

# HLA-DRA variants predict penicillin allergy in genome-wide fine-mapping genotyping

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**Background:** Immediate reactions to  $\beta$ -lactams are the most common causes of anaphylactic reactions and can be life-threatening. The few known genetic factors influencing these reactions suggest a link with atopy and inflammation. **Objective:** We performed a fine-mapping genome-wide association study of the genetic predictors of  $\beta$ -lactam allergy to better understand the underlying mechanisms. **Methods:** We studied 387 patients with immediate allergic reactions to  $\beta$ -lactams and 1124 paired control subjects from Spain. We replicated the results in 299 patients and 362 paired control subjects from Italy. **Results:** We found significant associations with the single nucleotide polymorphisms rs4958427 of *ZNF300* (c.64-471G>A,  $P = 9.9 \times 10^{-9}$ ), rs17612 of *C5* (c.4311A>C [p.Glu1437Asp],  $P = 7.5 \times 10^{-7}$ ), rs7754768 and rs9268832 of the *HLA-DRA* | *HLA-DRB5* interregion ( $P = 1.6 \times 10^{-6}$  and  $4.9 \times 10^{-6}$ ), and rs7192 of *HLA-DRA* (c.724T>G [p.Leu242Val],  $P = 7.4 \times 10^{-6}$ )

in an allelic model, with similar results in an additive model. Single nucleotide polymorphisms of *HLA-DRA* and *ZNF300* predicted skin test positivity to amoxicillin and other penicillins but not to cephalosporins. A haplotype block in *HLA-DRA* and the *HLA-DRA* | *HLA-DRB5* interregion encompassed a motif involved in balanced expression of the  $\alpha$ - and  $\beta$ -chains of MHC class II, whereas rs7192 was predicted to influence  $\alpha$ -chain conformation. *HLA-DRA* rs7192 and rs8084 were significantly associated with allergy to penicillins and amoxicillin ( $P = 6.0 \times 10^{-4}$  and  $P = 4.0 \times 10^{-4}$ , respectively) but not to cephalosporins in the replication study. **Conclusions:** Gene variants of *HLA-DRA* and the *HLA-DRA* | *HLA-DRB5* interregion were significant predictors of allergy to penicillins but not to cephalosporins. These data suggest complex gene-environment interactions in which genetic susceptibility of HLA type 2 antigen presentation plays a central role. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

**Key words:** Drug allergy, immediate-type reactions, anaphylaxis,  $\beta$ -lactams, penicillins, amoxicillin, genome-wide association, *HLA-DRA*

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Hypersensitivity reactions against drugs are produced by the release of cellular mediators through both specific and nonspecific immunologic mechanisms.<sup>1,2</sup> Drug allergy is a hypersensitivity reaction for which a definite immunologic mechanism has been demonstrated. It may be either IgE dependent in immediate-type reactions or T cell mediated in delayed reactions.<sup>3</sup> Immediate reactions occur mostly within the first hour after drug exposure and include urticaria, angioedema, rhinitis, bronchospasm, and/or anaphylaxis.

Immediate reactions, particularly anaphylactic reactions, to  $\beta$ -lactams are the most common allergic reactions against drugs and can be life-threatening. The genetic risk factors influencing these reactions are poorly known, except those previously identified as predictors of atopy and inflammation. Two polymorphisms of nucleotide-binding oligomerization domain 2 (*NOD2*), a gene associated with chronic inflammatory bowel disease, influence the risk of  $\beta$ -lactam allergy (BLA) in Italy and Spain.<sup>4</sup> *G>A* at –308 of *TNFA*, a key gene of inflammation, is a significant independent predictor of the primary risk of BLA in central Italy.<sup>5</sup> *TNFA* is part of an extended haplotype, *HLA-A1-B8-DR3-DQ2*, suggesting a potential implication of drug recognition by the MHC. Several studies reported in Europe, the United States, and China have shown also that immediate-type allergic reactions to  $\beta$ -lactams are influenced by 3 genes that influence

**Abbreviations used**

|                 |                                             |
|-----------------|---------------------------------------------|
| BLA:            | $\beta$ -Lactam allergy                     |
| FDR:            | False discovery rate                        |
| GWAS:           | Genome-wide association study               |
| <i>IL4RA</i> :  | <i>IL-4</i> receptor $\alpha$               |
| NF- $\kappa$ B: | Nuclear factor $\kappa$ B                   |
| <i>NOD2</i> :   | Nucleotide-binding oligomerization domain 2 |
| SNP:            | Single nucleotide polymorphism              |
| UTR:            | Untranslated region                         |

IgE production, *IL13*, *IL4*, and *IL-4* receptor  $\alpha$  (*IL4RA*).<sup>6–11</sup> A polymorphism in the gene promoter of the high-affinity receptor for IgE (Fc $\epsilon$ RI $\beta$ ) is a predictor for increased IgE sensitization to cephalosporins in exposed health care workers from Korea.<sup>12</sup>

We have performed a genome-wide association study (GWAS) in participants with immediate-type BLA and matched control subjects from Spain and a replication study in an Italian population. This work was designed to perform fine mapping of the influence of genetics in the risk prediction of  $\beta$ -lactam-induced immediate-type allergic reactions. The use of the Immunochip Illumina array (Illumina, San Diego, Calif) allowed us to better understand the genetic predictors of BLA and the underlying mechanisms, in particular those related to antigen presentation, atopy, and inflammation.

## METHODS

### Case participants and control subjects

We studied 2 already published populations.<sup>4,5,10</sup> The first study, which was performed in Spain, included 436 patients with immediate-type BLA reactions and 1218 paired control subjects. The replication study was performed in 299 Italian patients with BLA and 362 paired control subjects. The allergic work-up was performed according to the European Network for Drug Allergy guidelines.<sup>13,14</sup> Patients with an age ranging between 16 and 60 years were included in the study. They had to have a history compatible with an immediate reaction to  $\beta$ -lactams. Skin tests were made with major and minor determinants of benzyl penicillin (penicilloyl-polylysine and minor determinant mixture [MDM] containing benzyl-penicillin, sodium benzyl-penicilloate, and benzyl-penicilloic acid at a final concentration of 1.5 mmol/L) and amoxicillin (Diater, Madrid, Spain) and the culprit drug when a cephalosporin was involved.<sup>13</sup> If skin test results were negative, controlled administration was carried out for establishing the diagnosis, as previously reported.<sup>13</sup> Those patients with severe anaphylactic reactions were not included if they had negative skin test results but the risk of a severe reaction existed. Age- and sex-paired volunteers were recruited in preventive care consultations. All of them tolerated amoxicillin and other  $\beta$ -lactams on different occasions. Control participants who never had taken  $\beta$ -lactams were not included in the study. They had no history of allergic, dermatologic, or respiratory diseases. Participants with a C-reactive protein blood concentration of greater than 5 mg/L were excluded. All patients and control subjects were white. Written informed consent for participating in the study and providing DNA samples was obtained from all patients and control subjects. Subjects younger than 16 years and pregnant women were not included in the study. The local ethics committees approved the protocols, as described previously.<sup>4,5,10</sup>

### Genotyping

We extracted genomic DNA from blood buffy coats (Qiagen, Courtaboeuf, France). The Spanish population was genotyped with the Immunochip custom array (Illumina France Holding, Paris, France), according to the recommendations of the STrengthening the REporting of Genetic Association studies (STREGA, <http://www.med.uottawa.ca/public-health-genomics/web/eng/strega.html>). Bead intensity data were processed and normalized for

each sample by using the GenomeStudio Software Genotyping Module (Illumina). This module enables normalization of genotyping data, genotype calling, clustering, and data intensity analysis. The normalization was performed for adjustment of raw microarray data to remove systematic variation of nonbiological origin. Genotype calling determined the genotype for each subject at each variation site studied on the Immunochip array. Illumina's proprietary genotype-calling algorithm GenCall uses normalized measures of DNA binding to allele-specific probes to ascertain the genotype of a subject at a given single nucleotide polymorphism (SNP). Genotypes were called for each sample by their signal intensity (norm R, y-axis) and allele frequency (norm Theta, x-axis) relative to canonical cluster positions for a given SNP marker (Illumina data sheet, [www.illumina.com](http://www.illumina.com)). We used build 36 (hg18 assembly) of the National Center for Biotechnology Information as the reference assembly of the human genome for mapping variant positions (Illumina manifest file Immuno\_BeadChip\_11419691\_B.bpm). From the initial samples of the 436 patients and 1218 control subjects, 387 and 1124 passed quality control, respectively, and were retained in the final statistical analyses. All data sets underwent the same rigorous quality checks (see the **Methods** section in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). SNPs that had greater than 5% missing data, a minor allele frequency of less than 1%, or deviated from Hardy-Weinberg equilibrium per sample study were excluded. Sample quality control measures included sample call rate, overall heterozygosity, and relatedness testing. All participants included in the study also had a genotyping success rate of greater than 90%. We assessed duplicated samples and those showing cryptic relatedness by calculating identity by descent. Principal component analysis was performed on the samples merged with HapMap phase 3 populations (Utah residents with Northern and Western European ancestry from the CEPH collection [CEU], Han Chinese in Beijing, China [CHB], Chinese in Metropolitan Denver, Colorado [CHD], Japanese in Tokyo, Japan [JPT], Mexican ancestry in Los Angeles, California [MEX], Toscani in Italia [TSI], Yoruba in Ibadan, Nigeria [YRI]) as reference populations to identify ancestry outliers (see **Fig E1** in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). All quality control and genotypic analyses were performed with SNP & Variation Suite 7.7.8 (Golden Helix, Bozeman, Mont). We repeated the genotyping of polymorphisms rs7192, rs8084 (*HLA-DRA*), rs17612 (*C5*), and rs4058427 (*ZNF300*) by means of real-time PCR (Roche Molecular Biochemical, Lyon, France) in the Italian population by using the primers and probes listed in **Table E1** in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org), as previously described.<sup>4,5,10</sup>

### Statistical analysis

All quantitative variables are described as means and SDs. All proportions are expressed as percentages with 95% CIs. In the GWAS stage SNP associations were determined by using the Fisher test for the allelic model and logistic regression analysis for the additive model, with false discovery rate (FDR) correction for multiple testing. Statistical analyses were performed with the SNP & Variation Suite 7.7.8 (Golden Helix) and STATA 12.1 software (StataCorp, College Station, Tex). Haploview version 4.1 (Cambridge, Mass) was used to calculate linkage disequilibrium coefficients ( $r^2$ ).<sup>15</sup> The minimal size of our sample was estimated at 280 patients, with a study power of 1- $\beta$  value of 0.8 and  $\alpha$  value of 0.05, assuming a 2.0-fold difference in the less frequent alleles between control subjects and patients. *P* values of less than .05 were considered significant after FDR correction for multiple testing. A *P* value of  $7.4 \times 10^{-6}$  was the threshold of significance before correction that corresponded to a *P* value of less than .05 after FDR correction in the allelic model.

## RESULTS

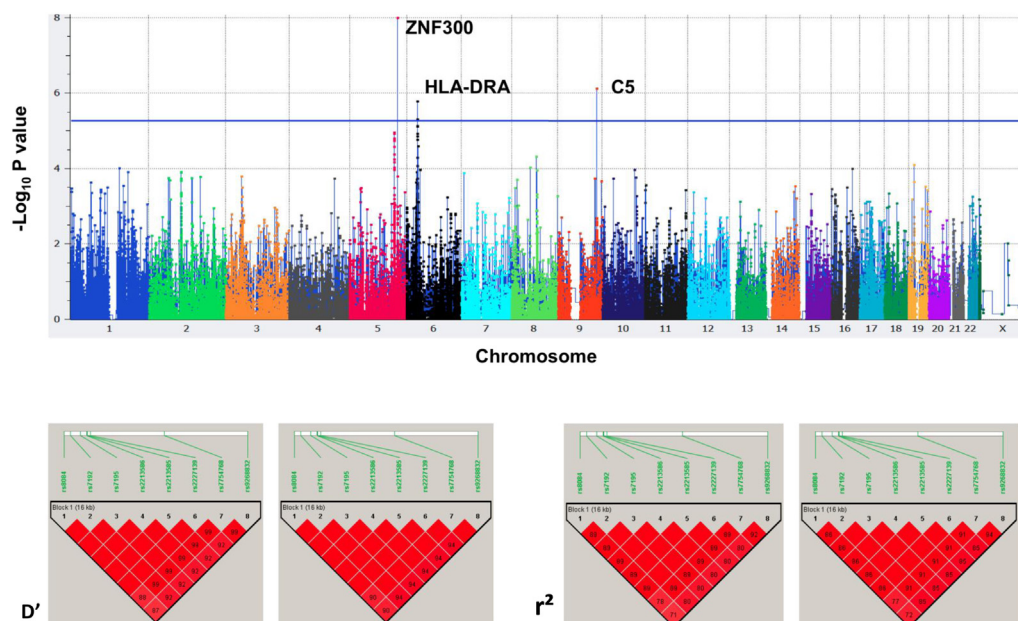
The characteristics of the Spanish and Italian populations are summarized in **Table I**. Part of these data have been published previously.<sup>4,5,10</sup> After quality control, 107,398 SNPs with a

**TABLE I.** Clinical and biological characteristics of control subjects and patients with immediate-type BLA in Spain and Italy

| Characteristic                    | Spain                                      |                                            |                                                      | Italy                                     |                                            |                                                      |
|-----------------------------------|--------------------------------------------|--------------------------------------------|------------------------------------------------------|-------------------------------------------|--------------------------------------------|------------------------------------------------------|
|                                   | Control subjects<br>(n = 1124),<br>no. (%) | Patients with<br>BLA (n = 387),<br>no. (%) | P value, patients<br>with BLA vs<br>control subjects | Control subjects<br>(n = 362),<br>no. (%) | Patients with<br>BLA (n = 299),<br>no. (%) | P value, patients<br>with BLA vs<br>control subjects |
| Age (y)                           | 43 ± 1                                     | 47 ± 1                                     | <.001                                                | 48 ± 1                                    | 45 ± 1                                     | .105                                                 |
| Male sex                          | 453 (40.3)                                 | 166 (42.9)                                 | .264                                                 | 134 (37.1)                                | 107 (35.9)                                 | .383                                                 |
| Time interval (mo)                | NA                                         | 20 ± 4                                     | NA                                                   | NA                                        | 20 ± 4                                     | NA                                                   |
| Personal history of atopy         | 147 (13.1)                                 | 87 (22.4)                                  | .001                                                 | 0                                         | 79 (26.5)                                  | NA                                                   |
| Total serum IgE (kIU/L)           | 118 ± 14                                   | 246 ± 42                                   | <.001                                                | 84 ± 10                                   | 281 ± 32                                   | <.001                                                |
| IgE >130 kIU/L                    | 231 (20.6)                                 | 141 (36.4)                                 | <.001                                                | 50 (13.7)                                 | 140 (46.5)                                 | <.001                                                |
| Atopy*                            | 344 (30.6)                                 | 239 (61.7)                                 | <.001                                                | 64 (17.8)                                 | 176 (59.0)                                 | <.001                                                |
| Allergy to amoxicillin            | NA                                         | 330 (85.4)                                 | NA                                                   | NA                                        | 142 (47.2)                                 | NA                                                   |
| Allergy to cephalosporins         | NA                                         | 22 (5.8)                                   | NA                                                   | NA                                        | 100 (33.2)                                 | NA                                                   |
| Allergy to other $\beta$ -lactams | NA                                         | 34 (8.8)                                   | NA                                                   | NA                                        | 59 (19.6)                                  | NA                                                   |
| Shock                             | NA                                         | 196 (50.7)                                 | NA                                                   | NA                                        | 236 (78.5)                                 | NA                                                   |

NA, Not available.

\*Atopy was defined based on a personal history of atopy and/or a total IgE level of greater than 130 kIU/L and/or a positive specific IgE level against prevalent allergens.

**FIG 1.** Top, Manhattan plot of the Immuchip association statistics highlighting genes associated with immediate-type BLA. The blue horizontal line indicates a threshold of genome-wide significance at a P value of  $5 \times 10^{-5}$ . Each dot represents a P value for the comparison of the 387 case participants and 1124 healthy control subjects resulting from the allelic genetic model that tests associations with individual alleles. Bottom, Linkage disequilibrium of *HLA-DRA* variants in patients and control subjects. The  $r^2$  and  $D'$  values among SNPs are reported by using Haploview software.<sup>15</sup>

minor allele frequency of greater than 1% were available for analysis. Three haplotype blocks were significant predictors of BLA among a total of 661. These blocks were in locus 5q33.1 (top SNP, rs4958427 [*ZNF300*];  $P = 9.8 \times 10^{-9}$ ), locus 9q33.2 (top SNP, rs17612 [*C5*];  $P = 7.5 \times 10^{-9}$ ), and locus 6p21.32 (top SNP, rs7754768 [*HLA-DRA* | *HLA-DRB5*];  $P = 1.6 \times 10^{-6}$ ), respectively (Fig 1). The gene variants at position rs4958427 of *ZNF300* (c.64-471G>A [intronic]), rs17612 of *C5* (c.4311A>C [p.Glu1437Asp]), rs7754768 and rs9268832 of the *HLA-DRA* | *HLA-DRB5* interregion, and rs7192 of *HLA-DRA* (c.724T>G [p.Leu242Val]) were the top 5 SNPs listed by order of significance, which predicted the risk of BLA in the Spanish population (Table II and see genotype counts in Table E2

in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The top 5 SNPs, rs4958427, rs17612, rs7754768, rs9268832, and rs7192, passed FDR correction (Table II). We identified a strong signal at the 6p21.32 locus. Indeed, 8 of the 10 top SNPs belonged to the intronic, exonic, and intergenic regions of *HLA-DRA* within this locus, including a second exonic variant, rs8084 (c.402A>C [p.Ile134=], Table II). In addition, rs7192 was in strong linkage disequilibrium with the other top SNPs (Fig 1). The minor alleles of genotypes at position rs17612 of *C5* and position rs7192 of *HLA-DRA* predicted a lower risk of BLA (odds ratios, 0.27 [95% CI, 0.15-0.49] and 0.64 [95% CI, 0.53-0.78], respectively), whereas those at position rs4958427 of *ZNF300* predicted an increased risk (odds ratio, 1.88 [95% CI, 1.52-2.33]; Fig 2).

**TABLE II.** Variants associated with primary risk of  $\beta$ -lactam hypersensitivity (full SNP array) in the Spanish population

| Variant   | Gene†                            | Chromosome | Position* | HGVS                          | MAF, $\beta$ -lactams | MAF, control subjects | Regression <i>P</i> value‡ | Regression FDR <i>P</i> value | OR   |
|-----------|----------------------------------|------------|-----------|-------------------------------|-----------------------|-----------------------|----------------------------|-------------------------------|------|
| rs4958427 | <i>ZNF300</i>                    | 5          | 150258780 | c.64-471G>A<br>(intronic)     | 0.288                 | 0.176                 | 1.8E-08 (9.8E-09)§         | 9.8E-04                       | 1.85 |
| rs17612   | <i>C5</i>                        | 9          | 122765747 | c.4311A>C<br>(p.Glu1437Asp)   | 0.018                 | 0.062                 | 4.4E-07 (7.5E-07)          | .009                          | 0.27 |
| rs7754768 | <i>HLA-DRA</i>   <i>HLA-DRB5</i> | 6          | 32528157  | g.32420179C>T<br>(intergenic) | 0.223                 | 0.313                 | 1.4E-06 (1.6E-06)          | .017                          | 0.63 |
| rs9268832 | <i>HLA-DRA</i>   <i>HLA-DRB5</i> | 6          | 32535767  | g.32427789T>C<br>(intergenic) | 0.214                 | 0.299                 | 4.1E-06 (4.9E-06)          | .040                          | 0.64 |
| rs7192    | <i>HLA-DRA</i>                   | 6          | 32519624  | c.724T>G<br>(p.Leu242Val)     | 0.208                 | 0.290                 | 8.1E-06 (7.4E-06)          | .050                          | 0.65 |
| rs2227139 | <i>HLA-DRA</i>   <i>HLA-DRB5</i> | 6          | 32521437  | g.32413459G>A<br>(intergenic) | 0.208                 | 0.290                 | 8.2E-06 (7.7E-06)          | .048                          | 0.65 |
| rs2213586 | <i>HLA-DRA</i>   <i>HLA-DRB5</i> | 6          | 32521072  | g.32413094A>G<br>(intergenic) | 0.208                 | 0.288                 | 1.4E-05 (1.2E-05)          | .054                          | 0.66 |
| rs2213585 | <i>HLA-DRA</i>   <i>HLA-DRB5</i> | 6          | 32521128  | g.32413150G>A<br>(intergenic) | 0.208                 | 0.288                 | 1.5E-05 (1.4E-05)          | .056                          | 0.66 |
| rs7195    | <i>HLA-DRA</i>                   | 6          | 32520517  | g.32412539A>G<br>(3'UTR)      | 0.209                 | 0.287                 | 2.0E-05 (1.7E-05)          | .072                          | 0.66 |
| rs8084    | <i>HLA-DRA</i>                   | 6          | 32519013  | c.402A>C<br>(p.Ile134=)       | 0.234                 | 0.310                 | 4.7E-05 (1.7E-05)          | .120                          | 0.68 |

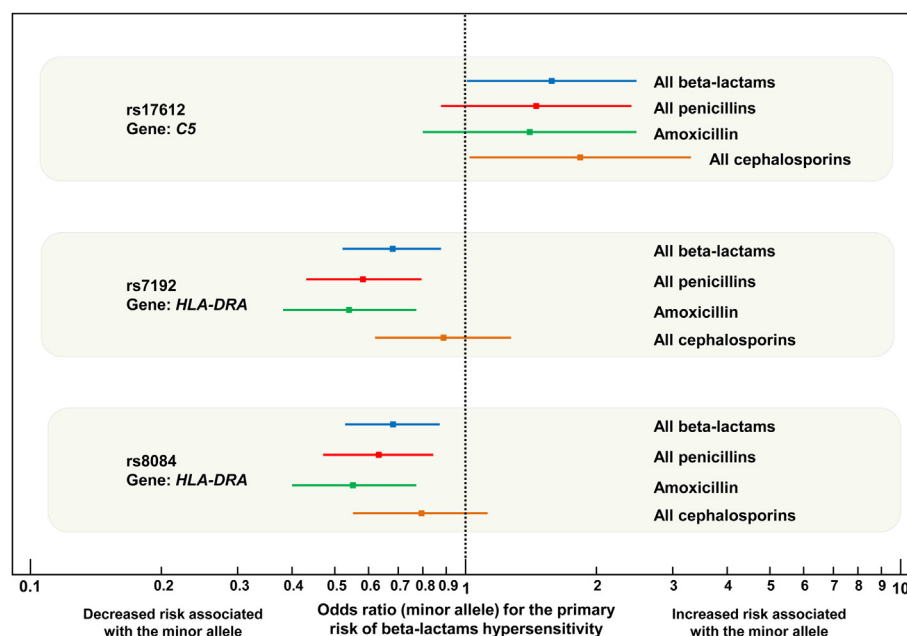
HGVS, Human Genome Variation Society; MAF, minor allele frequency; OR, Odds ratio.

\*Position according to National Center for Biotechnology Information build 36 (Hg18) coordinates.

†Flanking genes are reported in case of intergenic SNPs.

‡Regression *P* values in the additive model.

§*P* values of exact Fisher test in the allelic model.

**FIG 2.** Forest plots showing the effect size of associations between the primary risk of hypersensitivity to  $\beta$ -lactams and replicated variants on *HLA-DRA*, *C5*, and *ZNF300* genes in a case-control population from Italy. For each analysis, the odds ratio of the association is reported with the 95% CI (solid line).

We investigated whether other variants in linkage with *HLA-DRA* variants could influence gene expression and protein function, respectively (see Table E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). For this purpose, we performed Sanger sequencing of the promoter region and reading frame of the *HLA-DRA* gene in 20 patients with minor alleles and 20 patients with dominant alleles in rs74134796, rs17612, and

rs7192 compared with 10 control subjects. We found additional polymorphisms but none that could directly influence either promoter activity or coding of the transmembrane and peptide-binding domains.

Also, we evaluated whether associations between *ZNF300*, *C5*, and *HLA-DRA* gene variants and BLA were replicated in an Italian case-control population. In this replication rs7192 and

**TABLE III.** Replication of associations between *HLA-DRA*, *C5*, and *ZNF300* gene variants and BLA in the Italian case-control population

| Variant   | Gene           | Genotype | Control subjects (n = 362) | Patients with BLA (n = 299) | P value* (FDR P value) | PA (n = 199) | P value* (FDR P value) |
|-----------|----------------|----------|----------------------------|-----------------------------|------------------------|--------------|------------------------|
| rs7192    | <i>HLA-DRA</i> | G allele | 530 (73.2)                 | 479 (80.1)                  | .003 (.008)            | 328 (82.4)   | 5.3E-04 (.002)         |
|           |                | TT       | 26 (7.2)                   | 14 (4.7)                    | .003 (.008)            | 5 (2.5)      | 3.9E-04 (.001)         |
|           |                | TG       | 142 (39.3)                 | 91 (30.3)                   |                        | 60 (30.2)    |                        |
|           |                | GG       | 194 (53.5)                 | 194 (64.7)                  |                        | 134 (67.3)   |                        |
| rs8084    | <i>HLA-DRA</i> | C allele | 501 (69.1)                 | 459 (76.5)                  | .002 (.012)            | 311 (78.1)   | .001 (.002)            |
|           |                | AA       | 33 (9.1)                   | 17 (5.7)                    | .002 (.008)            | 7 (3.5)      | .001 (.002)            |
|           |                | AC       | 157 (43.5)                 | 105 (35.0)                  |                        | 73 (36.7)    |                        |
|           |                | CC       | 172 (47.4)                 | 177 (59.0)                  |                        | 119 (59.8)   |                        |
| rs17612   | <i>C5</i>      | A allele | 687 (94.9)                 | 551 (92.2)                  | .053 (.089)            | 369 (92.7)   | .146 (.195)            |
|           |                | AA       | 326 (90.1)                 | 253 (84.6)                  | .040 (.053)            | 71 (85.9)    | .144 (.192)            |
|           |                | AC       | 35 (9.6)                   | 45 (15.1)                   |                        | 27 (13.6)    |                        |
|           |                | CC       | 1 (0.3)                    | 1 (0.3)                     |                        | 1 (0.5)      |                        |
| rs4958427 | <i>ZNF300</i>  | G allele | 594 (82.3)                 | 511 (85.2)                  | .180 (.225)            | 336 (84.4)   | .406 (.406)            |
|           |                | AA       | 12 (3.3)                   | 3 (1.0)                     | .164 (.164)            | 3 (1.5)      | .363 (.363)            |
|           |                | AG       | 104 (28.8)                 | 83 (27.7)                   |                        | 56 (28.1)    |                        |
|           |                | GG       | 246 (67.9)                 | 213 (71)                    |                        | 140 (70.4)   |                        |

PA, Penicillin allergy.

\*The allelic model was analyzed by using the Fisher exact test, and the additive model was analyzed by using regression analysis.

rs8084 of *HLA-DRA* were the most significant predictors of BLA. Association of rs17612 of *C5* with BLA was at the limit of statistical significance, and no significant association of rs4958427 of *ZNF300* with BLA was reported (Table III). The prediction of rs7192 and rs8084 of *HLA-DRA* was much more significant when restricting the analysis to the patients with BLA against amoxicillin ( $P = 5.5 \times 10^{-4}$  and  $P = 4.1 \times 10^{-4}$ , respectively) or against all penicillins (Fig 2 and Table III). Conversely, we observed no association of *HLA-DRA* variants and a weak association of *C5* rs17612 with BLA when considering only the patients who reacted against cephalosporins (Fig 2 and see Table E4 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). We observed significant associations of the SNPs of *ZNF300* and *HLA-DRA* with skin test positivity to amoxicillin and penicillins but not cephalosporins (rs4958427,  $P = .005$  and rs7192 and rs8084,  $P = .023$  and  $P = .010$ , respectively).

## DISCUSSION

To our knowledge, this study is the first GWAS with fine-mapping genotyping that evaluates the genetic predictors of immediate-type drug allergy. We found that immediate-type BLA is influenced by gene variants of *HLA-DRA*, a class II MHC gene, and, to a much lesser extent, by the *C5* gene in 2 populations from Spain and Italy.

The variants of *HLA-DRA* are significant predictors of BLA risk. They belonged to the same haplotype block, and their minor alleles predicted a 1.6-fold reduction of BLA risk. Class II MHC proteins are membrane-anchored  $\alpha/\beta$  heterodimers encoded by 3 different  $\alpha$ -chain loci (*DRA*, *DQA1*, and *DPA1*) and by corresponding  $\beta$ -chain loci (*DRB1*, *DRB3/4/5*, *DQB1*, and *DPB1*).<sup>16</sup> *HLA-DRA* is expressed in antigen-presenting cells, such as B lymphocytes, dendritic cells, and macrophages. It exerts a central role in the immune system by presenting peptides derived from extracellular proteins that are degraded in the endocytic pathway.<sup>16</sup> Our study suggests that *HLA-DRA* variants can influence the presentation of  $\beta$ -lactam-derived antigenic motifs and the subsequent mechanisms leading to BLA. The

recognition of  $\beta$ -lactams by *HLA-DRA* did not cover the whole molecular spectrum of these antibiotics because the association was significant for patients with penicillin allergy but not for those allergic to cephalosporins (Fig 2 and see Table E4). We confirmed the influence of *HLA-DRA* on the specific risk of penicillin allergy in Spanish and Italian populations by the significant association of rs7192 and rs8084 with skin test positivity to amoxicillin and penicillins but not with those positive to cephalosporins. The specificity association of *HLA-DRA* exonic variants with penicillins, but not with cephalosporins (Fig 2), was consistent with the low rate of true cross-reactivity of cephalosporins with penicillin, which was recently estimated to be 6% in patients with a history of penicillin allergy and a positive penicillin skin test result.<sup>17</sup>

The *HLA-DRA* variants could influence BLA through 3-dimensional changes of  $\alpha/\beta$  heterodimers. The *DRA*  $\alpha$ -chain is the preferred partner of  $\beta$ -chains encoded by *DRB1*, *DRB3*, *DRB4*, and *DRB5*, respectively.<sup>16</sup> The *DR*  $\alpha$ -chain is highly constant, which is in contrast to the antigen-binding domains of *DRB1-5*  $\beta$ -chains.<sup>16</sup> The rs7192 polymorphism is located in exon 4 (codon 242), which encodes part of the *DRA*  $\alpha$ -chain cytoplasmic domain (240-254).<sup>16</sup> This polymorphism results in substitution of a Leu side chain with a smaller Val side chain. On the basis of the cytoplasmic location of this variant residue and established interactions with other proteins, the Leu>Val substitution in codon 242 predicts changes that might alter interactions with  $\beta$ -chain or ubiquitin E3 ligases, which control the cell-surface expression of class II MHC proteins (see Figs E2 and E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

The top 5 SNPs located in the *HLA-DRA* | *HLA-DRB5* interregion might exert an influence on the respective expression of *HLA-DRA* and *HLA-DRB1-5*. These top SNPs belong to the same haplotype block, which encompasses the 3' untranslated region (UTR) of *HLA-DRA* (see Fig E4 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). A fine level of transcription regulation ensures a balanced synthesis of *HLA-DRA* and *HLA-DRB* mRNAs and a consequent balanced surface expression

of related heterodimers through a mechanism that involves the 3'UTR.<sup>18</sup> This regulatory mechanism couples the transcription and processing of mRNAs encoding  $\alpha$ - and  $\beta$ -chains through the binding of secondary structure UTR motifs to ribonucleoprotein complex.<sup>18</sup> It is noteworthy that one of the top SNPs of the haplotype block is located in the 3'UTR sequence, which encodes one of the motifs (see Fig E5 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).<sup>18</sup>

There are potential interactions between *HLA-DRA/HLA-DRB* genes and the genes previously found to influence the risk of BLA: *IL4*, *IL4RA*, *NOD2*, and *IL10*.<sup>6-11,19,20</sup> MHC class II alleles at human chromosome 6p21.1 have been implicated in susceptibility to allergies and increased IgE levels.<sup>19-21</sup> MHC class II antigen presentation can trigger IgE production through increased *IL4* pathway. The *NOD2* polymorphisms could influence *HLA-DRA* expression through an impaired nuclear factor  $\kappa$ B (NF- $\kappa$ B) pathway.<sup>16,22</sup> They decrease the expression and release of IL-10 after stimulation with Toll-like receptor 2 ligands.<sup>23</sup> This cytokine affects antigen presentation by regulating MHC exocytosis and recycling.<sup>24,25</sup>

The rs17612 missense SNP in the *C5* gene was another predictor of BLA in the Spanish population and, to a much lesser extent, in the Italian population. The cleavage of the  $\alpha$  polypeptide of C5 protein by a convertase produces an activation peptide, C5a, which is an anaphylatoxin with potent spasmogenic and chemotactic activity.<sup>26</sup> This SNP was not associated with the severity of the reaction, suggesting the implication of another mechanism. More consistent with our data, the C5 deficit in mice increases ovalbumin-induced experimental asthma, whereas blockage of the C5a receptor (also known as CD88) increases T<sub>H</sub>2 sensitization and the serum IgE response to ovalbumin through increased production of T<sub>H</sub>2 cytokines.<sup>27</sup>

The GWAS identified *ZNF300* as a predictor of BLA in Spain but not in Italy. *ZNF300* is a gene encoding a 68-kDa zinc finger protein, which was originally cloned from a human early embryo.<sup>28</sup> In addition to *NOD1* and *NOD2*, it was identified as one of the genes most strongly associated with Crohn disease, a chronic disorder that causes inflammation of the digestive tract.<sup>29</sup> *ZNF300* binds to the promoter regions of genes that enhances NF- $\kappa$ B signaling.<sup>30</sup> Taken together, these data could suggest a role of *ZNF300* on *HLA-DRA* expression through its influence on NF- $\kappa$ B signaling, as discussed for *NOD2*.

Our study had some limitations. Genome-wide identification of additional genetic risk factors reaching significance might have been missed by the limited sample size of our study. In addition, the study was done in Spanish and Italian populations. Additional studies will be needed to extrapolate our findings to other ethnicities and geographic locations. Despite their limited size, our 2 case populations are the biggest among population studies on BLA. The Immunochip genome-wide microarray interrogates a number of loci adapted to this sample size and to the European origin of our study population. It is designed for the fine mapping of GWAS loci by using various sources of confirmed GWAS association intervals for immune-mediated diseases.

In conclusion, we found that *HLA-DRA* exonic variants are associated with immediate allergy to penicillins, but not cephalosporins, in 2 European populations. These data are consistent with the low rate of true cross-reactivity of cephalosporins with penicillins and the previous identification of predictors of BLA in Spanish and Italian populations, including *NOD2*

and *IL4RA* polymorphisms and sensitization against prevalent allergens. They suggest that immediate-type BLA results from complex gene-environment interactions in which genetic susceptibility of HLA type 2 antigen presentation plays a central role.

**Clinical implications: Gene variants of *HLA-DRA* and the *HLA-DRA* | *HLA-DRB5* interregion are predictors of allergy to penicillins but not to cephalosporins. These variants influence sensitization against prevalent allergens, which predicts penicillin allergy as well. This suggests gene-environment interactions in which genetic susceptibility of HLA type 2 antigen presentation plays a central role.**

## REFERENCES

1. Rawlins MD, Thompson JW. Pathogenesis of adverse drug reactions. In: Davis DM, editor. Textbook of adverse drug reactions. Oxford: Oxford University Press; 1977. p. 10.
2. Doña I, Blanca-López N, Torres MJ, García-Campos J, García-Núñez I, Gómez F, et al. Drug hypersensitivity reactions: response patterns, drug involved, and temporal variations in a large series of patients. *J Investig Allergol Clin Immunol* 2012;22:363-71.
3. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004;113:832-6.
4. Bursztejn AC, Romano A, Guéant-Rodríguez RM, Cornejo JA, Oussalah A, Chery C, et al. Allergy to betalactams and nucleotide-binding oligomerization domain (NOD) gene polymorphisms. *Allergy* 2013;68:1076-80.
5. Guéant-Rodríguez RM, Guéant JL, Viola M, Tramoy D, Gaeta F, Romano A. Association of tumor necrosis factor- $\alpha$  -308G>A polymorphism with IgE-mediated allergy to betalactams in an Italian population. *Pharmacogenomics* 2008;8:162-8.
6. Guéant-Rodríguez RM, Romano A, Béri-Dexheimer M, Viola M, Gaeta F, Guéant JL. Gene-gene interactions of IL13 and IL4RA variants in immediate allergic reactions to betalactam antibiotics. *Pharmacogenet Genomics* 2006;16:713-9.
7. Guglielmi L, Fontaine C, Gougat C, Avinens O, Eliaou JF, Guglielmi P, et al. IL-10 promoter and IL4-Ralpha gene SNPs are associated with immediate beta-lactam allergy in atopic women. *Allergy* 2006;61:921-7.
8. Qiao HL, Yang J, Zhang YW. Relationships between specific serum IgE, cytokines and polymorphisms in the IL-4, IL-4Ralpha in patients with penicillin allergy. *Allergy* 2005;60:1053.
9. Apter AJ, Schelleman H, Walker A, Addya K, Rebbeck T. Clinical and genetic risk factors of self-reported penicillin allergy. *J Allergy Clin Immunol* 2008;122:152-8.
10. Cornejo-García JA, Guéant-Rodríguez RM, Torres MJ, Blanca-Lopez N, Tramoy D, Romano A, et al. Biological and genetic determinants of atopy are predictors of immediate-type allergy to betalactams, in Spain. *Allergy* 2012;67:1181-5.
11. Guéant JL, Guéant-Rodríguez RM, Aimone Gastin I, Cornejo-García A, Viola M, Barbaud A, et al. Pharmacogenetic determinants of immediate and delayed reactions of drug hypersensitivity. *Curr Pharm Des* 2008;14:2770-7.
12. Nam YH, Kim JE, Kim SH, Jin HJ, Hwang EK, Shin YS, et al. Identifying genetic susceptibility to sensitization to cephalosporins in health care workers. *J Korean Med Sci* 2012;27:1292-9.
13. Torres MJ, Blanca M, Fernandez J, Romano A, Weck A, Aberer W, et al. Interest Group on Drug Hypersensitivity. Diagnosis of immediate allergic reactions to beta-lactam antibiotics. *Allergy* 2003;58:961-72.
14. Torres MJ, Mayorga C, Leyva L, Guzman AE, Cornejo-García JA, Juarez C, et al. Controlled administration of penicillin to patients with a positive history but negative skin and specific serum IgE tests. *Clin Exp Allergy* 2002;32:270-6.
15. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263-5.
16. Neefjes J, Jongstra ML, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol* 2011;11:823-36.
17. Park MA, Koch CA, Klemawesch P, Joshi A, Li JT. Increased adverse drug reactions to cephalosporins in penicillin allergy patients with positive penicillin skin test. *Int Arch Allergy Immunol* 2010;153:268-73.
18. Pisapia L, Cicatiello V, Barba P, Malanga D, Maffei A, Hamilton RS, et al. Co-regulated expression of alpha and beta mRNAs encoding HLA-DR surface

- heterodimers is mediated by the MHCII RNA operon. *Nucleic Acids Res* 2013;41:3772-86.
19. Ramasamy A, Curjuric I, Coin LJ, Kumar A, McArdle WL, Imboden M, et al. A genome-wide meta-analysis of genetic variants associated with allergic rhinitis and grass sensitization and their interaction with birth order. *J Allergy Clin Immunol* 2011;128:996-1005.
  20. Grammatikos AP. The genetic and environmental basis of atopic diseases. *Ann Med* 2008;40:482-95.
  21. Stephan V, Kuehr J, Seibt A, Saueressig H, Zingsem S, Dinh TD, et al. Genetic linkage of HLA-class II locus to mite-specific IgE immune responsiveness. *Clin Exp Allergy* 1999;29:1049-54.
  22. Yang Z, Gu P, Shao X, Li Z, Zhou Y, Lu L, et al. Mutated major histocompatibility complex class II transactivator up-regulates interleukin-33-dependent differentiation of Th2 subset through Nod2 binding for NLR (NOD-like receptor) signaling initiation. *J Biol Chem* 2012;287:9972-81.
  23. Thibodeau J, Bourgeois-Daigneault MC, Huppe G, Tremblay J, Aumont A, Houde M, et al. Interleukin-10-induced MARCH1 mediates intracellular sequestration of MHC class II in monocytes. *Eur J Immunol* 2008;38:1225-30.
  24. Buschow SI, van Balkom BW, Aalberts M, Heck AJ, Wauben M, Stoorvogel W. MHC class II-associated proteins in B-cell exosomes and potential functional implications for exosome biogenesis. *Immunol Cell Biol* 2010;88:851-6.
  25. Van den Hoorn T, Paul P, Jongsma ML, Neeffjes J. Routes to manipulate MHC class II antigen presentation. *Curr Opin Immunol* 2011;23:88-95.
  26. Zhang X, Köhl J. A complex role for complement in allergic asthma. *Expert Rev Clin Immunol* 2010;6:269-77.
  27. Köhl J, Baelder R, Lewkowich IP. A regulatory role for the C5a anaphylatoxin in type 2 immunity in asthma. *J Clin Invest* 2006;116:783-96.
  28. Gou D, Wang J, Gao L, Sun Y, Peng X, Huang J, et al. Identification and functional analysis of a novel human KRAB/C2H2 zinc finger gene ZNF300. *Biochim Biophys Acta* 2004;1676:203-9.
  29. Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nat Genet* 2007;39:830-2.
  30. Wang T, Wang XG, Xu JH, Wu XP, Qiu HL, Yi H, et al. Overexpression of the human ZNF300 gene enhances growth and metastasis of cancer cells through activating NF-kB pathway. *J Cell Mol Med* 2012;16:1134-45.

## METHODS

### Genotyping analysis

Bead intensity data were processed and normalized for each sample. National Center for Biotechnology Information build 36 (hg18) map was used (Illumina manifest file Immuno\_BeadChip\_11419691\_B.bpm), and normalized probe intensities were extracted for all samples that passed standard laboratory quality control thresholds. Genotype calling was performed with the Genome Studio Gen Train 2.0 algorithm (Illumina's Genome Studio data analysis software). From the genotyping of initial samples from 436 cases and 1218 control subjects, 387 and 1124 passed quality control, respectively. All data sets underwent the same rigorous quality checks. SNPs with greater than 5% missing data, a minor allele frequency of less than 1%, or deviation from Hardy-Weinberg equilibrium per sample study were excluded. Sample quality control measures included sample call rate, overall heterozygosity, and relatedness testing. All subjects included in the study also had a genotyping success rate of greater than 90%. We assessed duplicate samples and those showing cryptic relatedness by calculating identity by descent. Principal component analysis was performed on the samples merged with HapMap phase 3 populations (CEU, TSI, YRI, MEX, JPT, CHD, and CHB) as reference populations to identify ancestry outliers (Fig E1). All quality control and genotypic analyses were performed with the SNP & Variation Suite 7.7.8 (Golden Helix). After quality control, 107,398 SNPs with a minor allele frequency of greater than 1% were available for analysis.

### Sample quality control

A total of 1654 DNA samples from 436 cases and 1218 control subjects were genotyped by using the Immunochip array. From these samples,

39 had a call rate of less than 0.90, 39 were outliers in autonomic heterozygosity analysis (20 with overabundance of autonomic heterozygous SNPs and 22 with underabundance of autonomic heterozygous SNPs), 23 were excluded in the identity by using descent estimation analysis, and 39 were outliers in the primary component analysis (Fig E1). A total of 1511 samples were included in the final data set (387 cases and 1124 control subjects).

## REFERENCES

- E1. Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* 2006;22:195-201.
- E2. Kiefer F, Arnold K, Künzli M, Bordoli L, Schwede T. The SWISS-MODEL Repository and associated resources. *Nucleic Acids Res* 2009;37:D387-92.
- E3. Hartshorn MJ. Astex Viewer: a visualisation aid for structure-based drug design. *J Comput Aided Mol Des* 2002;16:871-81.
- E4. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* 1997;18:2714-23.
- E5. Schultz J, Milpetz F, Bork P, Ponting CP. SMART, a simple modular architecture research tool: identification of signaling domains. *Proc Natl Acad Sci U S A* 1998;95:5857-64.
- E6. Letunic I, Doerks T, Bork P. SMART 7: recent updates to the protein domain annotation resource. *Nucleic Acids Res* 2012;40:D302-5.
- E7. Lapaque N, Jahnke M, Trowsdale J, Kelly AP. The HLA-DRalpha chain is modified by polyubiquitination. *J Biol Chem* 2009;284:7007-16.
- E8. Pisapia L, Cicatiello V, Barba P, Malanga D, Maffei A, Hamilton RS, et al. Co-regulated expression of alpha and beta mRNAs encoding HLA-DR surface heterodimers is mediated by the MHCII RNA operon. *Nucleic Acids Res* 2013;41:3772-86.

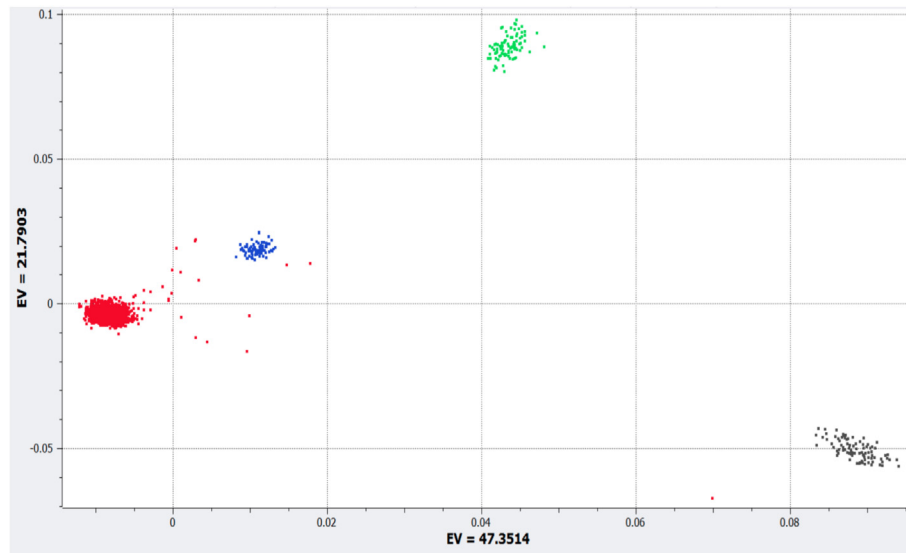


**FIG E1.** Principal component analysis was performed on the study samples merged with HapMap phase 3 populations as reference populations to identify ancestry outliers. *Red dots*, Study population subjects; *blue dots*, Utah Residents (CEPH) with Northern and Western European ancestry population subjects; *green dots*, Japanese in Tokyo, Japan, and Han Chinese in Beijing, China, populations subjects; *gray dots*, Yoruba in Ibadan, Nigeria, population subjects.

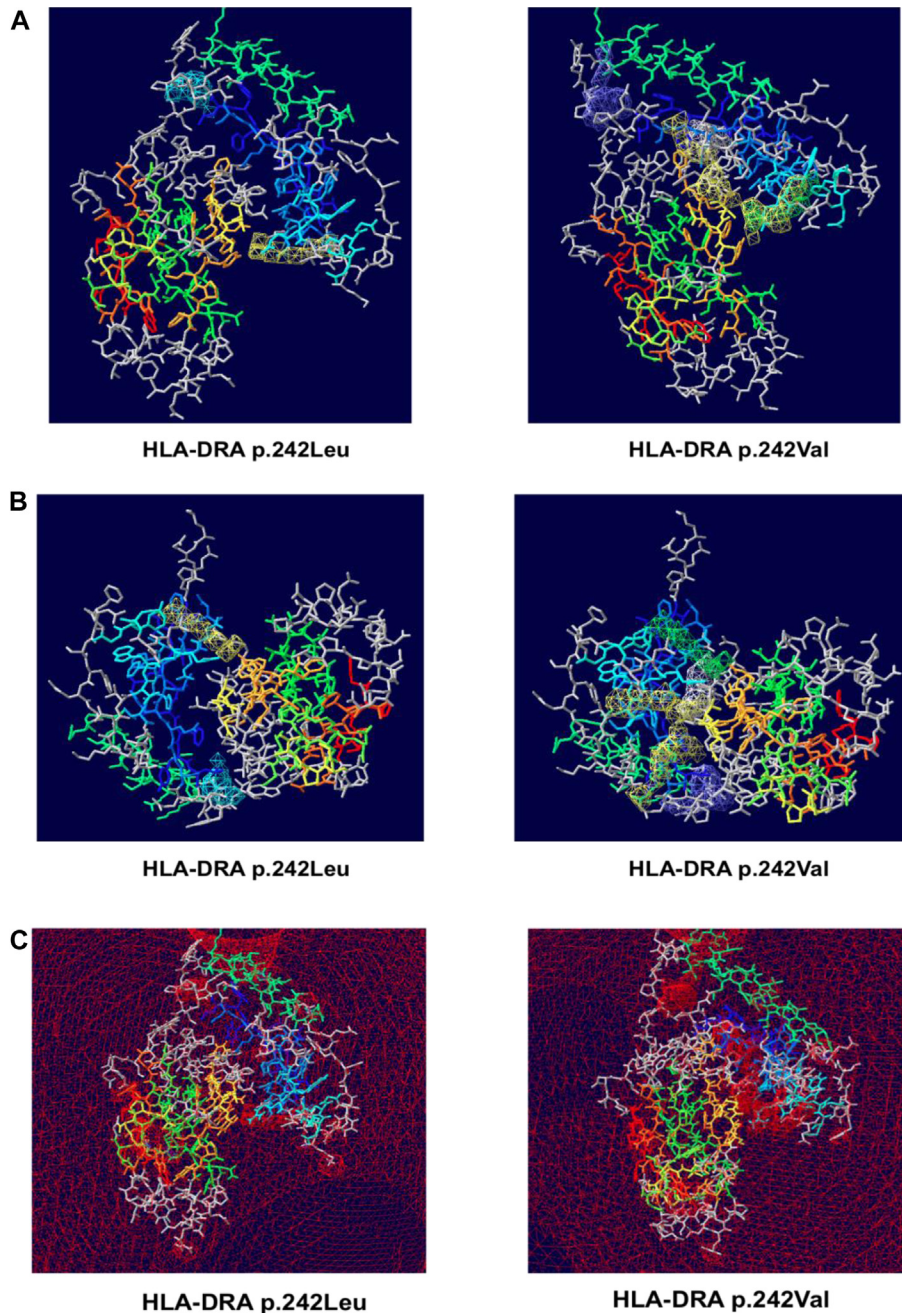


**FIG E2.** Three-dimensional modeling of human wild-type HLA-DRA (p.242Leu) and variant HLA-DRA (p.242Val). Secondary structure succession was colored by using the “rainbow” method. Narrow grooves in each model are depicted as a 3-dimensional network in a different color than the underlying secondary structure of the protein. **A** and **B**, HLA-DRA protein from 2 different angles of view. **C**, Negative electrostatic potential map, which is displayed as a red 3-dimensional network surrounding the protein. The 3-dimensional modeling of the human wild-type and variant proteins were performed by using an automated homology modeling program.<sup>E1,E2</sup> The homology-modeling server SWISS-MODEL was accessed at <http://swissmodel.expasy.org/>.<sup>E1,E2</sup> The 3-dimensional models were visualized with the DeepView/Swiss-PdbViewer 4.1.0 and OpenAstexViewer 3.0 software.<sup>E3,E4</sup> For each 3-dimensional model of the native and variant proteins, narrow grooves, hydrophobic patches, and electrostatic potential on charged residues using the Coulomb method were studied with DeepView/Swiss-PdbViewer 4.1.0 software.<sup>E4</sup>

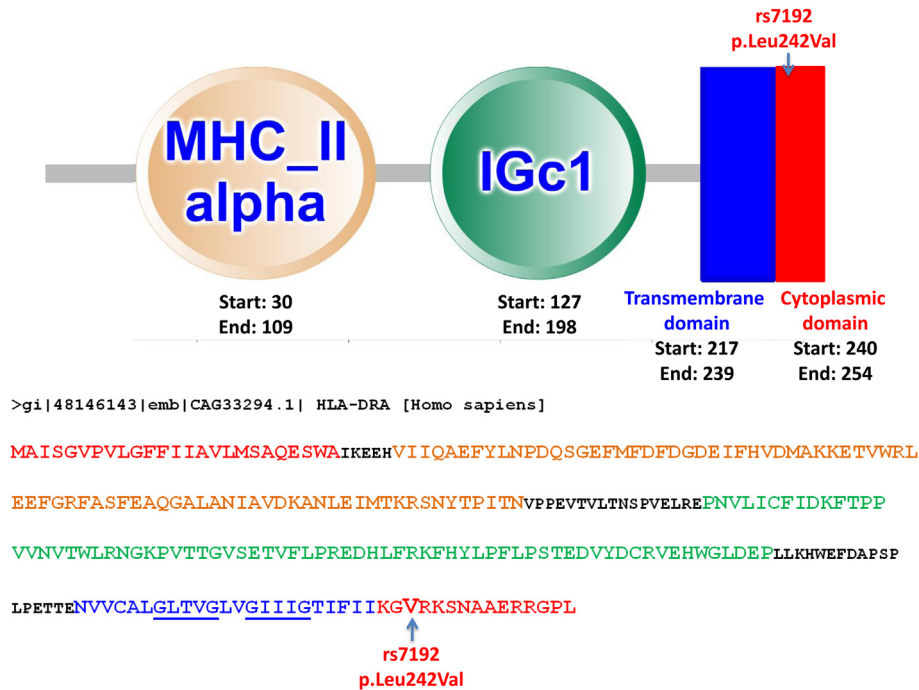


**FIG E3.** The Leu>Val substitution of rs7192 in codon 241 is located in the DRA  $\alpha$ -chain cytoplasmic domain.<sup>E5,E6</sup> The residues of this domain are considered important in regulation of cell-surface expression and intracellular localization of DR molecules.<sup>E7</sup> Two GxxxG motifs of the transmembrane domain are in the vicinity and mediate high-affinity helix-helix association. The conformational changes of the variant protein shown by using 3-dimensional modeling of the human wild-type HLA-DRA (Fig E2) might alter the interactions of the DRA  $\alpha$ -chain cytoplasmic domains with the DR  $\beta$ -chain or the ubiquitin E3 ligases that control the cell-surface expression of the antigen (<http://smart.embl-heidelberg.de/>). *MHC\_II\_alpha*, Class II histocompatibility antigen,  $\alpha$  domain; *IGc1*, Immunoglobulin C-type 1 domain.

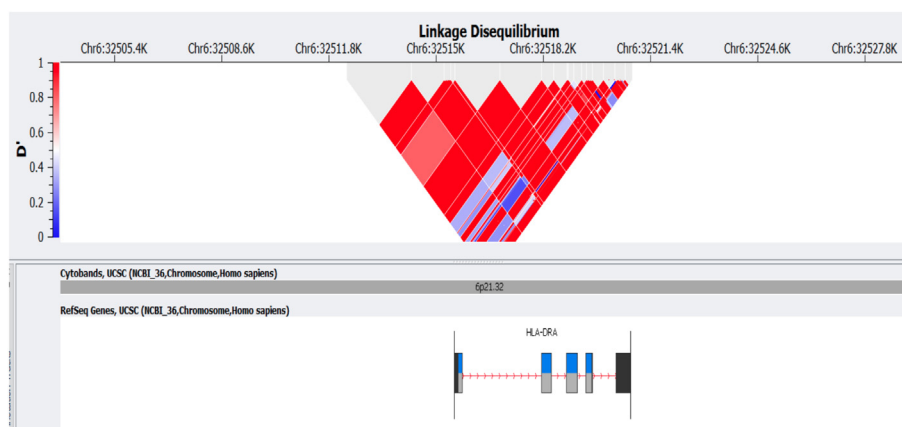


**FIG E4.** Regional association plots of all sequenced variants on the promoter and reading frame of the *HLA-DRA* gene. SNPs are plotted according to their chromosomal positions. Linkage disequilibrium ( $D'$  values) between the lead SNP and the other SNPs are indicated by color.

**5'UTR sequence:**

ATTTTAATGGTCAGACTCTATTACACCCACATTCTCTTTCTTTATTCTTGTCTGTTCTGCC

TCACTCCCGAGCTCTACTGACTCCCACAGAGCGCCCAAGAAGAAA  
 -35 rs14004 -15  
 DNA sequence corresponding to  
 RNA secondary structure motifs

**3'UTR sequence:**

GGTGATGGTGTTCTTAGAGAGAAGATCACTGAAGAACTTCTGCTTTAATGGCTTTACAAAGCTGGCAATATTACAATCC  
 rs3177928 44 rs7194 84  
 TTGACCTCAGTGAAAGCAGTCATCTTCAGCATTTTCCAGCCCTATAGCCACCCCAAGTGTGGATATGCCTCTTCGATTGCT  
 rs7195 rs1131541 rs7196  
CCGTACTCTAACATCTAGCTGGCTTCCTGTCTATTGCCTTTTCTGTATCTATTTCTCTATTTCTATCATTATTATTAT  
 rs7197 rs1051336  
 CACCATGCAATGCCTCTGGAATAAAACATACAGGAGTCTGTCTCTGCTATGGAATGCCCATGGGGCATCTCTGTGTACTTA  
 TTGTTTAAGGTTTCTCAAACGTGATTTTTCTGAACACAATAAACTATTTTGATGATCTTGGGTGGAATTT  
 rs1041885

**FIG E5.** 5'UTR and 3'UTR sequences of the *HLA-DRA* gene with DNA sequences corresponding to RNA secondary structure motifs (*red font*) and annotation of variants flanking these motifs (*underlined blue font*).<sup>E8</sup>

**TABLE E1.** Primer sequences for SNP genotyping and fluorescence resonance energy transfer analyses of the replication study

| SNP ID                      | Amplification PCR primer (5'-3')                                      | Probes (5'-3')                                                    |
|-----------------------------|-----------------------------------------------------------------------|-------------------------------------------------------------------|
| rs8084 ( <i>HLA-DRA</i> )   | Forward: CCAGTACCTCCAGAGGTAAC<br>Reverse: CAGGAAGACTGTCTCTGACAC       | Flu: TCCTCATCTGTTTCATCGACAAGT<br>Red: CACCCCACCAGTGGTCAATGTCA     |
| rs7192 ( <i>HLA-DRA</i> )   | Forward: CAGGAAGACTGTCTCTGACAC<br>Reverse: TCTGTTCCCTACCTTGTCTCTTAAAT | Flu: ATCTTCATCATCAAGGGATTGGCGC Red:<br>AAAGCAATGCAGCAGAACGCAGGGG  |
| rs17612 ( <i>C5</i> )       | Forward: TACAAGCCCAGCAGGGA<br>Reverse: TTCTGGGTGATTCTATAAAAGTGGTA     | Flu: TCAGTGCAAATGAAGACGACTT<br>Red: AAAGCCGTATGTGTTTTCTTTTAAATCGT |
| rs4958427 ( <i>ZNF300</i> ) | Forward: TCTCCTTGGCTTCTTTATACTA<br>Reverse: GTACCTCAAAATGTGATATGACTT  | Flu: CATGATCTGTGTCTCATCCAAAG<br>Red: GGGAAATTTGAACACAAAGACATGCA   |

**TABLE E2.** Genotype counts of the *ZNF300*, *C5*, and *HLA-DRA* gene variants associated with primary risk of  $\beta$ -lactam hypersensitivity in the additive model

| Variant   | Gene                             | DD genotype |                   |                  | Dd genotype |                   |                  | dd genotype |                   |                  |
|-----------|----------------------------------|-------------|-------------------|------------------|-------------|-------------------|------------------|-------------|-------------------|------------------|
|           |                                  | All         | Patients with BLA | Control subjects | All         | Patients with BLA | Control subjects | All         | Patients with BLA | Control subjects |
| rs4958427 | <i>ZNF300</i>                    | 64          | 22                | 42               | 425         | 120               | 305              | 898         | 143               | 755              |
| rs17612   | <i>C5</i>                        | 5           | 0                 | 5                | 138         | 12                | 126              | 1287        | 326               | 961              |
| rs7754768 | <i>HLA-DRA</i>   <i>HLA-DRB5</i> | 131         | 20                | 111              | 608         | 132               | 476              | 762         | 234               | 528              |
| rs9268832 | <i>HLA-DRA</i>   <i>HLA-DRB5</i> | 115         | 18                | 97               | 605         | 130               | 475              | 787         | 239               | 548              |
| rs7192    | <i>HLA-DRA</i>                   | 119         | 16                | 103              | 572         | 128               | 444              | 814         | 241               | 573              |
| rs2227139 | <i>HLA-DRA</i>   <i>HLA-DRB5</i> | 119         | 16                | 103              | 573         | 129               | 444              | 815         | 242               | 573              |
| rs2213586 | <i>HLA-DRA</i>   <i>HLA-DRB5</i> | 117         | 16                | 101              | 572         | 129               | 443              | 818         | 242               | 576              |
| rs2213585 | <i>HLA-DRA</i>   <i>HLA-DRB5</i> | 116         | 16                | 100              | 573         | 129               | 444              | 818         | 242               | 576              |
| rs7195    | <i>HLA-DRA</i>                   | 116         | 16                | 100              | 569         | 129               | 440              | 816         | 241               | 575              |
| rs8084    | <i>HLA-DRA</i>                   | 132         | 20                | 112              | 609         | 141               | 468              | 761         | 226               | 535              |

*D*, Minor allele; *d*, major allele; *DD*, minor homozygous genotype; *Dd*, heterozygous genotype; *dd*, major homozygous genotype.

**TABLE E3.** Association analysis in the allelic model on variants found in the sequencing of the promoter region and reading frame of the *HLA-DRA* gene

| Marker     | Chromosome | Position | Gene           | HGVS        | Location | AA change   | MAF, cases | MAF, control subjects | Fisher exact test, <i>P</i> value | Fisher exact test, FDR | Odds ratio (minor allele) |
|------------|------------|----------|----------------|-------------|----------|-------------|------------|-----------------------|-----------------------------------|------------------------|---------------------------|
| rs2395178  | 6          | 32513340 | <i>HLA-DRA</i> | g.32405362  | 5UTR     | Intronic    | 0.45       | 0.50                  | 7.82E-01                          | 8.66E-01               | 0.82 (0.27-2.47)          |
| rs2395179  | 6          | 32515280 | <i>HLA-DRA</i> | c.-426A>G   | 5UTR     | Intronic    | 0.23       | 0.06                  | 1.70E-01                          | 3.11E-01               | 4.57 (0.55-37.69)         |
| rs2395180  | 6          | 32515288 | <i>HLA-DRA</i> | c.-418T>G   | 5UTR     | Intronic    | 0.23       | 0.06                  | 1.70E-01                          | 3.11E-01               | 4.57 (0.55-37.69)         |
| rs2395181  | 6          | 32515382 | <i>HLA-DRA</i> | c.-324G>C   | 5UTR     | Intronic    | 0.23       | 0.06                  | 1.70E-01                          | 3.11E-01               | 4.57 (0.55-37.69)         |
| rs3129873  | 6          | 32515411 | <i>HLA-DRA</i> | c.-295G>C   | 5UTR     | Intronic    | 0.23       | 0.06                  | 1.70E-01                          | 3.11E-01               | 4.57 (0.55-37.69)         |
| rs3129874  | 6          | 32515418 | <i>HLA-DRA</i> | c.-288T>C   | 5UTR     | Intronic    | 0.23       | 0.06                  | 1.70E-01                          | 3.11E-01               | 4.57 (0.55-37.69)         |
| rs3129875  | 6          | 32515446 | <i>HLA-DRA</i> | c.-260T>C   | 5UTR     | Intronic    | 0.23       | 0.06                  | 1.70E-01                          | 3.11E-01               | 4.57 (0.55-37.69)         |
| rs16822584 | 6          | 32515515 | <i>HLA-DRA</i> | c.-191C>T   | 5UTR     | Intronic    | 0.03       | 0.00                  | 1.00E+00                          | 1.00E+00               |                           |
| rs14004    | 6          | 32515687 | <i>HLA-DRA</i> | c.-19C>A    | Intron   | Intronic    | 0.33       | 0.44                  | 5.58E-01                          | 6.66E-01               | 0.64 (0.21-1.98)          |
| rs3129884  | 6          | 32518188 | <i>HLA-DRA</i> | c.83-15T>C  | Intron   | Intronic    | 0.15       | 0.00                  | 1.91E-01                          | 2.95E-01               |                           |
| rs3129885  | 6          | 32518193 | <i>HLA-DRA</i> | c.83-10T>C  | Intron   | Intronic    | 0.15       | 0.00                  | 1.91E-01                          | 2.95E-01               |                           |
| rs9268659  | 6          | 32518919 | <i>HLA-DRA</i> | c.329-21T>C | Intron   | Intronic    | 0.52       | 0.06                  | 1.19E-03                          | 6.13E-03               | 16.03 (1.99-129.2)        |
| rs3135391  | 6          | 32518965 | <i>HLA-DRA</i> | c.354A>G    | Exon     | p.Thr118=   | 0.10       | 0.00                  | 3.33E-01                          | 4.30E-01               |                           |
| rs8084     | 6          | 32519013 | <i>HLA-DRA</i> | c.402A>C    | Exon     | p.Ile134=   | 0.48       | 0.06                  | 2.99E-03                          | 1.03E-02               | 14.03 (1.74-113.07)       |
| rs60110771 | 6          | 32519264 | <i>HLA-DRA</i> | c.610+43C>T | Intron   | Intronic    | 0.05       | 0.00                  | 1.00E+00                          | 1.00E+00               |                           |
| rs2239804  | 6          | 32519501 | <i>HLA-DRA</i> | c.611-10T>C | Intron   | Intronic    | 0.58       | 0.13                  | 1.47E-03                          | 6.51E-03               | 9.8 (2.04-47.01)          |
| rs11544315 | 6          | 32519551 | <i>HLA-DRA</i> | c.651C>T    | Exon     | p.Asn217=   | 0.02       | 0.06                  | 3.79E-01                          | 4.70E-01               | 0.25 (0.02-4.3)           |
| rs7192     | 6          | 32519624 | <i>HLA-DRA</i> | c.724T>G    | Exon     | p.Leu242Val | 0.50       | 0.00                  | 1.06E-04                          | 1.64E-03               |                           |
| rs16822632 | 6          | 32519690 | <i>HLA-DRA</i> | c.*11+14G>A | Intron   | Intronic    | 0.03       | 0.00                  | 1.00E+00                          | 1.00E+00               |                           |
| rs3129888  | 6          | 32519704 | <i>HLA-DRA</i> | c.*11+28G>A | Intron   | Intronic    | 0.22       | 0.00                  | 5.83E-02                          | 1.64E-01               |                           |
| rs4935356  | 6          | 32520366 | <i>HLA-DRA</i> | c.*12-41T>G | Intron   | Intronic    | 0.60       | 0.00                  | 1.45E-04                          | 1.49E-03               |                           |
| rs3135390  | 6          | 32520373 | <i>HLA-DRA</i> | c.*12-34C>A | Intron   | Intronic    | 0.15       | 0.00                  | 1.91E-01                          | 2.95E-01               |                           |
| rs4935354  | 6          | 32520376 | <i>HLA-DRA</i> | c.*12-31C>T | Intron   | Intronic    | 0.47       | 0.00                  | 2.72E-04                          | 1.69E-03               |                           |
| rs3177928  | 6          | 32520413 | <i>HLA-DRA</i> | c.*18G>A    | Intron   | Intronic    | 0.08       | 0.13                  | 6.34E-01                          | 7.27E-01               | 0.64 (0.11-3.63)          |
| rs7194     | 6          | 32520458 | <i>HLA-DRA</i> | c.*63G>A    | Intron   | Intronic    | 0.50       | 0.00                  | 1.06E-04                          | 1.64E-03               |                           |
| rs7195     | 6          | 32520517 | <i>HLA-DRA</i> | c.*122A>G   | Intron   | Intronic    | 0.47       | 0.00                  | 2.72E-04                          | 1.69E-03               |                           |
| rs1131541  | 6          | 32520544 | <i>HLA-DRA</i> | c.*149T>A   | Intron   | Intronic    | 0.13       | 0.00                  | 1.91E-01                          | 2.58E-01               |                           |
| rs7196     | 6          | 32520549 | <i>HLA-DRA</i> | c.*154A>T   | Intron   | Intronic    | 0.40       | 0.00                  | 1.63E-03                          | 6.31E-03               |                           |
| rs7197     | 6          | 32520558 | <i>HLA-DRA</i> | c.*163T>C   | Intron   | Intronic    | 0.22       | 0.00                  | 5.83E-02                          | 1.64E-01               |                           |
| rs1051336  | 6          | 32520570 | <i>HLA-DRA</i> | c.*175G>A   | Intron   | Intronic    | 0.13       | 0.00                  | 1.91E-01                          | 2.58E-01               |                           |
| rs1041885  | 6          | 32520787 | <i>HLA-DRA</i> | c.*392T>A   | Intron   | Intronic    | 0.13       | 0.00                  | 1.91E-01                          | 2.58E-01               |                           |

AA, Amino acid; HGVS, Human Genome Variation Society; MAF, minor allele frequency; OR, odds ratio.

**TABLE E4.** Replication of associations between *ZNF300* and *HLA-DRA* gene variants and BLA in an Italian case-control population, considering only patients with allergic reactions to cephalosporins

| Variant   | Gene           | Genotype | Control subjects (n = 362) | Patients with BLA (n = 100) | P value* |
|-----------|----------------|----------|----------------------------|-----------------------------|----------|
| rs7192    | <i>HLA-DRA</i> | G allele | 530 (73.2)                 | 151 (75.3)                  | .289     |
|           |                | TT       | 26 (7.2)                   | 9 (9.0)                     | .518     |
|           |                | TG       | 142 (39.3)                 | 31 (31.0)                   |          |
|           |                | GG       | 194 (53.5)                 | 60 (60.0)                   |          |
| rs8084    | <i>HLA-DRA</i> | C allele | 501 (69.1)                 | 148 (73.7)                  | .109     |
|           |                | AA       | 33 (9.1)                   | 10 (10.0)                   | .188     |
|           |                | AC       | 157 (43.5)                 | 32 (32.0)                   |          |
|           |                | CC       | 172 (47.4)                 | 58 (58.0)                   |          |
| rs17612   | <i>C5</i>      | C allele | 37 (5.1)                   | 18 (9.0)                    | .033     |
|           |                | ACCC     | 36 (9.9)                   | 18 (18.0)                   | .024     |
|           |                | AA       | 326 (90.1)                 | 82 (82.0)                   | .046     |
|           |                | AC       | 35 (9.3)                   | 18 (18.0)                   |          |
| rs4958427 | <i>ZNF300</i>  | CC       | 1 (0.3)                    | 0 (0.0)                     |          |
|           |                | G allele | 594 (82.3)                 | 173 (86.4)                  | .096     |
|           |                | AGGG     | 350 (96.6)                 | 100 (100.0)                 | .051     |
|           |                | AA       | 12 (3.3)                   | 0 (0.0)                     | .149     |
|           |                | AG       | 104 (28.8)                 | 27 (27.0)                   |          |
|           |                | GG       | 246 (67.9)                 | 73 (73.0)                   |          |

\*The allelic, dominant, and recessive models were analyzed by using the  $\chi^2$  test, and the additive model was analyzed by using regression analysis.