lack of association of HLA class I genes and TNF alpha-308 polymorphism in toluene diisocyanate-induced asthma. *Allergy* 59:61–64
- Bilollikar H, Nam AR, Rosenthal M, Davies JC, Henderson DC, Balfour-Lynn IM (2005) Tumour necrosis factor gene polymorphisms and childhood wheezing. *Eur Respir J* 26:637–646
- Buckova D, Holla LI, Vasku A, Znojil V, Vacha J (2002) Lack of association between atopic asthma and the tumor necrosis factor alpha-308 gene polymorphism in a Czech population. *J Investig Allergol Clin Immunol* 12:192–197
- Carroll MC, Katzman P, Alicot EM, Koller BH, Geraghty DE, Orr HT, Strominger JL, Spies T (1987) Linkage map of the human major histocompatibility complex including the tumor necrosis factor genes. *Proc Natl Acad Sci USA* 84:8535–8539
- Chagani T, Pare PD, Zhu S, Weir TD, Bai TR, Behbehani NA, Fitzgerald JM, Sandford AJ (1999) 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* 160:278–282
- Choi IW, Sun K, Kim YS, Ko HM, Im SY, Kim JH, You HJ, Lee YC, Lee JH, Park YM, Lee HK (2005) TNF-alpha induces the late-phase airway hyperresponsiveness and airway inflammation through cytosolic phospholipase A(2) activation. *J Allergy Clin Immunol* 116:537–543
- Daniels SE, Bhattacharya S, James A, Leaves NI, Young A, Hill MR, Faux JA, Ryan GF, le Souef PN, Lathrop GM, Musk AW, Cookson WO (1996) A genome-wide search for quantitative trait loci underlying asthma. *Nature* 383:247–250

- Di Somma C, Charron D, Deichmann K, Buono C, Ruffilli A (2003) Atopic asthma and TNF-308 alleles: linkage disequilibrium and association analyses. *Hum Immunol* 64:359–365
- Dizier MH, Besse-Schmittler C, Guillaud-Bataille M, Annesi-Maesano I, Boussaha M, Bousquet J, Charpin D et al (2000) Genome screen for asthma and related phenotypes in the French EGEA study. *Am J Respir Crit Care Med* 162:1812–1818
- Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315:629–634
- El Bahlawan L, Christensen M, Binaei S, Murphy C, Zhang Q, Quasney M (2003) 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* 123:374S–375S
- Gupta V, Sarin BC, Changotra H, Sehajpal PK (2005) Association of G–308A TNF- α polymorphism with bronchial asthma in a North Indian population. *J Asthma* 42:839–841
- Howarth PH, Babu KS, Arshad HS, Lau L, Buckley M, McConnell W, Beckett P, Al Ali M, Chauhan A, Wilson SJ, Reynolds A, Davies DE, Holgate ST (2005) Tumour necrosis factor (TNF α) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. *Thorax* 60:1012–1018
- Kirov G, Jones I, McCandless F, Craddock N, Owen MJ (1999) Family-based association studies of bipolar disorder with candidate genes involved in dopamine neurotransmission: DBH, DAT1, COMT, DRD2, DRD3 and DRD5. *Mol Psychiatry* 4:558–565
- Laitinen T, Daly MJ, Rioux JD, Kauppi P, Laprise C, Petays T, Green T, Cargill M, Hahtela T, Lander ES, Laitinen LA, Hudson TJ, Kere J (2001) A susceptibility locus for asthma-related traits on chromosome 7 revealed by genome-wide scan in a founder population. *Nat Genet* 28:87–91
- Li YF, Gauderman WJ, Avol E, Dubeau L, Gilliland FD (2006) Associations of tumor necrosis factor G–308A with childhood asthma and wheezing. *Am J Respir Crit Care Med* 173:970–976
- Li Kam Wa TC, Mansur AH, Britton J, Williams G, Pavord I, Richards K, Campbell DA, Morton N, Holgate ST, Morrison JF (1999) Association between –308 tumour necrosis factor promoter polymorphism and bronchial hyperactivity in asthma. *Clin Exp Allergy* 29:1204–1208
- Lin YC, Lu CC, Su HJ, Shen CY, Lei HY, Guo YL (2002) The association between tumor necrosis factor, HLA-DR alleles, and IgE-mediated asthma in Taiwanese adolescents. *Allergy* 57:831–834
- Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN (2003) Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 33:177–182
- Louis R, Leyder E, Malaise M, Bartsch P, Louis E (2000) Lack of association between adult asthma and the tumour necrosis factor α –308 polymorphism gene. *Eur Respir J* 16:604–608
- Makino S, Adachi M, Ago Y, Akiyama K, Baba M, Egashira Y, Fujimura M et al (2005) Epidemiology of asthma. *Int Arch Allergy Immunol* 136 [Suppl 1]:5–13
- Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ (1995) Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 332:133–138
- Migita O, Noguchi E, Koga M, Jian Z, Shibasaki M, Migita T, Ito S, Ichikawa K, Matsui A, Arinami T (2005) Haplotype analysis of a 100 kb region spanning TNF-LTA identifies a polymorphism in the LTA promoter region that is associated with atopic asthma susceptibility in Japan. *Clin Exp Allergy* 35:790–796
- Moffatt MF, Cookson WO (1997) Tumour necrosis factor haplotypes and asthma. *Hum Mol Genet* 6:551–554
- Nicolae D, Cox NJ, Lester LA, Schneider D, Tan Z, Billstrand C, Kuldanek S, Donfack J, Kogut P, Patel NM, Goodenbour J, Howard T, Wolf R, Koppelman GH, White SR, Parry R, Postma DS, Meyers D, Bleecker ER, Hunt JS, Solway J, Ober C (2005) Fine mapping and positional candidate studies identify HLA-G as an asthma susceptibility gene on chromosome 6p21. *Am J Hum Genet* 76:349–357
- NIH (1997) National Heart Lung and Blood Institute NIH guidelines for the diagnosis and management of asthma. US Government Printing Office, Washington
- Noguchi E, Yokouchi Y, Shibasaki M, Inudou M, Nakahara S, Nogami T, Kamioka M, Yamakawa-Kobayashi K, Ichikawa K, Matsui A, Arinami T (2002) Association between TNFA polymorphism and the development of asthma in the Japanese population. *Am J Respir Crit Care Med* 166:43–46
- Ober C, Cox NJ, Abney M, Di Rienzo A, Lander ES, Changyaleket B, Gidley H, Kurtz B, Lee J, Nance M, Pettersson A, Prescott J, Richardson A, Schlenker E, Summerhill E, Willadsen S, Parry R (1998) Genome-wide search for asthma susceptibility loci in a founder population. The Collaborative Study on the Genetics of Asthma. *Hum Mol Genet* 7:1393–1398
- Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV (1984) Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* 312:724–729
- Randolph AG, Lange C, Silverman EK, Lazarus R, Weiss ST (2005) Extended haplotype in the tumor necrosis factor gene cluster is associated with asthma and asthma-related phenotypes. *Am J Respir Crit Care Med* 172:687–692
- Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. *Science* 273:1516–1517
- Saetta M, Turato G (2001) Airway pathology in asthma. *Eur Respir J Suppl* 34:18s–23s
- Sandford AJ, Chan HW, Wong GW, Lai CK, Chan-Yeung M (2004) Candidate genetic polymorphisms for asthma in Chinese schoolchildren from Hong Kong. *Int J Tuberc Lung Dis* 8:519–527
- Schlesselman JJ (1974) Sample size requirements in cohort and case-control studies of disease. *Am J Epidemiol* 99:381–384
- Shin HD, Park BL, Kim LH, Jung JH, Wang HJ, Kim YJ, Park HS, Hong SJ, Choi BW, Kim DJ, Park CS (2004) Association of tumor necrosis factor polymorphisms with asthma and serum total IgE. *Hum Mol Genet* 13:397–403
- Thomas PS (2001) Tumour necrosis factor- α : the role of this multifunctional cytokine in asthma. *Immunol Cell Biol* 79:132–140
- Thomas PS, Pennington DW, Schreck RE, Levine TM, Lazarus SC (1996) Authentic 17 kDa tumour necrosis factor α is synthesized and released by canine mast cells and up-regulated by stem cell factor. *Clin Exp Allergy* 26:710–718
- Trabetti E, Patuzzo C, Malerba G, Galavotti R, Martinati LC, Boner AL, Pignatti PF (1999) Association of a lymphotoxin α gene polymorphism and atopy in Italian families. *J Med Genet* 36:323–325

- Wang TN, Chen WY, Wang TH, Chen CJ, Huang LY, Ko YC (2004) Gene–gene synergistic effect on atopic asthma: tumour necrosis factor- α -308 and lymphotoxin- α -NcoI in Taiwan's children. *Clin Exp Allergy* 34:184–188
- Wigginton JE, Cutler DJ, Abecasis GR (2005) A note on exact tests of Hardy–Weinberg equilibrium. *Am J Hum Genet* 76:887–893
- Winchester EC, Millwood IY, Rand L, Penny MA, Kessling AM (2000) Association of the TNF- α -308 (G $\rightarrow$ A) polymorphism with self-reported history of childhood asthma. *Hum Genet* 107:591–596
- Witte JS, Palmer LJ, O'Connor RD, Hopkins PJ, Hall JM (2002) Relation between tumour necrosis factor polymorphism TNF α -308 and risk of asthma. *Eur J Hum Genet* 10:82–85
- Wjst M, Fischer G, Immervoll T, Jung M, Saar K, Rueschendorf F, Reis A et al (1999) A genome-wide search for linkage to asthma. German Asthma Genetics Group. *Genomics* 58:1–8
- Yokouchi Y, Nukaga Y, Shibasaki M, Noguchi E, Kimura K, Ito S, Nishihara M, Yamakawa-Kobayashi K, Takeda K, Imoto N, Ichikawa K, Matsui A, Hamaguchi H, Arinami T (2000) Significant evidence for linkage of mite-sensitive childhood asthma to chromosome 5q31–q33 near the interleukin 12 B locus by a genome-wide search in Japanese families. *Genomics* 66:152–160
- Zhu S, Chan-Yeung M, Becker AB, Dimich-Ward H, Ferguson AC, Manfreda J, Watson WT, Pare PD, Sandford AJ (2000) Polymorphisms of the IL-4, TNF- α , and Fc ϵ s1 gene and the risk of allergic disorders in at-risk infants. *Am J Respir Crit Care Med* 161:1655–1659