

LTC₄-synthase A-444C polymorphism: lack of association with NSAID-induced isolated periorbital angioedema in a Spanish population

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Background: The mechanism of nonsteroidal anti-inflammatory drug (NSAID)-induced reactions is unknown. However, strong evidence supports the hypothesis of an enhanced production of cysteinyl-leukotrienes. The existence of a polymorphism (A-444C) in the promoter region of the leukotriene (LT)_{C₄}-synthase gene (the terminal enzyme in the LTC₄ production pathway) has been reported. This polymorphism has yielded contradictory results on its association with aspirin-induced asthma.

Objective: The present study was designed to investigate the possible genetic association of C₄₄₄ allele and a specific clinical phenotype of NSAID sensitivity, the NSAID-induced isolated periorbital angioedema, via a case/control study.

Methods: The polymorphism A-444C was analyzed in 58 patients with NSAID-induced periorbital angioedema and 61 control subjects, who had undergone single-blind, placebo-controlled oral challenge. Genotype was determined by polymerase chain reaction-restriction fragment length polymorphism.

Results: We have not found an association of C₄₄₄ allele with NSAID-induced isolated periorbital angioedema.

Conclusions: Further studies are needed to determine whether polymorphisms in the LTC₄-synthase gene or other leukotriene-forming enzymes are involved in the pathogenesis of the different subsets of NSAID sensitivity.

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INTRODUCTION

The reactions caused by nonsteroidal anti-inflammatory drugs (NSAIDs) may be classified according to three clinical criteria¹: 1) the syndrome that appears during controlled oral challenge; 2) the existence of concomitant diseases, such as rhinosinusitis, bronchial asthma, or chronic urticaria; and 3) the cross-reactivity pattern among different NSAIDs during challenge. The main clinical forms of NSAIDs sensitivity are the respiratory (bronchial asthma and/or na-

soocular reaction), the skin (mainly urticaria and angioedema), and the anaphylactoid reactions.^{1,2}

The mechanism of NSAID-induced reactions is unknown. Strong evidence supports the hypothesis that an enhanced production of cysteinyl-leukotrienes (Cys-LTs)^{3,4} plays a key role in aspirin-induced asthma (AIA), probably attributable to the inhibition of the activity of at least one of the cyclooxygenase (COX) isoforms.⁵ Cys-LTs are potent inflammatory mediators, synthesized from arachidonic acid through a sequential pathway that involves several enzymes. In patients with AIA, Cowburn et al⁶ reported a significant increase of leukotriene C₄-synthase (LTC₄S) expression in lung biopsy specimens of patients with respiratory reactions (the so-called acetylsalicylic acid [ASA] sensitivity),

when compared with NSAID-tolerant asthmatic patients and healthy subjects. LTC₄S, the terminal enzyme in the LTC₄ production pathway, was found to be overexpressed in eosinophils, mastocytes, and monocytes-macrophages. Increased expression of LTC₄S may be because of transcriptional upregulation. Sanak et al⁷ reported the existence of a polymorphism (A-444C) in the promoter region of the LTC₄S gene, which was shown to be associated with AIA in a population of Polish patients. This single base-pair mutation consists of an adenine (A) to cytosine (C) transversion that occurs 444 nucleotides upstream from the translation start site. The mutation creates an additional core motif for transcription factor AP2. Nevertheless, in a recent work, van Sambeek et al⁸ have not found differences in the distribution of this polymorphism in an aspirin-sensitive asthmatic population from the United States, nor have they found upregulatory effects of the C₄₄₄ allele in the transcription of LTC₄S gene. The authors suggest the mutation might not be related to the ASA sensitivity phenotype, but might represent a population-stratified polymorphism within patients of eastern European descent.

Patients with NSAID-induced cutaneous reaction have usually chronic urticaria.⁹ However, we have described a series of NSAID-induced cutaneous reactors who showed urticaria or angioedema, exclusively after the intake of the NSAID.^{1,10} The patients recruited for this study had challenge-proven periorbital angioedema induced by NSAIDs not involved in the previous reaction, nor chemically related

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Table 1. Drugs and Doses Used for SBPCOC

Drug	Dose (mg)	Interval between doses (min)
Lactose		
Paracetamol	100, 250, 500, 1000	60
Salsalate	500, 1000	60
Diflunisal	100, 500	60
Mefenamic acid	50, 125, 250	60
Clonixin	50, 125	60
Diclofenac	25, 50	120
Piroxicam	10, 20	120
Ketoprofen	10, 25, 50	120
Dipyron	10, 50, 125, 250, 575*	60
Acetylsalicylic acid	50, 100, 250, 500†	180

All drugs were administered in opaque gelatin capsules.

* Four doses (10, 25, 125, and 250 mg) were administered on the first day, and one dose (575 mg) on the second day.

† Two doses (50 and 100 mg) were administered on the first day, and other two doses (250 and 500 mg) on the second day.

with the initial one.^{1,10} The patients were younger than other NSAID-reactors; the first symptoms usually appeared during the first or second decade of life^{1,11}; and they showed a concomitant atopic respiratory disease in most cases.^{1,10,12}

The present study was designed to investigate the possible genetic association of the C₄₄₄ allele and NSAID-induced reactions. Unlike previous works which have studied only asthmatic patients, we have focused on a specific clinical phenotype of NSAID sensitivity: the cross-reactive NSAID-induced isolated periorbital angioedema.

METHODS

Selection of Patients and Control Subjects

Genetic analyses were performed on a population of European-born, white patients with NSAID sensitivity. The group of patients consisted of 58 unre-

lated individuals who showed isolated periorbital angioedema during controlled oral challenge with NSAIDs. The response to the challenge was considered positive if it fulfilled at least one of the following criteria: 1) pruritic and erythematous areas raised over normal skin in any location, and 2) swelling of skin and/or external mucosa. The most frequent response to the oral challenges was the isolated periorbital angioedema.¹ None of these patients showed asthmatic or nasocular reactions during the challenges. Similar analyses were performed on 61 control subjects matched with the patients for age and sex (patients who showed no evidence of clinical reaction during NSAID challenge).

Informed consent was obtained from the subjects participating in the study. The protocol was approved by the Investigation and Ethics Committee of our hospital.

Controlled Oral Challenge

All patients were included in a previously described single-blind, placebo-controlled oral challenge (SBPCOC).¹ Briefly, the order of the different drugs administered to each patient in the SBPCOC was paracetamol, salsalate, clonixin, diflunisal, piroxicam, diclofenac, dipyron, ketoprofen, and aspirin (Table 1). Each patient was challenged with the first drug not involved in a previous reaction. If no response occurred, a new challenge was performed with the next drug. Each SBPCOC was carried out separately, and at least 7 days were allowed to elapse between challenges. After informed consent was obtained, a venous blood sample was drawn from each subject.

Genotyping

Genomic DNA was isolated from blood samples by phenol/chloroform extraction. Genotyping was performed by the restriction fragment length polymorphism method, as described by Sanak et al.⁷ A product spanning 563 base pairs was amplified from the 5' flanking region of the LTC₄S gene, and subsequently digested with the restriction enzyme MspI. The primers used were the following: 5' TCC ATT CTG AAG CCA AAG GC 3', and 5' GTG ACA GCA GCC AGT AGA GC 3'. The presence of the mutation creates a second recognition site for MspI, allowing the distinction of the wild and the mutant allele because of the different pattern of restriction fragments.

Statistical Analysis

Allelic and genotypic frequencies were compared by Fisher's exact test. *P* values were corrected by multiplying them by the number of alleles tested. A *P* value <0.05 was considered to indicate a statistically significant difference between groups.

RESULTS

Patients

The clinical features of subjects included in the study are summarized in Tables 2 and 3. Fifty-eight patients had positive SBPCOC responses to one or

Table 2. Clinical Features of the Subjects Studied

	Patients	Controls
Number of subjects	58	61
Mean age $\pm$ SD (years)	20.1 $\pm$ 7.8	32.2 $\pm$ 15.9
Sex (M/F)	13/45	21/40
Atopic diseases (in %)	100	38.8
Concomitant diseases (in %)		
None	0	71.5
Rhinitis	81	22.5
Rhinitis and asthma	19	6

Table 3. Clinical Characteristics of the Patients with NSAID-Induced Skin Reaction

Patient	Age	Sex	Drug involved	Positive SBPCOC	Negative SBPCOC	Reaction
1	24	M	ASA	Dip	P, M, S, Pi	PA
2	20	M	ASA, Dip	Dip	P, M, S, D	PA
3	23	M	ASA	Dip	P, M, S	PA
4	21	F	ASA, M	M, Dip	P, S	PA
5	27	F	ASA, P, Pr	M, D	P, Dip	PA
6	33	F	ASA	D	P, M, S	PA
7	22	F	ASA, Pr, P, Nap	ASA, Dip	P, M, S, D	PA
8	19	F	ASA	Dip	P, S	PA
9	16	F	ASA	Dip	P, M, S	PA
10	34	F	ASA, P, D, Dip	P	M, S	PA
11	24	F	ASA	M, Pi	P, S	PA
12	22	F	ASA	Pi	P, M, S	PA
13	22	F	ASA, Dip, P, C	P	M, S	PA
14	19	F	ASA	D	P, M, S	PA
15	31	F	ASA	D	P, M, S	PA
16	26	F	ASA, Dip, C	Dip	P, M, S, Pi	PA
17	24	F	ASA, Dip, K	ASA	P, M, S, Pi	PA
18	26	F	ASA, P	Pi	M, S	PA
19	18	F	ASA	Dip	P, M, S	PA
20	15	M	ASA	Dip	P, M, S	PA
21	15	M	ASA, Dip	D	P, M, S	PA
22	19	M	ASA	Dip	P, M, S	PA
23	27	F	ASA	D	P, M, S	PA
24	8	F	ASA	D	P, M, S, Pi	PA
25	8	M	ASA	Dip	P, Pi	PA
26	18	M	ASA, Dip	ASA	P, M, S, Pi, D	PA
27	27	M	ASA	D	P, M, S, Pi	PA
28	19	F	ASA, Dip	D	P, M, S, Pi	PA
29	24	F	ASA, Ind, K, Dip	Pi	P, S	PA
30	20	F	ASA, K	M, Pi	P	PA
31	17	F	ASA	ASA	P, M, S, Pi, D	PA

ASA, aspirin; Dip, dipyrone; M, mefenamic acid; P, paracetamol; Pr, propyfenazone; D, diclofenac; Nap, naproxen; C, clonixin; K, ketoprofen; Pi, piroxicam; S, salsalate; Ind, indomethacin.

M, male; F, female. SBPCOC, single-blind, placebo-controlled oral challenge; PA, periorbital angioedema.

several NSAIDs (Table 3). These patients had 118 episodes of isolated periorbital angioedema. The drugs most commonly involved were ASA (57 instances, 48.8%), dipyrone (17 instances, 14.5%), and paracetamol (9 instances, 7.6%). A more heterogeneous group of drugs (mefenamic acid, propyfenazone, diclofenac, ibuprofen, ketoprofen, indomethacin, naproxen, clonixin, piroxicam) was involved in the remaining 35 episodes. As shown in Table 2, the group of patients included 45 women and 13 men, ranging in age from 5 to 34 years (mean age, 20.1 years). Forty-six patients had allergic rhinitis and 12 patients had rhinitis and bronchial asthma.

Controlled Oral Challenge

During the study, 314 SBPCOCs were carried out in 119 subjects. Of these challenges, 233 were performed in the patients with NSAID intolerance. All patients had isolated periorbital angioedema; none of them showed any asthmatic or urticarial reaction. Cross-reactivity to other NSAIDs not involved in previous adverse reactions was found in all patients (Table 3). The remaining 61 patients showed no symptoms during SBPCOCs.

Frequencies of the Polymorphism A-444C

In our study, the frequency of the C₄₄₄ allele showed no difference between patients and controls (Table 4). Similarly,

we found no evidence of association of any of the A-444C polymorphism genotypes with the cross-reactive NSAID-induced isolated periorbital angioedema in our population (Table 5).

DISCUSSION

It has been suggested that the series of biochemical reactions that result in the production of Cys-LTs from arachidonic acid, the 5-lipoxygenase pathway, is important in modulating the airway response in patients with ASA sensitivity.^{3,4} Thus, the existence of genetic polymorphism in the enzymes of this pathway has been explored in patients with ASA sensitivity, and LTC₄S gene has been suggested as a prime

Table 4. Allelic Frequencies

Allele	Patients N = 116	Controls N = 122	P	P _c
A	70.7 (82)	77.9 (95)	0.13	NS
C	29.3 (34)	22.1 (27)	0.13	NS

P_c, corrected P value; NS, not significant.

candidate for this specific subset of bronchial asthma.⁷ So far, a polymorphism consisting of an A to C transversion has been described in the promoter region of LTC₄S gene. The C allele was found to be associated with AIA in a Polish population.⁷ Recently, an independent group has reported a lack of correlation of A-444C polymorphism with the asthmatic phenotype in the United States.⁸

Although there is no direct evidence of the involvement of Cys-LTs in NSAID-induced skin reactions such as urticaria or angioedema, the high degree of cross-reactivity among NSAIDs in these patients (Table 2) suggests a mechanism dependent on COX inhibition.^{1,10} Another observation has provided additional information about this issue: in one patient with aspirin-induced urticaria, the treatment with leukotriene modifiers over the course of 3 weeks completely abolished the symptoms and prevented a clinical reaction after administration of a strong COX-inhibitor, piroxicam.¹³ In contrast, patients with aspirin-induced cutaneous reaction tolerated a desensitization-based protocol and treatment with daily aspirin for coronary artery disease without adverse effects for periods ranging from 1 to 24 months.¹⁴ Thus, although we do not exclude other pathophysiologic pathways and mechanisms from playing a role in this inhibition study and/or desensitization procedure, these findings could suggest the involvement

of Cys-LTs in the development of the reactions in some NSAID-induced skin reactors. Therefore, there is rationale to study the genetic polymorphism of LTC₄S in this specific NSAID-induced syndrome. Nevertheless, the presence and distribution of this polymorphism in this particular group of patients is largely unknown.

The present study was undertaken to determine the frequency of A-444C polymorphism of LTC₄S gene in patients with cross-reactive NSAID-induced isolated periorbital angioedema in a population of southern Europe (Canary Islands, Spain). Our study was based on a careful selection of patients and control subjects through a SBPCOC, evaluating the outcome with stringent criteria. Our patients (skin reactors) are different from those used in the previous case/control studies, which included patients with AIA exclusively. We selected specifically patients with isolated periorbital angioedema, as it is the most frequent phenotype of NSAID sensitivity in our population.¹

We have not found an association of the C₋₄₄₄ allele with the cross-reactive NSAIDs-induced isolated periorbital angioedema. The frequency of the C₋₄₄₄ allele in our patients (0.293) is substantially similar to that found in a US population (0.278), and much lower than the observed frequency in Polish patients (0.436). The population bias can not be ruled out, as the ethnic origin could be on the basis of the difference found among these studies. So far, the studies on the functional

effect of the A-444C polymorphism have yielded contradictory results.^{8,15} Therefore, there is not complete agreement on the role of this polymorphism on the expression of LTC₄S and its possible effect concerning LTC₄ levels. Further studies are needed to determine whether polymorphisms in the LTC₄S gene or other leukotriene-forming enzymes are involved in the pathogenesis of the different subsets of NSAID sensitivity.

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Table 5. Genotypic Frequencies

Genotype	Patients (N = 58)	Controls (N = 61)	P	P _c
AA	0.448 (26)	0.574 (35)	0.12	NS
AC	0.517 (30)	0.410 (25)	0.16	NS
CC	0.034 (2)	0.016 (1)	0.48	NS

P_c, corrected P value; NS, not significant.

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