

Short communication

The association between tumor necrosis factor, HLA-DR alleles, and IgE-mediated asthma in Taiwanese adolescents

Background: Human leukocyte antigen (HLA) DR genes and the tumor necrosis factor (TNF) gene locus are associated with asthma and IgE production. TNF α -308G/A frequencies between Japanese and Caucasians in the UK have been found to be different. The roles of HLA-DRB1 and TNF genotypes are unknown in Taiwanese adolescents with IgE-mediated asthma (I-asthma).

Methods: From the population of a 1996 nation-wide survey, we recruited a random sample for a physical examination, determination of total serum IgE (sIgE), dust-mite-specific IgE, and HLA-DRB1, TNF α -308, and LT α NcoI polymorphisms by polymerase chain reaction.

Results: A total of 80 I-asthmatics and 69 non-asthmatics completed the study. We suggested that I-asthmatics had a higher frequency of the DR13 gene (OR = 8.6, 95% CI = [1.6–161]). The DR13 gene was associated with high sIgE and high dust-mite-specific IgE, especially *Dermatophagoides farinae*. No TNF haplotype or genotype was associated with I-asthma. The DR13 gene was linked to the LT α NcoI*1 allele. When sIgE was adjusted by multiple logistic regression, the risk of I-asthma was much higher for the DR13(+)/LT α NcoI*1 haplotype (OR = 25.6, 95% CI = [2.2–1378]) than for the others.

Conclusions: In Taiwanese children sensitized to *Der f*, the DR13(+)/LT α NcoI*1 haplotype was associated with a much higher risk of having clinical asthma than any other DR13/LT α NcoI haplotype.

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IgE is known to play an important role in the pathogenesis of IgE-mediated asthma (I-asthma) (1). HLA-DR genes have been associated with high total serum IgE (sIgE) and IgE against allergens (2,3), and lymphotoxin- α (LT α ; TNF β) with IgE production (4). Even though HLA-DR genes and the tumor necrosis factor (TNF) gene locus, TNF α -308 (promoter variant at position-308) genotypes, and LT α NcoI genotypes in the first intron were associated with asthma and located on chromosome 6p21.1–21.3 (5,6), the relationships between HLA-DR, the TNF gene locus, and sIgE remained unclear.

Caucasian families in England and Australia have been used to prove that TNF α -308*A (TNF2)/LT α NcoI*1 haplotypes with or without an HLA-DR allele were associated with allergic (A-asthma) (7,8). While the subjects were independent, the A-asthma risks for the

two identified bi-allelic polymorphisms (TNF1 and LT α NcoI*2) were different (9). Another study reported that the allele frequency of TNF α -308*A/G was different between Japanese and Caucasians in the UK (10). A study in Singapore collected unrelated subjects and showed that TNF haplotypes or alleles were not associated with A-asthma in Malays and local Chinese (11). However, it did not present any data linking TNF genes or alleles and HLA-DRB1. The roles of HLA-DRB1 and TNF genotypes are unknown in Taiwanese adolescents. Therefore, a case-control was designed to determine the roles of HLA-DRB1 and the TNF gene locus on I-asthma. In a previous study, we found that sensitization to dust mite was an important risk factor for Taiwanese children with I-asthma (12). Our next endeavor, the present study, was to [i] determine the relationship between HLA-DRB1 alleles and I-asthma or dust-mite-specific IgE/sIgE, [ii] determine the association between TNF α -308/LT α NcoI polymorphisms and I-asthma as well as HLA-DRB1 alleles, and [iii] statistically analyse the relationship between [i] and [ii] in asthmatic vs. non-asthmatic children.

Abbreviations. ci: confidence interval, HLA: human leukocyte antigen, IgE: immunoglobulin E, l: liter, LT: lymphotoxin, ml: milliliter, OR: odds ratio, PCR-SSP: polymerase chain reaction with sequence specific primers, s: second, sIgE: total serum IgE, SE: standard error, TNF: tumor necrosis factor, μ l: microliter

Material and methods

Study population and subjects

A total of 149 independent subjects were identified from the population of an October 1996 nation-wide survey in Tainan city and county, as previously described (12). The mean age of all children was 13.9 years (range 13–15 years). The study protocols were approved by our institution's review board.

Immunological assays and genotyping

Total serum IgE was determined by Pharmacia Phadiatop assay (Pharmacia Diagnostics, Uppsala, Sweden). The RAST-FEIA-CAP system (Pharmacia) was used to determine the IgE against two common allergens in Taiwan, including *Dermatophagoides pteronyssinus* (*Der p*), and *Dermatophagoides farinae* (*Der f*).

After standard genomic DNA extraction from whole blood, the HLA-DRB1 genotyping by polymerase chain reaction (PCR) with sequence-specific primers was modified from those described previously (13,14). A total reaction volume of 25 µl with 0.1 µl of Taq DNA polymerase (5 U/µl, supertherm; Hoffmann-La Roche, Nutley, NJ, USA) conformed to the manufacturer's instructions. A pair of primers that amplified the human growth hormone gene was also included in each reaction as the internal positive control for PCR amplification. The PCR reaction program was 5 min at 94°C followed by 15 cycles at 94°C for 20 s, 30 s at 66°C, 30 s at 72°C, 25 cycles at 94°C for 20 s, 30 s at 61°C, 30 s at 72°C followed by 2 min at 72°C. The 12 µl of PCR products were analysed in 2% agarose gel. The PCR conditions of the TNFα-308/LTαNcoI polymorphisms were adapted from previous studies (8,15) with minor modification. Twenty percent of the genotype population was randomly checked for TNFα, LTα, and HLA-DR genes. When there was disagreement about a genotype, the samples were repeatedly tested until agreement was reached.

Statistical analysis

The Pearson chi-squared test or Fisher's exact test was applied to analyse the difference in frequency between two groups. The Wilcoxon rank sum test was used to determine the difference in sIgE and allergen-specific IgE between two groups. Multiple logistic regression was applied to test the association between predictors and outcome. *P*-values or *P_c*-values (*P*-values corrected for the number of alleles observed) < 0.05 were considered statistically significant.

Results

No differences in the IgE or frequencies of HLA-DRB1 alleles and TNF genotypes were found between I-asthmatics and those in remission. For further analysis, we combined the data of these two groups. I-asthmatics had a higher HLA-DR13 allele frequency and a lower HLA-DR7 allele frequency than non-asthmatics, but the *P_c*-value was not statistically significant (Table 1). However, no similar difference was found in the other HLA-DRB1 alleles.

By the Wilcoxon rank sum test, only children with the DR13 gene had a higher sIgE (mean ± SE; 1172.7 ± 396.0 vs. 412.8 ± 37.0, *P* = 0.015, *P_c* = NS), and IgE against *Der p* (73.3 ± 12.4 vs. 34.8 ± 3.3, *P* = 0.0083, *P_c* = NS) and *Der f* (70.2 ± 12.3 vs. 29.1 ± 2.9, *P* = 0.0034,

Table 1. Distribution of HLA-DRB1 alleles in asthmatic and non-asthmatic children

HLA-DRB1	Asthmatic children <i>n</i> = 80 (%)	Non-asthmatic children <i>n</i> = 69 (%)	<i>P</i>	<i>P_c</i>
DR4	16 (20.0)	17 (24.6)	NS	NS
DR7‡	1 (1.3)	8 (11.6)	0.012	NS
DR8	18 (22.5)	15 (21.7)	NS	NS
DR9	24 (30.0)	14 (20.3)	NS	NS
DR11	17 (21.3)	13 (18.8)	NS	NS
DR12	14 (17.5)	15 (21.7)	NS	NS
DR13‡	9 (11.3)	1 (1.5)	0.021	NS
DR14	14 (17.5)	11 (16.0)	NS	NS
DR15	13 (16.3)	11 (16.0)	NS	NS
DR16	12 (15.0)	13 (18.8)	NS	NS
DR17	15 (18.8)	12 (17.4)	NS	NS

For DR7, Odds ratio (OR) = 0.1, 95% confidence interval (CI) = [0.05–0.6].

For DR13, OR = 8.6, 95% CI = [1.6–161].

Those genes with <5% frequency were not shown (DR1 and DR10).

‡ *P_c*, corrected for the number of alleles tested (13).

‡ *P*-value was calculated by Fisher's exact test. All others were calculated with Pearson's chi-squared test.

NS, not significantly different.

P_c = 0.044) than those without, but only the *P_c*-value for *Derf*-specific IgE was statistically significant. Children with the DR10 gene had a higher level of IgE against *Der p* (*P* = 0.023, *P_c* = NS) but the *P_c*-value was not statistically significant. No such association was found in the other HLA-DRB1 alleles.

No TNF2.2 genotype was found in the present study. TNF1 was genetically linked to LTαNcoI*1 (Table 2). No association was found between I-asthma and genotypes of the TNF gene locus or TNF haplotype. The DR13 gene was not genetically linked to TNF1/2, but it was linked to LTαNcoI*1 (Table 2). However, the DR13/LTαNcoI*1 haplotype was not associated with TNF1/2.

By multiple logistic regression, the risk of I-asthma was compared for children with the DR13 gene and LTαNcoI genotypes while adjusting for sIgE. The DR13(+)/LTαNcoI*1 haplotype had an OR nearly 26-fold that of DR13(-)/LTαNcoI*2 (Table 3). DR13(-)/LTαNcoI*2 had an OR of 0.1 (95% CI = [0.01–0.5]), 10% that of DR13(-)/LTαNcoI*1 (data not shown).

Table 2. Relationship between TNFα-308, the LTαNcoI polymorphism, and the DR13 gene

	<i>N</i>	TNF-α		LTα	
		-308*1.2 (%)	-308*1.1 (%)	NcoI*1.1/1.2 (%)	NcoI*2.2 (%)
DR13(+)	10	40.0	60.0	100.0	0.0
DR13(-)	139	18.7	81.3	68.4	31.6
LTαNcoI*1.1/1.2	105	27.6	72.4		
LTαNcoI*2.2	44	2.3	97.7		

The DR13 gene was linked to the LTαNcoI*1 allele, *P* = 0.034 (Fisher's exact test). The LTαNcoI*1 allele was linked to the TNF1 allele, *P* = 0.0002 (Fisher's exact test).

Table 3. Risk factors for asthma by multiple logistic regression

	Asthmatic children (n = 80)	Non-asthmatic children (n = 69)	Odds ratio	95% Confidence interval
Total IgE				
≤ 100 IU/ml	9	29	1.0	
> 100 IU/ml	71	40	6.0	[2.6–15.4]
DR13/LT α				
(-)/Ncol*2	18	26	1.0	
(-)/Ncol*1	53	42	0.4	[0.04–1.5]
(+)/Ncol*1	9	1	25.6	[2.2–1378]

Discussion

Our study suggested that the frequency of the DR7 gene (DR7) is lower and that of the DR13 gene (DR13) is higher in I-asthmatics than in non-asthmatics. DR13 was associated with high dust-mite-specific IgE levels and sIgE. No TNF haplotypes or genotypes were associated with I-asthma. LT α Ncol*1 was linked to DR13. The DR13/LT α Ncol*1 haplotype was an important risk factor for I-asthmatics sensitized to *Der f*.

The frequencies of HLA-DRB1 alleles in this study were compatible with those reported by a Marrow Donor Registry in Taiwan (16,17). A study of that Registry showed that DR7 was less common in Taiwanese (1.9%) than in Caucasians (15.1%) and African-Americans (9.1%). In the present study, I-asthmatics had a lower DR7 frequency than non-asthmatics, but the *P*-value was not statistically significant. No association was found between DR7 and sIgE or dust-mite-specific IgE. A study in Germany (18) showed DRB1*0701 was associated with high IgE against *Hev b 1* and the frequency was higher in sensitized patients. A study in the UK (19) reported a strong association between DRB1*0701 and high sIgE (*P* < 0.001). In addition, a Korean study (20) showed that the frequency of DRB1*07 was higher in citrus red mite-sensitive asthmatics than in controls (17.6% vs. 4.1%, *P* = 0.01). Various genetic and environmental backgrounds might account for different findings associated with DR7 in different studies.

In the present study, the association between DR13 and high *Der f*-specific IgE and sIgE was compatible with previous studies reporting that HLA-DRB1 (2) or HLA-DRB (3) genes could control the mite-specific IgE levels and sIgE. This is the first paper reporting a relationship between DR13 and dust-mite-specific IgE. In atopic subjects with sIgE > 8000 IU/ml, DR13 prevalence was higher than in non-atopic subjects (21). The association between DR13 and elevated *Der f*-specific IgE in this

study supports the finding of the relationship between this gene and occurrence of asthma.

The association between the LT α Ncol genotype and A-asthma has been shown (5), but we found no such relationship. We tested the hypothesis that the association was caused by the association between DR13 and I-asthma, and DR13 in linkage disequilibrium with the LT α Ncol*1 allele. In theory, there should be four groups of haplotypes: DR13(+)/LT α Ncol*1, DR13(+)/LT α Ncol*2, DR13(-)/LT α Ncol*1, and DR13(-)/LT α Ncol*2. In the present study, all children with the DR13 gene had at least one LT α Ncol*1 allele. Therefore, no children with DR13(+)/LT α Ncol*2 were found. When sIgE was adjusted by multiple logistic regression (Table 3), the risk of I-asthma was much higher with DR13(+)/LT α Ncol*1 (OR = 25.6, 95% CI = [2.2–1378]) than with the others. This association could be contributed by DR13 and synergy between two alleles. However, it was impossible for us to distinguish between the effects of the HLA-DR13 allele and the LT α Ncol*1 alleles.

This study found no TNF2.2 genotype. This was compatible with two previous studies which demonstrated that the frequency of TNF2.2 was very rare in Asian populations (10,11). No association was found between the TNF1/2 allele and I-asthma but TNF1 was in linkage disequilibrium with LT α Ncol*1. Chagani et al. (22) reported that prevalence of TNF α -308 genotypes was associated with A-asthma severity. In order to narrow down the research for the relationship between TNF haplotypes or alleles and gene functions, further investigation on the association between the haplotype (TNF1/LT α Ncol*1) or alleles and the phenotype of moderate to severe asthma is necessary.

In conclusion, we suggest that I-asthmatics have a higher allele frequency of DR13 than non-asthmatics. DR13 was associated with *Der f*-specific IgE level and was in linkage disequilibrium with the LT α Ncol*1 allele. In Taiwanese children sensitized to *Der f*, the DR13(+)/LT α Ncol*1 haplotype was associated with a much higher risk of having clinical asthma than any other DR13/LT α Ncol haplotype.

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