

The human leucocyte antigen-DRB1*1302-DQB1*0609-DPB1*0201 haplotype may be a strong genetic marker for aspirin-induced urticaria

S.-H. Kim*, J.-H. Choi*, K.-W. Lee†, S.-H. Kim*, E.-S. Shin‡, H.-B. Oh§, C.-H. Suh*, D.-H. Nahm* and H.-S. Park*

*Department of Allergy and Rheumatology, Ajou University Hospital, Suwon, Korea, †Department of Laboratory Medicine, Hallym Institute for Genome Application, Hallym University, Anyang, Korea, ‡Statistical Research Institute and §Department of Laboratory Medicine, University of Ulsan College of Medicine, Seoul, Korea

Summary

Background Urticaria/angioedema is a common aspirin-induced allergy; however, its pathogenic mechanism is not understood.

Objective In order to uncover the genetic mechanism, we studied the associations of the human leucocyte antigen (HLA) genotypes in patients with aspirin-induced urticaria compared with aspirin-intolerant asthma and normal control in a Korean population.

Methods Ninety-four aspirin-induced urticaria patients presenting urticaria/angioedema-induced by both ASA and NSAID (50 had underlying chronic urticaria) and showing positive responses on oral aspirin challenge test, 76 aspirin-intolerant asthmatics with positive responses on lysine–aspirin bronchoprovocation test, and 185 normal healthy controls were enrolled. HLA-DRB1, DQB1, and DPB1 genotyping were performed by direct DNA sequencing analysis.

Results The allele frequencies of HLA-DRB1*1302 (18.1%) and HLA-DQB1*0609 (10.1%) in aspirin-induced urticaria were significantly higher than in aspirin-intolerant asthma (5.3%, $P = 0.0004$; 2.0%, $P = 0.0024$) and in normal controls (8.1%, $P = 0.0005$; 3.2%, $P = 0.0008$), and they remained significant after correcting for multiple comparisons. The patients with these two HLA markers had a significantly younger age than patients without, while no associations were found in with respect to atopic status, a history of previous allergic diseases, total IgE level, or presence of underlying chronic urticaria ($P > 0.05$, respectively). In haplotype analysis, the HLA-DRB1*1302-DQB1*0609-DPB1*0201 was significantly higher in the aspirin-induced urticaria (8.0%) than in the aspirin-intolerant asthma (0.7%, $P = 0.0014$) and normal controls (2.0%, $P = 0.0006$).

Conclusion These findings suggest that the HLA-DRB1*1302-DQB1*0609-DPB1*0201 may be a strong genetic marker to determine the aspirin-induced urticaria phenotype.

Keywords aspirin, HLA-DPB1*0201, HLA-DQB1*0609, HLA-DRB1*1302, urticaria

Submitted 2 July 2004; revised 6 December 2004; accepted 14 January 2005

Introduction

The prevalence of urticaria/angioedema because of aspirin (ASA)/nonsteroidal anti-inflammatory drugs (NSAIDs) in the general populations is not known, but cutaneous reactions because of NSAIDs are estimated to occur in 0.1–3% of patients consuming these drugs [1, 2]. In susceptible individuals, ingestion of ASA/NSAIDs can induce acute urticaria or aggravate urticaria in chronic urticaria patients [3].

Although the pathogenesis of ASA-induced urticaria is not fully understood, a cyclooxygenase (COX) theory has been suggested [4, 5]. This theory proposes that urticaria can be triggered by the inhibition of the COX-1 enzyme. An IgE-mediated response was reported in a case of ASA-induced urticaria [6].

Regarding the genetic mechanism of urticaria, one report demonstrated an association between human leucocyte antigen (HLA) and chronic urticaria [7]. However, no suggestion has been made concerning HLA association in specific patient groups, such as those with urticaria-induced by ASA/NSAIDs.

In this study, to explore the association between HLA and the development of ASA-induced urticaria in a Korean population, we performed HLA-DRB1, DQB1, and DPB1 genotyping in 94 ASA/NSAID-induced urticaria patients and compared the results obtained to those of ASA-intolerant asthma patients and normal controls. Clinical characteristics were compared with HLA genotypic associations.

Materials and methods

Subjects

Ninety-four patients with urticaria sensitive to both ASA and NSAIDs (42 male subjects; mean age: 34.5 years; 50 had

Correspondence: Dr Hae-Sim Park, Department of Allergy and Rheumatology, Ajou University Hospital, Yongtonggu Woncheondong San-5, Suwon 442-749, Korea.

E-mail: hspark@ajou.ac.kr

underlying chronic urticaria with more than 6 weeks duration), 76 patients with ASA-intolerant asthma (21 male subjects, mean age: 46.5 years), and 185 normal healthy controls enrolled from the department of Allergy and Rheumatology, Ajou University Hospital, Suwon, Korea were enrolled in the study. In this study, ASA-induced urticaria group was defined as patients having a certain history of urticaria/angioedema development after the ingestion of more than two kinds of NSAIDs and positive responders on oral ASA challenge test (classified as cross reacting group by Sanchez-Borges et al. [3]). Also NSAID sensitivity could be confirmed because the patients visited our Allergy Clinic or emergency room presenting current urticaria/angioedema after taking NSAIDs. In order to exclude a single ASA-induced urticaria, we performed skin prick test (SPT) with 10 mg/mL of lysine-ASA (L-ASA) and none of them had positive SPT. ASA-intolerant asthma was diagnosed by a positive result to L-ASA bronchoprovocation testing. 185 normal controls were enrolled at random from a local population. They were healthy and had no history of atopic diseases or drug allergies including ASA and NSAIDs. Twenty-nine (38.2%) patients among the ASA-induced urticaria group and 38 (30.5%) in ASA-intolerant asthma patients were atopic. All subjects provided informed consent and the protocol used were approved by the ethics committee of Ajou University Hospital, Suwon, Korea.

Allergy skin prick test

SPTs were performed using 12 common aeroallergens (Bencard, Middlesex, UK) including *Dermatophagoides pteronyssinus*, *Dermatophagoides farine*, cat, dog, cockroach, tree pollen mixture, grass pollen mixture, mugwort, ragweed, *Hop Japanese*, *Aspergillus*, *Alternaria*, histamine, and a saline control. SPT with L-ASA solution (10 mg/mL, Althargyl[®], Arthromedica, Ilyang Co., Seoul, Korea) was performed in all the study subjects to exclude a possible IgE-mediated response. Reactions were read after 15 min, and wheals were measured in two directions. A positive reaction was defined when a mean wheal diameter exceeded 3 mm. Atopy was defined as one or more positive reactions by skin prick testing.

Oral ASA challenge and L-ASA bronchoprovocation tests

All medications, including theophyllines, β_2 -agonists, leukotriene receptor antagonist, antihistamine and steroids, were withdrawn 72 h before these procedures. After placebo challenge, 500 mg of ASA in a tablet was orally administered, and subjects were observed for 4 h and monitored for urticaria and changes in lung functions every 30 min. Appearance of urticaria within 4 h without any changes in forced expiratory volume in 1 s (FEV₁) was considered a positive result. The L-ASA bronchoprovocation test was performed using a modification of a previously described method [8]. Test solutions were delivered using a DeVilbiss 646 nebulizer (DeVilbiss Co, Somerset, PA, USA) and connected to a compressed air source (5 L/min). L-ASA powder (Althargyl[®], Arthromedica) containing 900 mg of L-ASA and 100 mg of aminoacetic acid was diluted in normal saline to produce a stock solution of 300 mg/mL L-ASA. A placebo solution of

normal saline was used as a control. Subjects inhaled the aerosol via a mouthpiece during normal tidal breathing. The L-ASA solution was inhaled every 30 min. The FEV₁ and the maximum mid expiratory flow (MMEF) were measured 10 and 30 min after each dose. The provocation was stopped when the FEV₁ reduced by 20% or more from the baseline. The FEV₁ and the MMEF were measured at 10, 30, 60 min after the last dose, and then hourly for 7 h.

Genomic DNA preparation and high-resolution human leucocyte antigen genotyping

The HLA-DRB1, DQB1, and DPB1 types of the samples were obtained by direct DNA sequencing. The procedures and primers for this PCR amplification and sequencing of HLA-DRB1, DQB1, and DPB1 have been described previously [9–11].

Statistical analysis

Allele frequencies were calculated using the allele procedure in SAS/Genetics software (SAS Institute Inc, Cary, NC, USA), and frequencies and haplotypes of the two groups were compared using the χ^2 test. *P*-values were corrected for multiple comparisons by multiplying by allele numbers to test for an association with ASA-induced urticaria and ASA-intolerant asthma groups. However in haplotype, a *P*-value of less than 0.005 was considered significant arbitrarily because haplotypes are so numerous that Bonferroni correction was unjustified and ten most frequent haplotypes in ASA-induced urticaria were tested. The clinical characteristics of two groups (ASA-induced urticaria vs. ASA-intolerant asthma) were compared using the χ^2 test and the Student's *t*-test. A *P*-value of 0.05 or less was regarded as significant. Values for all measurements are expressed as means $\pm$ SD.

Results

Human leucocyte antigen-DRB1 alleles (Table 1)

Thirty DRB1 alleles were found during the present study. The frequency of HLA-DRB1*1302 (18.1%) in the ASA-induced urticaria group was significantly higher than in the ASA-intolerant asthma group (5.3%, *P* = 0.0004, odds ratio (OR) = 4.0) and in the normal control group (8.1%, *P* = 0.0005, OR = 2.5). Furthermore, this remained significant after *P*-values were corrected for multiple comparisons (*P*_c = 0.0120, 0.0150). When the ASA-induced urticaria group was divided into two subgroups according to the presence of underlying chronic urticaria, the frequencies of HLA-DRB1*1302 was similar (18.0% vs. 18.2%) and they were significantly higher than those of ASA-intolerant asthma (*P* = 0.001, 0.002) and normal control groups (*P* = 0.005, 0.009). The frequency of HLA-DRB1*0901 (8.0%) in the ASA-induced urticaria group was significantly lower than in the ASA-intolerant asthma group (18.4%, *P* = 0.0040, OR = 0.4), this was not significant noted after correction for multiple comparisons. No significant differences were found for the other DRB1 alleles.

Table 1. Allele frequencies of HLA-DRB1 in the study subjects

DRB1 allele*	ASA-induced urticaria (%) <i>n</i> = 188	ASA-intolerant asthma (%) <i>n</i> = 152	Normal control (%) <i>n</i> = 370	P-value (OR)	
				ASA-induced urticaria vs. AIA	ASA-induced urticaria vs. NC
DRB1*0101	7 (3.7)	3 (2.0)	25 (6.8)	0.0040 (0.4)	
DRB1*0405	17 (9.0)	10 (6.6)	29 (7.8)		
DRB1*0701	13 (6.9)	8 (5.3)	28 (7.6)		
DRB1*0803	14 (7.4)	11 (7.2)	29 (7.8)		
DRB1*0901	15 (8.0)	28 (18.4)	38 (10.3)		
DRB1*1101	14 (7.4)	9 (5.9)	19 (5.1)		
DRB1*1201	7 (3.7)	5 (3.3)	16 (4.3)	0.0004 (4.0)	0.0005 (2.5)
DRB1*1202	7 (3.7)	2 (1.3)	18 (4.9)		
DRB1*1302	34 (18.1)	8 (5.3)	30 (8.1)		
DRB1*1501	7 (3.7)	11 (7.2)	26 (7.0)		

*Ten frequent alleles in the ASA-induced urticaria group are listed.

OR, odds ratio; AIA, aspirin-intolerant asthma; NC, normal control; ASA, aspirin; HLA, human leucocyte antigen.

Table 2. Allele frequencies of HLA-DQB1 in the study subjects

DQB1 allele*	ASA-induced urticaria (%) <i>n</i> = 188	ASA-intolerant asthma (%) <i>n</i> = 152	Normal control (%) <i>n</i> = 370	<i>P</i> -value (OR)	
				ASA-induced urticaria vs. AIA	ASA-induced urticaria vs. NC
DQB1*0501	10 (5.3)	5 (3.3)	30 (8.1)	0.0139 (4.3)	0.0008 (3.4)
DQB1*0503	9 (4.8)	6 (3.9)	15 (4.1)		
DQB1*0601	16 (8.5)	13 (8.6)	34 (9.2)		
DQB1*0604	15 (8.0)	3 (2.0)	18 (4.9)		
DQB1*0609	19 (10.1)	3 (2.0)	12 (3.2)	0.0024 (5.6)	0.0182 (0.5)
DQB1*02	15 (8.0)	16 (10.5)	34 (9.2)		
DQB1*0301	31 (16.5)	20 (13.2)	56 (15.1)		
DQB1*0302	14 (7.4)	21 (13.8)	44 (11.9)		
DQB1*0303	19 (10.1)	29 (19.1)	45 (12.2)	0.0182 (0.5)	
DQB1*0401	16 (8.5)	10 (6.6)	28 (7.6)		

*Ten frequent alleles in the ASA-induced urticaria group are listed.

OR, odds ratio; AIA, aspirin-intolerant asthma; NC, normal control; ASA, aspirin; HLA, human leucocyte antigen.

Human leucocyte antigen-DQB1 alleles (Table 2)

Seventeen DQB1 alleles were noted in this study. The frequency of HLA-DQB1*0609 in ASA-induced urticaria (10.1%) was significantly higher than in ASA-intolerant asthma (2.0%, $P = 0.0024$, OR = 5.6) and than in normal controls (3.2%, $P = 0.0008$, OR = 3.4), this remained significant after P -values were corrected for multiple comparisons ($P_c = 0.0408$, $= 0.0120$). Also the frequencies of HLA-DQB1*0609 in the two subgroups of ASA-induced urticaria depending on underlying chronic urticaria were similar (10.1% vs. 11.1%) and they were significantly higher than those of ASA-intolerant asthma and normal control ($P < 0.05$, respectively). Although HLA-DQB1*0604 was significantly higher in ASA-induced urticaria (8.0%) than in ASA-intolerant asthma (2.0%, $P = 0.0139$, OR = 4.3), this was not significant after the correction for multiple comparisons. The frequency of DQB1*0303 (10.1%) in the ASA-induced urticaria group was significantly lower than in ASA-intolerant asthma (19.1%, $P = 0.0182$, OR = 0.5), this was not significant after correction for multiple comparisons. No significant differences in other DQB1 alleles were observed.

Human leucocyte antigen-DPB1 alleles (Table 3)

Nineteen DPB1 alleles were found. The frequency of HLA-DPB1*0301 was 5.9% in the ASA-induced urticaria group and 13.8% in the ASA-intolerant asthma group ($P = 0.0124$, OR = 0.4), and the frequencies of DPB1*0501 (28.7%, $P = 0.0280$, OR = 0.7) and DPB1*1301 (3.2%, $P = 0.0324$, OR = 0.4) were significantly lower in ASA-induced urticaria than in the normal controls (38.1%, 7.8%) however, this was not significant after correction for multiple comparisons. No significant differences were observed in the other DPB1 alleles.

Comparisons of the clinical characteristics of patients with HLA-DRB1*1302 and/or DQB1*0609 and those without (Table 4)

To investigate the relation between these two gene markers and the characteristics of the ASA-induced urticaria group, the clinical features of patients carrying or not carrying DRB1*1302 and/or DQB1*0609 were compared. Within the ASA-induced urticaria group patients with HLA-DRB1*1302

Table 3. Allele frequencies of HLA-DPB1 in the study subjects

DPB1 allele*	ASA-induced urticaria (%) <i>n</i> = 188	ASA-intolerant asthma (%) <i>n</i> = 152	Normal control (%) <i>n</i> = 370	<i>P</i> -value (OR)	
				ASA-induced urticaria vs. AIA	ASA-induced urticaria vs. NC
DPB1*0201	54 (28.7)	39 (25.7)	93 (25.1)	0.0124 (0.4)	
DPB1*0202	11 (5.9)	5 (3.3)	16 (4.3)		
DPB1*0301	11 (5.9)	21 (13.8)	11 (3.0)		
DPB1*0401	18 (9.6)	8 (5.3)	26 (7.0)		
DPB1*0402	18 (9.6)	10 (6.6)	29 (7.8)	0.0280 (0.7)	
DPB1*0501	54 (28.7)	48 (31.6)	141 (38.1)		
DPB1*0901	3 (1.6)	3 (2.0)	8 (2.2)		
DPB1*1301	6 (3.2)	8 (5.3)	29 (7.8)		
DPB1*1401	4 (2.1)	3 (2.0)	3 (0.8)	0.0324 (0.4)	
DPB1*1701	6 (3.2)	5 (3.3)	5 (1.4)		

*Ten frequent alleles in the ASA-induced urticaria group are listed.

OR, odds ratio; AIA, aspirin-intolerant asthma; NC, normal control; ASA, aspirin; HLA, human leucocyte antigen.

Table 4. Comparison of the clinical findings depending on two HLA gene markers (DRB1*1302, DQB1*0609) in 94 ASA-induced urticaria patients

Clinical parameter	DRB1*1302		<i>P</i> -value	DQB1*0609		<i>P</i> -value
	Positive (<i>n</i> = 30)	Negative (<i>n</i> = 64)		Positive (<i>n</i> = 19)	Negative (<i>n</i> = 75)	
Age*	28.2 ± 7.0	38.0 ± 13.6	<0.0001	29.0 ± 6.9	36.4 ± 13.4	0.001
Sex (M/F)	17/13	25/39	0.11	12/7	30/45	0.08
Atopy (%)	25 (86.2%)	44 (77.2%)	0.39	17 (94.4%)	52 (76.5%)	0.11
Total IgE (IU/mL)*	392.0 ± 343.4	267.2 ± 290.5	0.10	436.5 ± 360.4	276.0 ± 293.5	0.06
History of allergic diseases (%)	15 (50.0%)	26 (40.6%)	0.39	8 (42.1%)	33 (44.0%)	0.88
Underlying chronic urticaria (%)	16 (53.3%)	34 (53.1%)	1.00	9 (47.4%)	41 (57.4%)	0.49

*Mean ± SD.

M, male; F, female; ASA, aspirin; HLA, human leucocyte antigen.

and DQB1*0609 had a significantly younger age ($P < 0.0001$) and tended to have a higher serum total IgE level than those without (Table 4). No significant differences were observed for atopy, gender, a history of previous allergic diseases, or the presence of underlying chronic urticaria in the two subgroups ($P > 0.05$, respectively).

Haplotype analysis

The 10 most common haplotypes at the HLA-DRB1-DQB1-DPB1 loci with more than 2.5% of the prevalence in the ASA-induced urticaria group are shown in Table 5. The HLA-DRB1*1302-DQB1*0609-DPB1*0201 haplotype was significantly higher in the ASA-induced urticaria group (8.0%) than in the ASA-intolerant asthma group (0.7%) and in the normal control group (2.0%) ($P = 0.0014$, 0.0006), while DRB1*0901-DQB1*0303-DPB1*0501 was significantly lower (4.0%) in the ASA-induced urticaria group than in the ASA-intolerant asthma group (12.0%, $P = 0.0050$).

Discussion

Acute and chronic urticaria are common among the various manifestations of ASA/NSAIDs-induced allergic symptoms [3]. Although no published data are available on the prevalence of ASA sensitivity among patients with acute urticaria, it has

been reported that one-third of patients with chronic urticaria was associated with ASA sensitivity [12]. In this study, in order to investigate the genetic mechanism involved, we studied the association between HLA genotypes in confirmed cases of ASA-induced urticaria who had cross-sensitivity to ASA and NSAIDs, and these findings were compared with those of ASA-intolerant asthma and normal controls.

The expressions of MHC class II molecules were increased on the surfaces of infiltrating cells found in the skin tissue of urticaria patients [13]. HLA class II (HLA-DR, DQ, and DP) antigens on chromosome 6 are central to the immune processing of exogenous antigens [14] and many investigators have tried to identify HLA gene markers in ASA related allergies. We recently reported that HLA-DPB1*0301 as a strong gene marker in the Oriental population with ASA-intolerant asthma [15], which may be in accord with observations in Caucasian population [16]. Another study of patients with an anaphylactic reaction induced by NSAID [17], also reported a positive association with HLA-DR11.

The present study is the first to demonstrate an association between HLA genotype and ASA-induced urticaria by using high-resolution analysis technique, and it confirms a strong positive association between the HLA-DRB1*1302 and DQB1*0609 alleles in ASA-induced urticaria. In this study, when the ASA-induced urticaria group was divided into two subgroups depending on presence of underlying chronic urticaria, the frequencies of these two HLA gene markers

Table 5. Haplotype analysis of HLA-DRB1, DPB1, DQB1 in ASA-induced urticaria patients compared to ASA-intolerant asthmatics and normal controls

					P-value	
Haplotypes*		AIU (%) (n = 188)	AIA (%) (n = 152)	NC (%) (n = 370)	AIU vs. AIA	AIU vs. NC
DRB-DQB-DPB						
1.	1302-0609-0201	8.0	0.7	2.0	0.0014	0.0006
2.	1302-0604-0401	6.8	1.3	2.9	0.0137	0.0285
3.	0405-0401-0501	5.0	3.3	4.9	0.4320	0.7744
4.	0803-0601-0501	4.6	1.5	4.4	0.1056	0.4271
5.	0901-0303-0501	4.0	12.0	5.6	0.0050	0.2177
6.	0701-02-1701	3.2	2.6	1.1	0.7607	0.0850
7.	0901-0303-0201	2.8	4.9	2.9	0.3150	0.4016
8.	1202-0301-0501	2.7	0.0	2.3	0.1360	0.6718
9.	0101-0501-0402	2.7	0.0	0.6	0.1050	0.0511
10.	0701-02-0201	2.7	0.0	2.9	0.1480	0.7524
DRB-DQB						
1.	1302-0609	10.1	2.0	3.2	0.0024	0.0008
2.	1302-0604	8.0	2.0	4.9	0.0139	0.1405
3.	0901-0303	8.0	17.8	10.3	0.0064	0.3829
DRB-DPB						
1.	1302-0201	8.4	1.4	3.1	0.0037	0.0067
2.	1302-0401	7.4	1.9	3.1	0.0192	0.0196
3.	0901-0501	4.1	11.6	5.6	0.0070	0.2353
DQB-DPB						
1.	0609-0201	7.8	1.3	2.1	0.0057	0.0010
2.	0604-0401	6.9	0.8	2.9	0.0064	0.0253
3.	0303-0501	5.8	12.7	5.4	0.0236	0.7733

*Ten frequent 3-locus haplotypes in the ASA-induced urticaria group are listed in frequency order.

AIU, aspirin-induced urticaria; AIA, aspirin-intolerant asthma; NC, normal control; ASA, aspirin; HLA, human leucocyte antigen.

were identical. Thus, these alleles are potential risk markers of susceptibility to ASA-induced urticaria, particularly in patients having cross sensitivity to ASA/NSAIDs, which may be clinically useful in the early recognition of potential ASA sensitivity in adult patients suffering from acute and chronic urticaria. Also, these findings suggest that among patients suffering from ASA related allergic symptoms, those carrying the HLA-DPB1*0301 could present the ASA-intolerant asthma phenotype, while those carrying DRB1*1302 and/or DQB1*0609 could present the ASA-induced urticaria phenotype, while not being associated with asthma or anaphylaxis in the Korean population. These HLA differences between two major ASA related allergies may represent the target organ specificity rather than underlying mechanism. Further studies will be needed to investigate the molecular mechanism to be involved.

The clinical findings of patients carrying these two HLA markers showed a younger age and a tendency to have a higher serum total IgE level. Other clinical parameters could not be differentiated between these two subgroups. These findings suggest that patients with these gene markers may develop ASA-induced urticaria at an earlier age.

Regarding the relationship between these two HLA markers, in the study subjects, 63.3% of the ASA-induced urticaria patients carrying HLA-DRB1*1302, had DQB1*0609, while all patients carrying DQB1*0609 had DRB1*1302, thus showing strong associations between these

two markers (data not shown, $P < 0.0001$), which is due these two HLA markers having a strong linkage disequilibrium in the Korean population [18]. Further studies will be needed to investigate how these HLA markers are involved in the clinical manifestation of ASA-induced urticaria.

The present HLA haplotype analysis also demonstrates that the HLA-DRB1*1302-DQB1*0609-DPB1*0201 haplotype presents a significant risk for the development of ASA-induced urticaria. DPB1*0201 alone was not significantly associated with ASA-induced urticaria. This may be because of the increased power of detecting associations by haplotype analysis [19, 20] or to an additive effect of the two alleles in terms of the development of ASA-induced urticaria.

In conclusion, we suggest that HLA-DRB1*1302-DQB1*0609-DPB1*0201 may be a strong genetic marker of the ASA/NSAID-induced urticaria phenotype.

Acknowledgements

This study was supported by a grant of the Korean Health 21 R&D project, Ministry of Health & Welfare, Republic of Korea (01-PJ10-PG6-01GN14-0007).

References

- 1 Stevenson DD. Diagnosis, prevention, and treatment of adverse reactions to aspirin and nonsteroidal anti-inflammatory drugs. *J Allergy Clin Immunol* 1984; 74:617–22.
- 2 Samter M, Beers Jr RF. Concerning the nature of intolerance to aspirin. *J Allergy* 1967; 40:281–93.
- 3 Sanchez-Borges M, Capriles-Hulett A, Caballero-Fonseca F. Cutaneous reactions to aspirin and nonsteroidal anti-inflammatory drugs. *Clin Rev Allergy Immunol* 2003; 24:125–36.
- 4 Szczeklik A. The cyclooxygenase theory of aspirin-induced asthma. *Eur Respir J* 1990; 3:588–93.
- 5 Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis and management. *J Allergy Clin Immunol* 1999; 104:5–13.
- 6 Blanca M, Perez E, Garcia JJ et al. Angioedema and IgE antibodies to aspirin: a case report. *Ann Allergy* 1989; 62:295–8.
- 7 O'Donnell BF, O'Neill CM, Francis DM et al. Human leucocyte antigen class II associations in chronic idiopathic urticaria. *Br J Dermatol* 1999; 140:853–8.
- 8 Park HS. Early and late onset asthmatic responses following lysine-aspirin inhalation in aspirin-sensitive asthmatic patients. *Clin Exp Allergy* 1995; 25:38–40.
- 9 Blasczyk R. Sequence-based typing for HLA-DRB1 and DRB3/4/5 separating alleles. In: Tilanus MGJ, Hansen JA, Hurley CK, eds. *IHWG technical manual*. Seattle: International Histocompatibility Working Group, 2000, TM10C 1–10.
- 10 Van der Zwan, Griffith B, Rozemuller E, Williams T, Tilanus MG. Sequence-based typing for HLA-DQB1 strategy for ABI sequencing equipment. In: Tilanus MGJ, Hansen JA, Hurley CK, eds. *IHWG technical manual*. Seattle: International Histocompatibility Working Group, 2000, TM12A 1–4.
- 11 Van der Zwan, Rozemuller E, Tilanus MG. Sequence-based typing for HLA-DPB1 strategy for ABI sequencing equipment. In: Tilanus MGJ, Hansen JA, Heuley CK, eds. *IHWG technical manual*. Seattle: International Histocompatibility Working Group, 2000, TM14A 1–4.
- 12 Genton C, Frei PC, Pecoud A. Value of oral provocation tests to aspirin and food additives in the routine investigation of asthma and chronic urticaria. *J Allergy Clin Immunol* 1985; 76:40–5.

- 13 Haas N, Iwen W, Grabbe J, Uchanska-Ziegler B, Czarnetzki BM. MHC class II antigen expression is increased in different forms of urticaria. *Int Arch Allergy Immunol* 1996; 109:177–82.
- 14 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; 29:1024–8.
- 15 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.
- 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 Quirarte J, Sanchez-Garcia F, Torres MJ et al. Association of HLA-DR11 with the anaphylactoid reaction caused by nonsteroidal anti-inflammatory drugs. *J Allergy Clin Immunol* 1999; 103:685–9.
- 18 Song EY, Park MH, Kang SJ et al. HLA class II allele and haplotype frequencies in Koreans based on 107 families. *Tissue Antigens* 2002; 59:475–86.
- 19 Akey J, Jin L, Xiong M. Haplotypes vs single marker linkage disequilibrium tests: what do we gain? *Eur J Hum Genet* 2001; 9:291–300.
- 20 Fallin D, Cohen A, Essioux L et al. Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. *Genome Res* 2001; 11: 143–51.