

# Clinical and genetic risk factors of self-reported penicillin allergy

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**Background:** Patients with self-reported penicillin allergy are frequently denied  $\beta$ -lactam antibiotics.

**Objective:** To identify and correlate clinical and genetic risk factors of self-reported penicillin allergy.

**Methods:** We conducted a case-control study of adults recruited from allergists' offices. Cases had a history of urticaria, angioedema, wheeze, hypotension, vomiting, or anaphylaxis after a dose of penicillin. DNA from buccal swabs was genotyped for variants associated with candidate genes linked to immediate hypersensitivity (*IL4*, *IL4R*, and *IL10*) and penicillin metabolism (*LACTB*). Logistic regression was used to calculate the association between penicillin allergy and clinical and genetic factors.

**Results:** Seventeen allergists identified 76 adults. Complete data were available for 23 cases and 39 controls. Penicillin allergy was associated with a history of penicillin allergy in first-degree relatives ( $P = .002$ ), a history of other adverse drug reactions ( $P = .008$ ), and atopy ( $P = .039$ ). However, in the multivariable analysis, only family history of penicillin allergy remained significant. *IL4* single nucleotide polymorphisms (SNPs) rs11740584 ( $P = .012$ ), rs10062446 ( $P = .021$ ), and rs2070874 ( $P = .035$ ) were associated and *LACTB* SNP rs2729835 ( $P = .058$ ) was marginally associated with penicillin allergy. Adding rs11740584 or rs10062446 individually improved the clinical multivariable model ( $R^2$  increased from 0.23 to 0.33). Haplotype analysis did not provide additional information to the SNP analysis.

**Conclusion:** Self-reported penicillin allergy may be influenced by clinical and genetic factors such as *IL4*. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

**Key words:** Penicillin, drug allergy, adverse drug reaction, immediate hypersensitivity, genetics, *IL-4*

Penicillin is a first-line antibiotic whose use is contraindicated in patients allergic to it. Although 10% of hospitalized patients report penicillin allergy, and allergic events are reported to occur as frequently as 2% per treatment, fatality is thankfully infrequent, estimated at 1 death per 50,000 to 100,000 treatment courses.<sup>1-3</sup> Penicillin allergy is a clinical diagnosis,<sup>4</sup> and it is usually presented in the literature based on patient report, sometimes supported by skin testing results. Unfortunately, skin testing is unavailable because of lack of commercial testing reagents. Although it was the best available test, its positive predictive value is limited by very small numbers of penicillin challenges in patients with positive histories and tests.<sup>5-7</sup> Thus, in the absence of a clinical or laboratory diagnostic gold standard or well defined risk factors,<sup>2</sup> many persons are misclassified as allergic and denied access to penicillin and other  $\beta$ -lactam antibiotics.<sup>8</sup> Misclassification may occur more frequently when patients' history of events is nonspecific or inconsistent with an allergic reaction.<sup>8,9</sup>

The clinical presentation of penicillin allergy and the detection of IgE antibodies in individuals with allergy have led to the hypothesis that the immunologic mechanism is immediate hypersensitivity.<sup>3,10-13</sup> However, some observations are not entirely consistent with this mechanism. For example, some patients who have had symptoms characteristic of an allergic reaction had no adverse reaction when re-exposed to penicillin.<sup>14</sup> In addition, in the few patients rechallenged with penicillin who had both a clinical history of allergy and detectable IgE antibodies, not all had a subsequent reaction, and some of the associated adverse events did not occur immediately after administration of the antibiotic.<sup>5,6</sup> Also, although patients with penicillin allergy are theoretically at increased risk of a reaction after exposure to structurally related antibiotics like cephalosporins, such reactions, depending on how they are defined, occur only about 1% of the time.<sup>15</sup> Finally, patients with penicillin allergy have been found to be at increased risk for reactions after exposure to unrelated antibiotics.<sup>15,16</sup>

These observations led us to hypothesize that penicillin allergy is a complex trait that may be under genetic influences, including genes that regulate immediate hypersensitivity. The molecular mechanism for an IgE-mediated immediate hypersensitivity reaction is triggered by antigens cross-linking preformed IgE antibody that is bound to Fc $\epsilon$ R1 on mast cell surfaces. After activation of the mast cells, histamine, leukotrienes, and other inflammatory mediators are released.<sup>1,17</sup> Therefore, genes that might be associated with penicillin allergy include those coding for cytokines and their receptors that promote IgE production, Fc $\epsilon$ R1, signal transduction proteins, and mast cell products released after antigen cross-linking.<sup>17,18</sup> With our limited resources,

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**Abbreviations used**

|        |                                |
|--------|--------------------------------|
| HWE:   | Hardy-Weinberg equilibrium     |
| IL4R:  | IL-4 receptor gene             |
| LACTB: | $\beta$ -Lactamase gene        |
| LD:    | Linkage disequilibrium         |
| OR:    | Odds ratio                     |
| SNP:   | Single nucleotide polymorphism |
| WGA:   | Whole genome amplification     |

we elected to study the IL-4 gene, because IL-4 is an essential cytokine for isotype switch of B cells to IgE. In mice and human beings, *IL4* has been associated with drug allergy in a small number of genetic studies of penicillin allergy.<sup>19–21</sup> The IL-4 gene is located on chromosome 5 near other genes whose products are associated with immediate hypersensitivity (ie, IL-13, IL-3, IL-5, IL-9, and IL-4 receptor [IL-4R]). IL-4, through IL-4R, activates signal transducer and activator of transcription 6, which in turn is one of the inducers of GATA-3, the key transcription factor promoting T<sub>H</sub>2 cell differentiation.<sup>18</sup> Polymorphisms of *IL4R* have been implicated in drug allergy.<sup>22</sup> Besides IL-4 and IL-4R, IL-10 might also be a factor that is involved in the hypersensitivity reaction. Polymorphisms of the IL-10 promoter have been associated with  $\beta$ -lactam allergy.<sup>19,22,23</sup> IL-10, produced by T<sub>H</sub>2 cells and macrophages, is thought to inhibit T<sub>H</sub>1 cytokine production and to promote B-cell survival, proliferation, and differentiation.<sup>18</sup> We included *IL10* in our analysis.

In addition, we considered genes whose products participate in penicillin metabolism, because polymorphisms of drug metabolism enzymes have been associated with heterogeneity of treatment effects.<sup>24</sup> We hypothesized that polymorphisms of  $\beta$ -lactamase gene, *LACTB*, the enzyme governing penicillin metabolism, which converts penicillin to penicilloate,<sup>25</sup> influences penicillin's allergenicity. Both penicillin and penicilloate are among the molecules most highly associated with penicillin-induced anaphylaxis.<sup>2,3</sup>

Our aim was to identify clinical and genetic risk factors of penicillin allergy to diagnose penicillin allergy better. We hypothesized that polymorphisms in *IL4*, *IL4R*, *IL10*, and *LACTB* are associated with penicillin allergy and that correlation may exist between these genetic factors and clinical risk factors for penicillin allergy.

**METHODS****Study design and protocol summary**

We undertook a case-control study of adults with and without a history of clinical penicillin allergy. Allergist-immunologists identified patients with penicillin allergy (cases) and comparison patients without penicillin allergy with similar demographic characteristics (controls). All participants completed a brief questionnaire assessing the history of the allergic reaction in cases and the presence of risk factors of penicillin allergy hypothesized in the literature: a history of atopy, adverse reactions to drugs other than penicillin, and penicillin allergy among first-degree relatives.<sup>1,2</sup> Genomic DNA was collected by using buccal swabs (Medical Packaging Corp, Camarillo, Calif), extracted, and genotyped for variants associated with the chosen candidate genes. The protocol was approved by the University of Pennsylvania Institutional Review Board.

**Penicillin allergy**

Allergists identified a patient with penicillin allergy as one answering affirmatively the following question: "Are you allergic to penicillin or its

derivatives (for example, penicillin, amoxicillin, Augmentin (GlaxoSmith Kline, Middlesex, United Kingdom), ampicillin, dicloxacillin, ticarcillin, imipenem)? By allergic reaction, we mean did you have urticaria, angioedema, wheeze, hypotension, vomiting, or anaphylaxis following a dose of penicillin." Although it is not known how many affirmatively answering adults truly have penicillin allergy, asking for specific events is more likely to identify persons with allergy than simply asking if there is a history of penicillin allergy.<sup>5,7,9</sup> Thus, it is more likely this group is enriched for true penicillin allergy compared with the control group who had a history of exposure to penicillin but no adverse events after exposure. This definition is characteristic of the actual descriptions frequently presented to clinicians.

**Study population**

A convenience sample of allergist-immunologists from throughout the United States was contacted and asked to identify patients with penicillin allergy by the question discussed, using a standardized script. For each patient with allergy, allergists were asked to identify 2 comparison patients within 5 years of age and the same sex and race/ethnicity who had tolerated penicillin. All participants were mailed a consent form, Health Insurance Portability and Accountability Act of 1996 form, brief questionnaire, and 3 brushes to swab cheeks to collect extractable DNA. The questionnaires and swabs were returned by mail.

**Candidate genes**

We considered genes whose products participate in IgE-mediated reactions and genes whose protein products have been cited in the literature as associated with penicillin allergy. *IL4*, *IL4R*, and *IL10* were selected as candidate genes.

We also selected *LACTB*, as described. There are 2  $\beta$ -lactamase genes identified in humans beings, *LACTB* and *LACTB2*, but *LACTB2* is less studied.<sup>26</sup> Cytochrome P450 enzymes do not appear to participate significantly in penicillin metabolism and were therefore not chosen for study.

**Single nucleotide polymorphism selection and validation**

We chose Tag single nucleotide polymorphisms (SNPs) on the basis of linkage disequilibrium patterns for *IL4* and *LACTB*. We searched public databases including dbSNP and HapMap for SNPs that provide the best coverage of candidate gene regions, particularly in the coding or regulatory regions—that is, the 5' and 3' ends—of candidate genes.<sup>27</sup> We also examined the publicly available haplotypes containing these genes. SNPs were examined if they fell into a region defined by 20 kb upstream and 10 kb downstream from the coding region of each gene. Using the SNPbrowser software ([http://marketing.appliedbiosystems.com/mk/get/snpb\\_landing](http://marketing.appliedbiosystems.com/mk/get/snpb_landing); Applied Biosystems, Foster City, Calif), we identified validated SNPs with Linkage Disequilibrium Unit 0.5, minor allele frequency 10% for white subjects because our participants were almost entirely white subjects. We identified 1 nonsynonymous SNP in *IL4* and 2 in *LACTB*. We selected 7 SNPs for *IL4* (rs2070874, rs734244, rs2243291, rs12186803, rs10062446, rs2299010, rs11740584) and 5 for *LACTB* (rs1126309, rs1566660, rs2652820, rs2729835, rs34536322). We chose 2 SNPs from the *IL10* promoter (rs1800871, rs1800872) and 1 from *IL4R* (rs1805010) identified by published reports.<sup>22,23</sup> Probes were obtained from Applied Biosystems. Once obtained, the SNP probes were tested in a standardized test population to ensure the frequencies of alleles using these probes were similar to those reported in the literature.

**DNA extraction**

Extraction of genomic DNA was performed by using the QIAamp 96 DNA Buccal Swab Biorobot Kit and a 9604B Biorobot (Qiagen, Inc, Valencia, Calif). For 8 samples for which the amount of DNA was insufficient, whole genome amplification (WGA; GenomePlex; Sigma Aldrich, St Louis, Mo) was performed.<sup>28,29</sup> WGA was completed on 26 additional samples, and concordance with the unamplified genotype assay was compared.

**TABLE I.** Demographic and clinical characteristics of 62 participants with and without a history of penicillin allergy

|                                                          | Penicillin allergy<br>N (% of 23) | No penicillin allergy<br>N (% of 39) | Association of clinical characteristic<br>with penicillin allergy†<br>P value |
|----------------------------------------------------------|-----------------------------------|--------------------------------------|-------------------------------------------------------------------------------|
| Age (y)*                                                 | 41 ± 12                           | 47 ± 12                              | .08§                                                                          |
| Female                                                   | 21 (91)                           | 36 (92)                              | 1.0                                                                           |
| White                                                    | 22 (96)                           | 38 (97)                              | 1.0                                                                           |
| Doctor-diagnosed asthma                                  | 13 (57)                           | 19 (49)                              | .61                                                                           |
| Doctor-diagnosed allergic rhinitis                       | 16 (70)                           | 24 (62)                              | .59                                                                           |
| Doctor-diagnosed eczema                                  | 4 (17)                            | 10 (26)                              | .54                                                                           |
| History of atopy‡                                        | 17 (74)                           | 18 (46)                              | .039                                                                          |
| History of adverse drug reactions, other than penicillin | 16 (70)                           | 13 (45)                              | .008                                                                          |
| First-degree relatives with penicillin allergy           | 16 (70)                           | 10 (26)                              | .002                                                                          |
| Family history of atopy‡                                 | 18 (78)                           | 30 (77)                              | 1.0                                                                           |
| Taking 2 or more medications regularly                   | 16 (70)                           | 22 (56)                              | .42                                                                           |
| More than 2 current medical diagnoses                    | 8 (35)                            | 12 (31)                              | .78                                                                           |

\*Mean ± SD.

†Fisher exact test.

‡Atopy: doctor's diagnosis of asthma, allergic rhinitis, or eczema.

§Logistic regression.

## Genotyping

Single nucleotide polymorphism analysis was performed on an ABI Prism 7900HT instrument (Applied Biosystems) using Assays on Demand or Assays by Design reagents from Applied Biosystems. Coriell DNA controls (Coriell Institute for Medical Research, Camden, NJ) representing each genotype for each variant and a no template (water) control were included in each 384-well plate. End-point fluorescence levels of 6-carboxyfluorescein and VIC were measured automatically in each well by using the SDS 2.1 manufacturer's custom software (Applied Biosystems). Allelic discrimination results were then graphed on a scatter plot contrasting reporter dye fluorescence. Clustering boundaries for each variant-specific genotype were assigned automatically most of the time. Manual calls were based on the position of the positive control within each cluster and verified by the real-time data. Genotypes were called on the basis of their corresponding location within each cluster. Sequence variants were called undetermined if they clustered in proximity to the negative (water) controls (<1 fluorescence units), showed an abnormal spectral plot (probe-specific fluorophore signal to internal control signal <1), or did not cluster tightly (outliers). Percentage of undetermined data for each genotype varied between the different sequence variants, and the ranges of failure rates were between 2.86% and 11.43% for the genomic DNA and between 0% and 13.54% for WGA DNA. If there was discordance between genotype derived from WGA compared with genomic genotyping, the data were considered missing.

## Statistical analyses

We tested for Hardy-Weinberg equilibrium (HWE) using STATA command genhwi (Release 9; StataCorp, College Station, Tex) and removed SNPs if the *P* value <.005. The rare homozygote and the heterozygote genotype groups were combined for the analyses and show the effect of the variant allele versus the most common homozygous genotype group. Odds ratios (ORs) and 95% CIs were calculated to evaluate the relationship of clinical risk factors, individual SNPs, and haplotypes with penicillin allergy in univariable analysis. We repeated this analysis by using the Fisher exact test. We then adjusted for sex for all significant univariable analyses; because almost all participants were white (97%), the models could not be adjusted for race or ethnicity. We did not adjust for age (age of enrollment) because it is not a confounder—that is, it is not correlated with genotype. We also repeated the analyses omitting the 2 subjects who were not white.

A multivariable model was created using unconditional logistic regression to account simultaneously for sex and the clinical risk factors of drug allergy. Then, models were created that added 1 significant SNP at a time to the clinical model to assess whether there were genetic contributions to the OR above the contribution of clinical factors. We did not construct a model of all clinical

variables and SNPs together to avoid the potential for multicollinearity that would influence our results. An additional model tested whether significant SNP information from different genes—that is, *IL4* and *LACTB*—independently influenced the OR.

*IL4*, *LACTB*, *IL4R*, and *IL10* haplotypes for each individual sample were estimated with use of the Arlequin program (version 3.11; reference = <http://cmppg.unibe.ch/software/arlequin3/>). By using unconditional logistic regression, ORs and 95% CIs were calculated for the association between each common haplotype and study outcome. The goal of the haplotype analysis was to confirm the single SNP associations.

## RESULTS

### Participants

Seventeen allergists identified 76 adults; 65 patients returned all required study information. Three patients were excluded because the buccal swabs contained no detectable DNA. The 62 remaining patients, 23 (37%) with and 39 (63%) without penicillin allergy, were predominantly female and white (Table I).

Of the 23 adults with penicillin allergy, 21 (91%) reported urticaria, 9 (39%) angioedema or swelling, and 7 (30%) shortness of breath or chest tightness or wheeze after receiving penicillin. Seven described both dermatologic and respiratory symptoms. Sixteen (70%) sought immediate medical advice. One was evaluated in an emergency department, and 1 reported experiencing the reaction during a hospitalization. For 5 of 23 (22%) cases, the reaction occurred within 3 to 5 years of enrollment. The reaction started within 2 hours of the most recent dose for 11 (48%) and within 48 hours for all but 1 (96%). The adverse event occurred within the first 1 to 2 days of the antibiotic course for 18 (78%).

### SNP validation and genotyping

One *IL4* probe, rs2299010, failed in the validation assay. The total genotype call rate was greater than 91% for all but 1 SNP: rs34536322, a custom-made *LACTB* probe for which the call rate was 89%.

The allele frequencies of *IL4* SNP rs734244 deviated significantly from HWE (*P* <.001). It was therefore excluded from further analysis. WGA was unsuccessful for 3 of the 8 samples for which there was insufficient DNA for direct genotyping.

**TABLE II.** Candidate genes and evaluated SNPs: evaluation of HWE, observed polymorphism frequency, and univariable association of SNPs with penicillin allergy

| Gene                 | Chromosome | dbSNP rs*  | HWE†  | Alleles (a/b)‡ | Observed frequencies, no. of cases |    |    | Observed frequencies, no. of controls |    |    | Association of SNP with penicillin allergy |      |
|----------------------|------------|------------|-------|----------------|------------------------------------|----|----|---------------------------------------|----|----|--------------------------------------------|------|
|                      |            |            |       |                | aa                                 | ab | bb | aa                                    | ab | bb | OR (95% CI)                                | P    |
| <i>IL4</i> §         | 5q31       | rs2070874  | 0.49  | C/T            | 11                                 | 10 | 1  | 30                                    | 7  | 2  | 3.33 (1.09, 10.21)                         | .035 |
|                      |            | rs2243291  | 0.89  | C/G            | 1                                  | 10 | 12 | 2                                     | 10 | 27 | 2.06 (0.71, 5.98)                          | .18  |
|                      |            | rs12186803 | 0.38  | A/G            | 2                                  | 8  | 13 | 1                                     | 8  | 29 | 2.48 (0.81, 7.54)                          | .11  |
|                      |            | rs10062446 | 0.22  | A/T            | 8                                  | 12 | 3  | 25                                    | 9  | 4  | 3.61 (1.21, 10.71)                         | .021 |
|                      |            | rs11740584 | 0.091 | C/G            | 7                                  | 13 | 3  | 25                                    | 8  | 6  | 4.08 (1.35, 12.30)                         | .012 |
| <i>LACTB</i>         | 15q22      | rs34536322 | 0.58  | A/C            | 21                                 | 1  | 0  | 32                                    | 7  | 0  | 0.22 (0.025, 1.90)                         | .17  |
|                      |            | rs1126309  | 0.43  | C/T            | 1                                  | 15 | 7  | 9                                     | 18 | 11 | 0.93 (0.30, 2.89)                          | .90  |
|                      |            | rs1566660  | 0.91  | C/G            | 2                                  | 11 | 9  | 3                                     | 13 | 22 | 1.99 (0.68, 5.77)                          | .21  |
|                      |            | rs2652820  | 0.72  | A/G            | 2                                  | 10 | 11 | 1                                     | 13 | 23 | 1.79 (0.62, 5.14)                          | .28  |
|                      |            | rs2729835  | 0.60  | C/T            | 6                                  | 12 | 5  | 19                                    | 14 | 4  | 2.99 (0.96, 9.28)                          | .058 |
| <i>IL4R</i>          | 16p11      | rs1805010  | 0.98  | A/G            | 5                                  | 12 | 4  | 11                                    | 17 | 9  | 1.35 (0.40, 4.62)                          | .63  |
| <i>IL10 promoter</i> | 1q31-32    | rs1800871  | 0.08  | C/T            | 2                                  | 8  | 12 | 7                                     | 6  | 22 | 1.41 (0.48, 4.17)                          | .53  |
|                      |            | rs1800872  | 0.049 | G/T            | 12                                 | 8  | 2  | 23                                    | 9  | 5  | 1.37 (0.47, 3.99)                          | .57  |

\*dbSNP rs number.

†HWE.

‡a/b, Polymorphism with A, adenine; C, cytosine; G, guanine; T, thymine.

§One SNP probe, rs2299010, failed in the validation assay. For rs734244, the genotype result was not in HWE.

### Genotype concordance between methods

For 26 subjects, we genotyped the panel of 16 SNPs under investigation using both WGA and unamplified DNA. After removing the 13 genotype pairs for which 1 of assays failed to give a genotype call, genotyping concordance among the remaining 403 genotype pairs was 97.0% ( $\kappa = 0.95$  [0.92, 0.98]). The majority of the discordant calls resulted from apparent loss of heterozygosity in the WGA sample, which is consistent with previous observations.<sup>30,31</sup>

### Univariable clinical associations

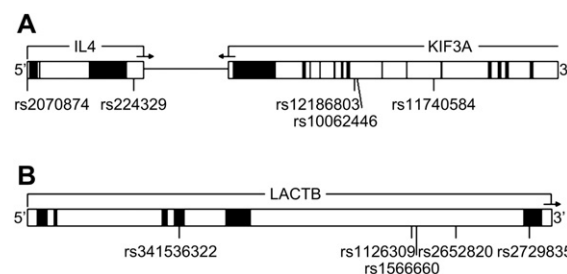
Penicillin allergy was associated with a history of penicillin allergy in first-degree relatives ( $P = .002$ ) and a personal history of having other adverse drug reactions ( $P = .008$ ). Penicillin allergy was also associated with a history of atopy ( $P = .033$ ) and younger age at entry into the study ( $P = .08$ ), although the latter was not considered a significant clinical difference (Table I).

### Univariable genetic associations

Penicillin allergy was associated with 3 SNPs identified at the *IL4* locus: rs11740584 ( $P = .012$ ), rs10062446 ( $P = .021$ ), and rs2070874 ( $P = .035$ ; Table II; Fig 1). The first 2 of these SNPs are located in another gene (*KIF3A*) but are in the same linkage disequilibrium block as *IL4* (LDU = 0.5; SNPbrowser 3.5.2).<sup>32</sup> Penicillin allergy was marginally associated with a *LACTB* SNP, rs2729835 ( $P = .058$ ), but not with the SNPs related to *IL4R* or *IL10*. Because there was only 1 case with a variant allele, the data for SNP rs34536322 are less reliable. Repeating this analysis omitting the 2 nonwhite subjects yielded the same results (data not shown).

### Multivariable models

In a model simultaneously assessing the contribution of the significant clinical factors from the univariable analysis, only a

**FIG 1.** Schematic diagram of *IL4* (A) and *LACTB* (B).

history of first-degree relatives with penicillin allergy remained a significant risk factor (Table III, "Adjusted clinical" column).

In adding SNPs found significant on univariable analysis to the clinical model, 2 *IL4* SNPs, rs10062446 (AA vs any T allele: OR, 6.27; 95% CI, 1.37, 28.69) and rs11740584 (GG vs any C allele: OR, 6.23; 95% CI, 1.36, 28.63), were individually associated with penicillin allergy and equally informative after controlling for the clinical factors (Table III, right columns). In these models with genetic factors, 2 clinical factors in addition to the genetic factors were associated with penicillin allergy: personal history of other adverse drug reactions, and family history of penicillin allergy. The addition of each SNP improved the adjusted model  $R^2$ , suggesting that *IL4* polymorphisms contribute information to drug allergy beyond that contributed by the clinical risk factors alone. *LACTB* SNP, rs2729835 (TT vs any C allele: OR, 1.42; 95% CI, 0.33, 6.13), and the other *IL4* SNP, rs2070874 (CC vs any T allele: OR, 2.70; 95% CI, 0.60, 12.11), did not independently contribute to the model above the clinical risk factors.

In a model assessing the contribution of SNPs, we began with the *IL4* SNP most strongly associated with penicillin allergy on univariable analysis, rs11740584. Adding the other *IL4* SNPs did not add to the model, but when *LACTB* SNP rs2729835 was added, it contributed marginally to the model ( $P = .07$ ; Table IV). The relatively low  $R^2$  of these models suggests there are other

**TABLE III.** Multivariable modeling of association of clinical characteristics with penicillin allergy and the contribution of SNPs that significantly contributed to the model beyond the clinical characteristics

| Characteristic                                                       | Unadjusted<br>OR (95% CI) | Adjusted clinical<br>OR (95% CI) | Adjusted clinical<br>and rs10062446<br>OR (95% CI) | Adjusted clinical<br>and rs11740584<br>OR (95% CI) |
|----------------------------------------------------------------------|---------------------------|----------------------------------|----------------------------------------------------|----------------------------------------------------|
| Personal history of atopy*                                           | 2.83 (0.96, 8.36)         | 1.91 (0.47, 7.67)                | 1.91 (0.45, 8.11)                                  | 1.90 (0.45, 8.12)                                  |
| Personal history of adverse<br>drug reactions, other than penicillin | 4.57 (1.51, 13.87)        | 3.43 (0.88, 13.41)               | 5.72 (1.19, 27.35)                                 | 5.61 (1.16, 27.11)                                 |
| First-degree relatives with penicillin allergy                       | 6.67 (2.02, 21.93)        | 7.03 (1.94, 25.41)               | 5.37 (1.36, 21.20)                                 | 5.89 (1.50, 23.06)                                 |
| rs10062446 ( <i>IL4</i> )                                            | 3.61 (1.21, 10.71)        |                                  | 6.21 (1.38, 27.98)                                 |                                                    |
| rs11740584 ( <i>IL4</i> )                                            | 4.08 (1.35, 12.30)        |                                  |                                                    | 6.21 (1.37, 28.08)                                 |
| Adjusted $R^2$                                                       |                           | 0.23                             | 0.32                                               | 0.31                                               |

\*Atopy, Doctor's diagnosis of asthma, allergic rhinitis, or eczema.

**TABLE IV.** Contribution to predictive model of penicillin allergy from *IL4* and *LACTB*

| Model                      | Unadjusted<br>OR (95% CI) | Stepwise analysis*<br>OR (95% CI) |
|----------------------------|---------------------------|-----------------------------------|
| rs11740584 ( <i>IL4</i> )  | 4.08 (1.35, 12.30)        | 4.27 (1.35, 13.54)                |
| rs2729835 ( <i>LACTB</i> ) | 2.99 (0.96, 9.28)         | 3.04 (0.92, 10.09)                |
| Adjusted $R^2$             |                           | 0.13                              |

\*Stepwise backward procedure, probability .10.

genetic or environmental factors to this association not considered here.

### Haplotype analysis

When comparing 1 risk-reducing *IL4* haplotype (CGGAC) versus 2 risk-reducing haplotypes, the OR was 3.82 (95% CI, 1.13, 12.95). Comparing a no risk-reducing *IL4* haplotype to 2 risk-reducing *IL4* haplotypes yielded an OR of 2.8 (95% CI, 0.57, 13.8). Similar results (data not shown) were seen with more combinations of the common risk-reducing (CGGAC) and risk-increasing (TCATG) haplotypes. In other haplotype analyses of a no risk-reducing *LACTB* haplotype (ATCAC) versus 1 or 2 risk-reducing haplotypes, the OR was 2.79 (95% CI, 0.91, 8.58). The ORs of all the haplotype analyses were of the same order of magnitude or lower than the single SNP analyses (Table II) and suggest that haplotype analysis did not add information to the single SNP analyses.

### DISCUSSION

There is very little current research addressing the diagnosis of drug allergy. We explored clinical and genetic risk factors of self-reported penicillin allergy to understand the underlying complex, multifactorial etiology of this trait, including the clinical, immunologic, and genetic contributions. This is a unique approach for dissecting a clinical diagnosis. We used self-reported allergy not only because penicillin skin testing reagents are unavailable but also because currently it is by history alone that most patients are evaluated.

We found that a history of penicillin allergy in first-degree relatives, a personal history of adverse drug reactions to drugs other than  $\beta$ -lactams, and atopy are associated with penicillin allergy. The finding that patients with penicillin allergy are at risk for adverse reactions to unrelated medications supports our earlier work<sup>15,16</sup> and our hypothesis that penicillin allergy is a complex

trait with several genetic and also environmental (eg, exposure to other medications) influences. When these factors were placed in a clinical and genetic model, family history of penicillin allergy and personal history of other adverse reactions remained significant. These findings add further support to our hypothesis that penicillin allergy is multifactorial.

Our hypothesis that clinical penicillin allergy has genetic influences including contributions from immediate hypersensitivity is supported by the associations of penicillin allergy with polymorphisms of *IL4*, one of the most important cytokines regulating immediate hypersensitivity. That polymorphisms of *IL4* remained significant in models with clinical factors suggests that *IL4* contributes to penicillin allergy independently of the contribution of other factors, including those reflected by family history of penicillin allergy and personal history of other drug reactions.

We found a marginally significant (univariable) association with a *LACTB* polymorphism (rs2729835) and penicillin allergy ( $P = .058$ ). However, this SNP did not significantly contribute to the model containing clinical risk factors, suggesting multicollinearity with at least 1 of these factors. When this SNP was included in a model with an *IL4* SNP, it attained marginal significance ( $P = .07$ ). Small sample size cannot be excluded as an explanation for lack of significance. Haplotype analysis of *IL4* (9 kb) and *LACTB* (20 kb) did not add any information to the single SNP analyses and confirmed the results from single SNP analyses.

Our findings did not agree with recent studies that found an association with SNPs in the promoter region of *IL10* and *IL4R* with penicillin allergy.<sup>22,23</sup> Although our small sample size raises the possibility of a type II error, there was no marginal association to suggest a larger size would yield different results. In addition, our sample size was comparable to those studies in which an association was observed. It is noteworthy that in those studies, patients with penicillin allergy who came from different parts of the world were identified by skin testing rather than clinical criteria.<sup>22,23</sup> However, these patients also had a clinical history of penicillin allergy, although the definition and collection of this history was not described.

Many of the issues raised concerning the question of an IgE mechanism for penicillin allergy could also be made about *Hymenoptera* sensitivity—that is, the lack of 100% concordance with the presence of IgE and reaction with re-exposure.<sup>33</sup> It is possible that other forms of anaphylaxis are also multifactorial, and the mechanism may involve factors, including genetic influences, in addition to those involving IgE-related mechanisms. Future

studies repeating our methodology in *Hymenoptera*-sensitive patients may demonstrate generalizability of our findings to other related disorders.

Our study has a number of limitations. First, as noted, our sample size is small. Nevertheless, we were able to develop successfully a method for recruiting participants and obtaining genetic material from all over the country. Importantly, we obtained statistical significance for a number of clinical and genetic risk factors, suggesting sufficient power was available to address many of our hypotheses.

Second, participants were recruited from allergists' offices, raising the possibility that these patients are different from other patient groups. We believe recruiting in this way allowed a more precise definition of drug allergy because allergists are accustomed to taking such a history. Recruiting from allergists' offices also enriched the study population for patients with atopic diseases, permitting better study of the impact of atopy on drug allergy. Thus, this is an appropriate population in which to make inferences, because it represents the types of individuals who may come to clinical attention and thereby ultimately benefit from knowledge of clinical and genetic information about their allergies. In addition, we were able to recruit a diverse geographic sample and one from many different practices, thus minimizing any contribution to penicillin allergy from a single practice or geographic region. Nevertheless, these results cannot be generalized to all subjects without confirmation in the general population.

As noted, it is possible that patients are misclassified with respect to penicillin allergy. It is known that the majority of patients who consider themselves to be allergic are not.<sup>8,34</sup> In addition, the use of self-report for diagnosis could result in a differential misclassification bias, because it is unlikely the controls were misclassified, whereas there is a greater chance that cases were misclassified. Differential misclassification of penicillin allergy may lead to bias either toward or away from the null hypothesis. It is difficult to judge the direction or magnitude of this bias, but it is a consideration for the results presented here.

Another limitation is that the population was almost entirely white and female. The present results may therefore not be applicable to other ethnic groups. There is some evidence that drug allergy is reported more commonly in female subjects,<sup>35-37</sup> and female subjects are more likely to receive prescriptions for antibiotics.<sup>15,38</sup> Further research is necessary to establish if this sex difference has determinants that are genetic or environmental (ie, exposure) or both. It is of note that the genes studied here are autosomal, and therefore sex differences would not be expected. However, sex differences in other risk factors may exist, and therefore inferences from this study may be more relevant to female than predominantly male populations. Finally, the participants had reported significant reactions to penicillin but did not appear to have life-threatening reactions. Nevertheless, we described the patients who most commonly report significant adverse drug reactions.

Finally, we chose Tag SNPs based on linkage disequilibrium (LD) patterns in this region. It is possible that the LD in this region may require more SNPs than we typed. In fact, the limited number of SNPs suggests we may have missed some associations. However, the positives we found suggest that we did at least choose some relevant SNPs that are themselves associated with self-reported penicillin allergy or are in LD with variants that are causative of it.

In conclusion, self-reported penicillin allergy likely has several genetic influences. These include contributions from immediate hypersensitivity, but there are probably others. Our data suggest that both clinical and genetic factors influence the complex, multifactorial etiology of penicillin allergy. Our results require additional confirmation in larger and more diverse sample sets. If confirmed, this research may provide an improved clinical definition of this trait and aid in identification of individuals with penicillin allergy.

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**Clinical implications: Identifying genetic risk factors may lead to improved diagnosis and understanding of the pathophysiology of penicillin allergy.**

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