

## **Atopy can be an interfering factor in genetic association studies of beta-lactam allergy**

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0441

**Key words:** Beta-lactam antibiotics, allergy, atopy, polymorphisms, genetics

**Palabras clave:** antibióticos beta-lactámicos, alergia, atopia, polimorfismos, Genética

Genetic and environmental factors are involved in immediate hypersensitivity reactions to beta-lactams antibiotics (BL). Several genes have been associated with BL immediate hypersensitivity reactions, including those encoding cytokines and receptors involved in IgE production, the high affinity receptor for IgE (*FCER1*), signal transduction proteins and products released by mast cell. Nevertheless, when analyzing the publications involving genetic association studies in BL allergic patients, most of them have been performed in three main populations, in most cases by the same groups of investigators, which progressively increased the population sample in successive studies. In addition, some concerns were present in most of them: (i) the diagnosis was not always performed by skin or challenge tests; or (ii) tolerance to BL in controls was not proved; or (iii) atopy was not considered.

The first group of studies was conducted in a Chinese population [1-3]. Overall, some patients were diagnosed by the medical history and immediate and non-immediate reactions were mixed. The number of patients and controls increased with the successive studies, although samples were not generally large. Usually, controls had not proven tolerance to penicillin: they were included because they had a negative history (or questionnaire) to BL or negative specific IgE or skin tests. The percentages of atopy were not controlled in patients and controls. The genes finally involved were *IL4*, *IL13*, *IL4RA*, *IFNR1*, *IL18* and *STAT6*.

The second group of studies were conducted in a Korean population. In one of them [4], different types of reactions (urticaria, angioedema, maculopapular rash and exudative erythema) and antibiotics (BL, quinolones and others) were assessed from a database of adverse drug reactions, whereas controls came from a previous study on the PGE2 receptor, and their characteristics were not described. In another study [5], patients professionally exposed to cephalosporins and unexposed non-atopic controls with negative skin tests to 3 frequently prescribed cephalosporins were evaluated. However, only four patients reported work-related symptoms and only one patient had positive skin tests and symptoms upon exposure to a cephalosporin. Although atopy was considered in one of the studies, it was not taken into account in genetic analyses. The involved genes were *FCERB1* and *CD40*.

The third group of studies were conducted in European populations. Initially in Italian patients, evaluated in collaboration with French researchers [6,7]. Later, a Spanish population was analyzed [8]. In the first of the studies [6], patients were diagnosed by a history of an immediate reaction and positive skin tests or specific IgE, while controls were selected from age matched volunteers in a query of an allergist, on the basis of absence drug reactions (tolerance was not confirmed). Although atopy was not considered, total IgE levels were higher in patients than in controls. In the study involving Italian and Spanish patients [8], both immediate and delayed reactions were simultaneously considered. Patients were diagnosed according to the recommendations of the ENDA group, but controls came from a consultation of preventive care. In these studies, total IgE levels were higher in

patients than in controls. In another Spanish study [9], patients were diagnosed by positive skin or controlled drug exposure tests, and controls were selected on the basis of not having reported a history of BL allergy. A significantly higher percentage of atopy was also observed in BL allergic patients, according to total IgE levels and specific IgE to prevalent allergens, which were significantly increased compared with controls. Genes involved were *IL4RA*, *IL13*, *TNFA*, and *NOD* (Nucleotide-binding Oligomerization Domain).

In short, the review of these studies suggests that larger size populations are needed, diagnostic criteria need to be homogeneous and not based only on the clinical history, tolerance to BL should be verified in control groups, different reactions should be analyzed separately, and atopy considered as a possible confounding factor.

We have evaluated whether atopy could be a confounding factor in genetic studies on BL allergy. Following the European Network for Drug Allergy (ENDA) protocol, we evaluated 98 patients that were diagnosed with immediate hypersensitivity to BL and 104 controls that had a negative result and tolerated a full dose of a BL (see the methods section in supplementary material). Skin tests with a locally adapted battery of common aeroallergens were performed to both patients and controls. Atopy was defined by the presence of at least one positive skin test from the battery. No statistically significant differences were observed between patients and controls regarding age, sex, atopy, or total IgE levels (Supplementary table 1).

By analyzing 22 polymorphisms in patients with BL hypersensitivity compared to controls, only statistically significant differences were identified for the *c25 TGFBI* SNP, with the C allele at codon 25 of *TGFBI* gene being significantly more frequent in

BL positive patients (11.8%) than in controls (4.5%),  $p = 0.029$  (see Supplementary material). When patients were classified according to atopy, irrespectively of their BL sensitization, statistically differences were found between control and patient groups for the following SNPs: *pst1 +1970 IL1R*, *+874 IFNG* and *-33 IL4* (table 1). To confirm whether these differences were due to BL hypersensitivity or to atopy, we compared atopic and non-atopic patients with BL allergy, and we found the same statistically significant differences above mentioned. In addition, we found an association with the SNP *+1902 IL4RA* (Supplementary table 3). On the other hand, considering only non-atopic patients irrespectively of their BL sensitization no differences were found between BL allergic and BL tolerant patients.

In summary, in our study, in which proven tolerance to BL and atopic status were taken into account, we could detect significant differences between atopic and non-atopic patients allergic to BL, as well as between atopic patients allergic to BL and controls, but not between non atopic patients. Therefore, we suggest that atopy is a confounding factor, being overrepresented in BL allergic patients, and that the previously described associations could have been due to atopy.

**Conflict of interests**

The authors have no conflict of interests or financial sources to disclose

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**Table 1.** Allelic and genotypic frequencies of polymorphisms that showed a significant association in the group of atopic patients with hypersensitivity to BL versus non-atopic controls

	Genotype					Allele		
<b>pst1+1970 <i>IL1R</i></b>	<b>Number</b>	<b>CC</b>	<b>TC</b>	<b>TT</b>	<b>p- Value</b>	<b>C</b>	<b>T</b>	<b>p- Value</b>
<b>Controls</b>	77	0.456	0.465	0.079		0.689	0.311	
<b>Patients</b>	19	0.326	0.435	0.239	<b>0.017</b>	0.543	0.457	<b>0.014</b>
<b>+874<i>IFNG</i></b>		<b>AA</b>	<b>AT</b>	<b>TT</b>	<b>p- Value</b>	<b>A</b>	<b>T</b>	<b>p- Value</b>
<b>Controls</b>	77	0.324	0.353	0.324		0.500	0.500	
<b>Patients</b>	19	0.571	0.286	0.143	0.15	0.714	0.286	<b>0.027</b>
<b>-33 <i>IL4</i></b>	<b>N°</b>	<b>CC</b>	<b>CT</b>	<b>TT</b>	<b>p- Value</b>	<b>C</b>	<b>T</b>	<b>p- Value</b>
<b>Controls</b>	77	0.692	0.280	0.028		0.832	0.168	
<b>Patients</b>	18	0.909	0.091	0.000	<b>0.017</b>	0.955	0.045	<b>0.004</b>