

ORIGINAL PAPER

Association of TNF- α genetic polymorphism with HLA DPB1*0301

S. -H. Kim*, Y. -M. Ye*, S. -K. Lee†, J. -H. Choi‡, J. W. Holloway§, C. -S. Park¶ and H. -S. Park*

*Department of Allergy and Rheumatology, Ajou University School of Medicine, Suwon, †Department of Internal Medicine, Dong-A University School of Medicine, Busan, ‡Department of Pulmonology and Allergies, Bundang Jesaeng General Hospital, Seongnam, Korea, §University of Southampton, Southampton, UK, and ¶Asthma Genomic Center, Soonchunhyang University School of Medicine, Bucheon, Korea

Clinical and
Experimental
Allergy

Summary

Background We speculated TNF- α can be one of candidate gene for aspirin-intolerant asthma (AIA) because TNF- α is pro-inflammatory cytokine and known to be increased level in asthmatic airways. In addition, genetic interaction between TNF- α and human antigen leucocyte (HLA) DPB1*0301, which is a strong genetic marker for AIA, was examined for its close location within chromosome 6.

Method To investigate genetic association of TNF- α with an AIA phenotype, three study groups (163 patients with AIA, 197 patients with aspirin-tolerant asthma (ATA), 257 normal control subjects) were enrolled. Single nucleotide polymorphisms (SNPs) were genotyped using a single-base extension method and HLA DPB1 genotyping was determined by high-throughput sequencing method.

Results All five SNPs of TNF- α were tested; there were no significant differences in allele and genotype frequencies among the three groups. However, significant association between TNF- α - 308G > A polymorphism and atopy status was noted ($P < 0.05$). Gene to gene interaction between TNF- α - 1031T > C (or - 863C > A or - 857C > A) and HLA DPB1*0301 could synergistically increase the susceptibility to AIA with odds ratio (OR) to 7.738 (or OR = 8.184 for - 863C > A, OR = 7.500 for - 857C > T, $P < 0.001$, respectively).

Conclusion TNF- α promoter polymorphism may significantly increase susceptibility to AIA by gene-to-gene interaction with HLA DPB1*0301.

Keywords aspirin-intolerant asthma, genetic polymorphism, HLA allele, tumour necrosis factor

Submitted 11 April 2006; revised 11 July 2006; accepted 2 August 2006

Correspondence:

Dr Hae-Sim Park, Ajou University
School of Medicine, Suwon, Korea.
E-mail: hspark@ajou.ac.kr

Introduction

It is widely recognized that ingestion of aspirin (acetylsalicylic acid) can induce bronchoconstriction in patients with aspirin-intolerant asthma (AIA) by triggering cysteinyl-leukotriene overproduction [1] and leukotriene-related genes, including 5-lipoxygenase and leukotriene C4 synthase, have been implicated in the pathogenesis of AIA. However, TNF- α has also been found to be increased level in asthmatic airways after segmental allergen provocation [2], associated with airway hyper-responsiveness (AHR) [3] and the phenotype of severe asthma [4]. A recent asthma cohort study demonstrated that AIA was associated with the increased asthma severity [5]. TNF- α is potent pro-inflammatory cytokine involved with airway inflammation and expressed in macrophages, T cells, mast

cells and bronchial epithelial cells [6–8]. In addition, TNF- α gene lies adjacent to each other within class III region of the human major histocompatibility complex (MHC) on chromosome 6p21 [9–11], so we speculate that gene to gene interaction between TNF- α and human antigen leucocyte (HLA) may contribute to development of AIA.

Several single-nucleotide polymorphisms (SNPs) have been reported in the TNF- α locus, including SNPs at - 1031T > C, - 863C > A, - 857C > T, - 308G > A and - 238G > A [12–14]. Furthermore, many association studies of these polymorphisms have been reported in asthmatic patients, but their association results have been remained controversial. We previously reported that TNF- α - 308G > A was significantly associated with atopic asthma in a Korean asthma cohort [15]. To date, there has been no published data on genetic polymorphism of

TNF- α in AIA, with this study being the first to investigate the genetic effects of TNF- α gene on AIA, and to evaluate gene to gene interaction between TNF- α and HLA DPB1*0301 which is known to be a strong genetic marker of AIA [16, 17].

Materials and methods

Study subjects

Three groups of study subjects [163 patients with AIA, 197 patients with aspirin - tolerant asthma (ATA), and 257 normal controls] were enrolled from Ajou University Hospital in Korea. Diagnosis of AIA was based on positive asthmatic reactions with more than 20% fall of forced expiratory volume in 1 s (FEV₁) after inhalation with lysine aspirin and ATA was defined as asthmatic patients showing negative response to lysine aspirin bronchoprovocation test. AHR was determined by methacholine bronchial challenge test. Atopy was defined by positive skin prick test response to more than one allergen among 12 common aeroallergens (Bencard, Brentford, UK). Serum total IgE was measured using the UniCAP system (Pharmacia Diagnostics, Valinge, Sweden) according to the manufacturer's instruction. The study protocol was approved by the institutional review board of Ajou University Hospital, Korea and all subjects were fully aware of the

scope of this study and each signed an informed consent form.

Genotyping

In total, five SNPs in the TNF- α gene (– 1031T > C, – 863C > A, – 857C > T, – 308G > A, – 238G > A) were genotyped by a single base extension method [15]. HLA-DPB1 high-resolution genotyping was obtained from amplified gene products with the use of locus-specific primers [18] and direct sequencing with the AB13100 Genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

A significant departure of genotype frequency from the Hardy-Weinberg equilibrium (HWE) at each SNP was tested by a χ^2 goodness-of-fit test. The distributions of the TNF- α promoter genotypes were not deviated from Hardy-Weinberg equilibrium ($P > 0.05$). A difference in genotype frequency between case and control was assessed by χ^2 test and calculation of odds ratios (OR) with 95% confidence intervals (CI). Haplotypes of TNF- α gene were analysed using Haploview v2.0 based on the EM algorithm [19]. Linkage disequilibrium (LD) between loci was measured using the absolute value of Lewontin's $|D'|$ [20]. Five SNPs of TNF- α gene were

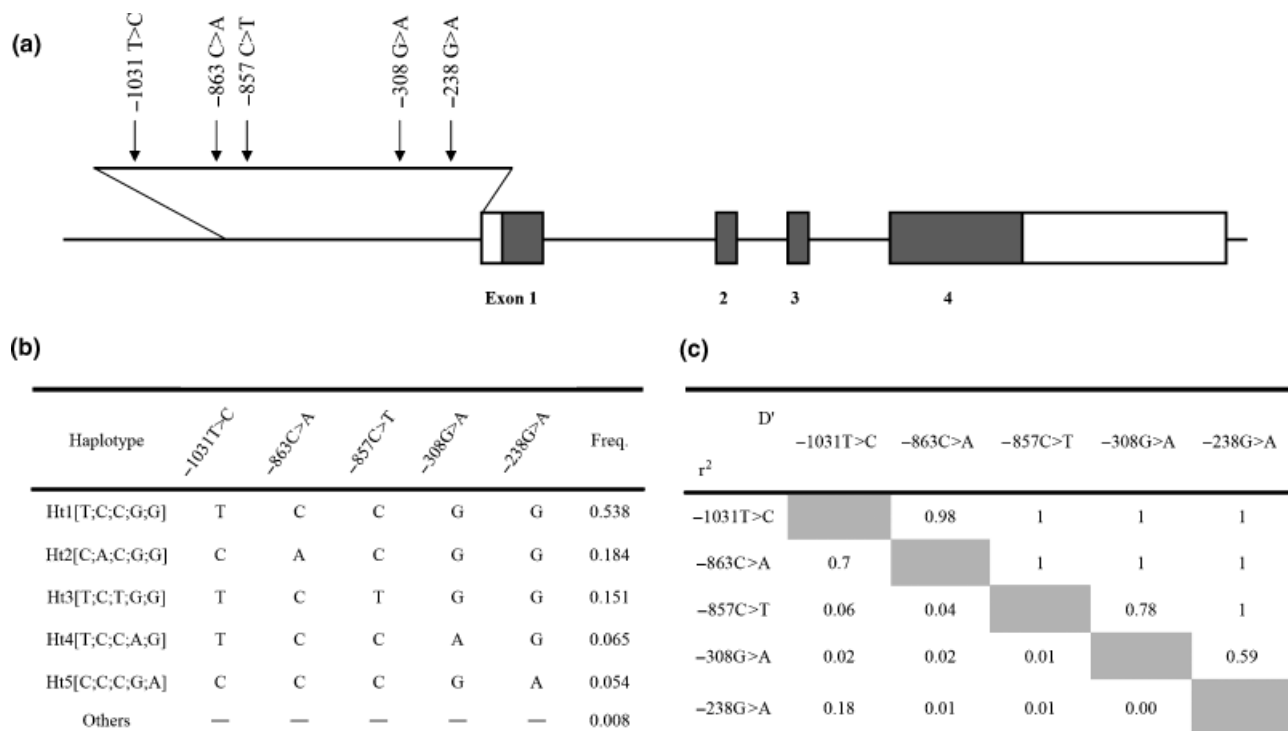


Fig. 1. Gene Map and linkage disequilibrium (LD) coefficients in TNF- α . (a) Gene Map and single nucleotide polymorphisms (SNPs) in TNF- α on chromosome 6p21. Coding exons are marked by black blocks and untranslated regions by white blocks. Transcription start site was denoted as nucleotide +1. (b) Haplotypes of TNF- α with frequency > 0.05 were presented. (c) LD coefficient ($|D'|$) of SNPs in TNF- α .

Table 1. Clinical characteristics of study subjects

	AIA (<i>n</i> = 163)	ATA (<i>n</i> = 197)	NC (<i>n</i> = 197)	<i>P</i> -value	
				AIA vs. ATA	AIA vs. NC
Sex (male/total)	62/163 (38.04%)	84/197 (42.64%)	110/257 (42.8%)	0.390	0.360
Atopy (presence/total)	84/151 (55.63%)	132/192 (68.75%)	33/224 (14.73%)	0.013	< 0.001
Age (years)*	43.3 $\pm$ 13.6	40.5 $\pm$ 14.1	36.7 $\pm$ 15.6	0.057	< 0.001
log[serum total IgE (IU/mL)]*	2.2 $\pm$ 0.6	2.2 $\pm$ 0.7	1.7 $\pm$ 0.6	0.707	< 0.001
Asthma duration (year)*	6.5 $\pm$ 5.8	4.9 $\pm$ 5.9	NA	0.021	NA
FEV ₁ (%)*	79.3 $\pm$ 27.2	87.6 $\pm$ 22.2	NA	0.002	NA
% fall of FEV ₁ by aspirin*	22.8 $\pm$ 11.3	7.0 $\pm$ 3.9	NA	< 0.001	NA
PC20 methacholine*	4.6 $\pm$ 7.9	8.8 $\pm$ 10.0	NA	< 0.001	NA
Paranasal sinusitis (presence/total)	59/128 (46.83%)	9/191 (4.71%)	NA	< 0.001	NA
Nasal polyp (presence/total)	67/126 (51.94%)	10/191 (5.24%)	NA	< 0.001	NA
Rhinitis (presence/total)	103/129 (80.47%)	140/191 (73.3%)	NA	0.180	NA

*Data are expressed as the mean $\pm$ SD. Bold indicates significant *P*-value.

AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; NC, normal control; NA, not applicable; FEV₁, forced expiratory volume in 1 s.

Table 2. Genotype frequency of TNF- α in the study subjects

Loci	Genotype	AIA (<i>n</i> = 163)	ATA (<i>n</i> = 197)	NC (<i>n</i> = 257)	<i>P</i> -value*	
					AIA vs. ATA	AIA vs. NC
TNF- α – 1031T > C	TT	88 (56.4%)	101 (54.6%)	161 (62.6%)	0.796	0.428
	CT	59 (37.8%)	70 (37.8%)	85 (33.1%)	0.666	0.489
	CC	9 (5.8%)	14 (7.6%)	11 (4.3%)	0.744	0.215
	q.	0.247	0.265	0.208	0.599	0.197
TNF- α – 863C > A	CC	104 (64.2%)	126 (64.6%)	182 (71.7%)	0.920	0.274
	AC	51 (31.5%)	59 (30.3%)	64 (25.2%)	0.806	0.594
	AA	7 (4.3%)	10 (5.1%)	8 (3.1%)	1.000	0.129
	q.	0.201	0.203	0.157	1.000	0.112
TNF- α – 857C > T	CC	112 (73.2%)	147 (78.2%)	166 (68.3%)	0.149	0.236
	CT	33 (21.6%)	38 (20.2%)	69 (28.4%)	0.070	0.433
	TT	8 (5.2%)	3 (1.6%)	8 (3.3%)	0.309	0.312
	q.	0.160	0.117	0.175	0.116	0.627
TNF- α – 308G > A	GG	139 (85.3%)	177 (89.8%)	217 (84.4%)	0.274	0.422
	AG	23 (14.1%)	20 (10.2%)	40 (15.6%)	0.453	0.388
	AA	1 (0.6%)	0 (0%)	0 (0%)	0.199	0.890
	q.	0.077	0.051	0.078	0.166	1.000
TNF- α – 238G > A	GG	140 (89.2%)	174 (89.2%)	231 (90.9%)	0.425	0.568
	AG	17 (10.8%)	19 (9.7%)	22 (8.7%)	0.504	1.000
	AA	0 (0%)	2 (1%)	1 (0.4%)	1.000	0.609
	q	0.054	0.059	0.047	0.870	0.742

*Each *P*-value was calculated with co-dominant, dominant, and recessive models. Logistic regression analysis was applied to control for age and sex as covariables.

AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; NC, normal control; q, minor allele frequency.

in LD with each other and partial LD was also observed between the TNF- α – 1031T > C and – 863C > A polymorphisms (D' = 0.97 and r^2 = 0.69, Fig. 1). Logistic regression models were used for analyses of SNPs and haplotypes controlling age and sex as covariates with three alternative models (co-dominant, dominant and recessive models). Differences in the mean value of the phenotypic characteristics within AIA patients were compared using ANOVA and *t*-tests. Statistical analyses were undertaken using SPSS v11 (SPSS Inc., Chicago, IL, USA). Contingency tables (2 $\times$ 2) and χ^2

tests were used to assess differences in TNF- α and HLA DPB1*0301 genotypes between case and control. The significance level was set at 0.05.

Results

Clinical features of the study subjects

Table 1 showed the clinical characteristics of the study subjects. The mean ages between AIA and normal healthy

Table 3. Comparison of the clinical characteristics according to the genotype of TNF- α in AIA patients

	- 1031T > C			- 863C > A			- 857C > T			- 308G > A			- 238G > A		
	TT	CT or CC	P-value [†]	CC	AC or AA	P-value [†]	CC	CT or TT	P-value [†]	GG	AG or AA	P-value [†]	GG	AG or AA	P-value [†]
Atopy (presence/total)	41 (50%)	41 (63.08%)	0.134	49 (51.04%)	35 (62.5%)	0.181	54 (50.94%)	26 (65%)	0.140	78 (60.47%)	7 (30.43%)	0.011	76 (56.3%)	7 (46.67%)	0.587
Rhinosinusitis	53 (80.3%)	63.08 (48%)	0.642	63 (79.75%)	62.5 (41%)	0.822	79 (84.04%)	65 (26%)	0.593	89 (80.91%)	30.43 (15%)	0.763	93 (80.87%)	46.67 (11%)	1.000
Paranasal sinusitis	29 (43.94%)	28 (50.91%)	0.322	35 (44.3%)	23 (47.92%)	0.600	49 (53.26%)	10 (30.3%)	0.067	52 (48.15%)	7 (36.84%)	0.035	50 (44.25%)	8 (61.54%)	0.458
Nasal polyp	34 (52.31%)	32 (54.24%)	0.859	39 (50%)	27 (51.92%)	0.859	51 (53.13%)	14 (43.75%)	0.417	57 (51.82%)	10 (50%)	1.000	59 (50.86%)	8 (61.54%)	0.564
Log [serum total IgE (IU/mL)]*	2.22 $\pm$ 0.53	2.22 $\pm$ 0.62	0.976	2.22 $\pm$ 0.49	2.21 $\pm$ 0.67	0.969	2.18 $\pm$ 0.59	2.28 $\pm$ 0.48	0.370	2.27 $\pm$ 0.54	1.97 $\pm$ 0.64	0.025	2.25 $\pm$ 0.56	1.94 $\pm$ 0.51	0.038
Asthma duration (years)*	6.88 $\pm$ 6.44	6.09 $\pm$ 5.22	0.462	6.78 $\pm$ 6.12	5.94 $\pm$ 5.27	0.424	6.77 $\pm$ 6.43	6.19 $\pm$ 4.19	0.628	6.67 $\pm$ 5.97	5.56 $\pm$ 4.76	0.445	6.18 $\pm$ 5.58	9.67 $\pm$ 7.22	0.047
FEV ₁ (%)	79.53 $\pm$ 27.1	77.25 $\pm$ 26.88	0.608	80.26 $\pm$ 26.11	76.82 $\pm$ 28.29	0.442	81.47 $\pm$ 25.4	76.78 $\pm$ 28.31	0.331	78.8 $\pm$ 26.8	82.14 $\pm$ 29.19	0.580	78.92 $\pm$ 27.29	82.78 $\pm$ 23.1	0.587
% fall of FEV ₁ by aspirin*	24.33 $\pm$ 12.45	20.6 $\pm$ 8.42	0.076	24.22 $\pm$ 12.62	20.58 $\pm$ 7.61	0.069	21.91 $\pm$ 9.72	23.38 $\pm$ 12.99	0.575	22.42 $\pm$ 11	25.28 $\pm$ 13.45	0.412	22.87 $\pm$ 11.14	22.55 $\pm$ 13.07	0.930
Log PC20, methacholine (mg/mL)*	0.14 $\pm$ 0.69	0.07 $\pm$ 0.7	0.595	0.1 $\pm$ 0.67	0.15 $\pm$ 0.72	0.71	0.1 $\pm$ 0.7	0.15 $\pm$ 0.67	0.748	0.08 $\pm$ 0.7	0.3 $\pm$ 0.62	0.228	0.14 $\pm$ 0.69	- 0.22 $\pm$ 0.66	0.112

*Data are expressed as the mean $\pm$ SD. [†]Logistic regression analysis was applied to control for age and sex as covariables. FEV₁, forced expiratory volume in 1 s.

control group were statistically different ($P < 0.001$), so we adjusted for age by multiple logistic regressions in order to adjust the confounding factor. There were significant differences in the prevalence of nasal polyp, paranasal sinusitis, atopy, asthma duration, FEV₁%, PC20 methacholine, and fall percent of FEV₁ after aspirin provocation between AIA and ATA groups (each $P < 0.05$); that is, it was more frequent prevalence of nasal polyp and paranasal sinusitis in AIA compared with ATA. AIA patients in this study showed lower atopic status, longer asthma duration, lower FEV₁% and lower PC20 dose of methacholine in comparison with ATA. Between AIA and normal healthy control groups, there were significant differences in atopy and serum total IgE level.

Association of tumour necrosis factor- α promoter polymorphisms with aspirin-intolerant asthma phenotype

Allele and genotype frequencies of TNF- α promoter polymorphisms among the three groups are shown in Table 2. All five promoter polymorphisms showed no significant association with AIA. Moreover, no significant differences in haplotype and diplotype frequencies were noted among the three groups (data not shown). However, among five SNPs, - 308G > A polymorphism was significantly associated with atopy, serum total IgE and the presence of paranasal sinusitis; AIA patients with homozygous GG genotype of - 308G > A had higher atopy rate, higher serum total IgE level and higher frequency of paranasal sinusitis than those without this genotype ($P = 0.011$, 0.025, 0.035 by logistic regression analysis for age and sex as covariables). Moreover, - 238G > A polymorphism showed associated with serum total IgE ($P = 0.038$). However, after Bonferroni correction, the association of - 308G > A polymorphism with atopy was only remained to be significant (Table 3).

Genetic interaction between tumour necrosis factor- α promoter polymorphism and HLA DPB1*0301

Synergistic contribution of both TNF- α and HLA DPB1*0301, which is a strong genetic marker of AIA, was examined and the combined genotypes of TNF- α promoter polymorphism, including - 1031T > C, - 863C > A and - 857C > T, and HLA DPB1*0301 appeared to synergistically contribute to the development of AIA; subjects carrying both TNF- α - 1031 TT (or - 863CC or - 857 CT or TT) and HLA DPB1*0301 alleles were significantly higher in AIA group than those without these genotypes ($P < 0.001$, respectively). In comparison between AIA and NC groups, gene to gene interaction between TNF- α - 1031T > C (or - 863C > A or - 857C > A) and HLA DPB1*0301 could synergistically increase the susceptibility to AIA with OR to 7.738 (or OR = 8.184 for - 863C > A, OR = 7.500 for - 857C > T) as shown in Table 4.

Table 4. The frequency of combined genotype of TNF- α promoter polymorphism and HLA DPB1*0301

				<i>P</i> -value*	
	AIA	ATA	NC	AIA vs. ATA	AIA vs. NC
<i>TNF-α</i> – 1031T > C					
TT					
HLA (+)	19 (25.7%)	6 (6.3%)	5 (4.3%)	0.001	< 0.001
DPB1*0301 (–)	55 (74.3%)	90 (93.8%)	112 (95.7%)	OR = 5.182	OR = 7.738
<i>n</i>	74	96	117		
CT or CC					
HLA (+)	18 (29.5%)	6 (8.0%)	9 (11.8%)	0.001	0.016
DPB1*0301 (–)	43 (70.5%)	69 (92.0%)	67 (88.2%)	OR = 4.814	OR = 3.116
<i>n</i>	61	75	76		
<i>TNF-α</i> – 863C > A					
CC					
HLA (+)	26 (30.6%)	9 (8.0%)	7 (5.1%)	< 0.001	< 0.001
DPB1*0301 (–)	59 (69.4%)	104 (92.0%)	130 (94.9%)	OR = 5.092	OR = 8.184
<i>n</i>	85	113	137		
AC or AA					
HLA (+)	11 (20.8%)	3 (4.8%)	7 (12.1%)	0.011	0.303
DPB1*0301 (–)	42 (79.2%)	59 (95.2%)	51 (87.9%)	OR = 5.151	OR = 1.908
<i>n</i>	53	62	58		
<i>TNF-α</i> – 857C > T					
CC					
HLA (+)	27 (27.0%)	9 (6.9%)	11 (8.3%)	< 0.001	< 0.001
DPB1*0301 (–)	73 (73.0%)	122 (93.1%)	122 (91.7%)	OR = 5.014	OR = 4.102
<i>n</i>	100	131	133		
CT or TT					
HLA (+)	9 (29.0%)	3 (8.1%)	3 (5.2%)	0.030	0.003
DPB1*0301 (–)	22 (71.0%)	34 (91.9%)	55 (94.8%)	OR = 4.636	OR = 7.500
<i>n</i>	31	37	58		
<i>TNF-α</i> – 308G > A					
GG					
HLA (+)	31 (26.5%)	11 (7.0%)	10 (6.0%)	< 0.001	< 0.001
DPB1*0301 (–)	86 (73.5%)	147 (93.0%)	157 (94.0%)	OR = 4.817	OR = 5.659
<i>n</i>	117	158	167		
AG or AA					
HLA (+)	7 (31.8%)	1 (5.9%)	4 (14.8%)	0.106	0.185
DPB1*0301 (–)	15 (68.2%)	16 (94.1%)	23 (85.2%)	OR = 7.467	OR = 2.683
<i>n</i>	22	17	27		
<i>TNF-α</i> – 238G > A					
GG					
HLA (+)	31 (25.8%)	9 (5.8%)	12 (6.9%)	< 0.001	< 0.001
DPB1*0301 (–)	89 (74.2%)	147 (94.2%)	161 (93.1%)	OR = 5.689	OR = 4.673
<i>n</i>	120	156	173		
AG or AA					
HLA (+)	6 (40.0%)	3 (16.7%)	2 (10.0%)	0.239	0.051
DPB1*0301 (–)	9 (60.0%)	15 (83.3%)	18 (90.0%)	OR = 3.333	OR = 6.000
<i>n</i>	15	18	20		

AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; NC, normal control; OR, odds ratio. *Each P-value was calculated with X³ test.

Discussion

To our knowledge, many association studies have been shown that genetic polymorphisms of TNF- α may be associated with a risk of asthma [3, 15, 21–26]. TNF- α – 308 A allele might confer genetic predisposition to

asthma [21–26] but, in childhood asthma [27, 28] and Korean atopic asthmatics [15], TNF- α – 308 A allele play a protective role in asthma pathogenesis. In a recent study, TNF- α – 863C > A polymorphism was found as a reduced risk of asthma by reducing serum TNF- α level [29]. Moreover, negative associations of TNF- α polymorphisms

have also been reported in relation with asthma [30–33]. Many association studies of TNF- α promoter polymorphisms have been reported in asthmatic patients, but their association results have been remained controversial.

In Korean population, TNF- α –308G > A was significantly associated with atopic asthma in a Korean asthma cohort [15]. However, there has been no published data on genetic polymorphism of TNF- α in Korean population with AIA, so we designed association study of TNF- α polymorphisms in AIA to investigate whether genetic variants of TNF- α gene can contribute to risk of AIA, and to evaluate gene to gene interaction between TNF- α and HLA DPB1*0301, a strong genetic marker of AIA in Korea population [15].

In the present study, no significant associations between TNF- α promoter polymorphisms and AIA were noted in allele and genotype frequencies. However, –308G > A polymorphism was associated with the intermediate phenotypes of AIA including paranasal sinusitis, serum total IgE, atopy status. Significant relation of TNF- α –308G > A polymorphism with atopy and serum total IgE was consistent with previous study of atopic asthma in Korean population [15]. Associations of –308 G > A polymorphism with the presence of paranasal sinusitis was firstly found in this study.

In terms of its close location within the class III region of the MHC, it has been investigated whether TNF- α polymorphisms, mainly TNF- α –308G > A and LT α NcoI, may contribute to the HLA association [22, 34]. The TNF- α –1031T > C, –863C > A, –857C > T polymorphisms were found to be in significant LD with HLA B and HLA DRB1 allele [14]. In this study, gene-to-gene interaction between TNF- α promoter polymorphism and HLA DPB1*0301 was also found and this interaction can synergistically contribute to the susceptibility to AIA. This is the first study to suggest a possible synergistic interaction between the TNF- α promoter polymorphism, including –1031T > C, –863C > A, –857C > T, and HLA DPB1*0301, as the susceptible risk for AIA in patients carrying combined genotypes was increased (OR = 7.7 for –1031T > C, OR = 8.2 for –863C > A, OR = 7.5 for –857C > T) compared with those carrying the HLA DPB1*0301 allele alone (OR = 4.8, data not shown). Further studies will be needed to investigate the mechanism how TNF- α genetic polymorphisms could interact with HLA DPB1*0301 allele in AIA.

In conclusion, TNF- α may be a potentially important gene in AIA susceptibility in the Korean population and TNF- α promoter polymorphisms interacting with HLA DPB1*0301 significantly increases susceptibility to AIA.

Acknowledgements

The study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of

Korea (01-PJ3-PG6-01GN04-0003, 01-PJ10-PG6-01GN14-0007, and A050571).

References

- 1 Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis, diagnosis, and management. *J Allergy Clin Immunol* 1999; 104:5–13.
- 2 Virchow JC, 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; 151:960–8.
- 3 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; 29:1204–8.
- 4 Holgate ST, Holloway J, Wilson S *et al*. Understanding the pathophysiology of severe asthma to generate new therapeutic opportunities. *J Allergy Clin Immunol* 2006; 117:496–506.
- 5 Mascia K, Haselkorn T, Deniz YM, Miller DP, Bleecker ER, Borish L. Aspirin sensitivity and severity of asthma: evidence for irreversible airway obstruction in patients with severe or difficult-to-treat asthma. *J Allergy Clin Immunol* 2005; 116:970–5.
- 6 Vassalli P. The pathophysiology of tumor necrosis factors. *Ann Rev Immunol* 1992; 10:411–52.
- 7 Ware CF, VanArsdale TL, Crowe PD, Browning JL. The ligands and receptors of the lymphotoxin system. *Curr Top Microbiol Immunol* 1995; 198:175–218.
- 8 Bradding P, Roberts JA, Britten KM *et al*. Interleukin-4, -5, and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994; 10:471–80.
- 9 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; 84:8535–9.
- 10 Nedospasov SA, Shakhov AN, Turetskaya RL *et al*. Tandem arrangement of genes coding for tumor necrosis factor (TNF α) and lymphotoxin (TNF- β) in the human genome. *Cold Spring Harb Symp Quant Biol* 1986; 51:611–24.
- 11 Nedwin GE, Jarrett-Nedwin J, Smith DH *et al*. Structure and chromosomal localization of the human lymphotoxin gene. *J Cell Biochem* 1985; 29:171–81.
- 12 D'Alfonso S, Richiardi PM. A polymorphic variation in a putative regulation box of the TNF α promoter region. *Immunogenetics* 1994; 39:150–4.
- 13 Ugialoro AM, Turbay D, Pesavento PA *et al*. Identification of three new single nucleotide polymorphisms in the human tumor necrosis factor- α gene promoter. *Tissue Antigens* 1998; 52:359–67.
- 14 Higuchi T, Seki N, Kamizono S *et al*. Polymorphism of the 5'-flanking region of the human tumor necrosis factor (TNF)- α gene in Japanese. *Tissue Antigens* 1998; 51:605–12.
- 15 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.
- 16 Dekker JW, Nizankowska E, Schmitz-Schumann M *et al*. Aspirin-induced asthma and HLA-DRB1 and HLA-DPB1 genotypes. *Clin Exp Allergy* 1997; 27:574–7.

- 17 Choi JH, Lee KW, Oh HB *et al.* HLA association in aspirin-intolerant asthma: DPB1*0301 as a strong marker in a Korean population. *J Allergy Clin Immunol* 2004; 113:562–4.
- 18 Tilanus MGJ, Hansen JA, Hurley CK. IHWG Technical Manual. Seattle: International Histocompatibility Working Group; 2000.
- 19 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21:263–5.
- 20 Hedrick PW. Gametic disequilibrium measures: proceed with caution. *Genetics* 1987; 117:331–41.
- 21 Moffatt M, Cookson W. Tumour necrosis factor haplotypes and asthma. *Human Mol Genet* 1997; 6:551–4.
- 22 Gupta V, Sarin BC, Changotra H, Sehajpal PK. Association of G-308A TNF-alpha polymorphism with bronchial asthma in a North Indian population. *J Asthma* 2005; 42:839–41.
- 23 Noguchi E, Yokouchi Y, Shibasaki M *et al.* Association between TNF α polymorphism and the development of asthma in the Japanese population. *Am J Respir Crit Care Med* 2002; 166:43–6.
- 24 Witte JS, Palmer LJ, O'Connor RD, Hopkins PJ, Hall JM. Relation between tumour necrosis factor polymorphism TNF alpha-308 and risk of asthma. *Eur J Hum Genet* 2002; 10:82–5.
- 25 Gao J, Shan G, Sun B, Thompson PJ, Gao X. Association between polymorphism of tumour necrosis factor {alpha}-308 gene promoter and asthma: a meta-analysis. *Thorax* 2006; 61:466–71.
- 26 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. *Human Genet* 2000; 107:591–6.
- 27 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; 28:578–84.
- 28 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; 173:970–6.
- 29 Sharma S, Sharma A, Kumar S, Sharma SK, Ghosh B. Association of TNF haplotypes with asthma, serum IgE levels and correlation with serum TNF-{alpha} levels. *Am J Respir Cell Mol Biol* 2006, May 25; [Epub ahead of print].
- 30 Buckova D, Holla LI, Vasku A, Znojil V, Vacha J. Lack of association between atopic asthma and the tumor necrosis factor alpha-308 gene polymorphism in a Czech population. *J Invest Allergol Clin Immunol* 2002; 12:192–7.
- 31 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: 604–8.
- 32 Zhu S, Chan-Yeung M, Becker AB *et al.* Polymorphisms of the IL-4, TNF-alpha, and Fcepsilon RIbeta genes and the risk of allergic disorders in at-risk infants. *Am J Respir Crit Care Med* 2000; 161:1655–9.
- 33 Lin YC, Lu CC, Su HJ, Shen CY, Lei HY, Guo YL. The association between tumor necrosis factor, HLA-DR alleles, and IgE-mediated asthma in Taiwanese adolescents. *Allergy* 2002; 57:831–4.
- 34 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; 54:757–61.