Check for updates

DR. ABDERRAHIM OUSSALAH (Orcid ID : 0000-0002-9731-4529)

DR. JOSÉ ANTONIO CORNEJO-GARCÍA (Orcid ID : 0000-0003-3465-8735)

DR. CRISTOBALINA MAYORGA (Orcid ID : 0000-0001-8852-8077)

PROF. MARÍA JOSÉ TORRES (Orcid ID : 0000-0001-5228-471X)

PROF. JEAN LOUIS GUEANT (Orcid ID : 0000-0002-5067-042X)

Article type : Original article

Next-Generation Sequencing and Genotype Association Studies Reveal the Association of *HLA-DRB3*02:02* With Delayed Hypersensitivity to Penicillins

Manuscript Acceptance Date: 09-Oct-2021

Antonino Romano, MD[1,2,*,†]; Abderrahim Oussalah, MD, PhD[1,3†]; Celine Chery, PhD[1,3†]; Rosa-Maria Guéant-Rodriguez, MD, PhD[1,3]; Francesco Gaeta, MD[4]; Jose-Antonio Cornejo-Garcia, PhD[5]; Pierre Rouyer, MSc[1]; Thomas Josse, BSc[3]; Cristobalina Mayorga, PhD[5]; Maria-Jose Torres, MD[5]; and Jean-Louis Guéant, MD, PhD[1,3*]

[†] Equal contribution

*Corresponding authors

1 University of Lorraine, INSERM UMR_S 1256, Nutrition, Genetics, and Environmental Risk Exposure (NGERE), Faculty of Medicine of Nancy, F-54000 Nancy, France.

2 Oasi Research Institute-IRCCS, Troina, Italy.

3 Department of Molecular Medicine, Division of Biochemistry, Molecular Biology, and Nutrition, University Hospital of Nancy, F-54000 Nancy, France.

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: 10.1111/ALL.15147

This article is protected by copyright. All rights reserved

Accepted Article

4 Allergy Unit, Columbus Hospital, Fondazione Policlinico A. Gemelli, IRCCS, Rome, Italy.
5 Allergy Unit, Hospital Regional Universitario de Málaga-UMA-ARADyAL, Malaga, Spain.

Word count (not including abstract, figure legends and references): 2830
Number of tables: 4
Number of figures: 1

Short title: *HLA-DRB3*02:02* and delayed hypersensitivity to penicillins

Keywords (in alphabetic order): Amoxicillin; Beta-lactams; Drug allergy; Genome-wide association; HLA-DRB3; Nonimmediate reactions; Penicillins.

Corresponding Authors:
Prof. Jean-Louis Guéant, MD, DSc, AGAF and Antonino Romano, MD
Department of Molecular Medicine, Division of Biochemistry, Molecular Biology, and Nutrition, University Hospital of Nancy, and INSERM UMR_S 1256, Nutrition, Genetics, and Environmental Risk Exposure (NGERE), Faculty of Medicine of Nancy, 9 Avenue de la Forêt de Haye, F-54000, Nancy, France.
Telephone: +33 3 83 68 32 92
Fax: +33 3 83 15 35 91
E-mail addresses: jean-louis.gueant@univ-lorraine.fr and aromano.allergy@gmail.com

Abbreviations (alphabetical order)
AGEP: Acute generalized exanthematous pustulosis; DRESS: Drug reaction (or rash) with eosinophilia and systemic symptoms; EM: Expectation maximization; GWAS: Genome-wide association study; LD: Linkage disequilibrium; MPE: Maculopapular exanthema; NGS: Next-generation sequencing; OR: Odds ratio; QTL: Quantitative trait loci; SNP: Single nucleotide polymorphism.

This article is protected by copyright. All rights reserved

ABSTRACT

Background: Nonimmediate (delayed) allergic reactions to penicillins are common and some of them can be life-threatening. The genetic factors influencing these reactions are unknown/poorly known/poorly understood. We assessed the genetic predictors of a delayed penicillin allergy that cover the HLA loci.

Methods: Using next-generation sequencing (NGS), we genotyped the MHC region in 24 patients with delayed hypersensitivity compared with 20 patients with documented immediate hypersensitivity to penicillins recruited in Italy. Subsequently, we analyzed *in silico* Illumina Immunochip genotyping data that covered the HLA loci in 98 Spanish patients with delayed hypersensitivity and 315 with immediate hypersensitivity compared to 1,308 controls.

Results: The two alleles DRB3*02:02:01:02 and DRB3*02:02:01:01 were reported in twenty cases with delayed reactions (83%) and ten cases with immediate reactions (50%), but not in the Allele Frequency Net Database. Bearing at least one of the two alleles increased the risk of delayed reactions compared to immediate reactions, with an OR of 8.88 (95% CI, 3.37–23.32; P <0.0001). The haplotype (ACAA) from rs9268835, rs6923504, rs6903608, and rs9268838 genetic variants of the HLA-DRB3 genomic region was significantly associated with an increased risk of delayed hypersensitivity to penicillins (OR, 1.7; 95% CI: 1.06–1.92; P=0.001), but not immediate hypersensitivity.

Conclusion: We showed that the *HLA-DRB3* locus is strongly associated with an increased risk of delayed penicillin hypersensitivity, at least in Southwestern Europe. The determination of *HLA-DRB3*02:02* alleles in the risk management of severe delayed hypersensitivity to penicillins should be evaluated further in larger population samples of different origins.

This article is protected by copyright. All rights reserved

INTRODUCTION

Penicillin "allergy" is the most common drug-class allergy identified in electronic medical records of large healthcare systems.[1-3] Specifically, penicillin allergy was reported by 12.8% of the entire patient population (n = 1,766,328) of a study by Zhou et al.[2] Urticaria (hives) and rash are the reactions most commonly reported by patients presenting for a penicillin allergy evaluation.[4,5] In fact, natural penicillins (i.e., penicillin G and penicillin V) and aminopenicillins (i.e., ampicillin and amoxicillin) were the causative drugs counting for 100,943 (37.5%) of 269,493 "rash/dermatitis" cases and for 61,457 (40.8%) of 150,450 "hives/urticaria" instances listed in a US-based electronic-health-record analysis.[6]

Based on their chronology, hypersensitivity reactions to penicillins are classifiable as immediate or nonimmediate (also called delayed).[7,8] The former occurs within 6 hours after the last drug administration, though typically within one hour after the first dose of a new treatment course.[7,8] They usually manifest as isolated symptoms, such as urticaria, angioedema, bronchospasm, and hypotension, or as anaphylaxis, and are mostly associated with an IgE-mediated pathogenic mechanism. Nonimmediate reactions occur more than one hour after the initial drug administration, commonly after many days of treatment[7-9], are more frequent than immediate reactions[6] and are characterized by a wide range of clinical manifestations, including severe ones such as Stevens-Johnson syndrome/toxic epidermal necrolysis, drug reaction (or rash) with eosinophilia and systemic symptoms (DRESS), and acute generalized exanthematous pustulosis (AGEP). Maculopapular exanthema (MPE) and delayed-appearing urticaria are the most common clinical presentations of nonimmediate reactions.[7,8] In some nonimmediate reactions, especially DRESS, AGEP, and MPE, a T-cell-mediated pathogenic mechanism has been demonstrated on the basis of positive responses to patch tests and/or delayed-reading intradermal tests.[8,10]

Despite a high prevalence of nonimmediate reactions to penicillins, little is known about the influence of genetic determinants, in contrast to the pharmacogenetic data published on immediate reactions.[11-14] An Italian case-control study by Romano *et al.*[15] assessed the HLA-A2 and HLA-DRw52 alleles in 24 patients with MPE caused by delayed hypersensitivity to aminopenicillins compared to 522 subjects from the general population and found that they were significantly more prevalent among the former. However, it is noteworthy that no genome-wide association study (GWAS) or HLA genotyping with next-generation sequencing (NGS) has been performed to evaluate the contribution of the genetic determinants.[14,15] To address this issue, we evaluated the influence of HLA genetic determinants on the risk of nonimmediate reactions to

This article is protected by copyright. All rights reserved

penicillins by performing HLA NGS genotyping/ NGS-based HLA typing in 24 patients with delayed hypersensitivity and 20 patients with immediate hypersensitivity to penicillins recruited in Italy. Then we performed NGS-based HLA typing for a case-control study to estimate the association between the potentially enriched HLA alleles obtained and the risk of nonimmediate hypersensitivity to penicillins. We subsequently replicated the results using *in silico* data from the Illumina Immunochip genotyping array that covered the HLA loci in 98 Spanish patients with delayed hypersensitivity compared to 1,308 controls.

METHODS

Overview of the study design

We performed NGS-based HLA typing to assess potentially enriched HLA alleles in patients with thoroughly documented delayed hypersensitivity to penicillins. We also performed the HLA-based typing to assess the potentially enriched *HLA-DRB3* alleles in 20 patients with documented immediate hypersensitivity to penicillins. We compared the delayed-hypersensitivity cases with the immediate ones to estimate the risk of nonimmediate reactions related to *HLA-DRB3* among patients with hypersensitivity to penicillins. Finally, we replicated the results using GWAS data from our previous study of a large Spanish population.[11] We performed per-variant and per-haplotype association studies on the haplotype and variants significantly associated with the risk of delayed hypersensitivity.

Study sample description

Italian cases assessed by NGS

A total of 24 Italian cases with T-cell-mediated hypersensitivity to penicillins and 20 with immediate hypersensitivity were studied by NGS-based HLA genotyping. The participants were recruited in the allergy units of the Columbus Hospital, Rome, Italy and Oasi Research Institute-IRCCS, Troina, Italy between January 2000 and June 2016. Participants were evaluated by skin tests and patch tests with penicillin reagents, as previously described.[16,17] Skin tests and patch tests with five cephalosporins were also performed. The inclusion criterion of the 24 cases was a reported delayed reaction to penicillin and a positive patch test and/or delayed-reading skin test result to at least one penicillin reagent. The 20 cases with immediate hypersensitivity had typical clinical presentations and positive prick and/or intradermal reactions. The cases with negative cephalosporin allergy tests underwent controlled administrations of therapeutic doses of the

This article is protected by copyright. All rights reserved

cephalosporins concerned (**Supplemental Table 1**), as previously described.[16] The 24 subjects were classified into three groups according to the results of skin testing: group A, positive to ampicillin and amoxicillin; group B, positive to benzylpenicillin, ampicillin, and amoxicillin; and group C, positive to all three penicillins and aminocephalosporins.

Skin and patch tests of Italian cases

On the first day, prick and intradermal tests with immediate and delayed reading were carried out using penicilloyl-polylysine, minor determinant mixture , and benzylpenicillin as described previously.[16] Patch tests were administered with benzylpenicillin, ampicillin, amoxicillin, cephalexin, cefaclor, and cefadroxil in petrolatum as described.[17] All reagents of patch testing were applied to uninvolved skin on the interscapular region of the patient's back, using acrylate adhesive strips with small plates attached for test allergens (Curatest, Lohmann GmbH & Co. KG, Neuwied, Germany), as previously described.[17] Occlusion time was 48 hours. Readings of patch tests were made 15 minutes after removal of the strips and 48 hours later. Positive reactions were scored as: + (erythema, infiltration, possibly discrete papules); ++ (erythema, infiltration, papules, and vesicles); and +++ (intense erythema, infiltration, and coalescing vesicles). Participants who had negative results in the allergy tests with the alternative cephalosporins concerned underwent controlled administrations of therapeutic doses of ceftriaxone (1 g), intramuscularly, as well as cefuroxime axetil (500 mg), cephalexin (1 g), cefaclor (500 mg), and cefadroxil (500 mg), orally, each in a different day and in the above order, as previously described.[16] In case of a positive response to an aminocephalosporin, the others were not administered. Patients were carefully monitored during all allergy testing, and for six hours after challenges. They were also asked to return to the clinical department to show any positive responses.

Spanish cases and controls assessed by GWAS

Spanish patients were recruited in the Allergy Service, Carlos Haya Hospital, Malaga, Spain. From the initial samples of the 436 patients and 1218 control subjects, 413 and 1124 passed quality control for HLA loci, respectively, and were retained in the final statistical analyses. We used 184 additional control subjects from the same population of Malaga, which allowed us to perform the association study with 1308 controls, using the same inclusion and exclusion criteria. The retained population included 98 cases with delayed hypersensitivity to penicillins diagnosed as previously described[18] and 315 cases with immediate allergic reactions from the aforementioned study.[11] They ranged from 16 to 60 years old. The 1,308 controls were from Malaga and Salamanca (Spain), as previously described.[11] Age- and gender-paired volunteers

This article is protected by copyright. All rights reserved

were recruited in preventive-care consultations. All of them tolerated amoxicillin and other penicillins on different occasions, and they had no histories of allergic, dermatological, or respiratory diseases. Subjects who had never taken penicillins and those with a C-reactive protein blood concentration higher than 5 mg/L were excluded from the control group. Subjects with elevated C-reactive protein blood concentration were excluded as some of them had a history of cutaneous eruptions, which could have been related to allergic mechanisms. Since C-reactive protein is correlated with HLA haplotype, we decided to define the blood concentration of C-reactive protein above 5 mg/L as an exclusion criterion for patients, in order to avoid any bias in the comparison between patients and controls. All patients and controls were of Caucasian origin. Before allergy tests, all subjects received information about possible related risks, and written informed consent for participating in the study and providing DNA samples was obtained from each patient or the parents of those under 18 years of age. The respective institutional review boards approved the protocol.

NGS-based HLA genotyping

High-throughput genotyping of DNA samples was performed at the INSERM Unit UMR_S 1256 (NGERE, University of Lorraine, France) according to the manufacturer's recommendations. We used the Illumina TruSight HLA v2 (Illumina Inc. CA, USA) sequencing panel, which provides high-resolution sequencing of 11 HLA loci (*HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1/3/4/5*, *HLA-DQB1*, *HLA-DPB1*, *HLA-DQA1*, and *HLA-DPA1*), according to the manufacturer's instructions at the INSERM Unit UMR_S 1256 (NGERE, University of Lorraine, France). Libraries were run in parallel on a MiSeq system (Illumina Inc. CA, USA) using 250 bp paired-end reads. We performed HLA allele assignation on the generated FASTQ files, using the TruSight HLA Assign 2.0 Software (Illumina Inc. CA, USA) to align the sequence reads and referencing with the International ImMunoGeneTics Information System/HLA database. We assessed the allele frequency of each HLA allele in the 24 patients studied. We calculated the enrichment ratio for a given HLA allele by dividing its allelic frequency found in the study population by that observed in the control population, using the Allele Frequency Net Database (www.allelefrequencies.net).[19] We defined the potentially enriched HLA alleles in patients with delayed hypersensitivity to penicillins as those with a frequency in the study population of at least 20% and an enrichment ratio >2. In the second phase of the study, we used an allelic model to estimate the odds ratio (OR) and the absolute risk difference for the association between carrying at least one *DRB3*02:02*

This article is protected by copyright. All rights reserved

allele *(DRB3*02:02:01:02* or *DRB3*02:02:01:01*) and the risk of delayed hypersensitivity to penicillins. We reported the 95% confidence interval (95% CI) and the associated *P*-values for the OR and the absolute risk difference using z-statistic and Chi-squared tests, respectively.

Haplotype analysis and genetic association study

We used *in silico* data generated from a previously reported fine-mapping GWAS on genetic predictors of penicillin allergy using the Illumina Immunochip array (Illumina Inc. CA, USA) that covers the HLA loci.[11] We extracted from the full dataset the genotypic data corresponding to the genetic variants located in the *HLA-DRB3* locus and its vicinity. The *HLA-DRB3* locus is not referenced according to the GRCh38/hg38 build, but rather according to the *Homo sapiens* chromosome 6 genomic contig, GRCh38 reference assembly alternate locus group ALT_REF_LOCI_2[20,21] with the following coordinates: hg38 chr6_GL000251v2_alt:3,934,009-3,947,126. Since the genomic positions of the Illumina Immunochip array were initially reported according to the NCBI36 build, we used the Liftover tool[22] from the UCSC Genome Browser database to convert the genomic position of the *HLA-DRB3* locus from the GRCh38 reference assembly to the NCBI36 build (chr6:32,571,675-32,584,792). Because of the complex structure and high level of linkage disequilibrium (LD) in the HLA locus,[23] we considered all the genetic variants located in the intergenic region between the *HLA-DRA* and *HLA-DRB5* genes that included the *HLA-DRB3* gene. Sample quality-control measures included: sample call rate (>90%), overall heterozygosity, and relatedness testing. We assessed cryptic relatedness using identity-by-descent analysis. Genetic variants were removed from the primary analysis if they had a call rate <90%, a significant departure from Hardy-Weinberg equilibrium (exact HWE-$P < 10^{-4}$ among controls), or a minor allele frequency <5%. We performed the genetic association analysis according to the allelic model. We completed the haplotype association analysis using a moving window with a fixed width of 4 markers. We performed LD-pairwise analysis on all adjacent pairs of genetic variants using a matrix output for both the expectation-maximization (EM) algorithm and the composite-haplotype method.[24,25] We used D' values in the LD plots. We estimated haplotype frequencies using the EM algorithm with maximum EM iterations of 50 and an EM convergence tolerance of 0.0001.[26] We compared haplotype frequencies using the Chi-squared test and reported the corresponding OR, the 95% confidence interval, and the associated *P*-value for each haplotype. Given the exploratory nature of our analysis, we considered a genomic region as potentially relevant if it encompassed genetic variants that were significantly associated with the

This article is protected by copyright. All rights reserved

risk of delayed hypersensitivity to penicillins in both per-variant and per-haplotype association analyses. All statistical analyses were performed using the SNP & Variation Suite (Golden Helix, Inc., Bozeman, MT, USA).

RESULTS

HLA genotyping by NGS in cases with delayed hypersensitivity and cases with immediate hypersensitivity to penicillins

The clinical characteristics and test results of the 24 patients with delayed hypersensitivity to penicillins who were subjected to HLA NGS/ NGS-based HLA typing are detailed in **Supplemental Tables 1 and 2**. With the exception of patient 17 who had had a bullous exanthema with edema associated with bacampicillin treatment, none of the other patients in the cohort had a severe cutaneous adverse reaction (SCAR) as presentation. Twenty-two of the 24 patients had/displayed both positive patch test and delayed intradermal test responses, which indicate a delayed hypersensitivity mechanism **Supplemental Table 2**. This pattern of positivity does not need to be supported by challenges, as previously reported.[15,16] Of the remaining two patients with negative patch tests and positive delayed-reading intradermal tests, one (patient 7) underwent challenge with the amoxicillin experiencing a MPE and the other (patient 22) was not provoked as he had experienced mild urticaria a few minutes after benzylpenicillin intradermal tests (**Supplemental Tables 1 and 2)**. Taking into account the results of skin testing, subjects classified as group A (i.e., positive only to ampicillin and amoxicillin, n = 14) were more numerous than those with less selective responses classified as group B (i.e., positive to benzylpenicillin, ampicillin and amoxicillin, n = 6) and C (i.e., positive to penicillins and aminocephalosporins, n = 4). The clinical characteristics and allergy test results of the 20 subjects with IgE-mediated hypersensitivity are given in **Supplemental Table 3.** All patients were retained after the quality control step of NGS. On the HLA alleles retrieved in the 24 Italian patients, two HLA alleles, *DRB3*02:02:01:02* and *DRB3*02:02:01:01*, had a dramatic enrichment among the seven alleles with an allelic frequency >20%, compared to the control population of the Allele Frequency Net Database (**Table 1 and Supplemental Tables 4 and 5**). The alleles *DRB3*02:02:01:02* and *DRB3*02:02:01:01* predicted a very high risk of delayed reaction, which could not be evaluated precisely because such alleles had not been previously reported in the general European population. 83% of the patients (20/24) harbored at least one of the two reported *HLA-DRB3* alleles, and 71% (11/24) were homozygous for one of the two *HLA-DRB3* alleles or had a

This article is protected by copyright. All rights reserved

compound heterozygous status (**Table 2 and Supplemental Table 4**). We observed a higher frequency of *DRB3*02:02:01:02* and *DRB3*02:02:01:01* homozygous genotypes in group A (n=12, 69%) than in group B and C patients (n=3, 30%). However, no difference of allele frequency was observed among the 3 groups (**Table 4**).

The allele frequency of the *DRB3*02:02* alleles (*DRB3*02:02:01:02* and *DRB3*02:02:01:01*) was significantly higher in patients with delayed hypersensitivity than in those with immediate hypersensitivity to penicillins (77% vs. 28%, respectively). These figures corresponded to an increased risk of delayed reactions in penicillin hypersensitivity cases harboring *DRB3*02:02* alleles, with an OR of 8.9 (95% CI, 3.4–23.3; P <0.0001) and an absolute risk difference of 49% (29%–64%; P <0.0001).

The HLA-DRB3 association did not appear to be affected by the severity of the initial delayed reaction. Indeed, patients with mild reactions still maintained this association.

Association of the *HLA-DRB3* locus with delayed hypersensitivity to penicillins in the Spanish population

In order to confirm the potential enrichment in the *HLA-DRB3* locus showed by the HLA NGS/NGS-based HLA typing of the 24 Italian patients, we performed a fine-mapping genetic association study to assess the per-variant and per-haplotype risk associations with genetic variants of the *HLA-DRB3* genomic region in 98 Spanish patients with delayed hypersensitivity compared with 1,308 controls. The clinical and demographic characteristics of patients and controls are given in **supplemental tables 6, 7 and 8**, respectively. The haplotype block is shown in **Figure 1**. We found that a haplotype (*ACAA*) from four genetic variants – rs9268835, rs6923504, rs6903608, and rs9268838 – was significantly associated with the risk of delayed hypersensitivity to penicillins (OR, 1.7; 95% CI: 1.06–1.92; P=0.001) (**Table 3**). In the per-variant analysis, two variants (rs9268835, rs9268838) of the four included in the *ACAA* haplotype were significantly associated with the risk of delayed hypersensitivity to penicillins, with a similar effect size (**Table 4**). We subsequently assessed the risk association of the *ACAA* haplotype in 315 patients with immediate reactions compared with 1,308 controls. The haplotype and per-variant association studies revealed no significant associations with the *ACAA* haplotype or with the rs9268835 and rs9268838 variants (**Tables 3 and 4**).

DISCUSSION

This article is protected by copyright. All rights reserved

We sequenced the HLA region of 24 patients with delayed and 20 with immediate hypersensitivity to penicillins in order to analyze the risk association for all high-resolution alleles with delayed hypersensitivity. We observed a strong association of *DRB3*02:02:01:02* and *DRB3*02:02:01:01* alleles with the risk of penicillin delayed hypersensitivity in the 24 Italian patients. It is noteworthy that as many as 83% of the cases carried a *DRB3*02:02* allele. The frequency of homozygous cases for the variants of the *HLA-DRB3* locus was higher in group A (with selective hypersensitivity to ampicillin and amoxicillin) than in the less selective groups B and C, suggesting that the association reflects the specificity of hapten recognition. All 24 Italian patients were of Caucasian origin. Therefore, larger studies involving patients of different ethnicities with delayed hypersensitivity reactions to penicillins are needed to confirm these associations. The association of the *HLA-DRB3* locus with an increased risk of penicillin delayed hypersensitivity was confirmed by the *in silico* study of genetic variants of the *HLA-DRB3* genomic region in Spanish patients. The protein encoded by the *HLA-DRB3* gene belongs to the HLA class II beta chain paralogs, including DRB1, DRB4, and DRB5. It is part of a heterodimer consisting of an alpha chain (DRA) and a beta one (DRB) and is involved in the antigen presentation of peptides derived from extracellular proteins. The association of *HLA-DRB3*02:02* with the risk of penicillin delayed hypersensitivity has never been reported for any other drug category so far. The *HLA-DRB3*02:02* allele is independently and strongly associated with an increased risk of *PLA2R*-related idiopathic membranous nephropathy in a Chinese population.[23] Therefore, our results suggest investigating the increased risk of delayed reactions to penicillins in this disease.

Only one study had previously evaluated the genetic determinants that contribute to the risk of delayed hypersensitivity to penicillins. This Italian case-control study assessed *HLA-A2* and *HLA-DRw52* alleles in 24 patients with MPE caused by delayed hypersensitivity to aminopenicillins and 522 subjects from the general population.[15] The *HLA-A2* and *HLA-DRw52* alleles were significantly more prevalent among cases. However, we did not replicate this result in the NGS of the MHC region in our study. A French study assessed nine variants on genes controlling cytokine production (i.e., *IL1*, *IL1B*, *IL1RN*, *IL2*, *IL4*, *IL5*, *IL10*, *IL16*, and *TNF*) in 118 patients with well-defined cutaneous adverse drug reactions and 236 controls.[27] Several responsible drugs were considered in the study, including beta-lactams. The haplotype combining *IL1RN A2* and *IL1B 511C* alleles was associated with an increased risk of DRESS (OR = 3.22; 95% CI: 1.23-8.41).[27] However, the number of cases was too limited to evaluate the specific risk of reactions to penicillins.

This article is protected by copyright. All rights reserved

We compared our data with pharmacogenetic research on immediate penicillin allergy. Most studies have evaluated genetic determinants of immediate reactions to beta-lactams utilizing a candidate-gene approach. They found an association with genes involved in IgE production, atopy, and inflammation, including 3 genes validated by replications: *ILR4*, *NOD2*, and *LGALS3*.[13,28-30] It is noteworthy that no association with *HLA-DRB1*10:01*, *ILR4*, *NOD2*, or *LGALS3* was identified by GWAS in our patients with delayed hypersensitivity reactions to penicillins. Despite the vicinity of the *HLA-DRB1*/*3*/*4*/*5* loci in chromosome 6, it is noteworthy that we did not find any association between the risk of delayed reactions to penicillins and the rs7754768 and rs9268832 of the *HLA-DRA*|*HLA-DRB5* inter-region in a GWAS performed in Italian and Spanish populations.[11] Similarly, we found no risk association of delayed reactions with the rs71542416 within the Class II HLA region, which is associated with the risk of immediate beta-lactam allergy in GWAS performed in Italian and Spanish populations.[12]

In conclusion, we showed that the *HLA-DRB3*02:02* alleles are strongly associated with an increased risk of delayed hypersensitivity to penicillins, at least in Southwestern Europe. In regard to the severity of some of the reactions, the interest for using the determination of *HLA-DRB3*02:02* alleles in the risk management of delayed hypersensitivity to penicillins should be evaluated in further studies of larger populations of different origin.

Accepted Article

This article is protected by copyright. All rights reserved

TABLES

Table 1. Frequency of the HLA alleles found in the 24 Italian patients with delayed hypersensitivity to penicillins.

HLA allele	Allele frequency (study population)	Allele frequency (control population)*	Enrichment ratio
*DRB3*02:02:01:02*	0.54	Absent in Europe	High
*DRB3*02:02:01:01*	0.23	Absent in Europe	High
*DQB1*03:01:01:02*	0.42	0.35	1.21
*DPB1*04:01:01:01*	0.42	0.26	1.60
*DQA1*05:05:01:01*	0.40	0.30	1.34
*A*02:01:01:01*	0.21	0.26	0.80
*C*07:01:01:01*	0.21	0.18	1.14

*We calculated the enrichment ratio for a given HLA allele by dividing its allelic frequency observed in the study population by that observed in the control population using the Allele Frequency Net Database (www.allelefrequencies.net).[19]

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

Table 2. Distribution of the HLA DRB3*02:02 alleles in Italian patients with delayed and those with immediate hypersensitivity to penicillins

	Delayed (n = 24)		**Delayed, Group A* (n = 14)**		**Delayed, Group B† (n = 6)**		**Delayed, Group C‡ (n = 4)**		**Delayed, Groups B/C‡ (n = 10)**		**Immediate (n = 20)**	
***DRB3*02:02* alleles status**	*n*	%	*n*	%	*n*	%	*n*	%	*N*	%	*n*	**%**
*DRB3*02:02:01:02*, homozygous	11	46%	8	57%	2	33%	1	25%	3	30%	0	0%
*DRB3*02:02:01:01*, homozygous	3	13%	3	21%	0	0%	0	0%	0	0%	0	0%
*DRB3*02:02:01:01*/*DRB3*02:02:01:02*	3	13%	0	0%	1	17%	2	50%	3	30%	1	5%
*DRB3*02:02:01:01*, heterozygous	2	8%	0	0%	1	17%	1	25%	2	20%	0	0%
*DRB3*02:02:01:02*, heterozygous	1	4%	0	0%	1	17%	0	0%	1	10%	9	45%
No *DRB3*02:02* allele	4	17%	3	21%	1	17%	0	0%	1	10%	10	50%

Note. *n*: number of patients; %: percentage.

*Group A: reactive only to ampicillin and amoxicillin.

†Group B: reactive to benzylpenicillin, ampicillin, and amoxicillin.

‡Group C: reactive to both penicillins and cephalosporins.

This article is protected by copyright. All rights reserved

Accepted Article

This article is protected by copyright. All rights reserved

Table 3. Haplotype association study for the association between HLA-DRB3 haplotypes and the risk of delayed and immediate hypersensitivity to penicillins in the Spanish population

Haplotype*	Beta-lactam hypersensitivity type	Odds ratio (95% CI)	*P*-value
ACAA	Delayed	1.42 (1.06–1.92)	0.02
	Immediate	1.14 (0.95–1.37)	0.17
GGGG	Delayed	0.79 (0.58–1.08)	0.14
	Immediate	0.96 (0.80–1.15)	0.66
GCAG	Delayed	0.88 (0.64–1.21)	0.45
	Immediate	0.92 (0.76–1.11)	0.36

Note. 95% CI: 95% confidence interval.

**HLA-DRB3* markers included in the haplotype: rs9268835, rs6923504, rs6903608, rs9268838.

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

Table 4. Genetic association study for the association between *HLA-DRB3* genetic variants and the risk of delayed and immediate hypersensitivity to penicillins in the Spanish population

Name	Risk/Ref. allele	Penicillin hypersensitivity type	Cases (n); Controls (n)	Call rate	MAF (Cases)	MAF (Controls)	Odds ratio (95% CI)	Fisher's Exact *P*-value
rs9268835	A/G	Delayed	98; 1302	0.986	0.39	0.31	1.42 (1.05–1.91)	0.03
		Immediate	315; 1302	0.990	0.34	0.31	1.14 (0.94–1.37)	0.18
rs6923504	G/C	Delayed	98; 1300	0.985	0.31	0.37	0.78 (0.57–1.06)	0.12
		Immediate	315; 1300	0.990	0.36	0.37	0.95 (0.79–1.14)	0.61
rs6903608	G/A	Delayed	98; 1308	0.990	0.31	0.36	0.80 (0.59–1.09)	0.17
		Immediate	315; 1308	0.990	0.36	0.36	0.98 (0.81–1.17)	0.82
rs9268838	A/G	Delayed	98; 1303	0.987	0.39	0.31	1.43 (1.06–1.94)	0.02
		Immediate	315; 1303	0.990	0.34	0.31	1.15 (0.95–1.38)	0.15

Note. *n*: number of subjects; MAF: minor allele frequency; 95% CI: 95% confidence interval.

This article is protected by copyright. All rights reserved

Accepted Article

FIGURE LEGEND

Figure 1. (A) Manhattan plot on the *HLA-DRA|HLA-DRB5* intergenic region, including the *HLA-DRB3* locus and its vicinity for reporting the genetic association analysis for the primary risk of nonimmediate reactions to penicillins, according to the allelic model. **(B)** Linkage disequilibrium plot illustrating the D' values between the lead variants and the other variants in the genomic region is indicated by color. The green pentagon indicates the haplotype block formed by the four variants: rs9268835, rs6923504, rs6903608, and rs9268838. **(C)** genomic context of the *HLA-DRA|HLA-DRB5* genomic region.

CONFLICTS OF INTEREST AND FUNDING

The authors have no conflicts of Interest to declare. This work was supported by a grant from the French Ministry of Health (National Program for Clinical Research, PHRC), a "Chercheur d'excellence" grant from the Region of Lorraine (France), the research projects FHU ARRIMAGE and OMAGE from Region Grand-Est and FEDER and the French PIA project "Lorraine Université d'Excellence", reference ANR-15-IDEX-04-LUE.

This article is protected by copyright. All rights reserved

Accepted Article

REFERENCES

1. Macy E, Contreras R. Health care use and serious infection prevalence associated with penicillin "allergy" in hospitalized patients: A cohort study. *J Allergy Clin Immunol.* 2014;133(3):790-796.
2. Zhou L, Dhopeshwarkar N, Blumenthal KG, et al. Drug allergies documented in electronic health records of a large healthcare system. *Allergy.* 2016;71(9):1305-1313.
3. Macy E. Penicillin and beta-lactam allergy: epidemiology and diagnosis. *Curr Allergy Asthma Rep.* 2014;14(11):476.
4. Castells M, Khan DA, Phillips EJ. Penicillin Allergy. *N Engl J Med.* 2019;381(24):2338-2351.
5. Shenoy ES, Macy E, Rowe T, Blumenthal KG. Evaluation and Management of Penicillin Allergy: A Review. *JAMA.* 2019;321(2):188-199.
6. Wong A, Seger DL, Lai KH, Goss FR, Blumenthal KG, Zhou L. Drug Hypersensitivity Reactions Documented in Electronic Health Records within a Large Health System. *J Allergy Clin Immunol Pract.* 2019;7(4):1253-1260 e1253.
7. Demoly P, Adkinson NF, Brockow K, et al. International Consensus on drug allergy. *Allergy.* 2014;69(4):420-437.
8. Romano A, Atanaskovic-Markovic M, Barbaud A, et al. Towards a more precise diagnosis of hypersensitivity to beta-lactams - an EAACI position paper. *Allergy.* 2020;75(6):1300-1315.
9. Pichler WJ. Immune pathomechanism and classification of drug hypersensitivity. *Allergy.* 2019;74(8):1457-1471.
10. Barbaud A, Collet E, Milpied B, et al. A multicentre study to determine the value and safety of drug patch tests for the three main classes of severe cutaneous adverse drug reactions. *Br J Dermatol.* 2013;168(3):555-562.
11. Gueant JL, Romano A, Cornejo-Garcia JA, et al. HLA-DRA variants predict penicillin allergy in genome-wide fine-mapping genotyping. *J Allergy Clin Immunol.* 2015;135(1):253-259.
12. Nicoletti P, Carr DF, Barrett S, et al. Beta-lactam-induced immediate hypersensitivity reactions: A genome-wide association study of a deeply phenotyped cohort. *J Allergy Clin Immunol.* 2020.

This article is protected by copyright. All rights reserved

13. Oussalah A, Mayorga C, Blanca M, et al. Genetic variants associated with drugs-induced immediate hypersensitivity reactions: a PRISMA-compliant systematic review. *Allergy.* 2016;71(4):443-462.
14. Oussalah A, Yip V, Mayorga C, et al. Genetic variants associated with T cell-mediated cutaneous adverse drug reactions: A PRISMA-compliant systematic review-An EAACI position paper. *Allergy.* 2020;75(5):1069-1098.
15. Romano A, De Santis A, Romito A, et al. Delayed hypersensitivity to aminopenicillins is related to major histocompatibility complex genes. *Ann Allergy Asthma Immunol.* 1998;80(5):433-437.
16. Romano A, Gaeta F, Valluzzi RL, Maggioletti M, Caruso C, Quaratino D. Cross-reactivity and tolerability of aztreonam and cephalosporins in subjects with a T cell-mediated hypersensitivity to penicillins. *J Allergy Clin Immunol.* 2016;138(1):179-186.
17. Romano A, Quaratino D, Di Fonso M, Papa G, Venuti A, Gasbarrini G. A diagnostic protocol for evaluating nonimmediate reactions to aminopenicillins. *J Allergy Clin Immunol.* 1999;103(6):1186-1190.
18. Torres MJ, Sanchez-Sabate E, Alvarez J, et al. Skin test evaluation in nonimmediate allergic reactions to penicillins. *Allergy.* 2004;59(2):219-224.
19. Gonzalez-Galarza FF, McCabe A, Santos E, et al. Allele frequency net database (AFND) 2020 update: gold-standard data classification, open access genotype data and new query tools. *Nucleic Acids Res.* 2020;48(D1):D783-D788.
20. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature.* 2001;409(6822):860-921.
21. Horton R, Gibson R, Coggill P, et al. Variation analysis and gene annotation of eight MHC haplotypes: the MHC Haplotype Project. *Immunogenetics.* 2008;60(1):1-18.
22. Navarro Gonzalez J, Zweig AS, Speir ML, et al. The UCSC Genome Browser database: 2021 update. *Nucleic Acids Res.* 2021;49(D1):D1046-D1057.
23. Mladkova N, Kiryluk K. Genetic Complexities of the HLA Region and Idiopathic Membranous Nephropathy. *J Am Soc Nephrol.* 2017;28(5):1331-1334.
24. Remington DL, Thornsberry JM, Matsuoka Y, et al. Structure of linkage disequilibrium and phenotypic associations in the maize genome. *Proc Natl Acad Sci U S A.* 2001;98(20):11479-11484.

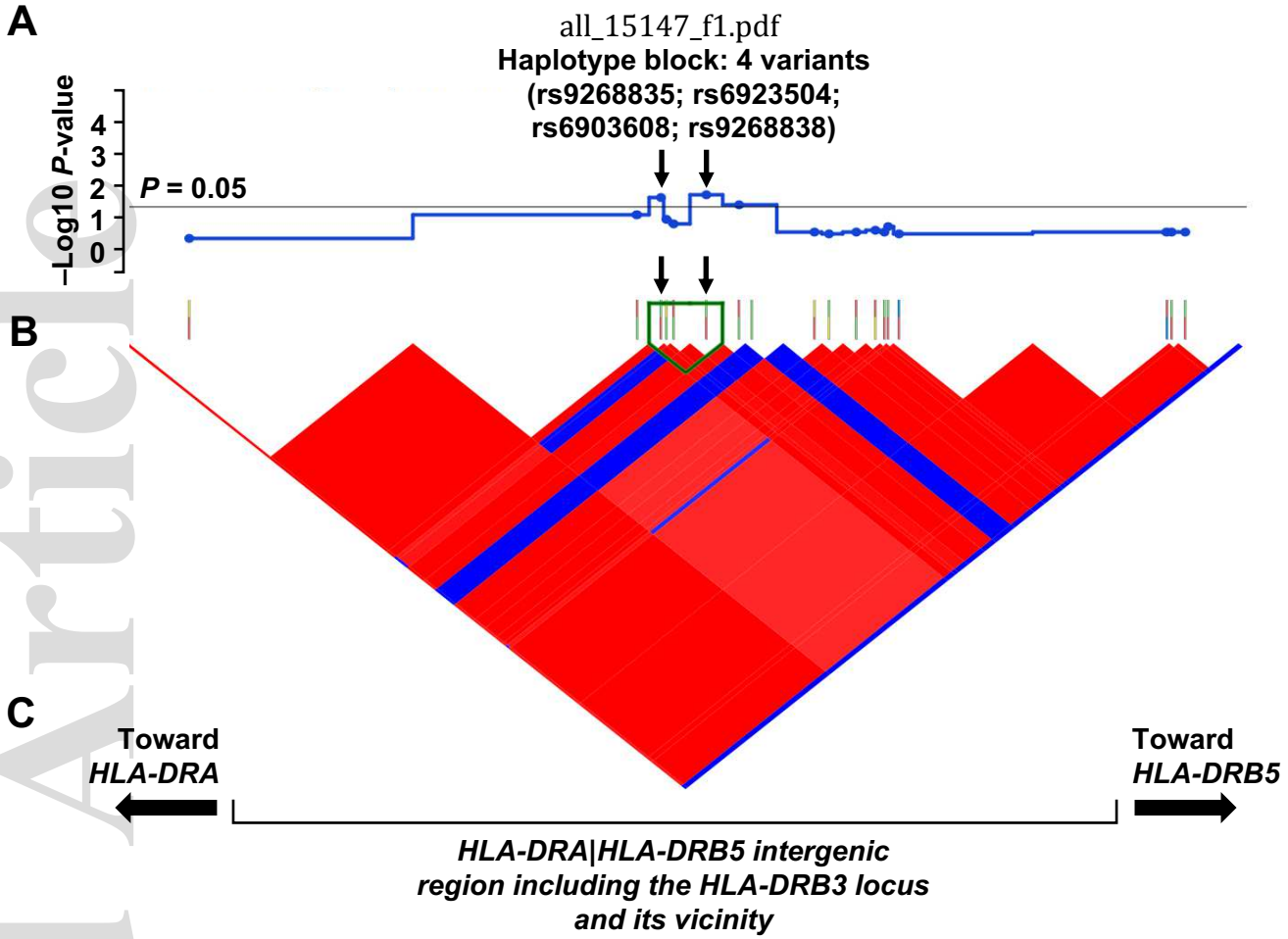
This article is protected by copyright. All rights reserved

25. Lewontin RC. The Interaction of Selection and Linkage. I. General Considerations; Heterotic Models. *Genetics.* 1964;49(1):49-67.
26. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. *Science.* 2002;296(5576):2225-2229.
27. Barbaud A, Waton J, Herbeth B, et al. Comparison of cytokine gene polymorphism in drug-induced maculopapular eruption, urticaria and drug reaction with eosinophilia and systemic symptoms (DRESS). *J Eur Acad Dermatol Venereol.* 2014;28(4):491-499.
28. Cornejo-Garcia JA, Romano A, Gueant-Rodriguez RM, et al. A non-synonymous polymorphism in galectin-3 lectin domain is associated with allergic reactions to beta-lactam antibiotics. *Pharmacogenomics J.* 2016;16(1):79-82.
29. Bursztejn AC, Romano A, Gueant-Rodriguez RM, et al. Allergy to betalactams and nucleotide-binding oligomerization domain (NOD) gene polymorphisms. *Allergy.* 2013;68(8):1076-1080.
30. Gueant-Rodriguez RM, Romano A, Beri-Dexheimer M, Viola M, Gaeta F, Gueant JL. Gene-gene interactions of IL13 and IL4RA variants in immediate allergic reactions to betalactam antibiotics. *Pharmacogenet Genomics.* 2006;16(10):713-719.

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article



This article is protected by copyright. All rights reserved

Figure 1

Supplemental Table 1. Clinical characteristics of the 24 Italian subjects with delayed hypersensitivity

Patient, n	Sex	Age (y)	Culprit drug	Route of administration	Eliciting dose*	Time interval§ (n of hours)	Type of reaction	Duration (n of days)
1	F	50	AX	Orally	12^{th}	3	MPE/ED/SS	30
2	F	53	AX/BC/AX	Orally	$3^{rd}/3^{rd}/1^{st}$	10/9/6	3 times MPE/ED/SS	6
3	F	27	AX+C	Orally	2^{nd}	2	MPE/ED/SS	8
4	M	28	BC/AX	Orally	$6^{th}/5^{th}$	4/3	2 times MPE/ED/SS	7
5	F	24	BC/AX+C	Orally	$13^{th}/1^{st}$	6/10	MPE/ED – MPE/SS	30
6	M	38	AM	Orally	14^{th}	10	MPE	30
7	F	34	AX	Orally	13^{th}	8	MPE/SS	4
8	M	71	AX	Orally	14^{th}	10	MPE/SS	20
9	M	47	AM	Orally	2^{nd}	6	MPE	6
10	F	44	AX/AM	Orally	$4^{th}/3^{rd}$	12/10	MPE - MPE/ED/SS	15
11	M	14	AX/AX	Orally	$14^{th}/3^{rd}$	12/4	MPE – MPE/ED/SS	15
12	F	26	AX+C/AX	Orally	$1^{st}/1^{st}$	6/5	MPE/ED – MPE/SS	6
13	F	63	AM/AX+C	Orally	$4^{th}/2^{nd}$	12/3	2 times MPE/ED/SS	20
14	F	68	AX/BC	Orally	$10^{th}/3^{rd}$	4/2	MPE – MPE/SS	10
15	M	68	BC	Orally	6^{th}	12	MPE	7
16	M	51	AX	Orally	3^{rd}	4	MPE/SS	30
17	F	45	BC	Orally	$13t^{h}$	2	BE/ED	30
18	F	28	BC	Orally	4^{th}	3	MPE/ED	10
19	F	56	AX/AM	Orally	$1^{st}/1^{st}$	6/5	2 times MPE/SS	30
20	F	53	BC/AM	Orally	$1^{st}/1^{st}$	12/8	2 times MPE	15
21	M	52	PG/PG	Topically	$3r^{d}/1^{st}$	6/3	EC – ER/ED	7
22	M	60	AM	Intramuscularly	2^{nd}	3	MPE/ED	21
23	F	20	PG	Intramuscularly	1^{st}	6	MPE	7
24	F	27	AX	Orally	3^{rd}	5	MPE/ED/SS	7

AM, ampicillin; AX, amoxicillin; BC, bacampicillin; BE, bullous exanthema; C, clavulanic acid; EC, eczema; ED, edema; ER, erythema; F, female; M, male; MPE, maculopapular exanthema; PG, penicillin G (or benzylpenicillin); SS, skin scaling.

*Last dose given after which symptoms appeared.

§Time elapsed between the last dose administered and the onset of symptoms.

Supplemental Table 2. Allergy test results of the 24 Italian subjects with delayed hypersensitivity

Patient, n	Patch tests						Delayed-reading intradermal tests										Cephalosporin challenges				
	PG	AM	AX	CH	CE	CD	PPL/ BP-OL	MDM/ MD	PG	AM	AX	CH	CE	CD	CU	CT	CT	CU	CH	CE	CD
1	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
2	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
3	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
4	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
5	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
6	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
7*	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
8	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
9	+	+	+	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–
10	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
11	–	+	–	–	–	–	+ (i)	+ (i)	–	+	+	–	–	–	–	+ (i)	np	np	–	–	–
12	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
13	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
14	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
15	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
16	+	+	+	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–
17	+	+	+	–	–	–	–	–	+	+	+	±	+	–	–	–	–	–	np	np	np
18	+	+	+	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–	np	np	np
19	–	+	+	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–	np	np	np
20	–	+	+	+	+	+	–	–	+	+	+	+	+	+	–	–	–	–	np	np	np
21	+	+	+	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–
22§	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–
23	+	+	+	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–
24	+	+	+	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–

AM, ampicillin; AX, amoxicillin; BP-OL, benzylpenicilloyl-octa-L-lysine; CD, cefadroxil; CE, cefaclor; CH; cephalexin; CT, ceftriaxone; CU, cefuroxime; F, female; (i), immediate reading; M, male; MD, sodium benzylpenilloate; MDM, minor determinant mixture; np, not performed; PG, penicillin G (or benzylpenicillin); PPL, penicilloyl-polylysine.

*She underwent challenge with AX (500 mg) and experienced MPE after 24 hours.

§He experienced mild urticaria few minutes after intradermal testing with benzylpenicillin which was negative at immediate reading.

Supplemental Table 3. Clinical characteristics and allergy test results of the 20 subjects with IgE-mediated hypersensitivity

Patient, *n*	Sex	Age	Type of reaction	ImmunoCAP					Skin tests									
				PG	PV	AMy	AXy	CE	BP-OL	MD	BP	AM	AX	CH	CE	CD	CT	CU
1	M	20	An	-	-	-	0.46	-	-	-	-	+	+	-	-	-	-	-
2	F	18	An	1.44	1.74	1.73	3.07	-	-	-	+	+	+	-	-	-	-	-
3	F	54	An	-	-	0.14	0.2	0.12	-	-	+	+	+	+	+	+	-	-
4	F	22	UA	-	-	-	0.27	-	-	-	-	+	+	-	-	-	-	-
5	F	49	An	0.18	3.54	5.27	0.24	-	-	-	+	+	+	+	+	+	-	-
6	F	73	An	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-
7	F	50	U An	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-
8	F	39	An	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-
9	F	59	An	-	-	-	0.63	-	-	-	-	-	+	-	-	-	-	-
10	M	42	UA	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
11	F	70	UA	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
12	F	52	An	-	-	-	0.28	-	-	-	-	+	+	-	-	-	-	-
13	F	61	UA	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-
14	F	35	An An	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
15	M	63	An	-	-	-	0.8	-	-	-	-	+	+	-	-	-	-	-
16	F	67	An	0.57	0.8	0.39	0.17	0.11	-	-	+	+	+	-	-	-	-	-
17	M	47	An	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
18	F	61	An An	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
19	F	58	U An	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-
20	F	62	An	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-

AM, ampicillin; AMy, ampicilloyl; An, anaphylaxis; AX, amoxicillin; AXy, amoxilloyl; BC, bacampicillin; BP, benzylpenicillin; BP-OL, benzylpenicilloyl-octa-L-lysine; C, clavulanic acid; CD, cefadroxil; CE, cefaclor; CH, cephalexin; CT, ceftriaxone; CU, cefuroxime; F, female; M, male; MD, minor determinant; PG, penicilloyl G; PV, penicilloyl V; U, urticaria; UA = urticaria and angioedema.

Supplemental Table 4. Frequency of the HLA alleles found in the 24 Italian patients with delayed hypersensitivity to penicillins

HLA alleles	Allele frequency
DRB3=DRB3*02:02:01:02	0.54
DQB1=DQB1*03:01:01:02	0.42
DPB1=DPB1*04:01:01:01	0.42
DQA1=DQA1*05:05:01:01	0.40
DRB3=DRB3*02:02:01:01	0.23
HLA_A=A*02:01:01:01	0.21
HLA_C=C*07:01:01:01	0.21
DRB1=DRB1*11:04:01	0.19
DRB1=DRB1*07:01:01:01	0.17
DRB1=DRB1*11:01:01:01	0.17
DRB4=DRB4*01:01:01:01	0.17
DQA1=DQA1*02:01:01:01	0.17
DPA1=DPA1*01:03:01:01	0.17
HLA_A=A*03:01:01:01	0.15
HLA_A=A*24:02:01:01	0.15
HLA_B=B*18:01:01:02	0.15
HLA_C=C*04:01:01:01	0.15
DPB1=DPB1*02:01:02:01	0.15
HLA_B=B*51:01:01:01	0.13
DPA1=DPA1*01:03:01:05	0.13
HLA_B=B*49:01:01:01	0.10
DRB1=DRB1*03:01:01:01	0.10
DRB4=DRB4*01:03:01:01	0.10
DRB4=DRB4*01:03:01:02N	0.10
DQB1=DQB1*02:01:01	0.10
HLA_A=A*01:01:01:01	0.08
HLA_C=C*12:03:01:01	0.08
DRB1=DRB1*13:02:01:02	0.08
DRB3=DRB3*03:01:01	0.08
DQB1=DQB1*06:04:01	0.08
DQB1=DQB1*02:02:01:01	0.08
DQB1=DQB1*03:03:02:01	0.08
DQA1=DQA1*01:02:01:04	0.08
DPB1=DPB1*04:02:01:01	0.08
HLA_B=B*38:01:01:01	0.06

HLA_C=C*15:02:01:01	0.06
HLA_C=C*05:01:01:02	0.06
HLA_C=C*04:01:01:06	0.06
DRB1=DRB1*04:03:01:01	0.06
DRB3=DRB3*01:01:02:01	0.06
DQB1=DQB1*03:02:01:01	0.06
DQA1=DQA1*03:01:01	0.06
DQA1=DQA1*05:01:01:03	0.06
HLA_A=A*25:01:01:01	0.04
HLA_A=A*32:01:01:01	0.04
HLA_A=A*66:01:01:01	0.04
HLA_A=A*23:01:01:01	0.04
HLA_A=A*30:01:01:01	0.04
HLA_A=A*31:01:02:01	0.04
HLA_B=B*07:02:01:01	0.04
HLA_B=B*18:01:01:01	0.04
HLA_B=B*44:02:01:01	0.04
HLA_B=B*35:01:01:02	0.04
HLA_B=B*07:05:01:01	0.04
HLA_B=B*35:08:01:01	0.04
HLA_B=B*44:03:01:01	0.04
HLA_B=B*57:01:01:01	0.04
HLA_C=C*07:02:01:03	0.04
HLA_C=C*05:01:01:01	0.04
HLA_C=C*15:05:02:01	0.04
DRB4=DRB4*01:03:02	0.04
DRB5=DRB5*01:02	0.04
DRB5=DRB5*01:01:01:01	0.04
DQB1=DQB1*05:01:01:02	0.04
DQA1=DQA1*01:01:01:01	0.04
DQA1=DQA1*05:01:01:01	0.04
DQA1=DQA1*05:05:01:13	0.04
DPB1=DPB1*104:01:01:01	0.04
DPB1=DPB1*04:02:01:02	0.04
DPA1=DPA1*02:01:01:02	0.04
HLA_A=A*01:03:01:02	0.02
HLA_A=A*30:02:01:01	0.02
HLA_A=A*03:02:01	0.02
HLA_A=A*68:01:01:02	0.02

HLA_A=A*68:02:01:01	0.02
HLA_A=A*29:01:01:01	0.02
HLA_A=A*33:01:01:01	0.02
HLA_A=A*03:01:01:05	0.02
HLA_B=B*73:01	0.02
HLA_B=B*15:17:01:01	0.02
HLA_B=B*35:02:01:01	0.02
HLA_B=B*41:48	0.02
HLA_B=B*50:01:01:01	0.02
HLA_B=B*53:01:01:01	0.02
HLA_B=B*15:01:01:01	0.02
HLA_B=B*41:01:01:01	0.02
HLA_B=B*14:02:01:01	0.02
HLA_B=B*35:03:01:01	0.02
HLA_B=B*08:01:01:01	0.02
HLA_C=C*15:05:01:01	0.02
HLA_C=C*07:01:02	0.02
HLA_C=C*02:02:02:01	0.02
HLA_C=C*03:08	0.02
HLA_C=C*17:03:01:01	0.02
HLA_C=C*06:02:01:02	0.02
HLA_C=C*03:03:01:01	0.02
HLA_C=C*17:01:01:05	0.02
HLA_C=C*08:02:01:01	0.02
HLA_C=C*07:02:01:01	0.02
HLA_C=C*06:02:01:01	0.02
HLA_C=C*01:02:01:01	0.02
DRB1=DRB1*01:03:01	0.02
DRB1=DRB1*01:01:01	0.02
DRB1=DRB1*13:05:01	0.02
DRB1=DRB1*15:02:01:01	0.02
DRB1=DRB1*15:01:01:01	0.02
DRB1=DRB1*14:54:01:01	0.02
DRB1=DRB1*13:03:01	0.02
DRB1=DRB1*12:01:01:01	0.02
DRB1=DRB1*11:03:01	0.02
DRB1=DRB1*11:02:01	0.02
DRB1=DRB1*13:01:01:01	0.02
DQB1=DQB1*06:01:01:01	0.02

DQB1=DQB1*06:02:01:01	0.02
DQB1=DQB1*05:03:01:01	0.02
DQB1=DQB1*03:01:01:05	0.02
DQB1=DQB1*03:19:01	0.02
DQB1=DQB1*06:03:01:01	0.02
DQA1=DQA1*01:03:01:01	0.02
DQA1=DQA1*01:04:01:03	0.02
DQA1=DQA1*01:02:01:01	0.02
DQA1=DQA1*05:05:01:03	0.02
DQA1=DQA1*01:03:01:02	0.02
DPB1=DPB1*13:01:01:01	0.02
DPB1=DPB1*04:01:01:03	0.02
DPB1=DPB1*23:01:01:01	0.02
DPB1=DPB1*17:01:01:01	0.02
DPB1=DPB1*04:01:01:28	0.02
DPB1=DPB1*03:01:01:01	0.02
DPB1=DPB1*14:01:01:01	0.02
DPB1=DPB1*02:02:01:01	0.02
DPB1=DPB1*04:01:01:10	0.02
DPB1=DPB1*124:01:02:01	0.02
DPB1=DPB1*10:01:01:01	0.02
DPB1=DPB1*104:01:01:03	0.02
DPB1=DPB1*02:01:02:10	0.02
DPA1=DPA1*02:01:01:06	0.02
DPA1=DPA1*02:01:01:03	0.02
DPA1=DPA1*01:03:01:03	0.02
DPA1=DPA1*01:03:04	0.02
DPA1=DPA1*01:03:01:12	0.02

Supplemental Table 5. Description of the *HLA-DRB3*02:02* alleles in the 24 Italian subjects.

ID (Group)	HLA status for the *DRB3*02:02* allele
1 (A)	No *DRB3*02:02* allele
2 (A)	No *DRB3*02:02* allele
3 (A)	*DRB3*02:02:01:01*, homozygous
4 (A)	*DRB3*02:02:01:02*, homozygous
5 (A)	*DRB3*02:02:01:02*, homozygous
6 (A)	*DRB3*02:02:01:02*, homozygous
7 (A)	*DRB3*02:02:01:02*, homozygous
8 (A)	*DRB3*02:02:01:01*, homozygous
9 (B)	*DRB3*02:02:01:01*, heterozygous
10 (A)	No *DRB3*02:02* allele
11 (A)	*DRB3*02:02:01:02*, homozygous
12 (A)	*DRB3*02:02:01:02*, homozygous
13 (A)	*DRB3*02:02:01:02*, homozygous
14 (A)	*DRB3*02:02:01:01*, homozygous
15 (A)	*DRB3*02:02:01:02*, homozygous
16 (B)	*DRB3*02:02:01:02*, homozygous
17 (C)	*DRB3*02:02:01:01*/*DRB3*02:02:01:02*
18 (C)	*DRB3*02:02:01:02*, homozygous
19 (C)	*DRB3*02:02:01:01*/*DRB3*02:02:01:02*
20 (C)	*DRB3*02:02:01:01*, heterozygous
21 (B)	*DRB3*02:02:01:02*, homozygous
22 (B)	*DRB3*02:02:01:02*, heterozygous
23 (B)	No *DRB3*02:02* allele
24 (B)	*DRB3*02:02:01:01*/*DRB3*02:02:01:02*

Supplemental Table 6. Descriptive statistics of the Spanish patients with non-immediate hypersensitivity to penicillins

Demographics — n/N, %, (95% CI)		
Age (years) — N, median (IQR, 25th – 75th)	98	43 (32–54)
Male sex — n/N, %, (95%CI)	42/98	42.9% (32.9–52.8)
Culprit drug — n/N, %, (95% CI)		
Amoxicillin	47/98	48.0% (37.9–58.0)
Amoxicillin-Clavulanic acid	27/98	27.6% (18.5–36.6)
Penicillin G	12/98	12.2% (5.6–18.9)
Other molecules*	12/98	12.2% (5.6–18.9)
Type of reaction — n/N, %, (95% CI)		
Urticaria	70/98	71.4% (62.3–80.5)
Urticaria and angioedema	25/98	25.5% (16.7–34.3)
Other types of reaction †	3/98	3.1% (0.7–8.7)

Note. N: total number of patients considered for the characteristics; n: number of observations with the characteristics; 95% CI: 95% confidence interval.
* Other molecules include ampicillin (n=2), cefuroxime (n=2), cefaclor (n=1), cefazolin (n=1), cefepime (n=1), cefixime (n=1), ceftazidime (n=1), ceftriaxone (n=1), cloxacillin (n=1), and piperacillin-tazobactam (n=1).
† Urticaria and upper airway reaction (n=2), angioedema without urticaria (n=1).

Supplemental Table 7. Descriptive statistics of the Spanish patients with immediate hypersensitivity to penicillins

Demographics		
Age (years) — N, median (IQR, 25th – 75th)	315	49 (38–58)
Male sex — n/N, %, (95%CI)	141/315	44.7 (39.2–50.4)
Type of reaction — n/N, %, (95% CI)		
Anaphylaxis	162/315	51.4 (45.7–57.1)
Anaphylactic shock	50/315	15.8 (12.0–20.4)
Urticaria	103/315	32.7 (27.5–38.2)
Culprit drug — n/N, %, (95% CI)		
Amoxicillin	173/315	54.9 (49.2–60.5)
Amoxicillin-Clavulanic acid	107/315	33.9 (28.7–39.5)
Penicillin G	15/315	4.8 (2.6–7.7)
Cefuroxime	7/315	2.2 (0.9–4.5)
Other molecules*	13/315	4.1 (2.2–6.9)
Prick test to culprit drug — n/N, %, (95% CI)		
Not available	92/315	29.2 (24.2–34.6)
Negative	144/315	45.7 (40.1–51.4)
Positive	79/315	25.1 (20.4–30.2)

Note. N: total number of patients considered for the characterictics; n: number of observations with the characteristics; 95% CI: 95% confidence interval.
*Other molecules include cefazoline (n=4), cefaclor (n=2), clavulanic acid (n=2), ampicillin (n=1), cefotaxime (n=1), ceftazidime (n=1), and cloxacillin (n=1).

Supplemental Table 8. Descriptive statistics of demographic origin of the Spanish control subjects without hypersensitivity to penicillins

Age (years) — N, median (IQR, 25th – 75th)	1308	42 (32–51)
Male sex — n/N, %, (95%CI)	577/1308	44.1% (41.4–46.9)
Geographical origin — n/N, %, (95%CI)		
Salamanca	846/1308	64.7% (62.1–67.3)
Malaga	462/1308	35.3% (32.7–37.9)
Personal history of atopy— n/N, %, (95%CI)	147/1124	13.1% (11.2-15.2)
Total serum IgE (kUI/L, mean ± SD)	118 ± 14	
IgE >130 kUI/L — n/N, %, (95%CI)	231/1124	20.6% (18.2- 22.9)

Note. N: total number of patients considered for the characteristics; n: number of observations with the characteristics; 95% CI: 95% confidence interval.