

Short communication

HLA DRB1*15-DPB1*05 haplotype: a susceptible gene marker for isocyanate-induced occupational asthma?

Background: There has been no study for evaluating the associations of human leukocyte antigen (HLA) class I and II alleles with toluene diisocyanate (TDI)-induced asthma in an Asian population.

Objective: The aim of this study was to investigate a susceptible or protective marker of HLA class I and II alleles in TDI-induced asthma.

Methods: Fifty-five patients with TDI-induced asthma patients (group I) showing positive responses on TDI bronchoprovocation test, 47 asymptomatic exposed subjects (group II) and 95 unexposed healthy nonatopic controls (group III) were enrolled in our study. HLA class I and II genotyping was done by the direct DNA sequencing method.

Results: The allelic frequency of C*09 (15.5%) was significantly higher in group I than in group III (6.8%, $P = 0.019$), but this statistical significance disappeared after correction was made for multiple comparisons. On two-locus and three-locus haplotype analysis, the allelic frequency of HLA DRB1*15-DPB1*05 in group I (10.6%) was significantly higher than that of group II (0%, $P = 0.001$) and group III (2.5%, $P = 0.003$). The allelic frequencies of HLA A*02-DRB1*15, A*02-DQB1*06, B*62-C*09 and A*02-DRB1*15-DQB1*06 were significantly higher in group I (8.5%, 10.3%, 8.2% and 6.8%, respectively) than those allelic frequencies of group III (1.3%, $P = 0.002$; 1.6%, $P = 0.001$; 0.6%, $P < 0.0001$; 0%, $P < 0.0001$, respectively). The allelic frequencies of HLA DQB1*06-DPB1*05 and DRB1*15-DQB1*06-DPB1*05 were significantly higher in group I (16.0% and 10.5%) than those in group II (2.5%, $P = 0.001$; 0%, $P = 0.001$), while the frequencies of DRB1*09-DPB1*05 and DRB1*09-DQB1*0303-DPB1*05 were significantly lower in group I (0% and 0%) than those of group II (7.4%, $P = 0.004$; 7.5%, $P = 0.004$). These differences remained statistically significant even after the correction for multiple comparisons.

Conclusions: The HLA haplotype DRB1*15-DPB1*05 can be a susceptibility gene marker for the development of TDI-induced asthma among the exposed workers in the Korean population.

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Toluene diisocyanates (TDI) are one of the most commonly identified causes of occupational asthma in far east-Asia (1). Previous studies have demonstrated that CD4⁺ and CD8⁺ T cells, as well as eosinophils and mast cells, could be involved in the pathogenic mechanism of TDI-induced asthma (2, 3). Regarding the genetic mechanisms, there has been a study demonstrating that the human leukocyte antigen (HLA) DQB1*0503 and DQB1*0201-0301 haplotypes are susceptibility gene markers for TDI-induced asthma in an Italian population (4, 5), while no significant associations were found in another studies, based on European populations, for the HLA class II and HLA class I alleles (6, 7).

In this study, we analyzed both the HLA class I and II alleles in TDI-induced Korean asthma patients compared with two Korean control groups, the asymptomatic

exposed control subjects and unexposed healthy control subjects in order to investigate the susceptible or protective markers. In addition, two-locus and three-locus haplotype analysis was also performed.

Material and methods

Subjects

Fifty-five patients with TDI-induced asthma, aged from 20 to 64 years, who showed positive responses to TDI bronchoprovocation tests were enrolled (group I) from three major medical centers (Ajou, Yonsei, and Inha University Hospitals). As control subjects, 47 asymptomatic exposed workers, aged from 27 to 61 years, who had worked in the same workplace and were exposed to TDI (group II), and 95 unexposed healthy nonatopic control subjects (group III)

Table 1. Clinical characteristics of the study subjects

	TDI (<i>n</i> = 55)	AEC (<i>n</i> = 47)	NC (<i>n</i> = 95)
Gender (male/female)	36/19	35/12	62/33
Age (years)*	42.3 ± 10.1	42.5 ± 7.6	42.4 ± 8.9
Duration of exposure (years)*	7.0 ± 3.9	12.0 ± 7.4	0
Smoking status (S/NS/EX)	33/17/5	15/29/3	32/54/9

*Mean ± SD.

TDI, toluene diisocyanate-induced asthma patients; AEC, asymptomatic exposed controls; NC, normal controls; S, smoker; NS, nonsmoker; EX, ex-smoker; NS, not significant.

were enrolled. Their clinical features are summarized in Table 1. DNA samples were obtained during the subjects' initial visit. We performed interviews for identifying work-related respiratory symptoms, and chest X-ray were also performed. All the subjects gave us their informed consents and this study was approved by the Institutional Review Board of Ajou Medical Center, Suwon, Korea.

Allergy skin prick test

Skin prick tests were performed using 12 common aeroallergens (Bencard Co., UK) including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, cockroach, tree pollen mixture, grass pollen mixture, mugwort, ragweed, Hop Japanese, *Aspergillus*, *Alternaria*, histamine and a saline control. The reactions were read after 15 min and the wheals were measured in two directions. A positive reaction was defined when a mean wheal diameter exceeded 3 mm. Atopy was defined when there was one or more positive reactions on skin prick testing.

Pulmonary function tests and inhalation challenges

If the patients complained of lower respiratory symptoms, pulmonary function tests and inhalation challenges with methacholine and TDI were performed to confirm the presence of TDI-induced asthma. The bronchoprovocation tests with methacholine and TDI were performed according to the protocol described previously (8).

Genotyping

The genomic DNA from the peripheral blood mononuclear cells of the patients was extracted using a G-DEX Genomic DNA Extraction Kit (Intron Biotechnology, Sungnam, Korea). The HLA class I (A, B, C) and II (DRB1, DQB1, DPB1) genotypes were obtained by direct DNA sequencing analysis using sequencing primers and BigDye Termination Cycle Reaction Kits on an ABI 3100 Genetic analyzer (Applied Biosystems, Foster City, CA) (9). Data analysis was performed using Sequence Navigator and MatchTool software (PE-Applied Biosystems Inc.).

Statistical analysis

The allelic frequencies were calculated using SAS/Genetics software (SAS Institute Inc., Cary, NC). The allelic frequencies and haplotypes between the two groups were compared using the chi-squared test. *P*-values were corrected for multiple comparisons by multiplying with the allele numbers. For the haplotype analysis, a *P*-value of <0.005 was arbitrarily considered as significant because the haplotypes are so numerous and Bonferroni's correction was not needed. A *P*-value of 0.05 or less was regarded as significant.

Results

Allele frequencies

The frequency of C*09 was significantly higher in group I (15.5%) than in group III (6.8%, *P* = 0.019, odds ratio = 2.5, 95% CI = 1.2–5.3); however, its statistical significance disappeared after correction was made for multiple comparisons. The frequencies of the other single HLA class I or class II alleles were not significantly different among the three study groups.

Haplotype frequencies

On the two-locus and three-locus haplotype analysis (Tables 2 and 3), the allelic frequency of HLA DRB1*15-DPB1*05 in group I (10.6%) was significantly higher than that in group II (0%, *P* = 0.001) and in group III (2.5%, *P* = 0.003). The allelic frequencies of HLA A*02-DRB1*15, A*02-DQB1*06, B*62-C*09 and A*02-DRB1*15-DQB1*06 were significantly higher in group I subjects (8.5%, 10.3%, 8.2%, 6.8%) than those in group III subjects (1.3%, *P* = 0.002; 1.6%, *P* = 0.001; 0.6%, *P* < 0.0001, *P* < 0.0001). The allelic frequencies of HLA DQB1*06-DPB1*05 and DRB1*15-DQB1*06-DPB1*05 were significantly higher in group I subjects (16.0%, 10.5%) than those in group II subjects (2.5%, *P* = 0.001; 0%, *P* = 0.001), while the frequencies of DRB1*09-DPB1*05 and DRB1*09-DQB1*0303-DPB1*05 were significantly lower in group I subjects

Table 2. Two-locus haplotype analysis of HLA alleles in TDI-induced asthma patients compared to asymptomatic exposed subjects and normal control subjects

Haplotypes	Haplotype frequencies (%)			<i>P</i> -value	
	TDI (<i>n</i> = 110)	AEC (<i>n</i> = 94)	NC (<i>n</i> = 190)	TDI vs. AEC	TDI vs. NC
A-B					
02-62	5.3	5.6	0.6	NS	0.010
A-C					
02-09	7.1	2.2	1.8	NS	0.021
02-10	6.8	6.4	2.2	NS	0.047
11-01	5.8	0.0	0.6	0.018	0.005
A-DRB					
02-15	8.5	5.3	1.3	NS	0.002
A-DQB					
02-06	10.3	7.8	1.6	NS	0.001
B-C					
62-09	8.2	3.6	0.6	NS	<0.0001
DQB-DPB					
06-05	16.0	2.5	11.6	0.001	NS
08-02	11.6	8.4	4.9	NS	0.032
DRB-DPB					
04-02	13.8	8.2	3.3	NS	0.049
09-05*	0.0	7.4	3.5	0.004	NS
15-05	10.6	0.0	2.5	0.001	0.003

*Protective allele.

TDI, toluene diisocyanate-induced asthma patients; AEC, asymptomatic exposed controls; NC, normal controls.

Table 3. Three-locus haplotype analysis of HLA alleles in TDI-induced asthma patients compared with asymptomatic exposed subjects and normal control subjects

Haplotypes	Haplotype frequencies (%)			P-value	
	TDI (n = 110)	AEC (n = 94)	NC (n = 190)	TDI vs AEC	TDI vs NC
A-DRB-DQB					
02-01-05	6.3	0.0	2.6	0.013	NS
02-12-0301*	0.0	4.3	5.1	0.029	0.017
02-15-06	6.8	3.2	0.0	NS	<0.0001
DRB-DQB-DPB					
09-0303-05*	0.0	7.5	3.3	0.004	NS
15-06-05	10.5	0.0	3.2	0.001	0.009

*Protective allele.

TDI, toluene diisocyanate-induced asthma patients; AEC, asymptomatic exposed controls; NC, normal controls.

(0%, 0%) than those in group II subjects (7.4%, $P = 0.004$; 7.5%, $P = 0.004$). These differences remained statistically significant even after correction was made for multiple comparisons.

Discussion

The pathogenic mechanism of TDI-induced asthma is currently not completely understood. Although there has been a few studies with Caucasian population on the genetic mechanism of TDI-induced asthma, the results are still controversial. The previous European study performed on 142 patients with TDI-induced asthma and 50 asymptomatic exposed controls demonstrated that HLA class I alleles were not significantly associated with the phenotype of TDI-induced asthma (7). Other studies have used high resolution technique to analyze HLA class II alleles for comparing TDI-induced asthma subjects and asymptomatic exposed controls. They have demonstrated that one HLA class II allele (DQB1*0503) and one haplotype (DQB1* 0201-0301) were significantly associated with the phenotype of TDI-induced asthma (4, 5), yet based on a German population, no association was found for the HLA class II allele (6). In this study, when we analyzed high resolution HLA markers: two class I alleles (C*0303, C*0602) and three class II alleles

(DRB1*0403, DRB1*1501, DQB1* 0602) seemed to be possible susceptibility gene markers for the development of TDI-induced asthma, but their statistical significances disappeared after correction was made for multiple comparisons. Moreover, based on high resolution data (data was not shown), significant alleles were not found on the two-locus or three-locus haplotype analysis. Therefore, we adopted the low resolution data on the HLA class I and II alleles for this study, and we extended the two and three-locus haplotype analysis to include the HLA class I and II alleles. Among all the HLA class I and II alleles, no single allele had any significant association with the TDI-induced asthma phenotype in our Korean study population, and this may have been because the number of study subjects was not large enough to present a strong single gene marker for this phenotype. Further investigation with a larger group of subjects will be needed to identify a single HLA allele for this condition.

The value of this study was that we analyzed both HLA class I and II alleles in all the study subjects; secondly, we enrolled two control groups: the asymptomatic exposed subjects and unexposed healthy nonatopic control subjects; thirdly, haplotype analysis was used to try to identify susceptible and protective gene markers. Although significant associations were not found on the single allele analysis, two and three locus haplotype analysis demonstrated several significant alleles as a potential susceptible or protective markers. Among them, we suggest that the HLA haplotype DRB1*15-DPB1*05 may be a useful marker for predicting the development of TDI-induced asthma in the Korean population.

Future studies will be needed to evaluate any HLA gene markers for their association with such immunologic markers as serum specific IgE and IgG antibodies to the TDI-human serum albumin conjugate in the exposed subjects, and how these gene markers could be involved in the pathogenic mechanisms of asthma.

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