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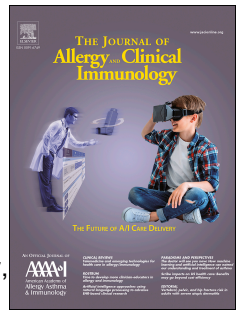

BETA-LACTAM-INDUCED IMMEDIATE HYPERSENSITIVITY REACTIONS: A GENOME-WIDE ASSOCIATION STUDY OF A DEEPLY PHENOTYPED COHORT

Paola Nicoletti, MD, PhD, Daniel F. Carr, PhD, Sarah Barrett, BSc, Laurence McEvoy, BSc, Peter S. Friedmann, FRCP, Neil H. Shear, MD, FRCPC, Matthew R. Nelson, PhD, Anca M. Chiriac, MD, Natalia Blanca-López, MD, PhD, Jose A. Cornejo, PhD, Francesco Gaeta, MD, PhD, Alla Nakonechna, MD, Maria J. Torres, MD, PhD, Cristiano Caruso, MD, Rocco L. Valluzzi, MD, Aris Floratos, PhD, Yufeng Shen, PhD, Rebecca K. Pavlos, PhD, Elizabeth J. Phillips, MD, Pascal Demoly, MD, Antonino Romano, MD, Miguel Blanca, MD, Munir Pirmohamed, FRCP, PhD

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ABSTRACT

Background: β-lactam antibiotics are associated with a variety of immune-mediated or hypersensitivity reactions, including immediate (Type I) reactions mediated by antigen-specific IgE.

Objective: To identify genetic predisposing factors for immediate reactions to β-lactam antibiotics.

Methods: Patients with a clinical history of immediate hypersensitivity reactions to either penicillins or cephalosporins, which were immunologically confirmed, were recruited from allergy clinics. A genome-wide association study (GWAS) was conducted on 662 patients (the discovery cohort) with a diagnosis of immediate hypersensitivity and the main finding was replicated in a cohort of 98 Spanish cases, recruited using the same diagnostic criteria as the discovery cohort.

Results: GWAS identified rs71542416 within the Class II HLA region as the top hit ($P = 2x10^{-14}$); this was in linkage disequilibrium with *HLA-DRB1*10:01* (OR = 2.93 $P = 5.4x10^{-7}$) and *HLA-DQA1*01:05* (OR=2.93, $P=5.4x10^{-7}$). Haplotype analysis identified that *HLA-DRB1*10:01* was a risk factor even without the *HLA-DQA1*01:05* allele. The association with *HLA-DRB1*10:01* was replicated in another cohort, with the meta-analysis of the discovery and replication cohorts showing that *HLA-DRB1*10:01* increased the risk of immediate hypersensitivity at a genome-wide level (OR = 2.96 $P=4.1x10^{-9}$). No association with *HLA-DRB1*10:01* was identified in 268 patients with delayed hypersensitivity reactions to β-lactams.

Conclusion: *HLA-DRB1*10:01* predisposed to immediate hypersensitivity reactions to penicillins. Further work to identify other predisposing HLA and non-HLA loci is required.

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Clinical implications: This novel insight into the mechanisms of immediate reactions associated with penicillins may be of use in risk stratifying patients where penicillin cannot be excluded as an etiological agent.

CAPSULE SUMMARY

Predisposition to immediate hypersensitivity reactions to penicillins is mediated by *HLA-DRB1*10:01*, and may help in risk stratifying patients where penicillin cannot be excluded as an etiological agent.

KEY WORDS: Type I hypersensitivity, β-lactams, penicillins, cephalosporins, allergy, anaphylaxis, pharmacogenomics.

ABBREVIATIONS

- ADR Adverse Drug Reaction
- AGEP Acute generalised exanthematous pustulosis
- BL β-Lactam
- DNA Deoxyribonucleic acid
- DRESS Drug reaction with eosinophilia and systemic symptoms
- GWAS Genome-wide association study
- HLA Human Leukocyte Antigen
- HPEPT1 human peptide transporter 1
- ITCH International Consortium on Drug Hypersensitivity
- OR Odds Ratio
- SJS/TEN Stevens-Johnson syndrome/toxic epidermal necrolysis
- SNP Single Nucleotide Polymorphism
- WTCCC Wellcome Trust Case Control Consortium

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131 **INTRODUCTION**

132

133 β-lactam (BL) antibiotics cause a wide spectrum of hypersensitivity reactions
134 (sometimes termed allergy). The self-reported incidence of BL allergy ranges from
135 1% to >10%[1], but in clinic populations most patients (~95%) are not found to be
136 truly allergic with validated skin testing and oral challenge. Indeed, a high proportion
137 are intolerant[2] as adverse effects such as diarrhea after the use BLs are often
138 mistakenly reported as allergy by patients.

139

140 True BLs hypersensitivity reactions are classified according to the time of onset of
141 the reaction following drug intake[3]. Immediate hypersensitivity reactions develop in
142 minutes or hours after drug intake and are due to cross-linking of specific IgE
143 molecules on the mast cell surface with release of vasoactive mediators such as
144 histamine leading to vasodilation, increased vascular permeability and smooth
145 muscle contraction[4]. Clinically this is manifested as urticaria, angioedema,
146 bronchospasm and hypotension. Anaphylaxis is the most severe and feared form of
147 immediate hypersensitivity. By contrast, delayed hypersensitivity reactions occurring
148 >6h after dosing are typically T-cell mediated and have variable manifestations
149 including maculopapular exanthem, DRESS (drug reaction with eosinophilia and
150 systemic symptoms) and Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis[3].

151

152 Medicines are amongst the main cause of fatal anaphylaxis with a mortality rate
153 higher than with other agents[5]. Penicillins and cephalosporins are still the two most
154 common drug classes associated with anaphylaxis[6], with penicillins having a higher
155 incidence (1-5 per 100,000)[7] compared to cephalosporins[1]. Cross-reactivity between
156 penicillins, cephalosporin and other BLs not sharing an R1 or R2 side-chain is now
157 thought to be <2%[8, 9].

158

159 Potential clinical risk factors for immediate hypersensitivity reactions are family
160 history, atopy, concomitant virus infections and the route of administration[10].
161 Genetic predisposing factors have also been identified[10]: the most comprehensive
162 was an analysis of 107,398 single nucleotide polymorphisms which identified that the
163 *HLA-DRA* locus may protect against penicillin-induced immediate hypersensitivity

reactions[11]. In order to further investigate the role of genetic factors in BL-induced immediate hypersensitivity reactions, we have undertaken a GWAS (genome-wide association study) of the largest deeply phenotyped patient cohort assembled so far.

METHODS

Cases

All subjects were recruited between 2009 and 2013 as part of International Consortium on Drug Hypersensitivity (ITCH), involving 5 recruitment centers worldwide (Australia, France, Italy, Spain, UK). The study was approved by ethics committees in all countries, and all patients gave written informed consent.

We recruited 662 patients (the discovery cohort) with a diagnosis of immediate hypersensitivity to BL antibiotics (table 1). The diagnosis of immediate hypersensitivity was made in specialist allergy clinics, as per published criteria[12]. All patients required immunological assessment (total and specific IgE, skin testing including skin prick and intradermal and/or oral provocation) as part of the inclusion criteria. Independent adjudication of all cases was undertaken by NS and PF. For replication of any signals, we separately recruited another 98 patients with immediate hypersensitivity from a clinic in Spain, diagnosed according to the same criteria.

To determine specificity of any signals identified in patients with immediate hypersensitivity, we also evaluated 268 patients with delayed hypersensitivity reactions across multiple beta-lactams. The diagnosis again was in accordance with published guidance[12], and all cases were adjudicated by NS and PF. We also included an additional 17 BL-induced delayed hypersensitivity reaction cases analyzed in Shen et al[13].

Controls

We used general population samples as study controls. This comprised 9217 European ancestry controls from multiple available sources enriching the group with Spanish, Italian and north European origin samples since cases were manly recruited from those countries. We used the Wellcome Trust Case Control Consortium

(WTCCC) (http://www.wtccc.org.uk), the population reference sample (POPRES)[14], PGX4000119[13], LAM30004[13] and Spanish Bladder cancer cohort (phs000346.v1)[15] from dbGAP, Hypergenes cohort (http://www.hypergenes.eu/), the National Spanish DNA Bank (http://www.bancoadn.org/), and TSI (Hapmap data). In addition, we also recruited a group of 137 penicillin tolerant controls from Italy.

Genotyping

Genome-wide genotyping of DNA extracted from whole blood was performed at the Broad Institute, Boston, for 662 cases with BL-induced immediate hypersensitivity and 268 cases with delayed hypersensitivity reaction, and from 137 penicillin tolerant controls from Italy. In 474 (354 BL-induced immediate and 120 BL-induced delayed) cases, the Illumina Infinium HumanCoreExome Bead Chip was used while for 439 (308 BL-induced immediate and 131 BL-induced delayed) cases, the Illumina HumanOmniExpress BeadChip was used. In this last batch, we also genotyped 137 Italian penicillin tolerant controls. In addition, the BL-induced delayed case group also included 17 β-lactam delayed hypersensitivity cases previously genotyped by the Illumina 1M Duo chip, extracted from a larger SJS/TEN study that included multiple drugs, as described by Shen et al.[13]. Other control cohorts were publicly available (Table S1). For each of the genotyping cohort, standard quality control was conducted at both single marker and subject levels as previously described[13]. This was followed by SNP and HLA imputation and amino acid analysis (see supplement).

Replication Cohort SNP and HLA genotyping

The top associated imputed single nucleotide polymorphisms (SNPs) were validated by SNP genotyping using either TaqMan, SNP genotyping assays (ThermoFisher Scientific, Paisley, UK) or iPLEX MassArray genotyping platform (Agena Biosciences, Hamburg, Germany). High resolution genotyping of *HLA-A, B, C, DRB1, DQA1* and *DQB1* was performed by Histogenetics (Ossining, New York). Sequencing data files were analyzed using Histogenetics' proprietary analysis software (Histomatcher and HistoMagic) for HLA genotype calling. Allele assignments are based on IMGT/HLA Database release version 2.21.0, dated April 2008 (http://www.ebi.ac.uk/imgt/hla/).

Statistical analysis

The effect of population structure was assessed through principal component analysis (PCA) using the smartPCA program from the EIGENSTRAT package (version 3.0)[16]. Single marker and haplotype association analyses and heterogeneity test analyses were carried out by PLINK 1.07[17]. The statistical association of each marker, HLA alleles and SNPs, was determined in a logistic regression framework with scores for the first seven principal components as covariates under an additive model using PLINK. We used the same statistical test for sub-population analyses, using two, seven and ten most significant principal components as covariates in Italian, Spanish and North European populations, respectively. We set the genome-wide traditional significance P-value threshold to 5.0×10^{-8} to correct for multiple testing and MHC-wide significance threshold to 2.0×10^{-4} to correct for total number of predicted alleles. When we obtained genome-wide significant signals, we tested for independent effects from the neighboring variants by including the most associated variants as a covariate and then testing the significance of others in the region. All detailed analyses and Manhattan plots were performed with R (Version 3.0.2). Regional plots were drawn by LocusZoom[18]. Meta-analysis was performed using a fixed-effect model in the *metafor* package (http://www.metafor-project.org/doku.php/metafor).

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RESULTS

Patient cohorts

The clinical characteristics of the patients are shown in table 1. Clinical manifestations in the discovery cohort included angioedema (35%), bronchospasm (24%) and urticaria (34%), while hypotension was reported in only 4% of cases. The length of reaction in patients with immediate hypersensitivity was 2-11 days, while it ranged from 21-26 days for patients with delayed hypersensitivity reactions. Patients were included if they had positive diagnostic assessment, as highlighted in table 1. Penicillins accounted for 75% of cases, with the most common culprit drug being amoxicillin accounting for 58% of cases in the discovery cohort.

Association with immediate reactions to beta-lactams

We first conducted a genome wide association study on 662 patients of European descent with immediate hypersensitivity reactions and 9217 previously genotyped population controls matched for ethnicity. The total number of SNPs which were included in the analyses after quality control was 4,265,742. The cases clustered within three major groups (Italian, Spanish and Northern European, Figure S1) in keeping with the self-reported ethnicity.

A genome-wide significant association was identified within the Class II HLA region, rs71542416 being the top hit (OR=5.17; 95% CI 3.40-5.17; $P=2x10^{-14}$; Table 2, Figure 1A and Figure S2). The frequency of rs71542416 in our control population was comparable with publicly available sources (Table 2). HLA allele imputation using HIBAG[19] showed the *HLA-DRB1*10:01* (OR=2.95; 95% CI 1.99-4.36; $P=6.0x10^{-8}$) and *HLA-DQA1*01:05* (OR=2.93 95%CI 1.92-4.45 $P=5.4x10^{-7}$) alleles to be significantly associated with the immediate reactions, with consistent odds ratios (Table 2) and were tagged by rs71542416 (r^2 0.76). Haplotype analysis identified that *HLA-DRB1*10:01* was a risk factor even without the *HLA-DQA1*01:05* allele (Table S2). *HLA-DRB1*10:01* was seen in 3% of cases and less than 1% of controls. The frequency of the HLA alleles within the Italian penicillin tolerant controls was 10 times less than in the Italian general population (0.1% vs 1%).

The HLA allele effect size was similar across the three major clusters (heterogeneity test P = 0.11) (Table 3). The positive predictions in cases were fully validated by direct HLA typing. An additional 67 cases with low quality predictions in both the loci were also typed. Among them, we found only one positive carrier for *HLA-DRB1*10:01*. All cases were also genotyped for rs71542416 – this showed a concordance of 99% between typed and imputed genotypes of rs71542416. *HLA-DRB1*10:01* co-occurred with rs71542416 in 89% of the *HLA-DRB1*10:01* positive patients, while 12% of all cases carried rs71542416 alone.

Including rs71542416 or the HLA alleles as covariates revealed a residual protective effect of the *HLA-DRA* locus, tagged by rs114632839, an intronic gene variant, in accordance with the findings of Gueant et al[11] (Table 2 and Figure S3A). Interestingly GTEx analysis revealed that this variant was a strong eQTL for *HLA-DRB5* (P = 5.3×10^{-23}) and sQTL for the *HLA-DRB1* (P = 1.1×10^{-16}), *HLA-DRB5* (P=1.1×10^{-16}) and *HLA-DRB6* (P = 1.1×10^{-16}) loci with the minor alleles showing a lower intron excision ratio. Both effects were detected in whole blood and shared across other tissues (Figure S3B).

A replication cohort of 98 patients with anaphylaxis induced by either amoxicillin or amoxicillin-clavulanate (table 1) was recruited separately from Spain. We identified 7 individuals who were positive for *HLA-DRB1*10:01*, as confirmed by HLA typing. Comparison using the 11 Spanish HLA typed cohorts reported in allelefrequency.net provided a total of 3137 Spanish subjects (Figure S4) as ethnically matched population controls. This analysis replicated the association with an odds ratio (OR) of 2.80 (95% CI 1.17-6.71; Fisher Exact Test P=0.016; Figure 1B).

Meta-analysis of the discovery and replication cohorts showed that *HLA-DRB1*10:01* increased the risk of immediate hypersensitivity at a genome-wide level (OR=2.96; 95% CI 1.99-4.37; P =4.1×10^{-9}) (Figure 2). The sensitivity and specificity of the allele is 0.06 and 0.98, respectively, while the positive and negative predictive values are 17% and 94% respectively.

316 The most significantly associated amino acid with immediate hypersensitivity
317 reactions was glutamate at position 10 (OR=2.72; 95% CI 1.81-4.08; P=1.4x10^{-6},
318 Table S3). Amoxicillin, amoxicillin-clavulanic acid and phenoxymethylpenicillin
319 showed the highest effect size (Table S4). Glutamate-10 co-occurred with other
320 amino acids (arginine-30, valine-31, alanine-38, tyrosine-40, proline-231, glutamine-
321 166) which had the same frequency in cases and controls as glutamate-10 and *HLA-*
322 *DRB1*10:01* (Table S3). However, association with these amino acids disappeared
323 after condition for either glutamate-10 or *HLA-DRB1*10:01* (Table S3). Interestingly,
324 glutamate-10 co-occurred with the shared epitope RRA at positions 70, 71 and 74,
325 previously associated with seropositive rheumatoid arthritis[20] and specific for the
326 *HLA-DRB1*10:01* allele. The ERRA haplotype increased risk (OR=2.72, P=1.4x10^{-6})
327 equivalent to that seen with glutamate-10 alone. None of the other risk/protective
328 amino acid motifs for seropositive rheumatoid arthritis[20] spanning positions 70 to 74
329 in the *DRB1* locus (such as "QRRAA" risk motif or "DERAA" and "DRRAA"
330 protective motifs) were associated with our phenotype.
331

332 **HLA analysis in patients with delayed hypersensitivity reactions**

333 To determine whether the association with *HLA-DRB1*10:01* was limited to patients
334 with immediate hypersensitivity reactions, we analyzed 268 patients with delayed
335 hypersensitivity to a variety of BLs (Table 1) using the same control set (Figure S1B).
336 No association was identified for *HLA-DRB1*10:01* (n=249; OR = 1.34; 95% CI 0.55-
337 3.26; P=0.5).
338

339 **Drug specific associations with immediate hypersensitivity**

340 *HLA-DRB1*10:01* was associated with penicillins as a class (OR=3.07), but not with
341 cephalosporins (Table 4). Among the penicillins, the strongest signals were for
342 amoxicillin (OR=3.48), amoxicillin clavulanic acid (OR=2.85) and phenoxymethyl
343 penicillin (OR=6.66) (Table 4). When we combined amoxicillin and amoxicillin
344 clavulanic acid cases (assuming that amoxicillin rather than clavulanic acid was the
345 culprit), the OR was 3.1 (95%CI 2.01-4.85; P=4.0x10^{-7}). Additional drug-specific HLA
346 allele associations that we identified will need confirmation (Tables S5 and S6).
347

In the drug-specific analysis, a genome-wide signal (rs71437970) on chromosome 13 upstream of *SLC15A1* (Figure S5) was identified for the amoxicillin cases (OR= 2.94 P =3.8x10^{-9}, Table S6). This association was shared across the European subpopulations and with amoxicillin-clavulanate cases (Tables S7 and S8). However, we failed to replicate the association, with an allele frequency which was lower than that observed in Spanish controls (0.007 vs 0.04).

DISCUSSION

We have identified an association between the SNP rs71542416 and immediate hypersensitivity reactions to penicillins. The SNP does not affect gene expression in GTEx but is in linkage disequilibrium with *HLA-DRB1*10:01* and *HLA-DQA1*01:05*. Haplotype analysis identified that *HLA-DRB1*10:01* was a risk factor even without the *HLA-DQA1*01:05* allele suggesting that *HLA-DRB1*10:01* may be the predominant driver of the association. However, 12% of cases carried rs71542416 but were negative for *HLA-DRB1*10:01* suggesting that the SNP may be a tag for other rare HLA alleles, which is consistent with the hypothesis of Heap at al[21] who showed an association between *HLA-DQA1-HLA-DRB1* variants and thiopurine-induced pancreatitis.

The association with *HLA-DRB1*10:01* and rs71542416 was most pronounced in the Spanish cohort (Table 3), but given that the odds ratios were of similar magnitude in all populations studied, there was overlap in the confidence intervals, and the prevalence of the SNP and HLA allele, our findings can be generalized across the European sub-ethnicities studied (Table 3). However, further studies will be needed in both European and non-European populations to determine the global relevance of this association. Additionally, the association was limited to immediate reactions and was not observed with the delayed hypersensitivity reactions highlighting the specific nature of the association. Evaluation of drug-specificity showed associations with amoxicillin, amoxicillin-clavulanate and phenoxymethylpenicillin. However, given the limited sample size with the other penicillins, we cannot exclude the possibility of an association with all penicillins (Table 4). Similarly, we did not find an association with cephalosporins, but this may also be because of a lower sample size.

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The clear strength of our study is that all patients were deeply phenotyped: there was a clear clinical history with a temporal relationship to drug intake, and the diagnosis was confirmed immunologically by skin testing and/or oral provocation. Such deep phenotyping is important because many patients claim to be penicillin allergic, but very few are: of those claiming to be allergic, less than 1 In 20 have an acute reaction to an oral challenge (the gold standard clinical test to confirm an IgE-mediated reaction)[22].

Our data adds to the increasing evidence of HLA in predisposing to different clinical phenotypes of drug hypersensitivity reactions[23]. The most well-known of these associations is *HLA-B*57:01* and abacavir hypersensitivity[24], which has been implemented into clinical practice and has resulted in a significant reduction in abacavir hypersensitivity[25]. It is important to note that most of the HLA associations identified to date have been with delayed hypersensitivity reactions[23]. However, more recent studies have identified HLA alleles as predisposing factors for immediate reactions. For instance, *HLA-DRB1*07:01* is a risk factor for the development of anti-asparaginase antibodies and immediate reactions[26]. Our data showing that *HLA-DRB1*10:01* predisposes to immediate hypersensitivity is also consistent with the pathogenesis of immediate reactions where the interaction between B cells and $CD4^+$/Th2-positive cells, through HLA class II alleles, is central to the immunoglobulin switching that leads to the generation of specific IgE antibodies. Different HLA alleles have been associated with other types of immune-mediated reactions caused by BLs. For example, *HLA-B*57:01* predisposes to flucloxacillin-induced cholestatic hepatitis[27], while liver injury caused by amoxicillin-clavulanate is associated with the class II HLA haplotype *HLA-DRB1*1501-DQB1*0602*[28]. Mechanistic studies undertaken in our laboratory have shown that drug-specific, HLA-restricted T cells can be isolated from patients with a past history of liver injury due to flucloxacillin[29] and amoxicillin-clavulanate[30]. It will be valuable to conduct similar studies in patients with a history of penicillin-induced immediate reactions to understand the mechanistic basis of the association with *HLA-DRB1*10:01*.

413 Another potentially interesting finding in this study was the association between
414 *SLC15A1* gene variants and amoxicillin-induced immediate reactions. SLC15A1
415 encodes the human peptide transporter 1 (HPEPT1) which is known to transport
416 amoxicillin[31]. Therefore, it is plausible that variation in the activity of HPEPT1 could
417 result in altered amoxicillin pharmacokinetics and thereby increase risk of a type I
418 reaction. However, we were not able to replicate this finding, and further work
419 (including functional studies) to understand whether this gene is important in
420 predisposing to immediate reactions will be required.
421
422 What are the clinical implications of this finding? Given the rarity of penicillin-
423 induced anaphylaxis, the low population prevalence and sensitivity of *HLA-*
424 *DRB1*10:01,* and the very wide usage of penicillins, the prospective use of this allele
425 in screening patients before penicillin prescription would not be practical or feasible
426 in terms of both high numbers needed to test to prevent one case and patients
427 unnecessarily excluded from therapy. However, this association of immediate
428 penicillin hypersensitivity with *HLA-DRB1*10:01* may provide much novel insights into
429 the mechanisms of immediate reactions associated with penicillins, including the
430 mechanisms of sensitization and natural loss or waning of penicillin which is known
431 to occur over time. Moreover, the higher negative predictive value of the allele
432 (94%) may be of use in risk stratifying patients where penicillin cannot be excluded as
433 an etiological agent in the setting of an immediate reaction.
434
435 Our study has limitations. First, the overall sample size is small compared to that
436 used in complex diseases but is larger than that used in many pharmacogenomic
437 studies. Our efforts to identify deeply phenotyped patients in this study was a result
438 of an extensive international collaboration highlighting the difficulties in achieving
439 large sample sizes in pharmacogenomic studies. Furthermore, we were unable to
440 perform permutation testing to validate the replication P value for *HLA-DRB1*10:01*.
441 Second, because we used population controls, we could not adjust for self-reported
442 ethnicity, but this is unlikely to have had a major impact as we accounted for this
443 through an analysis of population stratification (figure S1). Third, matching cases and
444 controls for age, gender and other co-morbidities was not possible because of the
445 use of population controls, and because gender could not be determined due to the

absence of X chromosome SNP data. Whether this impacts on the association with the genetic signals identified by us will require further study.

In summary, we have for the first time reported an association of *HLA-DRB1*10:01* carriage in deeply immunologically phenotyped European ancestry individuals with penicillin-induced immediate type I hypersensitivity reactions. However, we cannot exclude the possibility of other HLA alleles or HLA haplotypes also being important in conferring susceptibility in some patients, and therefore further work in both European and non-European patients is required to identify other HLA alleles, and also whether *HLA-DRB1*10:01* is universally important. It is also interesting to note that we also identified that rs114632839 which is a proxy for the HLA-DRA locus protected against the development of immediate hypersensitivity reactions to BLs, consistent with a previous study[11]. rs114632839 is an eQTL and sQTL for several HLA loci suggesting that predisposition to immediate hypersensitivity to penicillins is likely to be complex and mediated by a combination of susceptibility and protective HLA and non-HLA alleles. Clearly we have reported associations, and proof of causality will require a full understanding of the immunopathogenesis of initial sensitization to penicillin, and in particular, the mechanism of antigen presentation (including the relative importance of the BL ring vs the side chains) and interaction with $CD4^+$ T cells that ultimately leads to IgE-switching and the generation of hapten-specific IgE antibodies.

[3237 words]

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Author Contributions

PN, DC and MP wrote the manuscript. PN, MRN, DC and MP designed the research while PN, YS, AF undertook/supervised the data analysis. SB, LM, PSF,

NHS, AMC, NBL, JAC, FG, AN, MJT, CC, RLV, RKP, EP, PD, AR, MB and MP contributed to patient recruitment, adjudication and data acquisition. All authors contributed to, and approved, the final draft of the manuscript.

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Figure Legend

Figure 1 Genomic data in patients with immediate hypersensitivity reactions. **A:** Manhattan plot displaying the association analysis undertaken in patients with immediate hypersensitivity reactions to β-lactams (n=662). SNPs in green have a significance level less than $5x10^{-6}$ and red have a significance level less than $5x10^{-8}$. **B:** Forest plot showing the effect size of the association between *HLA-DRB1*10:01* and immediate reactions in the discovery and replication cohorts. For each analysis, the odd ratio of the association is reported with 95% CI. The dimension of the squares is proportional of the number of cases.

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Table 1: Causative drugs and clinical variables broken down across the discovery and replication cohorts

Clinical characteristics	Immediate hypersensitivity Discovery cohort (n = 662)	Immediate hypersensitivity Replication cohort (n = 98)	Delayed hypersensitivity cohort (n = 268)
Female (%)	416 (62%)	56 (57%)	174 (64%)
Age years: mean, SD* (%missing)	42.0, 16 (27%)	51.4, 12.3 (0%)	44.5, 20 (73%)
History of allergies (n with available information)	31% (658)	9% (98)	30.6% (268)
Number of ADRs*: mean, SD* (#available info)	1.1, 0.3 (659)	1.2 (0.5)	1, 0.2 (251)
Autoimmune disease diagnosis	9%	6%	7%
% positive skin test (of total number tested)	85% (578)	78% (67)	93% (204)
% positive prick test (of total number tested)	45% (142)	37% (82)	82% (207)
% positive oral provocation /re-challenge (of total number tested)	76% (106)	65% (20)	94% (17)
Clinical symptoms			
Immediate hypersensitivity manifestations¶	662 (100%)	98 (100%)	-
AGEP*	-	-	14 (5%)
DRESS*	-	-	7 (3%)
Mild reactions including maculopapular exanthem	-	-	212 (79%)
SJS/TEN*	-	-	36 (13%)
Drug Class			
Penicillin	501 (75%)	98 (100%)	246 (92%)
Cephalosporin	162 (25%)	-	20 (7.5%)
Other β-lactams	-	-	2 (0.01%)
Suspected causal drug			
Amoxicillin	165 (25%)	65 (66%)	77 (29%)
Ampicillin	36 (5%)	-	54 (20%)
Bacampicillin	20 (3%)	-	21 (8%)
Cefaclor	23 (3%)	-	-
Cefazolin	17 (3%)	-	4 (1.5%)
Cefotaxime	17 (3%)	-	-
Ceftazidime	18 (3%)	-	1 (0.4%)
Ceftriaxone	52 (8%)	-	4 (1.5%)
Cefuroxime	14 (3%)	-	2 (0.7%)
Co-amoxiclav	218 (33%)	26 (26%)	70 (26%)
Phenoxymethylpenicillin	24 (4%)	7 (7%)	5 (2%)
Piperacillin	18 (3%)	-	5 (2%)
Other	41 (6%)	-	25 (9%)

*ADRs: adverse drug reactions; AGEP: acute generalised exanthematous pustulosis, DRESS: drug reaction with eosinophilia and systemic symptoms; SD: standard deviation; SJS/TEN: Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis. ¶see text for nature of clinical manifestations

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Table 2: The most significantly associated variants for immediate hypersensitivity reactions to β-lactams

	Minor allele frequency			Association analysis		Association conditioned for HLA Haplotype¶		Association conditioned for rs71542416	
	Cases	**Controls**	**Population reference cohort**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**	**OR**	**P**
*HLA-DRB1*10:01*	0.03	0.008	0.008	2.95 (1.99-4.36)	6.0×10^{-8}	-	-	0.60 90.19-1.85)	0.37
*HLA-DQA1*01:05*	0.03	0.01	0.01	2.93 (1.92-4.46)	5.4×10^{-7}	-	-	0.79 (0.32-1.91)	0.60
rs71542416	0.03	0.006	0.008	5.17 (3.40-5.17)	1.2×10^{-14}	8.22 (2.68-25.23)	0.0002	-	-
rs114632839#	0.25	0.367	0.40	0.77 (0.67-0.89)	0.0003	0.69 (0.60-0.80)	1.1×10^{-6}	0.68 (0.59-0.79)	6.1×10^{-7}

Minor allele frequency for external data obtained from allelefrequncy.net for HLA alleles or GnomAD for SNPs; OR= Odd ratio of the logistic regression model correcting for population stratification; 95% CI= 95% confidence intervals of the Odd Ratio; P = logistic regression p-value; ¶HLA haplotype was *HLA-DRB1*10:01- HLA-DQA1*01:05.*
#The marker rs114632839 has merged with rs3135392.

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Table 3: The association between *HLA-DRB1*10:01* and rs71542416, and β-lactam induced immediate hypersensitivity reactions across the different nationalities

Ethnic cluster	Cases (n*)	Minor allele frequency Cases	Minor allele frequency Controls	OR (95% CI)	P
HLA-DRB1*10:01					
Italians	352	0.021	0.012	2.33 (1.15-4.73)	0.02
Spanish	226	0.049	0.014	3.81 (2.27-6.42)	4.74×10^{-7}
Northern Europeans	61	0.025	0.004	3.93 (1.17-13.21)	0.03
rs71542416					
Italians	352	0.02	0.007	4.33 (1.98-9.49)	0.0002
Spanish	226	0.05	0.008	6.80 (3.89-11.87)	1.69×10^{-11}
Northern Europeans	61	0.02	0.004	4.42 (1.29-15.13)	0.02

OR= Odd ratio of logistic regression model correcting for population stratification; 95%CI= 95% confidence intervals of the Odd Ratio; P = logistic regression p-value. *numbers represent homogeneous populations within clusters after PCA analysis.

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Table 4: Effect size of the association of *HLA-DRB1*10:01* with immediate hypersensitivity reactions broken down by drug classes and individual drugs

Drug	Ethnicity*	Cases (n)	Case MAF	OR (95% CI)	P
Cephalosporins	Caucasian	162	0.019	2.03 (0.82-5.07)	0.13
Cefaclor	Caucasian	23	0	-	-
Cefazolin	Caucasian	17	0.059	6.12 (1.32-28.30)	0.02
Cefotaxime	Italian	17	0	-	-
Ceftazidime	Italian	17	0	-	-
Ceftriaxone	Italian	48	0.010	1.05 (0.13-8.33)	0.96
Cefuroxime	Caucasian	14	0.050	2.90 (0.37-22.76)	0.31
Penicillins	Caucasian	501	0.036	3.07 (2.04-4.62)	7.42×10^{-8}
Amoxicillin	Caucasian	166	0.042	3.48 (1.92-6,28)	3.74×10^{-5}
Ampicillin	Italian	29	0.014	1.98 (0.25-15.79)	0.52
Co-Amoxiclav	Caucasian	218	0.034	2.85 (1.60-5.10)	0.0004
Phenoxymethylpenicillin	Caucasian	25	0.080	6.66 (2.14-20.79)	0.001
Piperacillin	Caucasian	18	0.028	2.32 (0.29-18.78)	0.43
Bacampicillin	Italian	21	0.024	2.09 (0.26-17.03)	0.49

*Ethnicity – Caucasian is applied to patients of Spanish, Italian and Northern European descent and confirmed by PCA analysis. Where only one nationality was available for a particular drug, this is indicated and only appropriate matching controls were chosen.

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OR= Odd ratio of logistic regression model correcting for population stratification; 95% CI= 95% confident intervals of the Odd Ratio; P = logistic regression p-value; MAF; minor allele frequency

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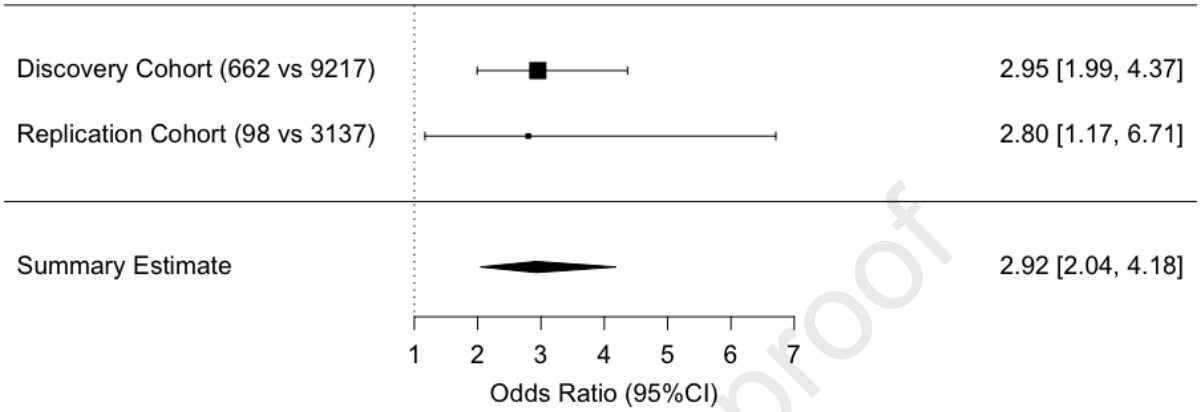

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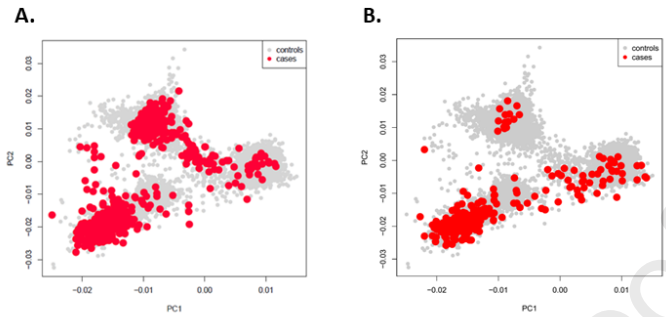

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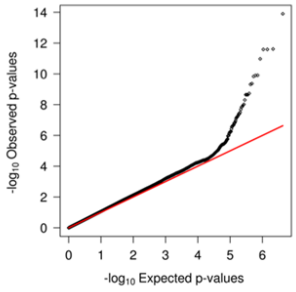

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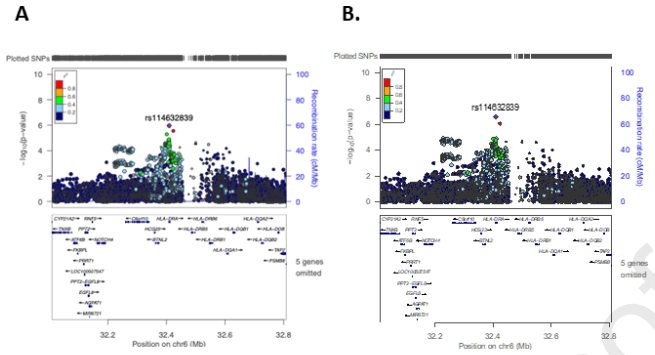

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Line	Allele	Population	% of individuals that have the allele	Allele Frequency (in_decimals)	Sample Size
1	DRB1*10:01	Germany DKMS - Spain minority		0.0131	1,107
2	DRB1*10:01	Spain Andalusia		0.0150	99
3	DRB1*10:01	Spain Andalusia Gipsy		0.0460	99
4	DRB1*10:01	Spain Arratia Valley Basque		0.0130	83
5	DRB1*10:01	Spain Barcelona	2.9	0.0140	941
6	DRB1*10:01	Spain Catalonia Girona		0.0000	88
7	DRB1*10:01	Spain Gipuzkoa Basque		0.0000	100
8	DRB1*10:01	Spain Granada		0.0200	280
9	DRB1*10:01	Spain Murcia		0.0080	173
10	DRB1*10:01	Spain North Cabuerniga		0.0100	95
11	DRB1*10:01	Spain North Cantabria		0.0120	83
12	DRB1*10:01	Spain Pas Valley		0.0160	88

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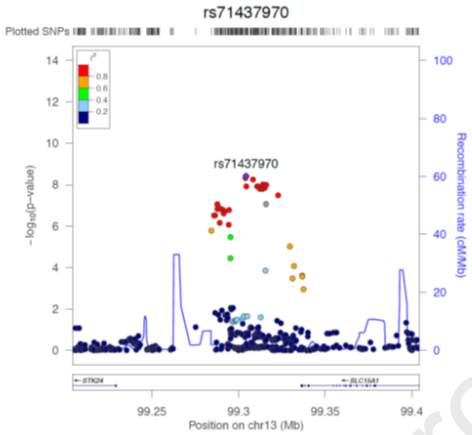

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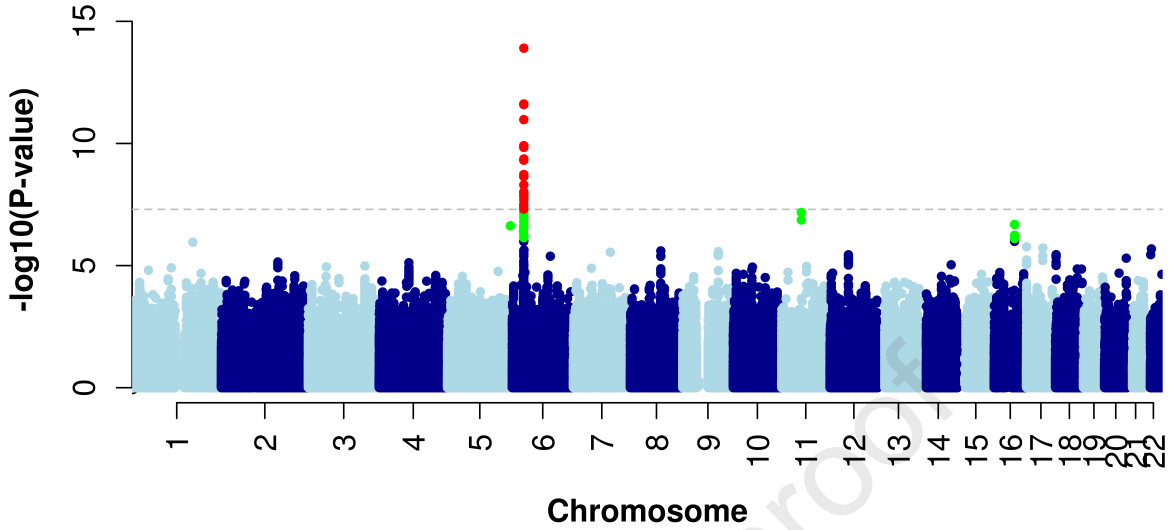

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