Susceptibility to Amoxicillin-Clavulanate-Induced Liver Injury Is Influenced by Multiple HLA Class I and II Alleles

BACKGROUND & AIMS: Drug-induced liver injury (DILI), especially from antimicrobial agents, is an important cause of serious liver disease. Amoxicillin-clavulanate (AC) is a leading cause of idiosyncratic DILI, but little is understood about genetic susceptibility to this adverse reaction. METHODS: We performed a genome-wide association study using 822,927 single nucleotide polymorphism (SNP) markers from 201 White European and US cases of DILI following AC administration (AC-DILI) and 532 population controls, matched for genetic background. RESULTS: AC-DILI was associated with many loci in the major histocompatibility complex. The strongest effect was with an HLA class II SNP (rs9274407, P = 4.8 × 10^{-19}), which correlated with rs3135388, a tag SNP of HLA-DRB1*1501-DQB1*0602 that was previously associated with AC-DILI. Conditioned on rs3135388, rs9274407 is still significant (P = 1.1 × 10^{-8}). An independent association was observed in the class I region (rs2523822, P = 1.8 × 10^{-10}), related to HLA-A*0201. The most significant class I and II SNPs showed statistical interaction (P = .0015). High-resolution HLA genotyping (177 cases and 219 controls) confirmed associations of HLA-A*0201 (P = 2 × 10^{-6}) and HLA-DQB1*0602 (P = 5 × 10^{-10}) and their interaction (P = .005). Additional, population-dependent effects were observed in HLA alleles with nominal significance. In an analysis of autoimmune-related genes, rs2476601 in the gene PTPN22 was associated (P = 1.3 × 10^{-6}). CONCLUSIONS: Class I and II HLA genotypes affect susceptibility to AC-DILI, indicating the importance of the adaptive immune response in pathogenesis. The HLA genotypes identified will be useful in studies of the pathogenesis of AC-DILI but have limited utility as predictive or diagnostic biomarkers because of the low positive predictive values.

Keywords: Hepatotoxicity; Genome-Wide Association Study; GWAS; Pharmacogenomics.

Diagnosyrcatic liver toxicity because of a prescribed drug is usually referred to as drug-induced liver injury (DILI). Most DILI involves reactions that appear unrelated to drug dose or concentration. Although comparatively rare, DILI is a serious clinical problem with up to 10% of cases with simultaneous severe elevations in alanine transaminase (ALT) and bilirubin developing liver failure. A prospective study estimated the standardized incidence rate of symptomatic hepatic adverse drug reactions at 8.1 per 100,000 people in France. In the United States, 13% of acute liver failure cases are because of idiosyncratic hepatotoxicity with 75% of those dying or requiring emergency liver transplantation.

Amoxicillin-clavulanate (AC) is among the most commonly prescribed antimicrobials worldwide. This drug is generally well tolerated, and, whereas liver injury can occur rarely, the overall risk benefit is favorable. DILI following AC administration (AC-DILI), which appears to be...
primarily due to the clavulanate component, is an important cause of idiosyncratic DILI in the United States and Europe, and represents 17% of all DILI-related hospitalizations. Whereas most patients with AC-DILI make a full recovery, cases of acute liver failure leading to death or liver transplantation have been reported. The mechanism of AC-DILI is unknown. Three previous studies from Northwestern Europe reported an association between AC-DILI and the HLA class II allele DRB1*1501, with odds ratios (OR) ranging between 2.6 and 10. A further study of 27 Spanish cases did not observe a significant association with DRB1*15 but did report a significantly higher frequency of DQB1*06. The differences in findings between the Spanish and Northwestern European studies may be due to use of low-resolution genotyping, population-specific linkage-dis-equilibrium patterns, population stratification, or to a larger proportion of hepatocellular cases in the Spanish study.

We conducted a genome-wide association (GWA) study to investigate whether additional common genetic variants affect susceptibility to AC-DILI. A group of well-phenotyped cases (n = 201) with high causality scores were assembled from several multicenter collections together with 532 genetically matched population controls. Our study confirms the DRB1*1501 association and identifies additional HLA class I and II associations.

Materials and Methods

Case Recruitment

Cases (n = 211) were recruited in 4 separate studies (DILIGEN, Spanish DILI Registry, Drug-induced liver injury network [DILIN], and EUDRAGENE) that used similar inclusion criteria. All participants provided written informed consent, and each study had been approved by the appropriate ethical review boards.

DILIGEN. Between October 2004 and April 2009, 78 cases of AC-DILI of European origin from centers throughout the United Kingdom were collected. Inclusion criteria were suspected liver injury because of AC with either (1) clinically apparent jaundice or bilirubin >2.4 mg/dL (after exclusion of cases because of hemolysis), (2) ALT >5X upper limit of normal (ULN), or (3) alkaline phosphatase (ALP) >2X ULN plus bilirubin above ULN. Cases were identified by searching histologic databases and discharge records at UK regional liver units for cases of DILI or cholestasis/hepatitis of unknown etiology. Direct contact with gastroenterologists was also made by advertising the study through professional societies.

EUDRAGENE. Adult (≥18 years of age) AC-DILI cases identified retrospectively and prospectively from European pharmacovigilance center adverse drug reaction reports were collected from November 2006 to August 2009. Of the 25 cases included in this study, 15 were from Spain, 7 from France, and 3 from Italy. Case definitions included (1) acute liver injury defined as presenting symptoms suggestive of liver disorder (nausea, vomiting, abdominal pain, and/or jaundice), referred to a specialist, or admitted to hospital; (2) ALT >2X ULN; or (3) aspartate aminotransferase (AST), ALP, and total bilirubin >2X ULN.

Spanish DILI registry. Fifty-two cases of AC-DILI patients ≥18 years of age were selected from those submitted to the Spanish DILI Registry, a collaborative network set up in 1994 to prospectively identify cases of DILI in a standardized manner. Inclusion laboratory criteria for AC-DILI cases in this study were (1) clinically apparent jaundice or bilirubin >2.4 mg/dL (after exclusion of cases because of hemolysis), (2) ALT ≥5X ULN, or (3) ALP ≥2X ULN. Detailed description of the operational structure of the registry, data recording, and case ascertainment has been reported elsewhere.

DILIN. Details of the DILIN prospective study have been described previously. A total of 65 eligible AC-DILI cases was recruited between August 2004 and April 2009 from 5 DILIN clinical sites in the United States. Of these, 56 cases of European ancestry and ≥18 years of age were included in the current study. Inclusion laboratory criteria were (1) serum AST or ALT >5X ULN on 2 separate occasions, (2) serum ALP >2X ULN on 2 consecutive occasions, or (3) serum total bilirubin >2.4 mg/dL in the absence of a competing cause of hyperbilirubinemia. Patients were excluded if there was known or suspected acetaminophen overdose, if there was a history of bone marrow or liver transplant prior to DILI onset, or if there was a prior history of immune-related liver disease such as autoimmune hepatitis.

Causality Assessment

Diagnosis of DILI was done by expert hepatologists at each of the collaborating centers. In addition, the cases were evaluated by application of the Council for International Organizations of Medical Science (CIOMS) scale, also called the Roussel Uclaf Causality Assessment Method (RUCAM). The pattern of liver injury was classified according to the International Consensus Meeting Criteria. Only cases having at least possible causality (score ≥3) were included in the study.

DNA Preparation From Cases

For DILIGEN and Spanish DILI Registry cases, DNA was prepared as described previously. EUDRAGENE DNA was extracted at Erasmus Medical Centre genotyping laboratory, The Netherlands, using standard procedures. DILIN DNA was extracted from lymphocytes and stored at the National Institute of Diabetes and Digestive and Kidney Diseases biosample repository at Rutgers University, Piscataway, NJ.

Controls

Genotyped controls (n = 532) from the Population Reference Sample (POPres) were matched to the cases using principal component analysis. Based on the first 2 principal components that capture much of the genetic substructure among Europeans, 306 controls were...
selected as Northwestern Europeans (predominantly POPRES UK), and 160 controls were selected as Spanish (predominantly POPRES Spanish and Portuguese).

**Genotyping**

**Genome-wide analysis.** Genome-wide genotyping of the European DILI cases and POPRES controls was performed by Expression Analysis, Inc (Durham, NC) and of the US cases by the Center for Human Genome Variation, Duke University. All subjects were genotyped using the Human1M-Duo BeadChip (Illumina, San Diego, CA), containing 1,072,820 markers. A total of 822,927 markers, genotyping with the Human1M-Duo BeadChip (Illumina, San Diego, CA), containing 1,072,820 markers. A total of 822,927 markers, including the first 2 principal component scores as covariates to control for population stratification. To test for independent effects within regions having multiple associated variants, we included 1 or more variants as a covariate in the logistic regression model. All detailed analyses were performed with R.23 Additional statistical analyses of HLA alleles were conducted by logistic regression using glm function (generalized linear model) and heterogeneity testing with meta.MH function (Mantel–Haenszel meta-analysis) from the rmeta package (http://cran.r-project.org/web/packages/rmeta/index.html) adjusting for gender and principal components when feasible.

**Results**

**Clinical Characteristics of the Cases**

Clinical details of the 201 cases included in this study are summarized in Table 1. As in previous studies of AC-DILI, there were more male than female cases. The average age at onset was 61 ± 14 years. Four cases underwent liver transplantation. Most cases (70%) were characterized as cholestatic or mixed at presentation, and 88% had bilirubin levels >2.4 mg/dL. Most causality scores (93%) suggested that DILI was either probable or highly likely because of AC. There were some significant differences in the pattern of disease and other phenotypic

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**Table 1. Clinical Characteristics of AC-DILI Cases**

<table>
<thead>
<tr>
<th>Cohorts</th>
<th>DILIGEN</th>
<th>EUDRAGENE</th>
<th>Spanish DILI Registry</th>
<th>DILIN</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>77</td>
<td>19</td>
<td>49</td>
<td>56</td>
<td>201</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>39 (51)</td>
<td>11 (58)</td>
<td>27 (55)</td>
<td>36 (64)</td>
<td>113 (56)</td>
</tr>
<tr>
<td>F</td>
<td>38 (49)</td>
<td>8 (42)</td>
<td>22 (45)</td>
<td>20 (36)</td>
<td>88 (44)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>61 (±14)</td>
<td>68 (±14)</td>
<td>60 (±18)</td>
<td>59 (±14)</td>
<td>61 (±14)</td>
</tr>
<tr>
<td>Total days on drug</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>8 (±6)</td>
<td>7 (±5)</td>
<td>11 (±9)</td>
<td>12 (±8)</td>
<td>10 (±7)</td>
</tr>
<tr>
<td>Days to DILI onset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>15 (±14)</td>
<td>11 (±9)</td>
<td>16 (±12)</td>
<td>32 (±19)</td>
<td>20 (±17)</td>
</tr>
<tr>
<td>Pattern of liver injury, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholestatic</td>
<td>36 (47)</td>
<td>6 (32)</td>
<td>17 (35)</td>
<td>20 (36)</td>
<td>79 (39)</td>
</tr>
<tr>
<td>Mixed</td>
<td>18 (23)</td>
<td>5 (26)</td>
<td>17 (35)</td>
<td>23 (41)</td>
<td>63 (31)</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>19 (25)</td>
<td>0</td>
<td>15 (31)</td>
<td>10 (18)</td>
<td>44 (22)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (5)</td>
<td>8 (42)</td>
<td>0</td>
<td>3 (5)</td>
<td>15 (7)</td>
</tr>
<tr>
<td>Causality (CIOMS), n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unlikely</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Possible</td>
<td>7 (9)</td>
<td>1 (5)</td>
<td>0</td>
<td>6 (11)</td>
<td>14 (7)</td>
</tr>
<tr>
<td>Probable</td>
<td>25 (32)</td>
<td>12 (63)</td>
<td>23 (47)</td>
<td>37 (66)</td>
<td>97 (48)</td>
</tr>
<tr>
<td>Highly probable</td>
<td>45 (58)</td>
<td>6 (32)</td>
<td>26 (53)</td>
<td>13 (23)</td>
<td>90 (45)</td>
</tr>
<tr>
<td>Peak Bilirubin, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>12.5 (±9.8)</td>
<td>10.8 (±12.2)</td>
<td>12.3 (±9.5)</td>
<td>16.5 (±11.8)</td>
<td>13.5 (±10.6)</td>
</tr>
<tr>
<td>Peak ALT, U/L</td>
<td>455 (±465)</td>
<td>342 (±268)</td>
<td>548 (±668)</td>
<td>575 (±713)</td>
<td>500 (±584)</td>
</tr>
<tr>
<td>Peak ALP, U/L</td>
<td>453 (±253)</td>
<td>438 (±171)</td>
<td>417 (±286)</td>
<td>456 (±293)</td>
<td>444 (±268)</td>
</tr>
</tbody>
</table>

F, female; M, male; SD, standard deviation.
characteristics among the 4 collections (Supplementary Table 1).

**Genome-Wide Analysis**

GWA analysis was carried out on 201 DILI cases and 532 controls for 822,927 markers that passed quality control. Principal component analysis showed that all cases clustered within Northwestern, Western, and Southern Europe (Supplementary Figure 1), consistent with previous studies on Europeans. Based on these genetic patterns, the US-derived DILIN samples were predominantly of Northwestern European origin with genotype patterns correlating well with both UK controls and cases. The genomic inflation factor ($\lambda$) of our study was 1.03, which indicates no problems with population stratification.

Case-control association analysis revealed 1 marked peak of association with several dozen genome-wide significant SNPs within the major histocompatibility complex (MHC) on chromosome 6p21.3 (Figure 1A). Quantile-quantile (Q-Q) plots (Supplementary Figure 2) suggested that no SNPs outside the MHC region showed a genome-wide significant association with DILI. The most significant associations localized within the class II and class I regions (Figure 1B). The top SNP associated with AC-DILI was rs9274407 ($P = 4.8 \times 10^{-14}$ with an estimated additive OR of 3.1 (95% confidence interval [CI]: 2.3–4.2); Table 2). This SNP is located within the HLA-DQB1 gene and had relatively high linkage disequilibrium (LD) ($r^2 = 0.76$) with rs3135388, one of the other top associated SNPs that is strongly correlated with (ie, a tag for) DRB1*1501 and the haplotype DRB1*1501-DQB1*0602. The top SNP (rs9274407) was significantly associated conditioned on the tag SNP (rs3135388) ($P = .0001$), whereas rs3135388 was not significantly conditioned on rs9274407 ($P = .93$).

Several SNPs from the HLA class I region were also genome-wide significant, the most significant being rs2523822 ($P = 1.8 \times 10^{-10}$, OR, 2.3; 95% CI: 1.8–2.9), previously reported as a tag for $A^*0201$. Conditioning on rs9274407, the association peak within class I remained genome-wide significant, whereas none of the other class II region SNPs remained significantly associated (Figure 1C, Supplementary Figure 2, Table 2). We found a statistically significant interaction ($P = .0015$, OR, 2.3; 95% CI: 1.4–3.8) between rs9274407 and rs2523822 indicating that the increased risk when both minor alleles were present was larger than expected based on their individual effects. Conditioned on both rs9274407 and rs2523822, no additional remarkable associations within the MHC were observed (Figure 2D).

A total of 14 of the 201 cases in the study had shown “possible” rather than “probable” or “highly probable” causality when scored by the CIOMS system. To assess whether exclusion of these cases from the analysis would affect the overall findings, we recalculated $P$ values for the 2 SNPs with the lowest $P$ values (Table 2). No significant alteration in these $P$ values was seen (data not shown).

To consider the potential for country-specific effects, genome-wide analyses were carried out on 3 separate subgroups within the original 201 cases, namely 74 UK, 51 US cases with Northwestern European genetic backgrounds, and 46 Spanish DILI Registry cases with Spanish genetic background (Supplementary Figure 2). Appropriately matched POPRES controls (306 Northwestern European for the United Kingdom and United States and 160 Spanish and Portuguese) were used. As with the combined samples, no SNPs outside the MHC were genome-wide significant, and most significant SNPs in the combined sample showed similar estimates of effects within each of the 3 groups (Table 3). Conditioning analysis on top SNPs, similar to that performed for the entire group shown in Table 2, was also performed on the different groups (Table 3). The association of rs9274407 conditioned on rs3135388 was observed only in the UK group with statistical significance ($P = 7.3 \times 10^{-5}$). Rs3135388 was not significantly associated conditioned on rs9274407 consistently in all 3 groups, but rs2523822 was significantly associated conditioned on either rs9274407 or rs3135388 in all 3 groups. The interaction between the class I rs2523822 and class II rs9274407, which was significant for the entire group, was also significant in the UK cases alone ($P = .013$) but not in the US and Spanish cases ($P = .14$ and $P = .31$, respectively). Although the Q-Q plots (Supplementary Figure 3) suggested that no SNPs outside the MHC region were significantly associated with AC-DILI genome wide, we also assessed whether there was any indication of a contribution from either genes concerned with drug absorption, distribution, metabolism and excretion (ADME) or, in view of the strong HLA associations, non-HLA genes known to be involved in autoimmune diseases generally.

We extracted all SNPs within 10 kilobase for a list of 130 ADME genes. A total of 4961 such SNPs were included in our analysis. Figure 2A shows the Q-Q plot of $P$ values for these SNPs and indicates that no significant associations occur. For the autoimmune disease genes, we extracted SNPs associated with these diseases with reported $P$ value smaller than $5 \times 10^{-8}$ (see Figure 2B). Among these SNPs (213 in total), 158 had been genotyped and analyzed in our study. Figure 2B shows the Q-Q plot of $P$ values of these SNPs, excluding SNPs from the MHC region. We found 2 SNPs, rs2476601 and rs6679677, in strong LD with each other, to be associated with AC-DILI (for rs2476601, $P = 1.3 \times 10^{-4}$, OR, 2.1; 95% CI: 1.5–3.2) (Supplementary Table 2). Rs2476601 is a nonsynonymous SNP in PTPN22, the gene encoding the lymphoid-specific protein tyrosine phosphatase, nonreceptor type 22 involved in T cell-receptor signaling, and has been reported to be associated with multiple autoimmune diseases. Although the association was not genome-wide significant, it had a $P$ value of .023 after Bonferroni correction for all the published GWA associations with autoimmune diseases that were genotyped in our study. The association of the PTPN22 SNPs with DILI appeared stronger in
Figure 1. AC-DILI genome-wide association results. Each point represents association analysis results for a single SNP with chromosome position on the x-axis and $-\log_{10} P$ value on the y-axis. All 201 DILI cases and 532 population controls were included in the analysis. SNPs with $P$ values smaller than $10^{-6}$ and $10^{-7}$ are highlighted in green and red, respectively. Panel A shows the results for the entire genome. Panels B–D show enlarged sections of the MHC region. Panel B is an enlargement of the results presented in A, panel C shows the analysis of each SNP in this chromosome region conditioned on the top class II SNP (rs9274407), and panel D shows analysis of each SNP conditioned on the top class I and II SNPs (rs9274407 and rs2523822). Positions of a range of MHC genes are shown in B–D.
the UK and US cases than in those from Spain (Supplementary Table 2).

**HLA Analysis**

To investigate further the observed associations within the MHC, high-resolution genotyping of HLA-A, -B, -DRB1, -DQA1, and -DQB1 was performed on a subset of 177 cases and 219 genetically matched controls. The relationship between HLA genotypes and the top associated SNPs within Northwestern European and Spanish cases and controls was investigated (Supplementary Table 3). There were substantial differences in LD between Northwestern Europeans and Spanish for rs9274407 and DQB1*0602 and for rs2523822 and A*0201 that were reflected in subsequent associations. In particular, rs2523822/C showed poor correlation with A*0201 in Spanish, especially among cases (r² = 0.64) compared with Northwestern Europeans (r² = 0.96 for cases) (Supplementary Table 3). DQB1*0602 was the most significantly associated HLA allele with DILI overall (P = 1.4 × 10⁻¹⁰, OR, 3.3; 95% CI: 2.0 –5.7) and within each group. DRB1*1501 was in near-perfect LD with DQB1*0602 in both Northwestern European and Spanish cases and controls, and the association of either allele with AC-DILI was statistically similar. A summary of key findings conditioned on DQB1*0602 is provided in Table 4.

<table>
<thead>
<tr>
<th>SNP</th>
<th>HLA allele tagged</th>
<th>HLA class</th>
<th>Chr Position (build 36)</th>
<th>P value</th>
<th>OR (95% CI)</th>
<th>P value conditioned on rs9274407</th>
<th>P value conditioned on rs3135388</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9274407</td>
<td>II</td>
<td>6</td>
<td>32740809</td>
<td>4.8 × 10⁻¹⁴</td>
<td>3.1 (2.3 –4.2)</td>
<td>N/A</td>
<td>.00011</td>
</tr>
<tr>
<td>rs9267992</td>
<td>II</td>
<td>6</td>
<td>32328375</td>
<td>6.8 × 10⁻¹³</td>
<td>3.1 (2.3 –4.2)</td>
<td>.07</td>
<td>.0034</td>
</tr>
<tr>
<td>rs3135388</td>
<td>DRB1<em>1501-DQB1</em>0602</td>
<td>II</td>
<td>32521029</td>
<td>3.5 × 10⁻¹¹</td>
<td>2.8 (2.1 –3.8)</td>
<td>.93</td>
<td>N/A</td>
</tr>
<tr>
<td>rs2523822</td>
<td>HLA-A*0201</td>
<td>I</td>
<td>29936639</td>
<td>1.8 × 10⁻¹⁰</td>
<td>2.3 (1.8 –2.9)</td>
<td>1.2 × 10⁻⁹</td>
<td>2.1 × 10⁻¹⁰</td>
</tr>
</tbody>
</table>

**NOTE.** The data shown are for all 201 drug-induced liver injury cases and 532 controls. Chr, chromosome; N/A, not applicable.
ergogeneity on the effect of \textit{A*0201} gave a \textit{P} value of .071, suggesting a population-dependent effect (Supplementary Figure 4). This inconsistency with the result of rs2523822 could be explained by the much lower LD between rs2523822 and \textit{A*0201} in Spanish cases (Supplementary Table 3). Additionally, \textit{B*1801} was associated in Spanish cases with nominal significance independently of \textit{A*0201} and \textit{DQB1*0602} but not in Northwestern Europeans.

The frequencies of the 5-gene HLA haplotypes in cases and controls are shown in Supplementary Table 4. The most significant association was observed for \textit{A*0201-B*0702-DRB1*1501-DQB1*0602} in Northwestern Europeans (\textit{P} = .0007; OR, 13) and Spanish (\textit{P} = .013; OR, 20). However, both \textit{DQB1*0602} and \textit{A*0201} were significantly associated conditioned on that haplotype. \textit{A*0201-B*1801} was significantly associated (\textit{P} = .015; OR, 6.4) in Spanish only.

**Relationship of Genotype With Clinical Features**

We investigated the relationship between the top associated HLA alleles and clinical features of AC-DILI, including age at onset, pattern of liver damage, and disease severity as assessed by magnitude of transaminase or bilirubin elevation (Supplementary Table 5). \textit{B*1801} carriage was significantly correlated with peak ALT values in Spanish (\textit{P} = .0056, Figure 3A) but not in Northwestern Europeans (\textit{P} = .90). We further investigated whether the effect of \textit{B*1801} varied in Spanish cases according to pattern of liver damage (Figure 3B). The estimated effect in hepatocellular cases (OR, 8) was much larger than cholestatic/mixed cases (OR, 2), but the apparent heterogeneity was not statistically significant (\textit{P} = .10). No other notable correlations were observed in either Spanish or Northwestern Europeans.

**Clinical Predictive Values**

Assuming the prevalence of AC-DILI is 0.014%,\textsuperscript{2} the best positive predictive value is 0.1% for Northwestern Europeans based on the presence of both \textit{A*0201} and \textit{DQB1*0602} (frequency of 41% in cases) and 0.13% for Spanish based on the presence of both \textit{B*1801} and \textit{DQB1*0602} (frequency of 8.5% in cases). In each case, these alleles are present at approximately 10-fold higher rates than in the population overall. The best negative predictive value (NPV), expressed for clarity as 1-NPV, is 0.006% for Northwestern Europeans based on the absence of \textit{A*0201} (carriage frequency 74% in cases) and 0.007% for Spanish based on the absence of rs2523822/C (carriage frequency 74% in cases), approximately half the assumed rate of AC-DILI in the population overall (Supplementary Table 6).

**Discussion**

This study was accomplished through an international cooperation to assemble a considerably larger and more diverse AC-DILI patient collection than previous
Table 4. Analysis of Combined SNP and HLA Data by Conditioning on DQB1*0602

<table>
<thead>
<tr>
<th>Conditioned on</th>
<th>Allele</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>DQB1*0602</td>
<td>4.2 (2.7–6.6)</td>
<td>4.6 × 10⁻¹⁰</td>
<td>3.3 (2.0–5.7)</td>
<td>1.4 × 10⁻⁶</td>
<td>7.4 (3.1–17.8)</td>
<td>7.4 × 10⁻⁶</td>
</tr>
<tr>
<td>+ DQB1*0602</td>
<td>DRB1*1501</td>
<td>8.0 (4.3–15)</td>
<td>.0002</td>
<td>1.4 (0.2–10)</td>
<td>.77</td>
<td>N/A</td>
<td>.26</td>
</tr>
<tr>
<td>+ DQB1*0602</td>
<td>DQA1*0102</td>
<td>2.0 (1.2–3.2)</td>
<td>.02</td>
<td>2.9 (1.1–7.2)</td>
<td>.02</td>
<td>1.8 (0.7–5)</td>
<td>.37</td>
</tr>
<tr>
<td>+ DQB1*0602</td>
<td>DQB1*0402</td>
<td>2.3 (0.9–5.5)</td>
<td>.1</td>
<td>7.5 (1.5–38)</td>
<td>4.9 × 10⁻³</td>
<td>0.9 (0.1–6)</td>
<td>.4</td>
</tr>
<tr>
<td>+ DQB1*0602</td>
<td>A*0201</td>
<td>2.2 (1.6–3.2)</td>
<td>1.9 × 10⁻⁶</td>
<td>2.9 (1.8–4.4)</td>
<td>7.9 × 10⁻⁷</td>
<td>1.6 (0.8–2.8)</td>
<td>.13</td>
</tr>
<tr>
<td>+ DQB1*0602</td>
<td>B*1801</td>
<td>2.0 (1.0–3.9)</td>
<td>.05</td>
<td>0.98 (0.36–2.6)</td>
<td>.95</td>
<td>4.0 (1.5–11)</td>
<td>.004</td>
</tr>
<tr>
<td>+ DQB1*0602</td>
<td>rs2523822/C</td>
<td>3.9 (1.5–11)</td>
<td>.01</td>
<td>0.86 (0.15–4.9)</td>
<td>.86</td>
<td>7.9 (2.1–30)</td>
<td>.004</td>
</tr>
<tr>
<td>+ DQB1*0602</td>
<td>rs9274407/Minor</td>
<td>2.4 (0.9–5.9)</td>
<td>.07</td>
<td>9.7 (1.9–49)</td>
<td>1 × 10⁻³</td>
<td>0.67 (0.11–4.1)</td>
<td>.65</td>
</tr>
<tr>
<td>+ DQB1*0602</td>
<td>DQA1*0102</td>
<td>1.8 (0.6–5.0)</td>
<td>.23</td>
<td>0.37 (0.07–1.9)</td>
<td>.2</td>
<td>11 (1.6–85)</td>
<td>.01</td>
</tr>
</tbody>
</table>

PC, principal component.

published studies on the subject.11–13 This resulted in the most powerful and comprehensive investigation into genetic risk factors for AC-DILI and the largest GWA for any rare serious adverse event conducted to date. We confirmed the previous associations of HLA-DRB1*1501 and DQB1*0602 with DILI susceptibility, and our larger sample size and GWA approach allowed the identification of additional HLA risk factors together with an apparent statistical interaction between 2 HLA alleles.

In particular, a novel contribution to disease susceptibility from a common variant (rs2523822) in the region of the HLA-A*0201 allele was found. This variant is a tag for the HLA-A*0201 allele in individuals of European ancestry, and the effect of rs2523822 and A*0201 were indistinguishable in the Northwestern European subset of this study. When considered as an individual risk factor, the effect of A*0201 was seen only in cases of Northwestern European, and not Spanish, origin. This is in spite of the most strongly associated SNP in this region (rs2523822) showing an effect of similar magnitude in both populations. Nevertheless, the OR for A*0201 in the Spanish subset was also in the same direction as for the other subjects. Comparison of the LD patterns in Northwestern versus Southern European subsets showed that rs2523822 is indeed strongly correlated with A*0201 in Northwestern Europeans but not in Spanish, similar to the most strongly associated SNP in this region (rs2523822) showing an effect of similar magnitude in both populations. Given this observation, and the high degree of functional variation in the MHC region, one plausible explanation is that A*0201 is not the actual causal variant underlying the association with rs2523822 but merely happens to be an adequate “tag” for the causal site(s) in the Northwestern Europeans but not in the Spanish. Similarly, because the most significant class II association in Northwestern Europeans rs9274407 was still significantly associated conditioned on HLA-DRB1*1501-DQB1*0602 (or its tag SNP rs3135388) but the opposite was not true: there is a possibility that the causal variant(s) may lie in another class II locus. Further investigation of genetic variation in the MHC region and functional assessment of these variants in the context of AC-DILI will be necessary to identify the causal sites securely. The differences among populations in terms of risk associated with class I HLA alleles are somewhat similar to the situation with serious skin rash induced by antiepileptic drugs in East Asians where HLA-B*1502 is a risk factor in Han Chinese but not in other groups such as Japanese.29 The complexity of the MHC region in terms of the number of variable sites, and
local differences in LD, make definitive claims of causality particularly difficult.

A relatively rare HLA haplotype positive for DRB1*1501 together with DQB1*0602, A*0201, and B*0702 was significantly associated with AC-DILI in both Northwestern Europeans and Spanish. However, conditioning analysis showed that the association was driven by the A*0201 and the DRB1*1501-DQB1*0602 alleles regardless of the cis or trans configuration. We found compelling evidence of a statistical interaction between rs9274407 and rs2523822 (or DQB1*0602 and A*0201). An interaction of this nature appears biologically plausible in view of the complementary roles for the class I and class II HLA gene products in the T-cell response. If validated, this demonstrates the kind of gene-by-gene interaction that has often been speculated on but rarely observed in studies of complex traits in humans. On the other hand, without knowing the true causal variants, especially for the class I allele, we cannot exclude the possibility that the interaction between classes I and II is due to imperfect tagging of the causal variants by SNPs or classical HLA alleles.

We found evidence for an increased frequency of DQB1*0402 in the Northwestern European cases. This allele is associated with increased susceptibility to the autoimmune liver disease, primary biliary cirrhosis (PBC). PBC occurs predominantly in females, whereas a majority of the DILI patients positive for DQB1*0402 in this study were males and therefore unlikely to be misdiagnosed PBC cases. Xenobiotic exposure is believed to have a role in triggering PBC, and it is possible that there are common susceptibilities to PBC and AC-DILI.

From previous reports, it appears that the HLA class II DRB1*1501-DQB1*0602 haplotype is also a risk factor for other forms of DILI. A second HLA class II haplotype, DRB1*0701-DQA1*0201, is a risk factor for hepatotoxicity relating to ximelagatran and lapatinib. However, apart from our reported association between fluoroacillin DILI and B*5701 and an association between A*3303 and ticlopidine DILI in Japanese, the role of HLA class I genotypes in susceptibility to DILI generally is still poorly understood and merits further investigation. Interestingly, in addition to being possibly associated with DILI in the Spanish, B*1801 was further associated with peak ALT values in these cases, suggesting a possible role for this allele in phenotypic expression. It is worth noting that the only 2 cases of fulminant liver failure in the Spanish cohort were both B*1801 carriers.

Additionally, the possibility that the genetic determinants of DILI risk detected here using common SNP markers may potentially represent contributions from a larger number of rare genetic variants should be considered. This is particularly relevant to rare adverse events such as DILI, for which rare genetic determinants are in principle one of the most parsimonious explanations. Detection of rare variants will require whole genome sequencing, which is increasingly feasible.

Although our GWA study failed to provide any genome-wide significant evidence of a role for non-HLA genes, including ADME genes, in AC-DILI susceptibility, it remains possible that other genes contribute with smaller effects. In particular, the connection between AC-DILI and autoimmune disease is further extended by the association of the PTTPN2 SNP rs2476001. This association could also apply to autoimmune-related DILI because of other drugs because the gene product has a general role in regulation of T-cell responses.

This unique study has demonstrated the ability to determine novel genetic risk factors of AC-DILI in diverse populations. We have shown there are improvements in the NPV for the combined HLA risk genotypes compared with our assumed incidence of AC-DILI of 0.014% with carriers of both class I and II risk alleles showing a nearly 10-fold increase in risk. A positive predictive value of just 0.1% means that HLA genotyping will not be an effective means of prospectively identifying those at risk of AC-DILI, but our findings have clinical utility in that HLA genotyping may be of value in strengthening AC-DILI diagnoses in view of the high NPVs seen.

**Supplementary Material**

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: [10.1053/j.gastro.2011.04.001](http://www.gastrojournal.org).

**References**


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Conflicts of interest
The authors disclose the following: Dr Fontana acted as a consultant to GlaxoSmithKline. Dr Nelson is an employee of GlaxoSmith Kline. The remaining authors disclose no conflicts.

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