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The A–444C Polymorphism in the Leukotriene C4 Synthase Gene Is Associated With Aspirin-Induced Urticaria

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Abstract

Background: Cysteinyl leukotriene production seems to be dysregulated in patients with hypersensitivity to aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). However, the underlying pathogenic mechanisms of these reactions are poorly understood. Previous studies have suggested a role for the A–444C polymorphism on the leukotriene C4 synthase gene (LTC4S) in aspirin-induced urticaria (AIU), but the results are controversial.

Objective: To evaluate in a case-control study whether the A–444C polymorphism in the promoter region of LTC4S is associated with AIU and atopic phenotypes in a Venezuelan population.

Methods: One hundred ten patients with AIU and 165 nonallergic controls were included. AIU was diagnosed by clinical history and confirmed by double-blind placebo-controlled oral provocation tests with NSAIDs. Genotyping of A–444C was performed by real-time polymerase chain reaction using Taqman probes. Atopy was defined as a positive skin test result to any of the 25 aeroallergens tested. Total and mite-specific immunoglobulin (Ig) E levels in serum were quantified using an enzyme-linked immunosorbent assay.

Results: A–444C was associated with AIU. The C allele was more frequent in patients with the cutaneous pattern of AIU and in patients with low skin reactivity to histamine. There was no association between A–444C and asthma, atopy, or total IgE levels.

Conclusion: The C allele of the A–444C polymorphism is a risk factor for AIU in our population and could be a genetic marker for this phenotype. Furthermore, this single-nucleotide polymorphism is mainly associated with the cutaneous clinical pattern and with low skin response to histamine.

Key words: LTC4S. Histamine. Aspirin-induced urticaria. Leukotrienes. NSAIDs.

Resumen

Antecedentes: La producción de Cisteinil leucotrienos parece estar alterada en los pacientes con hipersensibilidad a la aspirina y otros antiinflamatorios no esteroides (NSAIDs), sin embargo no se conocen bien los mecanismos subyacentes. Algunos estudios sugieren que el polimorfismo A–444C del gen de la enzima Leucotrieno C4 sintasa (LTC4S) juega un papel en la Urticaria Inducida por aspirina (AIU) pero esto aún es motivo de controversia. Objetivo: Evaluar mediante un estudio de casos y controles si el polimorfismo A–444C de la región promotora del gen LTC4S se asocia con AIU y fenotipos de atopía en una población de Venezuela.

Métodos: Se incluyeron 110 pacientes con AIU y 165 controles no alérgicos. La AIU se diagnosticó por la historia clínica y se confirmó mediante pruebas de provocación oral con NSAIDs hechas a doble ciego y controladas con placebo. La genotipificación de A–444C se hizo con PCR en tiempo real empleando sondas Taqman. Se consideró atopía cuando se encontró por lo menos una prueba cutánea positiva entre los 25 alérgenos probados. Los niveles totales y específicos de inmunoglobulina (Ig) E se cuantificaron usando un ensayo inmuno-enzimático.

Resultados: El polimorfismo A–444C se encontró asociado a la AIU. El alelo C fue más frecuente en los pacientes con el patrón cutáneo de la AIU y en aquellos con baja reactividad cutánea a la histamina. No hubo asociación entre A–444C y asma, atopía o niveles de IgE total.

Conclusión: El alelo C del polimorfismo A–444C es un factor de riesgo para AIU en nuestra población y podría ser un marcador genético para este fenotipo. Además, este SNP está asociado principalmente con el patrón clínico cutáneo y con baja respuesta cutánea a la histamina.

Palabras clave: LTC4S. Histamina. Urticaria inducida por aspirina. Leucotrienos. NSAIDs.
Introduction

Hypersensitivity reactions to acetylsalicylic acid (ASA or aspirin) and other nonsteroidal anti-inflammatory drugs (NSAIDs) include several clinical manifestations such as aspirin-exacerbated respiratory disease (AERD), also known as aspirin-intolerant asthma/rhininosinusitis), aspirin-induced urticaria and angioedema (AIU), and anaphylaxis [1,2]. Remarkably, the characteristics of AIU are different from other aspirin hypersensitivity (eg, asthma or urticaria) and exert different polymorphisms could determine different phenotypes [3]. Evidence indicates that these interactions are more complex and common alterations in AIU and asthma [5]. However, recent interactions underlie its pathogenesis. This disease affects 0.1% to 0.3% of the general population [3] and triggers or aggravates chronic urticaria in 20%-40% of patients [4]. Based on its clinical manifestations, NSAID hypersensitivity can be classified as cutaneous, mixed, respiratory, and systemic. The cutaneous pattern is characterized by rash, wheals, and/or angioedema, while upper respiratory tract and skin manifestations are found in the mixed pattern. Although the pathogenesis of AIU is poorly understood, it has been postulated that cross-reactions to ASA and NSAIDs are likely to be due to a pharmacological mechanism influenced by genetic polymorphisms involved in the metabolism of arachidonic acid.

Several lines of evidence strongly support that in sensitive individuals there is overexpression of the enzymes and receptors involved in the cysteinyl leukotriene metabolic pathway [4-6]; therefore, the inhibition of cyclooxygenase 1 (COX-1) by aspirin and NSAIDs shunts arachidonic acid metabolism towards the lipo-oxygenase pathway with subsequent overproduction of leukotrienes, as proposed for AERD in the cyclooxygenase hypothesis [7]. Thus, it has been postulated that polymorphisms that increase or dysregulate the expression of genes involved in leukotriene production could be risk factors for aspirin hypersensitivity [8,9] and influence the response to antileukotriene drugs. Some authors have described overproduction of leukotrienes and prostaglandin D2 as common alterations in AIU and asthma [5]. However, recent evidence indicates that these interactions are more complex and different polymorphisms could determine different phenotypes of aspirin hypersensitivity (eg, asthma or urticaria) and exert their effects in some populations but not in others [8,10].

Activation of cutaneous mast cells plays a central role in the pathophysiology of urticaria by releasing pro-inflammatory mediators including histamine, leukotrienes, prostaglandin D2, cytokines, and proteolytic enzymes. Leukotriene C4 synthase is a terminal enzyme in the leukotriene pathway and is responsible for the production of cysteinyl leukotrienes (LTC4, LTD4, LTE4), the levels of which are characteristically increased in urine, eosinophils, and bronchial biopsy specimens from patients with aspirin hypersensitivity [11]. Even though the results are controversial, transcriptional regulation of the LTC4S gene could be influenced by single-nucleotide polymorphisms (SNPs) in its promoter region, including a transversion of adenine for cytosine at 444 nucleotides of the transcription start site (A→444C) [12]. In white and Japanese individuals, the C allele is associated with a better therapeutic response to leukotriene receptor antagonists [13], and this polymorphism has been studied as a risk factor for aspirin hypersensitivity in conditions such as AERD and AIU. Nevertheless, the results are still contradictory [5,14-16].

In a Polish population, the frequency of the C allele of A→444C was significantly higher in patients with chronic idiopathic urticaria who exhibited a positive response to aspirin challenge [5] and in patients with aspirin-intolerant asthma (AIA) [12]. In addition, susceptibility to the AIU phenotype does not follow a clear Mendelian pattern, but the C allele is characteristically inherited in affected individuals [17]. By contrast, Kim et al [18] found no association between A→444C and AIU in Koreans, and no association was found between this SNP and the phenotype of NSAID-induced isolated periorbital angioedema in a Spanish population [19]. The objective of this study was to investigate the distribution of A→444C in a Venezuelan population and evaluate its association with AIU, the clinical pattern, drug hypersensitivity, and atopic phenotypes.

Methods

Study Population

One hundred ten patients with cross-reactive aspirin/NSAID-induced urticaria/angioedema were recruited from an outpatient allergy clinic in Caracas, Venezuela, between 2003 and 2007. Patients of any age or gender with a clinical history of urticaria and angioedema triggered by NSAIDs underwent double-blind placebo-controlled oral provocation tests with NSAIDs. Briefly, incremental doses of aspirin from 25 mg to 500 mg or placebo, given on different days, were concealed in identical opaque capsules, and administered 1 hour apart, with 4 hours of observation in the hospital and a telephone survey 24 hours later. Vital signs and pulmonary function (forced expiratory volume in 1 second [FEV1], forced vital capacity [FVC], forced expiratory flow, midexpiratory phase [FEF25-75], and peak expiratory flow [PEF]) were monitored at baseline and hourly for 4 hours, and the skin, nose, eyes, and thorax were examined at the same intervals. The presence of breathlessness, cough, wheezing, dysphonia, nasal or ocular itching, sneezing, rhinorrhea, nasal obstruction, and conjunctival erythema was specifically investigated at every hourly physical examination. For urticaria and angioedema, the percentage of skin involved was calculated as follows: head and neck, 30%; chest, 20%; abdomen, 20%; upper limbs, 15%; and lower limbs, 15%. The test result was regarded as positive for urticaria or angioedema if 20% or more of the body surface area was affected [20]. Antihistamines and leukotriene receptor antagonists were withheld for at least 96 hours before testing. Only patients who reacted to at least 2 chemically unrelated NSAIDs (cross-reactions) were included [21]. Patients with other phenotypes of aspirin hypersensitivity (eg, reactions to a single NSAID, AERD, chronic idiopathic urticaria, anaphylaxis induced by NSAIDs) and pregnant women were excluded. The clinical patterns of the reactions were classified as cutaneous (urticaria and/or angioedema), and mixed (urticaria and/or angioedema plus upper respiratory and/or ocular symptoms, including laryngeal angioedema), as previously described [1]. High-risk patients were defined as NSAID-sensitive individuals who reacted to weak COX inhibitors (acetaminophen) or COX-2 inhibitors (nimesulide, ...
meloxicam, celecoxib, rofecoxib), as proposed by Matucci et al [22]. Data from clinical records were collected (age, gender, drugs involved in previous reactions, and past or present history of allergic diseases). One hundred sixty-five unrelated, healthy, and randomly selected control participants were recruited from the blood bank, Hospital Universitario de Caracas. Patients and controls were from Caracas, whose population is a mixture of Spaniards, Africans, and Native Americans. The study was approved by the Institutional Review Boards of Hospital Universitario de Caracas and Clínica El Avila. A full verbal explanation of the investigation was given, and written informed consent was obtained from all participants.

Genotyping

Genomic DNA was extracted from peripheral white blood cells using the salting-out method [23]. The A→444C polymorphism was genotyped using the TaqMan 5′ exonuclease assay [24]. The validated assay (ID C_644967_10, rs.1800469) was designed and manufactured by Applied Biosystems (Foster City, California, USA). The chosen reporter fluorophores for the TaqMan GB probes were VIC for detecting the A allele and 6-carboxyfluorescein (FAM) for the C allele. The polymerase chain reaction was performed in a final volume of 10 μL containing 2 μL of DNA (10 ng), 5 μL of 2X Universal PCR Master Mix, 0.5 μL of the validated assay 20X, and 2.5 μL of distilled water. Thermal cycling conditions were as follows: AmpErase UNG activation at 50°C for 2 minutes followed by AmpliTaq Gold DNA pre-incubation at 95°C for 10 minutes, 50 cycles of 15 seconds at 92°C, and 60°C for 1 minute. After amplification, endpoint fluorescence detection was performed at 60°C. Automatic calling was performed using 7300 system SDS software in a 7300 Real Time PCR System (Applied Biosystems). Nontemplate controls were included in each run and the reproducibility of 30 samples repeated as genotyping controls was 100%.

Quantification of Total and Specific Immunoglobulin (Ig) E

Total IgE was determined using a commercial enzyme-linked immuno sorbent assay (ELISA) (RIDASCREEN, R-Biopharm AG, Darmstadt, Germany) in all participants according to the manufacturer’s instructions. Since Blomia tropicalis and Dermatophagoides pteronyssinus are the main sources of sensitization in tropical cycling conditions [25-27], and the prevalence of IgE sensitization to these allergens is frequent in allergic patients from Venezuela [91.6% and 97.2 %, respectively [28,29]], specific IgE (sIgE) against these 2 mites was determined by indirect ELISA, as described previously [30]. Absorbance was measured at 405 nm using a Spectrophotometer (Spectra MAX 250 Molecular Device Sunnyvale, California, USA) and expressed as optical density (OD) units. Samples were assayed in duplicate, and levels of sIgE above 0.142 OD (mean OD of 5 nonatopic participants + 3 SD) were considered positive.

Skin Tests

Specific IgE reactivity to common allergens was evaluated using skin prick tests to a battery of 25 aeroallergens including mites (D pteronyssinus and B tropicalis, CBF Leti, Madrid, Spain), cockroach, cat and dog dander, feathers, molds (Penicillium, Aspergillus, Cladosporium, Alternaria, Rhizopus), Bermuda (Cynodon dactylon), rye grass ( Lolium perenne), timothy (Phleum pratense), ragweed (Ambrosia artemisiifolia), Chenopodium album, Plantago lanceolata, mugwort (Artemisia vulgaris), Amaranthus, Pinus radiata, sycamore, cypress, juniper, acacia and eucalyptus (all from ALK-Abelló, Madrid, Spain). After 15 minutes, a wheal size of 3 mm or greater than that of the negative control was considered positive, and atopy was defined as a positive reaction to at least 1 allergen. Histamine phosphate 1 mg/mL (ALK-Abelló, Madrid, Spain) was used as a positive control, and glycerol saline solution as a negative control. Antihistamines were omitted at least 96 hours before skin testing.

Statistical Analysis

Statistical analyses for the case-control study were performed using SPSS v.13 software. Demographic characteristics between groups were compared using the χ² tests and t test as needed. The Hardy-Weinberg equilibrium was tested in patients and controls by the Markov Chain method using ARLEQUIN software (available at http://lgb.unige.ch/arlequin/). Allele and genotype frequencies were compared using the Fisher exact test and adjusted for covariates by logistic regression. Parametric and nonparametric tests were used to evaluate the association between the genotypes and total and specific IgE levels. All analyses were first conducted under a general genotype model and subsequently under an additive or recessive model that was most compatible with the general model. Considering a 0.3% frequency of AIU and including our sample size (110 patients), we obtained a 79.1% power to detect a genetic odds ratio of 1.9 with a 95% confidence interval (CI) at an α level of .05 under an additive model. The significance level was set at P<.05.

Results

The clinical characteristics of patients with AIU are shown in Table 1. Mean (SD) age was 31.4 (12.1) years in patients and 34.9 (10.1) years in controls (P=.05). There were more females in the patient group than in the control group (55.7% vs 44.3%, P=0.001), and consequently all the analyses were adjusted for these covariates. One hundred ninety-one patients (91.8%) had a history of atopic diseases, and most patients had positive skin test results to Aeroallergens. The clinical patterns of the reactions were mixed in 64 patients (58.2%) and cutaneous in 46 (41.8%). Fifty patients (45.5%) were classified as high-risk individuals reacting to weak COX-1 inhibitors and 5 had a history of anaphylaxis triggered by mite-contaminated foods. The medical history revealed that several NSAIDs were involved in the urticaria/angioedema reactions, the most common inducers being ibuprofen (70.9%), aspirin (67.3%), pyrazolone (44.5%), paracetamol (33.6%), diclofenac (30.9%), ketoprofen (20.9%), and nimesulide (13.6%).

Genotype distribution did not deviate from Hardy-Weinberg expectancy among patients or controls. The allele
The A–444C SNP and Aspirin-Induced Urticaria

Table 1. Clinical Characteristics of the Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n=110)</th>
<th>Healthy Controls (n=165)</th>
<th>OR (95% CI, P values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean (SD), range</td>
<td>31.4 (12.1)</td>
<td>31.4 (12.1)</td>
<td></td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>78 (70.9)</td>
<td>125 (75.8)</td>
<td>1.0 (reference category)</td>
</tr>
<tr>
<td>Atopic diseases</td>
<td>101 (91.8)</td>
<td>125 (75.8)</td>
<td>1.0 (reference category)</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>96 (87.3)</td>
<td>125 (75.8)</td>
<td>1.0 (reference category)</td>
</tr>
<tr>
<td>Rhinitis and asthma</td>
<td>23 (20.9)</td>
<td>125 (75.8)</td>
<td>1.0 (reference category)</td>
</tr>
<tr>
<td>Only asthma</td>
<td>2 (1.8)</td>
<td>125 (75.8)</td>
<td>1.0 (reference category)</td>
</tr>
<tr>
<td>Positive skin test results to inhaled allergens</td>
<td>91/92 (98.0)</td>
<td>125 (75.8)</td>
<td>1.0 (reference category)</td>
</tr>
</tbody>
</table>

Clinical pattern
Mixed 64 (58.2)
Cutaneous 46 (41.8)
High-risk patients 50 (45.4)
Oral mite anaphylaxis 5 (4.5)
Total IgE, IU/mL 305.3 (1475)

Table 2. Allele and Genotype Distribution of A–444C in Patients and Controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>AIU Patients (n=110)</th>
<th>Healthy Controls (n=165)</th>
<th>OR (95% CI, P values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>64 (58.2)</td>
<td>125 (75.8)</td>
<td>1.0 (reference category)</td>
</tr>
<tr>
<td>AC</td>
<td>38 (34.5)</td>
<td>34 (20.6)</td>
<td>3.8 (1.7-8.5; .001)</td>
</tr>
<tr>
<td>CC</td>
<td>8 (7.3)</td>
<td>6 (3.6)</td>
<td>4.9 (1.06-23.2; .04)</td>
</tr>
</tbody>
</table>

AC+CC vs AA 46/64 40/125 4.01 (1.88-8.5; .003)

Alleles
A 167 (75.9) 284 (86.1) 1.95 (1.26-3.03; .002)
C 24 (18.8) 29 (31.5) 1.99 (1.07-3.7; .02)

Abbreviations: AIU, aspirin-induced urticaria; CI, confidence interval; OR, odds ratio.

Table 3. Allele and Genotype Distribution of A–444C According to the Clinical Pattern

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Mixed</th>
<th>Cutaneous</th>
<th>OR (95% CI, P values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>42 (65.6)</td>
<td>22 (47.8)</td>
<td>1.0 (reference category)</td>
</tr>
<tr>
<td>AC</td>
<td>19 (29.7)</td>
<td>19 (41.3)</td>
<td>1.9 (0.83-4.4; .13)</td>
</tr>
<tr>
<td>CC</td>
<td>3 (4.7)</td>
<td>5 (10.9)</td>
<td>3.1 (0.66-14.1; .15)</td>
</tr>
</tbody>
</table>

AC+CC vs AA 22/42 24/22 2.1 (0.93-4.5; .072)

Alleles
A 104 (81.3) 63 (68.5) 1.99 (1.07-3.7; .02)
C 24 (18.8) 29 (31.5) 1.99 (1.07-3.7; .02)

Abbreviations: CI, confidence interval; OR, odds ratio.

A significant association was detected between the A–444C genotypes and AIU phenotype under a general model (Pearson $\chi^2=9.57, P=.008$), which remained significant after adjustment for age and gender when using logistic regression ($P=.001$, Table 3). This association was driven by a higher frequency of homozygotes to the wild-type A allele in controls and the presence of the AC and CC genotypes in patients with AIU. In addition, when analyzing allele distribution, we observed that the mutant C allele was significantly more frequent in AIU patients than in healthy controls ($24.1\%$ vs $13.9\%$, $P=.002$; Table 2).

A further evaluation of the A–444C distribution among patients according to their clinical pattern of AIU (cutaneous or mixed) showed that the AA genotype tended to be more frequent in patients with the mixed pattern (urticaria/angioedema plus respiratory symptoms), whereas the AC and CC genotypes were more frequent in patients with the cutaneous pattern (Table 3). A comparison of allele distribution according to
Histamine is an important mediator of urticarial reactions and histamine levels are characteristically higher in lesional skin than in nonlesional skin in all forms of urticaria, where and histamine levels are characteristically higher in lesional skin. It has been postulated that inhibition of prostaglandin D2 (PGD2) production by aspirin and other NSAIDs facilitates cutaneous mast cell degranulation in humans, since these lipid metabolites are natural inhibitors of histamine release in rat peritoneal mast cells [31]. Here, we used skin tests to evaluate the in vivo response to histamine in patients with AIU. The results revealed that wheal diameter after epicutaneous application of histamine was significantly lower in carriers of the C allele and the cutaneous pattern of AIU (P=0.02, Table 3).

Histamine is an important mediator of urticarial reactions and histamine levels are characteristically higher in lesional skin than in nonlesional skin in all forms of urticaria, where binding to H1 and H2 receptors induces erythema and whealing. It has been postulated that inhibition of prostaglandin D2 and E2 production by aspirin and other NSAIDs facilitates cutaneous mast cell degranulation in humans [4], since these lipid metabolites are natural inhibitors of histamine release in rat peritoneal mast cells [31]. Here, we used skin tests to evaluate the in vivo response to histamine in patients with AIU. The results revealed that wheal diameter after epicutaneous application of histamine was significantly lower in carriers of the C allele and the cutaneous pattern of AIU (P=0.02, Table 3).

The C allele tended to be more frequent in patients with a low skin response to histamine (28.9% vs 14.3%, P=0.03, Table 4). The effects were also observed when analyzing the degree of response to histamine according to the A–444C distribution, considering high reactivity a wheal diameter higher than 7 mm (75th percentile). In addition, the C allele was more frequent among patients with a low skin response to histamine compared to those with a high response (28.9% vs 14.3%, P=0.03, Table 4). The effects were significant after adjustment for clinical pattern (data not shown) and no association was observed with the wheal diameter of any of the 25 allergens tested.

In this population, the A–444C SNP was not associated with atopy, total IgE levels, or specific IgE levels to mites. We found no association when analyzing the degree of response to histamine according to the A–444C distribution, considering high reactivity a wheal diameter higher than 7 mm (75th percentile). In addition, the C allele was more frequent among patients with a low skin response to histamine compared to those with a high response (28.9% vs 14.3%, P=0.03, Table 4). The effects were significant after adjustment for clinical pattern (data not shown) and no association was observed with the wheal diameter of any of the 25 allergens tested.

We evaluated the frequency of the A–444C polymorphism of LTC4S in a Venezuelan population and its association with AIU in a well-characterized population of NSAID–cross-reactive patients. Based on one of the largest samples of patients for genetic studies of this disease, we replicated previous findings [5,17], suggesting that A–444C is a risk factor for AIU. In addition, we described for the first time that A–444C is associated with a particular clinical pattern, that is, the variant C allele is more frequent in patients with cutaneous adverse reactions to aspirin and other NSAIDs, a clinical picture restricted to the skin and devoid of respiratory or ocular manifestations.

An association between A–444C and aspirin-intolerance phenotypes (AIU and AIA) was first reported by Sanak et al [32]; however, studies including white and Asian populations have failed to replicate this finding [8,14]. This could be explained by differences in ethnic characteristics, definition of outcomes, criteria for defining hypersensitivity reactions, and different environmental and genetic backgrounds. Here, we found a significant association between A–444C and AIU in a group of cross-reactive nonwhite patients and showed that A–444C is an important risk factor for aspirin hypersensitivity.

In contrast to our results, Kim et al reported no association between A–444C and AIU in Koreans [16,33]. Interestingly, there is no significant difference in the frequency of the minor allele between Koreans and our population (q=0.13, P=2). In this case, a different genetic background could have been responsible for variations in the A–444C effect. Indeed, A–444C was only associated with intolerance to aspirin among Koreans in the setting of a gene-gene interaction with variants in CysLTR2 (cysteinyl leukotriene receptor 2) [34]. The role of the genetic component in the pathogenesis of AIU is not clear. Genes conferring specific susceptibility to AIU could exist while others could influence the AERD phenotype. Two strong positive associations have been reported in Koreans between HLA-DRB1*1302 and HLA-DQB1*0609 markers.

### Table 4. Allele and Genotype Distribution of A–444C According to Skin Histamine Reactivity

| Allele and Genotype Distribution of A–444C According to Skin Histamine Reactivity |
|---------------------------------|-------------------------------------------------|---------------------------------|---------------------------------|
| **Genotype** | **Wheal Diameter** | **Histamine Response** | **OR (95% CI, P value)** |
| | | **High** | **Low** | |
| AA | 6.5 (2.0) | 21 (75.0) | 32 (50.0) | 1.0 (reference category) |
| AC | 5.7 (1.0) | 6 (21.4) | 26 (40.6) | 2.8 (0.99-8.2; .05) |
| CC | 5.7 (1.1) | 1 (3.6) | 6 (9.4) | 3.9 (0.443-35.4; .21) |
| AC+CC vs AA | 6.5 vs 5.7 | 7/21 | 32/32 | 3.02 (1.112-8.16; .02) |
| Alleles | | | | |
| A | 6.3 (2.0) | 48 (85.7) | 91 (71.1) | 0.41 (0.16-1.01; .03) |
| C | 5.7 (1.0) | 8 (14.3) | 37 (28.9) | |

Abbreviations: CI, confidence interval; OR, odds ratio.

\* Mean, SD (adjusted linear regression)

\* According to wheal diameter >7 mm (75th percentile)

\* Age and sex adjusted by logistic regression

**Discussion**

In contrast to our results, Kim et al reported no association between A–444C and AIU in Koreans [16,33]. Interestingly, there is no significant difference in the frequency of the minor allele between Koreans and our population (q=0.13, P=2). In this case, a different genetic background could have been responsible for variations in the A–444C effect. Indeed, A–444C was only associated with intolerance to aspirin among Koreans in the setting of a gene-gene interaction with variants in CysLTR2 (cysteinyl leukotriene receptor 2) [34]. The role of the genetic component in the pathogenesis of AIU is not clear. Genes conferring specific susceptibility to AIU could exist while others could influence the AERD phenotype. Two strong positive associations have been reported in Koreans between HLA-DRB1*1302 and HLA-DQB1*0609 markers.
and AIU, although this association was not found with aspirin-induced asthma [35]. The findings of specific polymorphisms associated with AIU but not with other aspirin hypersensitivity phenotypes suggest different underlying alterations [9]. In this regard, our results support the idea that there are differences in the pathogenesis of these aspirin-hypersensitivity–induced syndromes, and that A→444C could be useful as a marker of the AIU phenotype, especially of the clinical cutaneous pattern. Our findings also suggest the possible influence of this SNP on other phenotypes, because the AC and CC genotypes, as well as the C allele, were significantly more common in patients with a lower cutaneous response to histamine. Logistic regression analysis showed that the effect of A→444C on histamine reactivity is independent of the clinical pattern (data not shown).

AIU responds to antihistamines, which are the first-line medication for this condition [4]. Our results show a significant association between A→444C and a differential response to exogenous histamine in patients with AIU. This could have implications for therapy. We do not know the biological basis of the association between A→444C and histamine response. Although patients were not under the effects of antihistaminic treatment by the time we performed the cutaneous skin tests, it is possible that different treatments could be confounding this result. Nevertheless, we found no association between low response to histamine and low response to any of the other allergens tested simultaneously. We speculate that patients carrying the C allele could produce more histamine and, consequently, are more resistant to the physiological effect of exogenous histamine in the skin. Indeed, differences in histamine levels have been described in patients with aspirin hypersensitivity [36].

Previous studies described an increase in the wheal and flare response to intradermal histamine in patients with chronic idiopathic urticaria compared to controls [37]. More studies are needed to evaluate the possible biological relationship between A→444C and the less intense response to histamine, as our results may reveal a particular feature in the pathogenesis of AIU.

The cutaneous phenotype of AIU has been reported to occur more frequently in atopic individuals [38,39], and we previously showed that the A→444C polymorphism is associated with the specific IgE response to mite among Colombian asthmatic patients [40]. Whether the association of A→444C with the cutaneous manifestations of AIU is related to atopy deserves further investigation; however, in the present study, we found no association between A→444C and atopy, or total IgE or specific IgE levels to mites, suggesting that A→444C has an independent effect on the cutaneous phenotype and that this effect involves mechanisms other than atopy pathways. Nevertheless, in specific genetic backgrounds, this SNP could co-segregate with other variants predisposing to AIU, atopy, or high total IgE levels, as found elsewhere for 2 promoter polymorphisms in the FCERIB gene [41].

Although we are aware that patients and controls shared common demographic characteristics, especially having been born in Caracas, there may still be a bias in our results because of genetic stratification of the population. However, it seems unlikely that stratification acts as a confounder, because, in addition to the associations between patients and controls, we also found associations between A→444C and specific phenotypes of AIU within subgroups of patients by statistically independent effects.

In summary, our data support the association between A→444C and AIU. This is the first report of an association between A→444C and the cutaneous clinical pattern of this phenotype, thus stressing the importance of this polymorphism as a genetic marker of AIU. Further studies are needed to evaluate the functional basis of the effect of A→444C on AIU and the cutaneous manifestations of this disease. The biological pathways underlying differences in histamine reactivity according to the A→444C SNP and its influence on antihistamine responsiveness in AIU patients deserve further investigation.

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