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A Missense Variant in *PTPN22* is a Risk Factor for Drug-induced Liver Injury

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BACKGROUND & AIMS: We performed genetic analyses of a multiethnic cohort of patients with idiosyncratic drug-induced liver injury (DILI) to identify variants associated with susceptibility. **METHODS:** We performed a genome-wide association study of 2048 individuals with DILI (cases) and 12,429 individuals without (controls). Our analysis included subjects of European (1806 cases and 10,397 controls), African American (133 cases and 1,314 controls), and Hispanic (109 cases and 718 controls) ancestry. We analyzed DNA from 113 Icelandic cases and 239,304 controls to validate our findings. **RESULTS:** We associated idiosyncratic DILI with rs2476601, a nonsynonymous polymorphism that encodes a substitution of tryptophan with arginine in the protein tyrosine phosphatase, nonreceptor type 22 gene (*PTPN22*) (odds ratio [OR] 1.44; 95% confidence interval [CI] 1.28–1.62; $P = 1.2 \times 10^{-9}$ and replicated the finding in the validation set (OR 1.48; 95% CI 1.09–1.99; $P = .01$). The minor allele frequency showed the same effect size (OR > 1) among ethnic groups. The strongest association was with amoxicillin and clavulanate-associated DILI in persons of European ancestry (OR 1.62; 95% CI 1.32–1.98; $P = 4.0 \times 10^{-6}$; allele frequency = 13.3%), but the polymorphism was associated with DILI of other causes (OR 1.37; 95% CI 1.21–1.56; $P = 1.5 \times 10^{-6}$; allele frequency = 11.5%). Among amoxicillin- and clavulanate-associated cases of European ancestry, rs2476601 doubled the risk for DILI among those with the HLA risk alleles A*02:01 and DRB1*15:01. **CONCLUSIONS:** In a genome-wide association study, we identified rs2476601 in *PTPN22* as a non-HLA variant that associates with risk of liver injury caused by multiple drugs and validated our finding in a separate cohort. This variant has been associated with increased risk of autoimmune diseases, providing support for the concept that alterations in immune regulation contribute to idiosyncratic DILI.

Keywords: Amino Acid Change; GWAS; Mutation; Inflammation.

Idiosyncratic drug-induced liver injury (DILI) is a rare adverse drug reaction that is an important cause of acute liver failure in the developed world.[1,2] DILI typically occurs in 10 to 20 of 100,000 treated patients, and although it can lead to death, most cases resolve with discontinuation

***Authors share co-first authorship; §Authors share co-senior authorship.**

Abbreviations used in this paper: **AC, amoxicillin-clavulanate; AF, allele frequency; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CI, confidence interval; DILI, drug-induced liver injury; DILIN, The Drug Induced Liver Injury Network; GWAS, genome-wide association study; HLA, human leukocyte antigen; iDILIC, International DILI Consortium; MHC, major histocompatibility complex; OR, odds ratio; SNP, single nucleotide polymorphism.**

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CLINICAL LIVER

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

It is not understood why otherwise safe drugs can rarely cause drug-induced liver injury (DILI), but mounting evidence supports inherited, or genetic, susceptibility factors.

NEW FINDINGS

The largest cohort of DILI patients yet studied revealed that risk of DILI was associated with a variant in a gene that has been previously associated with risk for a variety of autoimmune diseases.

LIMITATIONS

This study did not examine how this new information could be used to make drugs safer.

IMPACT

These findings add to growing evidence that DILI results from an immune response to the liver.

of the offending drug.[3,4] DILI is nonetheless one of the most frequent complications in the development and approval of new drugs, often leading to failure in the later stages of drug development or regulatory actions, including post-marketing withdrawals.[5]

A number of genome-wide association studies (GWAS) on DILI have been performed, leading to the discovery of significant associations with several human leukocyte antigen (HLA) alleles that are generally drug-specific. For example, HLA-B*57:01 is associated with DILI in response to flucloxacillin, HLA-A*02:01 and HLA-DRB1*15:01 are associated with amoxicillin-clavulanate (AC), HLA-B*35:02 is associated with minocycline, and HLA-A*33:01 is associated with terbinafine and probably several other drugs as well.[6–9] The association of DILI risk with HLA alleles supports a role for adaptive immunity in DILI. Although no confirmed associations outside the HLA region have yet been identified in a GWAS,[10] a trend toward association with all-cause DILI was recently observed with a single nucleotide polymorphism (SNP) (rs2476601) in the PTPN22 gene.[7] Because this variant has been associated with risk for a variety of autoimmune diseases, confirming this association would provide further support for the immune basis for DILI.

The Drug Induced Liver Injury Network (DILIN), in collaboration with the International Drug Induced Liver Injury Consortium (iDILIC), has assembled a cohort of 2048 DILI cases and 12,429 population controls across three major ethnic populations (European, African American, and Hispanic). After conducting a transethnic meta-analysis, we replicated our top associated SNPs on an independent European cohort of cases and performed multiple subset analyses to investigate their relationship with known HLA risk alleles for DILI and their effect sizes within a certain drug or injury type. Here, we confirm a significant association of DILI risk with rs2476601 in the PTPN22 gene. This is the first GWAS significant association outside the HLA region and the first that appears to hold across many different classes of drugs. Our finding supports immune mechanisms having a broad role in DILI.

Materials and Methods

We carried out a case/control association study in 3 different populations (European, Hispanic, and African American) and then performed a meta-analysis.

Cases

In the current study, we analyzed 2048 DILI cases due to multiple causal drugs, including amoxicillin-clavulonate (AC) and flucloxacillin, collected by the iDILIC and DILIN consortia. Causality assessment was performed as previously reported.[8,11] A total of 1149 European cases were previously genotyped and analyzed by Urban et al.[11] and/or Nicoletti et al.[8] and 899 had undergone GWAS genotyping for the first time. Supplementary Table 1 shows the breakdown of the case cohorts by recruitment center, genotyping chip, and ethnicity. Clinical characteristics of the DILI subjects are reported in Table 1.

DILIN Cases

A total of 1074 DILIN cases were included, of which 443 DILIN European cases had been previously reported,[8,11] and 631 cases, consisting of 389 European, 133 African American, and 109 Hispanic descendants were newly genotyped.[12] The DILIN protocol and entrance criteria have been previously published.[12] All participants provided written informed consent. Causality assessment was performed as previously described, and only cases considered probably, highly likely, or definitely related to the implicated drug were included.[13] DNA was extracted from lymphocytes and stored at the Nationa Institute of Diabetes and Digestive and Kidney Diseases biosample repository at Rutgers University, Piscataway, NJ. Genome-wide genotyping for the 564 DILIN cases was performed with the Multi Ethnic Genome Illumina Array at Duke University and for 32 African American and 35 Hispanic DILIN cases was performed with the 1Million Illumina duo Array at Duke University.

iDILIC European Cases

A total of 974 European iDILIC cases with a range of causal drugs and recruitment phases were included. Of these, 706 cases had been described previously,[8,11] and 268 cases due to flucloxacillin or AC were newly genotyped. The 268 patients were recruited between May 2009 and May 2013 as a part of an international collaborative study involving international recruitment centers. All participants provided written informed consent, and each study had been approved by the appropriate national or institutional ethical review boards. The clinical inclusion criteria for all cases were those described by Aithal et al.[14] The iDILIC cases were evaluated by application of the Council for International Organizations of Medical Science scale, also called the Roussel Uclaf Causality Assessment Method,[15] and by expert review by a panel of 3 hepatologists. Only cases having at least possible causality (score $\geq$3) were included in the study. DNA was prepared as described

Table 1. Clinical Characteristics of the Samples in the 3 Major DILI Case Population

CHARACTERISTICS	European n = 1806	Hispanic n = 109	African American n = 133
Clinical information			
Mean age, *yr*	55	41	47
Female, %	56.5	56.8	76.6
Median alanine aminotransferase (range), *U/L*	774 (9–15065)	843 (20–9108)	780 (47–7001)
Median alkaline phosphatase (range), *U/L*	290 (11–6239)	266 (79–2414)	265 (74–2399)
Median latency (range), *d*	28 (1–7046)	58 (3–2789)	51 (3–935)
Injury Type			
Cholestatic (%)	463 (26)	12 (11)	25 (19)
Hepatocellular (%)	747 (41)	73 (67)	80 (60)
Mixed (%)	465 (26)	19(17)	22 (16)
Not available (%)	130 (7)	5(5)	6 (5)
Total	1806	109	133

previously.[6] Genome-wide genotyping of the additional iDILIC cases was performed by the Broad Institute, Boston by Infinium HumanCoreExome BeadChip for 167 cases and by Infinium Human OmniExpress BeadChip for 101 cases.

Clinical Characteristics of the DILI Cases

We collected additional clinical information to further investigate the relevance of the most significant associations. Time from start of medication to DILI recognition, concomitant medications, and maximum serum levels of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were available for both DILIN and iDILIC cases. Also available on all cases was whether the injury was hepatocellular, cholestatic, or mixed (based on the initial R value).[15] Specific diagnosis of autoimmune diseases was recorded for iDILIC cases, while DILIN cases included reports on whether patients had a general history of autoimmune/collagen vascular disease (Yes/No).

Controls

As DILI has a very low incidence, we consider that a large set of general population control samples can overcome the potential bias of inclusion of people who may have experienced unrecorded DILI events. A total of 10,397 European controls described in our previous study[8] were used. Moreover, data for 1314 African American and 718 Hispanic controls were obtained from the MESA study (phs000420.v6.p3) in dbGAP.[16] Supplementary Table 1 also shows the breakdown of the control cohorts by genotyping chip and ethnicity.

Genetic Analysis

Quality control checks on the initial genotype data were performed as summarized in the Supplementary Materials and Methods. EIGENSTRAT analysis was used to identify the European, African-American, and Hispanic cohorts. Our final sample sizes were 1806 cases and 10,397 controls of European ancestry, 133 cases and 1314 controls of African American ancestry, and 109 cases and 718 controls of Hispanic ancestry. SNP imputation was performed in batches dividing the samples according to ethnicity and genotyping platforms. For each batch, imputation was carried out using the Michigan Imputation Server,[17] as described in the Supplementary Materials and Methods. For HLA genotypes, 4-digit HLA alleles were inferred using HIBAG.[18]

Association analyses were performed using logistic regression under the additive model in Plink.[19] EIGENSTRAT axes were used as covariates. A meta-analysis of the 3 ethnic groups was performed using GWAMA fixed effects. Variants that (1) had a concordant effect in at least 2 of the ethnic groups and that (2) showed final meta P values below 5×10^{-8} were considered statistically significant.[20,21] The top associated imputed SNPs were genotyped in the available cases using a TaqMan SNP genotyping assay (ThermoFisher Scientific, Waltham, MA) in accordance with the manufacturer's recommendations.

Multimarker association analysis among the combinations of carriage groups of known HLA risk alleles and the PTPN22 variant allele was performed by logistic regression using principal component as covariates and considering the joint negative carriers as the reference group in the drug-specific cohorts (such as AC, flucloxacillin, terbinafine, and flupirtine). Moreover, after transforming the quantitative clinical variables (latency, maximum ALP, and maximum ALT) to improve normality, we applied a linear regression model to test differences among known HLA risk alleles and PTPN22 variant in the AC cohort.

Epistasis analysis was performed by logistic regression, using principal component axes as covariates and considering an interaction term between being a carrier of any HLA risk alleles and being a carrier of the associated variant.

We also performed association analysis between the PTPN22 variant and reported clinical variables. First, we treated latency and other clinical variables as a quantitative trait. After transforming the quantitative variables to improve normality, we applied a linear regression model to test PTPN22 variant effect on clinical trait in a cases-only design. Epistasis, multimarkers, and multinomial logistic regression analyses were carried out using STATA15 (Stata Corp, College Station, TX).

Icelandic DILI Replication Cohort

An independent Icelandic DILI replication cohort was recruited at the National University Hospital of Iceland. The Icelandic DILI cases were evaluated in accordance with iDILIC causality assessment criteria.[8] Clinical characteristics of the

Icelandic DILI subjects are reported in Supplementary Table 2. The cohort included 113 DILI cases and 239,304 population controls. Geneotyping data from the Icelandic sample set was imputed as previously described.[22–24] HLA alleles were also imputed by Graphtyper.[25] Logistic regression under an additive model was used to test for association between variants and DILI. Detailed description of Icelandic analyses is reported in the Supplementary Materials and Methods.

Results

Overall Findings

Our final meta-analysis included 3,622,749 SNPs in 2048 cases and 12,429 controls (see QQ plots in Supplementary Figure 1). Clinical characteristics of the well-phenotyped DILI cases across 3 main ethnicities are reported in Table 1. We identified a significant association with rs2476601 (chr1:114377568>A/G), a polymorphism changing tryptophan to arginine at codon 620 of *PTPN22* (odds ratio [OR] 1.44; 95% confidence interval [CI] 1.28–1.62; $P = 1.2 \times 10^{-9}$; Figure 1). The enrichment was observed across all ethnic groups analyzed in our study, although the low number of African American and Hispanic cases limited the power to identify a significant association for variants with an OR < 2 (Table 2). Similar ORs were also evident within subgroups of European ancestry (Supplementary Table 3). Independent genotyping of available DILIN cases across ethnicities (N = 1070) confirmed the GWAS genotypes for rs2476601 with a concordance rate of 100% (Supplementary Table 4). rs2476601 was also found to increase the risk of DILI in the independent Icelandic replication cohort (allele frequency [AF] = 0.13 in 113 cases vs 0.09 in 239,304 controls), having an effect size that was comparable to that of the discovery cohort (OR 1.48; 95% CI 1.09–1.99; $P = .01$).

In addition to rs2476601, several variants in the MHC region were found to have genome-wide significant *P* values, led by rs3129880 (OR 1.48; 95% CI 1.36–1.60; $P = 1.2 \times 10^{-20}$). This variant is a proxy of HLA-DRB1*15:01 ($r^2 = 0.56$) consistent with the large number of AC DILI cases in the cohort. As expected based on inclusion of 195 flucloxacillin and 444 AC European DILI cases, the most significant HLA risk alleles were HLA-B*57:01, followed by HLA-DRB1*15:01 (OR 2.19; $P = 1.4 \times 10^{-18}$, see Table 2). Unlike the rs2476601 association, HLA association signals were specific for the European population in which flucloxacillin and AC cases were the most abundant.

Subsequent genome-wide conditional analysis incorporating the genotypes of the 4 well-established DILI HLA risk alleles (HLA-B*57:01, HLA-DRB1*15:01, HLA-A*02:01 and HLA-A*33:01)[6–8] as covariates was undertaken to identify novel independent risk factors. The analysis revealed that rs2476601 remained the most significant independent risk variant (OR 1.45; 95% CI 1.30–1.64; $P = 7.6 \times 10^{-10}$, Figure 1*B* and Supplementary Table 5). Similarly, the independence between rs2476601 and the main HLA risk alleles was confirmed in the Icelandic cohort in a multivariate regression model (OR 1.54; $P = .013$; Supplementary Table 6). When controlling for the 4 major known DILI HLA risk alleles, HLA-C*04:01 was the most significant independent HLA allele associated with DILI risk reaching near statistical significance when corrected for the total number of imputed HLA alleles (OR 1.21; 95% CI 1.09–1.37; $P = 6.3 \times 10^{-4}$). HLA-C*04:01 association showed consistent trends across all three ethnicity groups (European $P = .004$, OR 1.19; African American $P = .02$, OR 1.42; Hispanic $P = .53$, OR 1.13, Supplementary Table 7). Data for individual drugs in relation to this risk association are shown in Supplementary Table 8. It is notable that the greatest

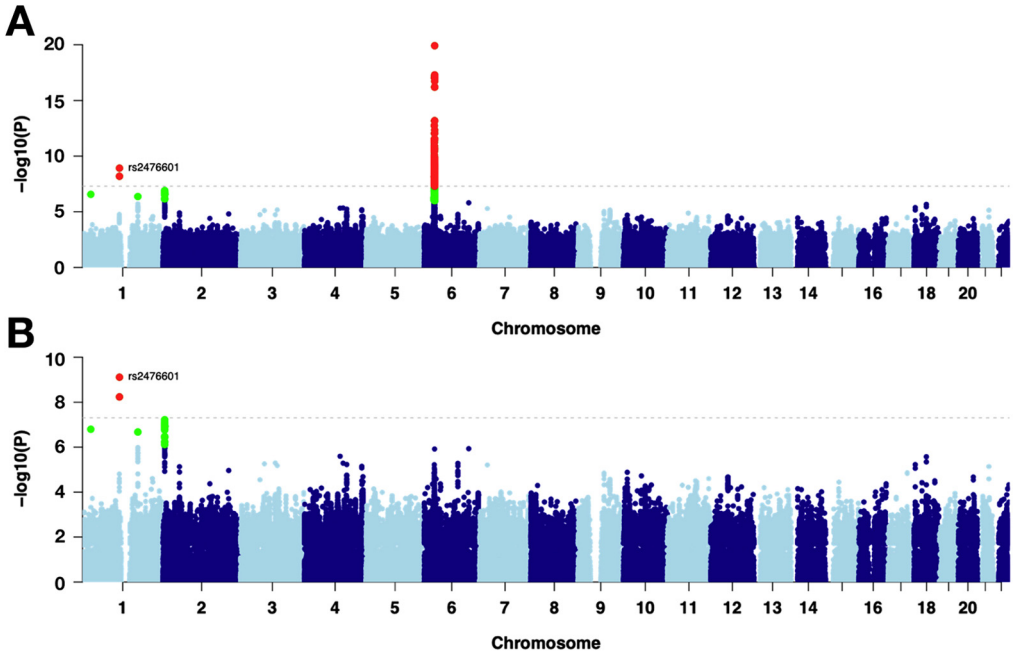

Figure 1. Manhattan plot displaying the association results of (*A*) the meta-analysis among the 3 major populations (European, African American, and Hispanic) and (*B*) the meta-analysis after conditioning on the 4 main known HLA DILI risk alleles among the 3 major populations (European, African American, and Hispanic). The results are reported for variants that had a consistent effect in Europeans and at least 1 of the 2 additional populations. SNPs shown in green have a significance level less than 5×10^{-6} and red have a significance level less than 5×10^{-8}.

association was seen with the 58 cases in whom DILI was attributed to herbal and dietary supplements (OR 2.24, $P = .0008$, individual agents listed in Supplementary Table 9).

We also found that rs72631546, an intergenic marker on chromosome 2, was the third most significant variant (OR 1.84; $P = 1.2 \times 10^{-7}$; Table 2). rs72631546 is in LD ($r^2 = 0.5$) with rs72631567, which was an SNP previously suspected to be associated with DILI risk.[8] The rs72631546 association was consistent between European and Hispanic individuals (OR 1.79, $P = 1.9 \times 10^{-6}$; OR 2.07, $P = .003$, respectively) and was independent of the known HLA risk associations (OR 1.87, $P = 6.0 \times 10^{-8}$).

Association With PTPN22 rs2476601

In the European cohort, the AC cases showed the most significant association with rs2476601 (OR 1.62; 95% CI 1.32–1.98; $P = 4.0 \times 10^{-6}$) with higher frequency than European controls (AF = 0.13 vs AF = 0.08, respectively). We also found evidence that the association was consistent among the remaining of European DILI cases (n = 1362; OR 1.37; 95% CI 1.21–1.56; $P = 1.5 \times 10^{-6}$, AF = 0.11; Supplementary Figure 2), and it did not appear to be driven by particular drugs or categories of drugs (Table 3). Significance of $P \leq .05$ was seen for cases due to several causal drugs including sulfamethoxazole-trimethoprim ($P = .01$) and terbinafine ($P = .01$). On the other hand, cases related to drugs such as flucloxacillin and diclofenac, which were well represented in the cohort as causal agents, showed smaller increases in minor AF compared with controls, which were not statistically significant ($P > .05$). We also evaluated the relationship of rs2476601 genotype to DILI phenotype. Of our European ancestry cases, 45% had a hepatocellular pattern of injury, and 55% were cholestatic or mixed. We found enrichment compared with controls for rs2476601 in both injury patterns, with similar AF and ORs (hepatocellular AF = 0.12, OR 1.38, $P = .0001$; cholestatic/mixed AF = 0.13, OR 1.50, $P = 6.5 \times 10^{-8}$; Supplementary Table 10). We also found that there was a trend for the frequency of rs2476601 to be higher in the DILI cases most confidently ascribed to the implicated drug (Supplementary Figure 2). We found that there was no significant association between rs2476601 and time of onset of DILI relative to starting treatment with the implicated drug as well as maximum ALP or maximum ALT values.

Assessment of Correlation With Autoimmune Diseases

As rs2476601 has been previously associated with numerous autoimmune diseases,[26] we investigated whether the presence of autoimmune diseases in our DILI cohort could have contributed to the associations observed. We identified 567 DILI subjects with evidence of autoimmune diseases; 135 of whom had a documented history of autoimmune/collagen vascular disease, and the remaining subjects were suspected to have an autoimmune disease because they had been treated with at least 1 drug commonly used in these conditions, usually in addition to the agent implicated as causing DILI (list of potential autoimmune treatments is presented in Supplementary Table 11). When all 567 samples with known or suspected diagnosis of autoimmune disease were excluded from our cohort, the rs2476601 association remained highly significant with the same effect size (n = 1245; OR 1.40; 95% CI 1.23–1.60; $P = 6.4 \times 10^{-7}$, Supplementary Table 12).

Assessment of Correlation With Known HLA Risk Alleles

We found an enrichment of rs2476601 among European DILI cases due to causal drugs known to have HLA alleles as the main genetic risk association (eg, flucloxacillin, terbinafine, fenofibrate, minocycline, sertraline, AC) compared with the rest of the cases (OR 1.52 vs OR 1.38, Supplementary Table 13). Among these drugs associated with HLA risk alleles, AC was the major causal drug (444 cases) and showed the strongest association with rs2476601 (OR 1.62; 95% CI 1.32–1.98; $P = 4.0 \times 10^{-6}$). AC-drug specific conditional analysis on HLA-A*02:01 and HLA-DRB1*15:01 confirmed that rs2476601 was an independently associated risk factor from the known HLA risk

Table 2. Summary Statistics for the Univariate Transethnic Meta-analysis of Genome-Wide Associated Variants

	All			European			African American			Hispanic		
Marker	OR	95%CI	*P*	OR	95%CI	*P*	OR	95%CI	*P*	OR	95%CI	*P*
rs2476601	1.44	1.28–1.62	1.2×10^{-9}	1.42	1.27–1.60	9.6×10^{-10}	1.94	0.73–5.18	.19	1.91	0.94–3.89	.07
rs72631546	1.84	1.47–2.31	1.2×10^{-7}	1.79	1.41–2.27	1.92×10^{-6}	—	—	—	2.07	1.27–3.36	.003
rs3129880	1.48	1.6–9.32	1.2×10^{-20}	1.56	1.44–1.69	5.09×10^{-26}	0.92	0.67–1.27	.62	0.97	0.68–1.4	.89
HLA-B*57:01	2.19	1.84–2.61	1.40×10^{-18}	2.24	1.94–2.6	4.53×10^{-27}	1.64	0.36–7.4	.52	0.72	0.17–3.02	.65
HLA-DQB1*03:03	1.67	1.41–1.99	7.48×10^{-9}	1.69	1.46–1.96	1.27×10^{-12}	—	—	—	0.97	0.29–3.16	.95
HLA-DRB1*15:01	1.37	1.22–1.53	1.03×10^{-7}	1.40	1.27–1.54	3.19×10^{-11}	0.85	0.43–1.69	.65	1.16	0.68–1.99	.58
HLA-C*06:02	1.40	1.23–1.59	1.88×10^{-7}	1.45	1.3–1.62	6.47×10^{-11}	0.92	0.58–1.48	.74	1.38	0.77–2.48	.28
HLA-DQB1*06:02	1.31	1.17–1.46	1.47×10^{-6}	1.40	1.27–1.55	3.15×10^{-11}	0.90	0.66–1.21	.48	1.11	0.64–1.91	.72

ORs, 95% Cis, *P* values are presented after correcting for population stratification with EIGENSTRAT axes within each major population.

Table 3. Association With rs2476601 for Drugs With at Least 3 Case Carriers in the European Cohort and OR >1

Drugs	No. cases	AF	OR	95% CI	*P*
Amoxacillin/Clavulanic acid	444	0.13	**1.62**	**1.32–1.98**	**.000004**
Terbinafine	15	0.20	**3.23**	1.29–8.1	**.01**
Sulfamethoxazole/Trimethoprim	42	0.17	2.07	1.16–3.71	.01
Methotrexate	9	0.22	3.34	1.09–10.16	.03
Rofecoxib	6	0.25	4.08	1.05–15.82	.04
Valproic acid	16	0.18	2.43	0.99–5.95	.05
Flupirtin	6	0.25	**4.43**	**0.98–20.05**	**.05**
Fenofibrate	10	0.20	2.93	0.97–8.87	.06
Erythromycin	11	0.18	2.89	0.95–8.78	.06
Doxycycline	6	0.25	3.21	0.85–12.1	.09
Pravastatin	6	0.25	3.21	0.83–12.47	.09
Nimesulide	20	0.12	2.10	0.81–5.41	.12
Cefuroxime	4	0.25	3.45	0.69–17.28	.13
Ethinylestradiol/Levonorgestrel	7	0.21	2.53	0.71–9.05	.15
Isoniazid	43	0.13	1.59	0.84–3.02	.16
Celecoxib	9	0.17	2.37	0.69–8.19	.17
Flucloxacillin	**195**	**0.11**	**1.24**	**0.90–1.71**	**.18**
Nitrofurantoin	74	0.12	1.40	0.85–2.32	.19
Piroxicam	5	0.20	2.85	0.60–13.68	.19
Gabapentin	5	0.20	2.79	0.58–13.39	.2
Cefazolin	21	0.14	1.59	0.67–3.8	.3
Mercaptopurine	10	0.15	1.72	0.50–5.97	.39
Imatinib	8	0.12	1.70	0.38–7.56	.49
Ticlopidine	**5**	**0.10**	**2.01**	**0.24–16.73**	**.52**
Atorvastatin	29	0.10	1.32	0.56–3.09	.53
Minocycline	**32**	**0.11**	**1.29**	**0.58–2.86**	**.53**
Interferon Beta-1a	4	0.12	1.90	0.21–16.79	.57
Amiodarone	5	0.20	1.64	0.28–9.73	.59
Diclofenac	66	0.10	1.17	0.67–2.04	.59
Ibuprofen	15	0.10	1.36	0.41–4.52	.62
Herbal and dietary products	58	0.10	1.19	0.65–2.17	.58
Disulfiram	8	0.12	1.40	0.32–6.13	.65
All other therapeutic products	9	0.11	1.33	0.30–5.93	.71
Nicotinic acid	4	0.12	1.45	0.17–12.17	.73
Lisinopril	5	0.10	1.45	0.17–12.17	.74
Phenytoin	10	0.10	1.18	0.27–5.11	.82
Rosuvastatin	4	0.12	1.27	0.14–11.29	.83
Sertraline	6	0.08	1.17	0.15–9.27	.88
Levofloxacin	17	0.09	1.05	0.32–3.44	.94

Drug results are ordered by *P* value. In bold are drugs previously known to be associated with at least 1 HLA risk allele.
No. cases, number of cases for each drug; *P*, logistic *P* value.

alleles (OR 1.6 and $P = 8.9 \times 10^{-6}$, Supplementary Table 5). Because the 3 markers were independent among each other, we looked for evidence of co-occurance of rs2476601 and the known HLA risk alleles. We therefore stratified AC cases and controls based on HLA allele carriage (Supplementary Table 14). There was evidence that carriers of rs2476601 were enriched in AC DILI patients who carried one or both the HLA. In agreement with this finding, multimarker analysis on the AC cohort confirmed that when rs2476601 co-occurred with either of the 2 HLA alleles, this consistently enhanced the association with DILI risk by almost 2-fold compared with risk associated with the HLA alleles alone or in combination (Table 4). Joint carriage of the 3 markers was associated with a 13-fold higher DILI risk compared with the negative carriers. We had only 12 AC cases carrying only rs2476601 and neither of the 2 HLA risk alleles. This limited number did not allow us to capture the association of rs2476601 alone with AC DILI risk, but the 95% CI reported in Table 4 included an OR of 1.5 . Moreover, when we compared the triple positive carrier against HLA-A*02:01 and HLA-DRB1*15:01 positive but rs2476601 negative group we found a significant 1.7-fold increase in the association with DILI risk (OR 1.8; 95% CI 1.24–2.60; $P = .002$). This confirmed that rs2476601 is independently associated with AC DILI risk. Finally, we tested the presence of a SNP-HLA interaction effect for AC DILI. The analysis showed that there was an epistatic effect between rs2476601 and the presence of at least one of the HLA risk alleles (OR 1.9; $P = .05$). In other words, the joint effect of one or both HLA risk alleles and rs2476601 was more than additive.

Table 4. Summary Statistics of the Multimarker Analysis Performed on the Carriage of HLA-DRB1*15:01, HLA-A*02:01 and rs2476601 in the European Amoxicillin-Clavulanate-related DILI Cohort

	Cases		Controls				
Carriage group	n	CF	n	CF	OR	95% CI	*P*
+/+/+	49	0.11	187	0.02	13.80	9.18–20.71	$1.1*10^{-36}$
−/+/+	35	0.08	625	0.06	3.03	1.99–4.63	$2.3*10^{-7}$
+/−/+	12	0.03	206	0.02	3.28	1.74–6.19	$2.5*10^{-4}$
−/−/+	12	0.03	663	0.07	0.95	0.51–1.77	.8
+/+/−	126	0.28	895	0.09	7.68	5.63–10.48	$8.8*10^{-38}$
−/+/−	101	0.23	3212	0.31	1.68	1.23–2.30	$1.1*10^{-3}$
+/−/−	41	0.09	1037	0.10	2.09	1.41–3.11	$2.7*10^{-4}$
−/−/−	68	0.15	3531	0.34	—	—	—

ORs, 95% Cis, and *P* values are presented after correcting for population stratification and considering the triple negative carriers as the reference group. The 3 letters in the first column (carriage group) reflects in order the HLA-DRB1*15:01 status, the HLA-A*02:01 status, and the rs2476601 status. The risk alleles status is represented by "+" = present or "–" = absent. CF, carriage frequency; n, number of samples in the group.

Multimarker analysis on the terbinafine, flucloxacillin, and flupirtine cohorts also supported rs2476601 as independently associated with DILI risk, showing consistent ORs in DILI due to flucloxacillin (OR 1.3), terbinafine (OR 3.4), and flupirtine (OR 2.5) in addition to the enhanced the DILI risk associated with the known HLA alleles (Supplementary Table 15). We also examined whether subjects in the AC cohort who carried any combination of the 3 risk alleles differed in clinical phenotype (see Materials and Methods) from each other or from individuals not carrying any of the 3 alleles. No differences were apparent.

Discussion

Here, we report the results of the largest DILI GWAS to date based on 2048 DILI cases and 12,429 controls. Our analyses identified a robust association with a variant in *PTPN22*, a tyrosine phosphatase that has been linked to numerous autoimmune disorders.[26] In addition, the association was not limited to a certain drug or pattern of injury, instead showing associations across the entire cohort. Moreover, we showed that the PTPN22 variant added to the DILI risk associated with known HLA alleles, increasing the association with AC DILI risk almost 2-fold and appearing to have a similar effect on other DILI events with known HLA risk alleles. This finding provides new insights into DILI etiology and highlights the potential role of non-HLA variants in immune-related genes as risk factors for DILI across a broad spectrum of causal drugs.

rs2476601 is associated with increased risk of type 1 diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus, vitiligo, and Graves' disease, among others, but is also associated with decreased risk of Crohn's disease and Behçet disease.[26] This is the first confirmed genome-wide association with DILI risk that lies outside the MHC locus and is the first variant that appears to generally predispose to DILI as opposed to DILI due to specific drugs. The replication of the association in separate Icelandic cases and controls where the PTPN22 variant is relatively common confirmed the association.

Our previous study also suggested that rs2476601 might be associated with risk for DILI, but the variant did not meet the criteria for genome-wide significance.[7] The present study confirmed that association, and with a larger sample size the strength of the association exceeded the required statistical threshold for a variant of this moderate effect size. It should be noted that the effect size seen for this variant with DILI was similar to those seen for other disease conditions in which rs2476601 is associated with increased risk.[27] The frequency of rs2476601 varies greatly from population to population, being as high as 15% in Finnish individuals and as low as <0.01% in East Asian individuals.[28] Here, we found that the frequency of the variant in DILI cases was higher in each population studied relative to the frequency in a matched control population, with the OR remaining similar among different ethnic and racial populations. Despite this consistency of the OR, the relatively small effect size of this variant means that larger sample sizes are needed to confirm the associations as real in all populations and with different agents responsible for causing DILI. The largest subset of the current study was Northern European individuals (n = 1107 cases and 5090 controls), in whom a very strong signal for this variant was seen ($P = 3.6 \times 10^{-6}$, OR 1.41, Supplementary Table 3). The other analyzed ethnicities, which had much smaller sample sizes, did not produce *P* values below .05 despite their similar ORs for the effect of this variant.

Because patients with autoimmune disease may take more medications than others, there was a possibility that the association we observed with rs2476601 could actually be due to an increased prevalence of autoimmune diseases in DILI patients. To address this possibility, we identified cases with a recorded or suspected (based on concomitant medications) diagnosis of autoimmune diseases. Although rs2476601 in these patients had a slightly higher effect size, the association remained comparably strong even among patients not known or suspected to have underlying autoimmune conditions. Because history of autoimmune diseases was not systematically collected in all subjects, and because patients with autoimmune diseases may not be

taking medication treatment for these conditions at the time of the DILI event, we cannot rule out the possibility that some of our DILI patients had undiagnosed and untreated autoimmune conditions.

The association with rs2476601 was consistent across various phenotypes in our cohort, including injury patterns (cholestatic or mixed vs hepatocellular), causal drugs, and strength of causality assessment. However, we did not find any association with other features, including DILI latency. We found that AC cases and other cases with the highest causality scores tended to have the highest frequencies for rs2476601. As DILI is a diagnosis of exclusion, it is unavoidable to have some uncertainty about the true cause of liver injury in certain patients, and so for real risk associations, we expect to see the strongest associations in those cases with the highest causality score. In our cohort, most AC cases were classified as having a high likelihood of DILI since the AC-DILI characteristic phenotypes have been well defined.[29] The higher allele frequency in AC cases likely reflects a higher proportion of patients who truly have DILI due to this drug. However, there may be an AC-specific genetic effect of rs2476601, as AC cases had a higher allele frequency than did other DILI cases even when restricting to the same causality probability categories.

Our analysis showed that rs2476601 appears to be associated with DILI risk regardless of which HLA alleles are associated with DILI risk. This is also the case with autoimmune diseases in which rs2476601 is associated across diseases that are themselves associated with different HLA alleles.[26] This effect is consistent with the fact that PTPN22 controls events downstream from HLA presentation of neoantigen as summarized in Burn et al.[30] PTPN22 encodes lymphoid protein tyrosine phosphatase (Lyp), which is expressed exclusively in immune cells. Although the mechanisms whereby the rs2476601 variant reduces immune tolerance are not clear, Lyp is involved in T-cell receptor signaling, acting at several intermediate points in the signaling cascade. Lyp also appears to influence regulatory T-cell function. Considerable data now support the concept that DILI can result from a T-cell–mediated immune attack on the liver, presumably directed at HLA-presented neoantigens. This response may be initiated relatively frequently during treatment with drugs capable of causing DILI. However, clinically important liver injury does not occur in most of these patients because immune tolerance is activated.[31,32] We therefore suggest that rs2476601 predisposes to DILI by reducing immune tolerance. Our finding supports this hypothesis, because we found a significant genetic interaction effect among HLA risk alleles and rs2476601, detecting that the AC DILI risk associated with the joint carriage of HLA risk alleles and the variant is more that the sum of the risks associated with each single risk factor. Most of the currently reported associations for other diseases and the PTPN22 variant also show HLA associations, particularly HLA class II associations which is consistent with the strong association seen with AC-DILI but only a slightly increased frequency of the variant with flucloxacillin-DILI in which the associated allele is class I. Moreover, the increased frequency of the rs2476601 variant in DILI cases where there was no apparent HLA association suggests the possibility of other HLA risk alleles yet to be discovered and/or a potential role for PTPN22 in non–T-cell–mediated forms of DILI in which other immune cells might be involved.

Although the association with rs2476601 was robust, its effect size was modest. The OR averaged approximately 1.3 in the various ethnic groups identified here. However, in the 10% of the subjects who also carried the 2 known HLA risk alleles (HLA-A*02:01 and HLA-DRB1*15:01), the risk of DILI due to AC was increased more than 13-fold. Given the rarity of serious liver injury due to AC, despite its widespread use, genotyping for risk management is probably not realistic. There may be instances when genotyping for this variant together with the identified HLA risk alleles could improve confidence in the causality assessment but this testing is not currently commercially available to our knowledge. It also should be noted that with drugs causing more frequent and severe liver injury, genotyping to identify 10% of a patient population at 13-fold increased risk of DILI might be reasonable.

In addition to the rs2476601 association, we also found evidence for a novel independent HLA risk factor in HLA-C*04:01. This association is interesting as the allele may be a risk factor across ethnicities and ADR clinical phenotypes. In fact, HLA-C*04:01 has previously been shown to be associated with nevirapine hypersensitivity in a Malawian population,[33] and in our cohort, the association was concordant across all three populations (Supplementary Table 8) and across multiple drugs and herbal preparations (Supplementary Table 7).

In conclusion, we have identified the variant rs2476601 in the PTPN22 gene as the first robust genetic risk association for DILI lying outside the MHC region. In addition, this association is the first to be associated across a broad range of implicated drugs and across different ethnic backgrounds. rs2476601 is therefore the first identified general risk association for DILI. The prior well-established association of this polymorphism with the risk of autoimmune diseases broadens the role of the immune system in DILI pathogenesis and may help inform future treatment and prevention efforts.

Appendix

Collaborators and Contributors to case recruitment

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DILIN investigators and coordinators can be found at http://www.dilin.org/publications/

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2019.01.034.

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Conflicts of interest
These authors disclose the following: Matthew R. Nelson is an employee of GlaxoSmithKline. Paola Nicoletti is an employee of Sema4. Naga Chalasani, Robert J. Fontana, and Paul B. Watkins report consulting agreements and research grants with several pharmaceutical companies but none represent potential conflicts for this paper. Thorunn Rafnar and Kari Stefansson are employees of deCODE genetics/Amgen. The DILIN causality committee considers potential conflicts while assigning cases for adjudication to individual investigators. The remaining authors disclose no conflicts.

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Supplementary Methods

Genome-Wide Association Study Quality Control for Each Cohort

Quality control was conducted at both single marker and subject levels before performing the SNP imputation. Any marker that did not pass the following criteria was excluded from analysis: (1) genotype call rate in the batch of subjects greater than 95%, (2) missing genotype rate greater than 5%, (3) P value for Hardy-Weinberg equilibrium greater than 10^{-7} in controls (if applicable). Any subject who did not pass the following criteria was excluded from analysis: (1) missing genotype rate < 0.05 among the SNPs that passed quality control; (2) not a sample duplicate or closely related based on estimated identity-by-descent using PLINK v 1.07

Imputation

We used the Michigan Imputation Server[1] to impute missing genotypes separately for each ethnicity and for each subset genotyped by the same genotyping platform. We used the options of SHAPEIT for phasing, the Haplotype Reference Consortium as the reference for European ancestry samples, and the 1000 Genomes Project as the reference for other ancestries.[1–5] Imputation methods are described in detail in the Supplementary Appendix. For HLA genotypes, 4-digit HLA alleles were inferred using HIBAG.[6] Sex chromosomes and mitochondria were not imputed. After imputation, the resulting calls were required to have $R^2 > 0.6$, maximum genotype posterior, and genotype missingness < 0.05 in each group and the overall cohort. Genotypes were discretized based on the probability (PP) > 0.9. Variants were also removed if they were found to be heavily influenced by genotyping chip, as determined by a logistic regression $P < .005$ for a difference between 2 chip types within the case or within the control cohort.

Icelandic Genetic Analysis

The current association analysis was done on 113 DILI cases and 239,304 population controls using software developed at deCODE genetics (Reykjavík, Iceland).[7] Genotypes of the Icelandic sample set were typed and then imputed as previously described.[7–9] The whole genomes of 15,220 Icelanders were sequenced, unveiling 40,780,213 SNPs and short indels. These variants were imputed into 151,677 Icelanders whose DNA had been genotyped with various Illumina SNP chips and phased using long-range phasing. Genealogical deduction of carrier status of 282,894 untyped relatives of chip-typed individuals further increased the sample size for association analysis. Logistic regression under an additive model was used to test for association between variants and disease, treating DILI as the response and expected genotype counts from imputation as covariates. Then those samples were used as reference for imputation of 155,250 Icelanders genotyped with chips. Using genealogic information, the sequence variants were also imputed into 282,894 relatives of the genotyped individuals.[10] HLA alleles were called for 28,075 Icelanders using whole-genome sequence data and Graphtyper.[11] Association testing with multiple explanatory variables was performed using the *glm* function in R.

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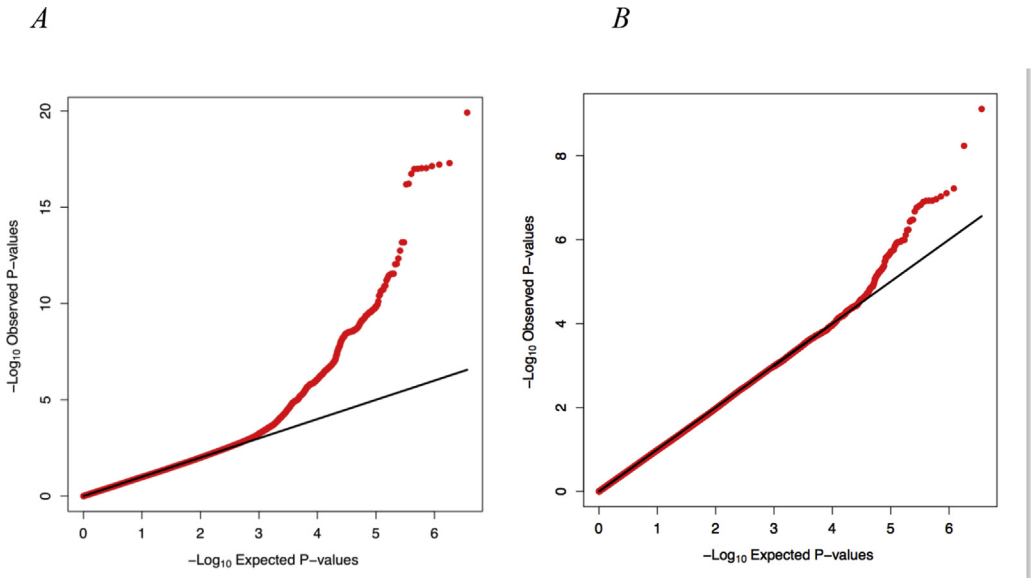
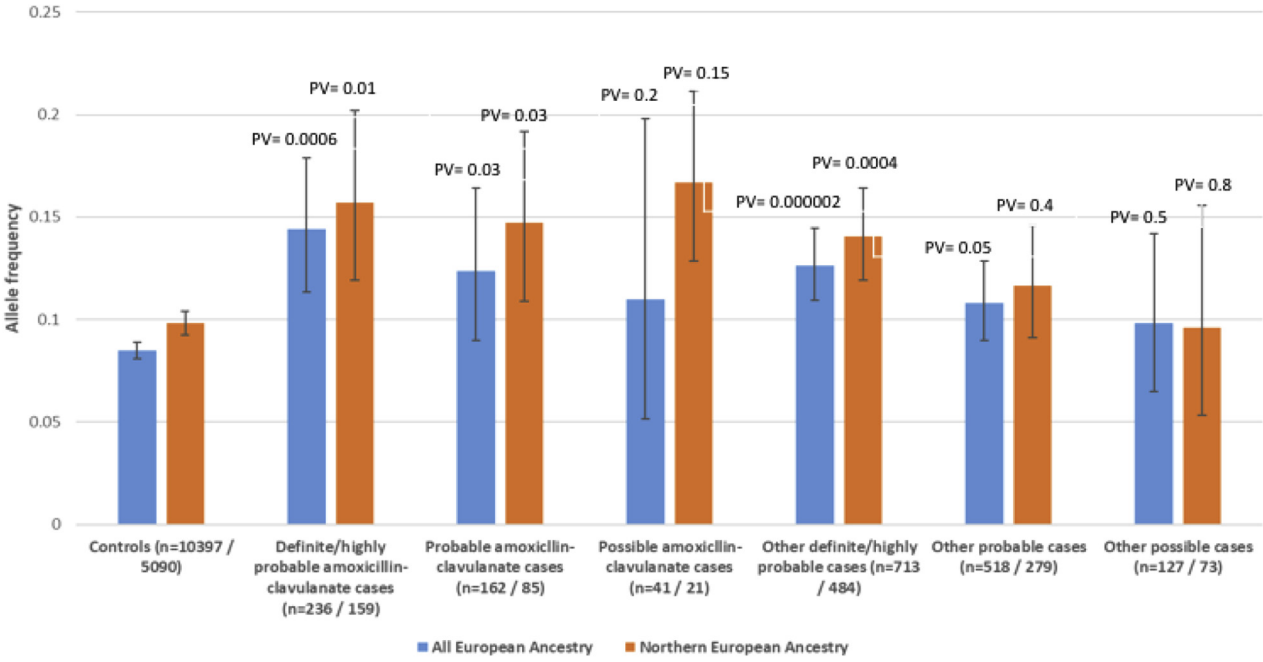


Supplementary Figure 1. QQ plots for (*A*) the overall original analysis and (*B*) conditional analysis.



Supplementary Figure 2. rs2476601 allele frequency across different subsets of our cohorts. Allele frequencies by causal drug and likelihood of DILI as described in methods. Error bars represent 95% CIs. The current stratification analysis based on causality score has been done dividing the cases by grouping DILIN "definite and highly likely" cases and iDILIC "highly probable" cases and grouping DILIN/iDILIC "probable" cases and grouping DILIN/iDILIC "possible" cases. PV, *P* value.