

Association of HLA-DR11 with the anaphylactoid reaction caused by nonsteroidal anti-inflammatory drugs

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Background: Several HLA alleles have been associated with asthma induced by nonsteroidal anti-inflammatory drugs (NSAIDs). The existence of HLA markers linked to other NSAID-induced reactions, such as cutaneous and anaphylactoid reactions, has not been established.

Objective: The purpose of our work was to study the HLA-DRB1 and HLA-DQB1 alleles in patients with cutaneous and anaphylactoid reactions caused by NSAIDs.

Methods: We have analyzed 114 HLA DRB1 and 26 HLA-DQB1 alleles in 21 patients with anaphylactoid reactions caused by NSAIDs, 47 patients who had exclusively cutaneous reactions during single-blind, placebo-controlled oral challenges with NSAIDs, and 167 tolerant control subjects (29 of whom had also had an IgE-mediated anaphylaxis to different agents). HLA-DRB1 and HLA-DQB1 alleles were typed by the polymerase chain reaction sequence-specific primers method with genomic DNA.

Results: The frequency of HLA-DR11 alleles was 58.8% in the anaphylactoid reaction group, compared with 15.9% in the NSAID-tolerant healthy control subjects (OR, 7.3; 95% confidence interval, 2.8-19.0; $P < .02$) and 6.3% in the group of the patients with a tolerance for NSAIDs and with IgE-mediated anaphylaxis (OR, 18.75; 95% confidence interval, 4.3-81.1; $P < .004$). No differences were observed among HLA-DR11 alleles analyzed. There were no significant HLA-DQB1 associations with NSAID-induced anaphylactoid reactions. Patients with cutaneous reactions had HLA frequencies that did not differ significantly from the tolerant control subjects.

Conclusion: The HLA-DRB1*11 alleles showed a positive association with NSAID-induced anaphylactoid reactions. (*J Allergy Clin Immunol* 1999;103:685-9.)

Key words: HLA, NSAID sensitivity, anaphylactoid reaction

Abbreviations used

AR:	Anaphylactoid reaction
ASA:	Acetylsalicylic acid
NSAID:	Nonsteroidal antiinflammatory drug
OR:	Odds ratio
PGS:	Prostaglandin-synthase
SBPCDC:	Single-blind, placebo-controlled drug challenge
SSP:	Sequence-specific primer

The reactions during controlled oral challenge with nonsteroidal anti-inflammatory drugs (NSAIDs) in patients with NSAID sensitivity include angioedema, urticaria, rhinoconjunctivitis, bronchial asthma, and anaphylactoid reactions (ARs).¹⁻³ An estimated 10% of patients with asthma⁴ and 30% of patients with chronic urticaria⁵ have reactions after NSAID exposure at some time in their lives.

The mechanisms of NSAID sensitivity is unknown. However, strong evidence supports the enhanced production of the cysteinyl leukotrienes,^{6,7} probably caused by activity inhibition of at least one of the different prostaglandin-synthase (PGS) isoforms,¹ as a cause of the most NSAID-induced reactions. Almost all patients with NSAID sensitivity are also intolerant to other NSAIDs, and this capability is related to their potency as inhibitors of PGS.¹

However, this hypothesis cannot explain all the adverse reactions to NSAIDs. We have described a well-defined group of patients who present selective reactions to NSAIDs, mainly to the pyrazol group, but tolerated other NSAIDs neither involved in the previous reaction nor structurally related,³ even those that were potent in vitro inhibitors of PGS activity. This patient group exhibited a clinical profile different from that of other NSAID reactors; most of them experienced ARs after specific NSAID exposure, and they appeared to be otherwise normal subjects without underlying diseases.² This evidence would indicate that PGS inhibition could not be implicated in these selective reactions, although the exact mechanism has not been elucidated at present. However, other authors^{1,8} suggest that the lack of cross-reactivity argues

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TABLE I. NSAIDs and doses used for the controlled oral challenge

Lactose*	Dose (mg)
Paracetamol	100, 250, 500†
Salsalate	500, 1000†
Piroxicam	10, 20‡
Diclofenac	25, 50‡
ASA	50, 100, 250, 500§

*All the drugs were administered in opaque gelatin capsules at the following intervals between each dose: †60 minutes, ‡120 minutes, §180 minutes.
§Two doses (50 and 100 mg) were administered on day 1, and the other 2 doses (250 and 500 mg) were administered on day 2.

TABLE II. HLA-DRB1 and HLA-DQB1 alleles

HLA-DRB1 alleles	HLA-DQB1 alleles
HLA-DRB1*0101 to *0104	DQB1*0501 to *0504
HLA-DRB1*1501 to *1504	DQB1*0601 to *0612
HLA-DRB1*1601 to *1606	DQB1*0201 to *0203
HLA-DRB1*0301 to *0304	DQB1*0301 to *0305
HLA-DRB1*0401 to *0419	DQB1*0401 to *0402
HLA-DRB1*1101 to *1119	
HLA-DRB1*1201 to *1203	
HLA-DRB1*1301 to *1324	
HLA-DRB1*1401 to *1417	
HLA-DRB1*0701	
HLA-DRB1*0801 to 0811	
HLA-DRB1*0901	
HLA-DRB1*1001	

against a direct inhibition of an enzymatic pathway and is more consistent with an immunologic mechanism.

The HLA class II genes of the MHC are central to immune processing of exogenous antigens, and the finding of an HLA association in this patient group with selective reactions to NSAIDs might favor an immune response as the underlying mechanism of this disease.

There have been several previous studies concerning NSAID sensitivity and MHC.⁹⁻¹² However, these association studies have been performed exclusively in the asthmatic population. The classic study by Mullarkey et al⁹ involved the HLA-DQw2 phenotype (or a disease susceptibility gene closely linked to DQw2) as the phenotype responsible for disease risk (relative risk of about 4) in aspirin-induced asthma. However, other authors have been unable to demonstrate this finding.¹⁰ Another attempt to define HLA markers was undertaken by Krishnamoorthy.¹¹ This author studied 2 groups of patients with NSAID-induced bronchial asthma with or without evidence of atopy. His data suggested that certain HLA-DQB1 and HLA-DPB1 alleles were associated with NSAID-induced asthma, but exclusively in atopic patients. Recently, Dekker et al¹² have shown a strong positive association between HLA-DPB1*0301 and aspirin-induced asthma.

The present study was designed to investigate the genetic association between NSAID sensitivity and HLA. Unlike previous works, we focused on other manifestations of NSAID sensitivity such as the cutaneous

TABLE III. Clinical features of the subjects studied

	NSAID-tolerant patients		NSAID-sensitive patients	
	Control subjects	IgE-mediated anaphylaxis	Cutaneous reactors	Anaphylactoid reactors
No of patients	138	29	47	21
Mean age ± SD (y)	32.2 ± 15.9	33.5 ± 12.9	20.4 ± 5.7	35.7 ± 12.8
Sex (M/F)	21/117	9/20	14/33	6/15
Atopic disease (%)	40.8	58.0	79.6	31.8
Concomitant diseases				
None (%)	72.4	41.3	1.2	63.6
Rhinitis (%)	24.6	31.0	67.4	22.7
Rhinitis/asthma (%)	3.0	27.7	26.5	9.0

reactions and ARs. Thus we have investigated a sample of patients with NSAID-induced cutaneous and anaphylactoid reactions from a Spanish population, comparing them with NSAID-tolerant control subjects.

METHODS

Selection of patients and control subjects

HLA-DRB1 and HLA-DQB1 analyses were performed on 2 different populations of European-born, white patients with NSAID sensitivity. The first group consisted of 21 unrelated patients with clinical evidence of ARs after NSAID administration who were admitted to the emergency unit of our hospital. AR was defined as the presence of urticaria and/or angioedema plus hypotension (systolic blood pressure below 90 mm Hg) and/or laryngeal edema. The second group consisted of 47 unrelated patients with cutaneous reaction during controlled oral challenge with NSAIDs. The challenge response was considered positive if it fulfilled at least 1 of the following criteria: pruritic and erythematous areas raised over normal skin, macular and/or papular areas in any localization, and swelling of skin and/or external mucosa. No patient showed asthmatic or naso-ocular reaction during challenges. Similar analyses were performed on 167 control subjects matched with the patients for age and sex, but who had no evidence of reaction during NSAID challenge. Twenty-nine of them also had IgE-mediated systemic anaphylaxis to different agents (latex, foods, or hymenoptera venom), and they fulfilled the same clinical criteria used for establishing the diagnosis of AR.

Controlled oral challenge

All patients were included in a previously described single-blind, placebo-controlled drug challenge (SBPCDC) protocol shown in Table I,² at least 1 month after resolution of the NSAID-induced reaction. The order of the SBPCDC of the different drugs administered to each patient was paracetamol, salsalate, piroxicam, diclofenac, and acetylsalicylic acid (ASA). Each patient was challenged with the first drug not involved in a previous reaction. If no response occurred, successive challenges with the next drug were performed. Each SBPCDC was carried out separately, and at least 7 days was allowed to elapse after a positive or negative response. In the patients with ARs, we did not use the drug involved or other structurally related NSAIDs.^{2,3} After informed consent was obtained, a venous blood sample (20 mL) was drawn from each subject.

TABLE IV. Clinical characteristics of the patients with NSAID-induced ARs

Patient	Sex	Age (y)	Previous reaction	Atopic disease	Drug involved*	Symptoms*
1	F	30	—	—	Indomethacin	U,AE,LE
2	F	42	—	—	Dipyrone	U,H
3	M	21	AR/ASA	R/mites	ASA	U,AE,LE
4	M	21	AE/propyphenazone AR/propyphenazone	R/cat	Propyphenazone	U,AE,LE
5	M	62	—	—	Dipyrone	U,AE,H
6	F	34	U/dipyrone	R/mites	Dipyrone	U,LE
7	F	43	—	—	Dipyrone	U,AE,LE
8	F	28	—	—	Propyphenazone	U,AE,LE
9	M	61	U/dipyrone	—	Diclofenac	U,AE,H
10	F	25	—	R/cat	Dipyrone	U,AE,H
11	F	27	—	R,A/mites	Diclofenac	U,LE,AB
12	F	52	—	R,A/mites	Paracetamol	U,AE,H
13	F	33	—	—	Dipyrone	U,AE,LE
14	F	60	—	—	Dipyrone	U,AE,LE
15	M	18	—	R/mites	Dipyrone	U,H,LE
16	F	36	—	—	Dipyrone	U,LE
17	M	22	U/dipyrone	—	Propyphenazone	U,AE,LE
18	F	33	AR/dipyrone	—	Dipyrone	U,AE,LE
19	F	20	—	—	Dipyrone	U,H
20	F	40	—	—	Dipyrone	U,AE,LE
21	F	37	—	—	Dipyrone	AE,LE

U, urticaria; AE, angioedema; LE, laryngeal edema; H, hypotension; R, rhinitis; A, asthma.

*Symptoms on admission to the emergency department and drug involved in the reaction.

PCR sequence-specific primer (SSP) method

Genomic DNA from each subject was isolated from whole blood samples by the phenol-chloroform method and ethanol precipitation, according to a standard procedure.¹³ HLA-DRB1 and HLA-DQB1 typing was performed by PCR-SSP method. In the determination of allelic polymorphism by this technique, oligonucleotide primers were used as previously described.¹⁴⁻¹⁶ These primers (Dynal AS, Oslo, Norway) have been designed to obtain amplification of specific alleles or groups of alleles. Briefly, 52 PCR reactions were used for each sample to determine low resolution DRB1 and DQB1 alleles. Additional high-resolution DRB1 reactions were carried out. One hundred fourteen HLA-DRB1 and 26 HLA-DQB1 alleles were tested for 13 and 7 specificities, respectively (Table II). In each reaction mix, a primer pair was included that amplified the human growth hormone as an internal positive amplification control. Assignment of alleles was based on the absence or presence of the amplified products, which were visualized by electrophoresis in agarose gels prestained with ethidium bromide and photographed under ultraviolet light.

Statistical analysis

The frequencies of each allele were compared with the chi-square or Fisher's exact test, as appropriate. A *P* value less than .05 after Bonferroni correction¹⁷ was considered to indicate a significant difference. Odds ratios (ORs) and 95% confidence intervals were calculated by the Woolf-Haldane method.^{18,19}

RESULTS

Patients

The clinical features of subjects included in the study are summarized in Table III. The patients with NSAID sensitivity had 88 episodes of cutaneous reactions and 26 episodes of ARs. In order of frequency, the drugs most

commonly involved were ASA (50 instances, 56.8%), dipyrone (27 instances, 30.6%), and paracetamol (8 instances, 9%). A more heterogeneous group of drugs (indomethacin, propyphenazone, ketoprofen, ibuprofen, and diclofenac) was involved in the remaining 29 episodes.

As shown in Table IV, the patients with NSAID-induced ARs included 15 women and 6 men, ranging in age from 18 to 62 years (mean age, 35.7 years). Six of 21 patients had experienced definite reactions to NSAIDs on previous occasions before being included in the study. Drugs involved in ARs were dipyrone in 13 patients (61.9%), propyphenazone in 3 patients (14.2%), acetic derivatives such as diclofenac and indomethacin in 3 patients (14.2%), ASA in 1 patient, and paracetamol in 1 patient. Five patients had allergic rhinitis, and 2 patients had rhinitis and bronchial asthma. The remaining patients had no evidence of any concomitant diseases.

Controlled oral challenge

During the study, 567 SBPCDCs were carried out in 235 subjects. Of the challenges, 180 were performed in the patients with NSAID sensitivity. In the cutaneous reaction group, 40 patients had periorbital angioedema, and 7 patients had urticaria. All patients with cutaneous reactions during SBPCDC cross-reacted to other NSAIDs not involved in previous reactions. Ninety-three SBPCDCs were performed in 21 subjects with anaphylactoid reactions. In 19 patients, a tolerance to all the drugs after completing SBPCDC protocol (except those reported as being responsible for the AR or structurally

TABLE V. Frequencies (%) of HLA-DR alleles

DR	CR	AR	Control	IgE-mediated anaphylaxis
01	14.9	17.6	27.5	25.0
03	27.7	1.8	15.9	28.1
04	17.0	23.5	26.8	31.3
07	27.8	29.5	24.6	18.8
08	8.5	0	5.1	9.4
09	2.1	0	0.7	3.1
10	0	17.6	0.7	0
11	27.7	58.8	15.9*	6.3†
12	0	0	5.1	0
13	34.0	11.8	34.8	43.8
14	0	0	5.8	0
15	6.4	17.6	18.8	9.4
16	12.8	11.8	5.8	3.1

CR, cutaneous reaction group.

**P* (corrected value) = .02; OR, 7.3; 95% confidence interval, 2.8-19.0.†*P* (corrected value) = .004; OR, 18.75; 95% confidence interval, 4.3-81.1.

related) was noted. The remaining 2 patients (patient 7 and patient 16) were challenged only with ASA, and no clinical reaction was observed.

HLA-DR alleles

The distribution of HLA-DR alleles among patients and control groups is summarized in Table V. The frequency of HLA-DR11 alleles was 58.8% in the AR group, compared with 15.9% in the NSAID-tolerant healthy control subjects (OR, 7.3; 95% confidence interval, 2.8-19.0; *P* < .001) and 6.3% in the group of the NSAID-tolerant patients with IgE-mediated anaphylaxis (OR, 18.75; 95% confidence interval, 4.381.1; *P* < .0001). These results were significant after the *P* value was corrected for multiple comparisons (*P* = .02 and *P* = .004, respectively). No differences were observed among the HLA-DRB1*11 alleles analyzed. Patients with cutaneous reactions had HLA-DR frequencies that did not differ significantly from those of tolerant control subjects.

HLA-DQ alleles

The distribution of HLA-DQ alleles among patients and control groups is summarized in Table VI. No significant differences were observed among patients and both healthy and IgE-mediated anaphylaxis control groups.

DISCUSSION

Failure to reproduce association results has been a common finding in most studies involving HLA and NSAID sensitivity caused by either genetic heterogeneity of the trait or to differences in the patient inclusion criteria^{9,10} and in the populations studied.⁹⁻¹² Our study was based on a careful selection of patients and control subjects through a single-blind, placebo-controlled oral challenge, evaluating the outcome with stringent criteria. Moreover, our patient group was quite different when compared with others used in the previous case/control

TABLE VI. Frequencies (%) of HLA-DQ alleles

DQ	CR	AR	Control	IgE-mediated anaphylaxis
02	46.8	23.5	37.7	35.6
04	6.4	0	2.9	4.1
05	29.8	35.3	43.5	42.4
06	34.0	23.5	38.4	37.4
07	40.4	58.8	35.5	35.1
08	17.0	17.6	19.6	22.3
09	4.3	17.6	3.6	4.3

CR, cutaneous reaction group.

studies because we have included other NSAID reactors, such as those with cutaneous reactions and ARs. To our best knowledge, the existence of HLA markers in these subsets of patients with NSAID sensitivity has not been established.

If these HLA markers represent a risk factor for experiencing these specific NSAID reactions, we would find a higher prevalence of these markers among subjects in whom these reactions occur, and this is what we have found. Our results show a strong association with NSAID-induced ARs in unrelated white patients of the HLA class II alleles DR11, which has not been previously reported.

The results of this study have provided evidence that HLA-DR and HLA-DQ alleles analyzed showed no association with patients with NSAID-induced cutaneous reaction. In addition, previous studies were unable to determine an association between HLA-DRB1 alleles and aspirin-induced asthma.^{10,12} Lympant et al¹⁰ did not find significant differences in the frequencies of any DRB1*, DQA1*, or DQB1* alleles between aspirin-sensitive patients and tolerant control subjects with asthma. However, there was a significant decrease in the incidence of DPB1*0401 in both aspirin-sensitive and aspirin-tolerant patients with asthma when they were compared with 43 control subjects. This negative association has also been detected in a recent study of 59 patients with aspirin-sensitive asthma by Dekker et al.¹² They found similar frequencies of the HLA-DRB1* alleles in the aspirin-sensitive asthma group compared with a control group. However, the patients with aspirin-sensitive asthma showed an increased occurrence of the HLA-DPB1*0301. They suggested that this allele may itself confer susceptibility to aspirin-induced asthma. However, this association has not always been replicated.^{10,11}

This study does not clearly show that HLA-DRB1*11 alleles themselves are responsible for disease risk, although the strength of association we have observed suggests that these alleles could be an important determinant of this specific reaction to NSAIDs in susceptible individuals. However, it seems likely that this MHC-encoded susceptibility in NSAID-induced ARs is a function of specific combinations on sequences on 1 or more locus products and even 1 or more haplotypes. It is of note that the HLA associations of NSAID-induced ARs

are different from those of IgE-mediated anaphylaxis; HLA-DR11 occurred at an increased prevalence only in NSAID-induced ARs. This different association is probably accounted for in part by the disease heterogeneity; the anaphylaxis is a clinically similar but genetically distinct syndrome with a multiplicity of inciting etiologic agents and a variety of pathogenetic mechanisms.

In summary, we have described that genetic susceptibility to NSAID-induced ARs is associated with the HLA-DRB1 genes encoding HLA-DR11 molecules. Our finding requires that confirmation and larger studies with data from other unrelated populations are needed. However, it is potentially of considerable importance that this marker would allow for the recognition of patients at risk for the development of future systemic reactions to NSAIDs.

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