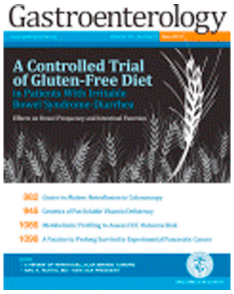

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Association of Liver Injury From Specific Drugs, or Groups of Drugs, With Polymorphisms in HLA and Other Genes in a Genome-wide Association Study

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on behalf of International DILI consortium (iDILIC), Drug-induced liver injury network (DILIN) investigators and International Serious Adverse Events Consortium (iSAEC)

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Abbreviations: DILI (drug-induced liver injury); genome wide association study (GWAS); Odd Ratio (OR); Roussel Uclaf Causality Assessment Method (RUCAM); Allele Frequency (AF); Hepatocellular (HC); Cholestatic-Mixed (CM); Expression quantitative trait loci (eQTL) Major Histocompatibility Complex (MHC); Minor allele frequency (MAF); Linkage Disequilibrium (LD); Human leukocyte antigen (HLA); Single Nucleotide Polymorphism (SNP)

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Abstract

BACKGROUND & AIMS: We performed a genome-wide association study (GWAS) to identify genetic risk factors for drug-induced liver injury (DILI) from licensed drugs without previously reported genetic risk factors.

METHODS: We performed a GWAS of 862 persons with DILI and 10588 population-matched controls. The first set of cases was recruited prior to May 2009 in Europe (n=137) or the USA (n=274). The second set of cases were identified from May 2009 through May 2013 from international collaborative studies performed in Europe, the USA and South America. For the GWAS, we included only cases of European ancestry associated with a particular drug (but not flucloxacillin or amoxicillin-clavulanate). We used DNA samples from all subjects to analyze human leukocyte antigen (HLA) genes and single nucleotide polymorphisms (SNPs). After the discovery analysis was concluded, we validated our findings using data from 283 European patients with diagnosis of DILI associated with various drugs.

RESULTS: We associated DILI with rs114577328 (a proxy for A*33:01 a HLA class I allele; odds ratio [OR], 2.7; 95% CI, 1.9–3.8; *P*=2.4x10–8) and with rs72631567 on chromosome 2 (OR, 2.0; 95% CI, 1.6–2.5; *P*=9.7x10–9). The association with A*33:01 was mediated by large effects for terbinafine-, fenofibrate-, and ticlopidine-related DILI. The variant on chromosome 2 was associated with DILI from a variety of drugs. Further phenotypic analysis indicated that the association between DILI and A*33:01 was significant, genome wide, for cholestatic and mixed DILI, but not for hepatocellular DILI; the polymorphism on chromosome 2 associated with cholestatic and mixed DILI as well as hepatocellular DILI. We identified an association between rs28521457 (within the *LRBA* gene) and only hepatocellular DILI (OR, 2.1; 95% CI, 1.6–2.7; *P*=4.8x10–9). We did not associate any specific drug classes with genetic polymorphisms, except for statin-associated DILI, which was associated with rs116561224 on chromosome 18 (OR=5.4; 95% CI, 3.0–9.5; *P*=7.1x10–9). We validated the association between A*33:01 terbinafine- and sertraline-induced DILI. We could not validate the association between DILI and rs72631567, rs28521457, or rs116561224.

CONCLUSIONS: In a GWAS of persons of European descent with DILI, we associated HLA-A*33:01 with DILI due to terbinafine and possibly fenofibrate and ticlopidine. We identified polymorphisms that appear to be associated with DILI from statins, as well as 2 non–drug-specific risk factors.

KEY WORDS: medication, liver damage, side effect, anti-fungal agent

Introduction

Hepatotoxicity is the second most common cause of drug attrition during development as well as for post-marketing withdrawal,[1] and idiosyncratic drug-induced liver injury (DILI) accounts for 11%-17% of cases of acute liver failure in the United States and Europe.[2, 3] The typical incidence of DILI varies from approximately 1% with the anti-tumor necrosis factor agents[4] to 0.04% with some widely used antimicrobials such as amoxicillin-clavulanate.[5] During the past 15 years, increasing progress on identifying genetic risk factors for DILI has been made. In particular, associations with HLA class I and II alleles have been reported for DILI caused by a range of drugs, though a particular HLA genotype does not appear to be relevant to all forms of idiosyncratic DILI.[6]

Previously, GWAS involving cohorts of DILI cases related to one particular drug only have resulted in identification of one or more drug-specific HLA risk alleles.[7-11] A large study involving 783 DILI cases due to a range of different drugs also resulted in a genome-wide significant HLA signal, but this association was abolished once 296 cases of DILI due to flucloxacillin and amoxicillin-clavulanate were excluded.[12] This partly reflects the fact that amoxicillin-clavulanate is a very common cause of DILI worldwide and flucloxacillin is an equally common cause in a number of Northern European countries.[13] Therefore, DNA collections from DILI cases generally will be highly enriched in cases relating to these two drugs, making detection of associations related to other compounds more difficult.

We have expanded our previous study of DILI caused by a range of different drugs,[12] and after excluding cases relating to amoxicillin-clavulanate and flucloxacillin, we have more than doubled the number of cases with additions from Europe, Australia, South America and the United States. We now report that HLA-A*33:01 is associated with risk of DILI, particularly due to terbinafine, fenofibrate and ticlopidine and especially with a cholestatic or mixed phenotype. We have also found novel non-major histocompatibility complex (MHC) related signals apparently shared across a range of different drugs; an intronic SNP, in the LPS-responsive vesicle trafficking, beach and anchor containing (LRBA) gene is associated with hepatocellular DILI and an intergenic SNP on chromosome 2, rs72631567, with DILI generally. An additional drug-specific genome-wide significant signal which could not be confirmed is also reported.

Materials and Methods

DILI discovery cohort

The cases in the study were from two separate recruitment phases. Phase I consists of 411 cases included in a previous study (from DILIN, DILIGEN and Eudragene)[12] and phase II more recently recruited cases (n=451) of which a small subset was included in a recent report.[14]

Phase I cases. These cases included 413 DILI cases not due to amoxicillin-clavulanate or flucloxacillin, with a defined casual drug and with causality score greater than possible (RUCAM score≥3) recruited in Europe (n=137) or the USA (n=274) prior to 2009. Clinical characteristics of these cases and methods used for genotyping have been described in detail previously.[12] Additional exome chip analysis (Illumina Infinium HumanCoreExome BeadChip) was performed on 150 of these 413 cases at the Broad Institute, Boston.

Phase II case recruitment-iDILIC. The iDILIC cases were recruited between May 2009 and May 2013 as part of an international collaborative study involving recruitment centers in the United Kingdom (Newcastle, Nottingham, Liverpool, London, Dundee), Sweden (Uppsala and Gothenburg), Spain (Malaga and Barcelona), France (Montpellier), the Netherlands (Utrecht), Germany (Kiel), Australia (Brisbane), Switzerland (Zurich), Finland (Helsinki), Argentina (Rosario), Uruguay (Montevideo) and Chile (Santiago). All participants provided written informed consent and each study had been approved by the appropriate national or institutional ethical review boards. For the GWAS, only cases of European ancestry where there were at least 2 cases due to a particular drug available (when phase I cases from Europe and the USA were also considered) and where the DILI was not due to either flucloxacillin or amoxicillin-clavulanate were included (n=339). Clinical inclusion criteria for all cases were those described by Aithal et al.[15]

Phase II case recruitment-DILIN. Details of the USA-based DILIN prospective study including IRB approval information have been described previously.[16] A total of 112 eligible new cases of European ancestry and ≥ 18 years were included in the current GWAS. These new cases were selected from the larger DILIN sample collection such that only cases relating to drugs also included among the iDILIC cases were represented. Laboratory inclusion criteria were as described previously.[16] 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.

Additional cases used for confirmation of associations

After the discovery analysis was concluded, we enrolled an additional 283 European patients with diagnosis of DILI across multiple causal drugs (6 from iDILIC and 277 from DILIN networks recruited subsequent to the GWAS). The causal drug distribution is reported in Table S1A. An additional 12 statin DILI samples from the Spanish iDILIC network and 3 UK-DILIGEN cases were recruited later in the study to confirm the class specific association (Table S1B).

Out of the 283, we used 272 DILI cases to directly type SNPs associated across multiple drugs or specific for drugs/drug classes and 11 DILI cases for HLA typing to confirm HLA associations. An additional Chinese terbinafine DILI sample was also HLA typed.

Causality assessment

The iDILIC 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)[15] and by expert review by a panel of three hepatologists. The pattern of liver injury was classified according to the International Consensus Meeting Criteria.[17] Only cases having at least possible causality (score ≥3) were included in the study. For all cases in DILIN, causality assessment was by expert consensus as previously described.[16]

Controls

Since DILI has a very low prevalence, we used general population samples as study controls. We selected 10588 European ancestry controls from multiple available sources; Welcome Trust Case Control Consortium (WTCCC) (http://www.wtccc.org.uk), the population reference sample (POPRES)[18], PGX40001[19] and Spanish Bladder cancer cohort (phs000346.v1) from dbGAP.[20] In order to increase the case/control ratio for Italian, Spanish and Swedish, we added samples from Hypergenes cohort (http://www.hypergenes.eu/dissemination.html#pub), the National Spanish DNA Bank (http://www.bancoadn.org/), Italian Penicillin Tolerant Controls (IPTC), and the Swedish Twin Registry (http://ki.se/en/research/the-swedish-twin-registry).

Genotyping

DNA preparation from Phase II cases. For iDILIC cases, DNA was prepared as described previously.[8] DILIN DNA was extracted from lymphocytes and stored at the NIDDK biosample repository at Rutgers University, Piscataway, NJ.

Genome-wide analysis. Genome-wide genotyping of the phase II and 150 phase I cases was performed by the Broad Institute, Boston by Illumina Infinium HumanCoreExome BeadChip. iDILIC and DILIN cases were genotyped in two separate batches. A total of 505740 markers shared across the batches passed quality control (QC) and no samples were excluded for low quality profile. (see Supplemental Materials and Methods). Details on the genotype data available for each control collection are reported in Table S2.

Imputation. SNP imputation was performed in batches dividing the cohorts according to genotyping platforms. Imputation methods are described in detail in the Supplementary Appendix. For HLA genotypes, four digit HLA alleles were inferred using HIBAG.[21]

SNP genotyping. The top associated imputed SNPs were validated by SNP genotyping in subsets of iDILIC cases and in the overall DILIN cohort (see Supplementary Appendix). The SNPs were further confirmed in additional cases using TaqMan® predesigned and custom SNP genotyping assays (ThermoFisher Scientific, Waltham, MA) in accordance with the manufacturer's recommendations.

HLA genotyping. High resolution genotyping of *HLA-A, B, C, DRB1, DQA1* and *DQB1* was performed on selected cases by Histogenetics (Ossining, New York). Sequencing data files were analyzed using Histogenetics’ proprietary analysis software (Histomatcher and HistoMagic) for HLA genotype calling. Allele assignments are based on IMGT/HLA Database release version 2.21.0, dated April 2008 (http://www.ebi.ac.uk/imgt/hla/).

Statistical analysis

The effect of population structure was assessed through principal components analysis (PCA) using the smartPCA program from the EIGENSTRAT package (version 3.0).[22] Single marker and haplotype association analyses and heterogeneity test analyses were carried out by PLINK.[23] The statistical association of each marker, HLA alleles and SNPs, was determined in a logistic regression framework with scores for the first seven principal components as covariates under an additive model using PLINK. We used the same statistical test for sub-population analyses, using two, seven and ten most significant principal components as covariates in Italian, Spanish and North European populations, respectively. We set the genome-wide traditional significance p-value threshold to $5.0\text{x}10^{-8}$ to correct for multiple testing.[24] When we obtained genome-wide significant signals, we tested for independent effects from the neighboring variants by including the most associated variants as a covariate and then testing the significance of others in the region. We also tested interaction effects among them by including interaction terms in the logistic regression. Differences in clinical characteristics among sample groups were tested by Fisher's exact test. All detailed analyses and Manhattan plots were performed with R (Version 3.0.2).[25] Regional plots were drawn by LocusZoom.[26]

Results

Clinical characteristics of the cases

Clinical details of the DILI cases included in the main GWAS are summarized in Table 1. A variety of different causative drugs were represented but the most common was diclofenac with 67 cases, followed by nitrofurantoin with 64 cases. A few drugs, including azathioprine, isoniazid, fenofibrate, and diclofenac had significantly disproportionate number of cases in one of the two recruitment phases. Details of all the causative drugs are shown in Table S3.

Overall analysis

The discovery cohort included 862 European ancestry DILI cases (411 from phase I[12] and 451 from Phase II) and 10,588 controls. PCA showed that all cases (including those from South America) clustered within three major groups (Italian, Spanish and Northern European) and matched with the population controls (Figure S1A). Consistent with the previous study,[12] phase I cases were predominantly Northwest European. The most significant genome-wide associated SNPs were rs72631567 on chromosome 2 (OR=2.0, 95% CI =1.6-2.5, p-value=$9.7x10^{-9}$) and rs114577328 in the MHC region of chromosome 6 (OR=2.7, 95% CI=1.9-3.8, p-value=$2.4x10^{-8}$)(See Figure 1A, Table 2 and Figures S2 and S3). Data for both SNPs had been obtained by imputation in cases and controls and subsequently validated by SNP typing (see Supplementary Methods). The associations were consistent among geographic clusters and study phases (Table S4) and not due to artefact/s of population structure, missing genotypes rate (Table S5) or variability in imputation quality among populations or genotyping platforms (see Supplementary Methods).

For the chromosome 2 SNP rs72631567, breakdown by drug showed that 10 unrelated drug causes had an OR greater than 2.0 with at least two carriers (Table S6). Ciprofloxacin-related cases showed the strongest association (n=21, OR=7.4, 95% CI=17.3-161, p-value = $4.0x10^{-6}$).

The chromosome 6 SNP rs114577328 is the SNP proxy of an uncommon HLA class I allele, HLA-A*33:01. Indeed this SNP was in near-perfect LD with A*33:01 (r^2= 0.98). From the imputed HLA allele assignments, a strong association with DILI for this allele is confirmed (OR=2.6; 95% CI=1.8-3.7, p-value=$8.0x10^{-8}$, Figure S4). Including rs114577328 or A*33:01 as a covariate removed any association in the MHC region, indicating that there is only one MHC association signal (Figure S5). The A*33:01 association appears independent of the chromosome 2 signal, since rs72631567 when conditioned on A*33:01 showed an almost unchanged effect size ($OR_{rs72631567}$ = 1.7, 95% CI= 1.25-2.2, p-value = 0.0006). There was no statistically significant interaction effect between the two signals (p-value = 0.5).

Breakdown by drug showed DILI due to terbinafine was most strongly associated with the HLA-A*33:01 signal (OR=40.5, 95% CI=12.5-131.4, p-value=$6.7x10^{-10}$) and a similarly strong association was seen with rs114577328 (OR=58.7, 95% CI=18.31-188.2, p-value=$7.3x10^{-12}$, Figure 1B and Figure S6). As summarized in Table 3, in addition to terbinafine cases, cases due to six additional drugs showed an association with A*33:01 with p-values lower than 0.01. The largest case subset related to terbinafine but we found that A*33:01 was also a risk factor for ticlopidine (OR=163.1, 95% CI=16.2-1642.0, p-value=0.00002), methyldopa (OR=97.8, 95% CI=12.3-743.0, p-value=0.00001) and fenofibrate DILI (OR=58.7; 95% CI=12.3-279.8; p-value=$3.2x10^{-7}$). Indeed, although fewer positive carriers were observed, A*33:01 also seems to be a common risk factor for enalapril (OR=34.8), sertraline (OR=29) and erythromycin DILI (OR=10.2). An erythromycin case was positive for A*33:03, an allele rare in European population controls (AF=0.002) which belongs to the A*33 group. Overall we found that 87% (n=36) of the A*33:01 positive carriers were also positive for HLA-B*14:02 and HLA-C*08:02. The haplotype showed a larger OR than A*33:01 as single marker in terbinafine ($OR_{haplotype}$ =49.2, p-value=$9.54x10^{-11}$), ticlopidine ($OR_{haplotype}$ =201; p-value= $7.2x10^{-6}$), fenofibrate ($OR_{haplotype}$ 68.5; p-value=$1.1x10^{-7}$) and erythromycin ($OR_{haplotype}$ = 13.1; p-value=0.002) DILI but not with DILI as a phenotype ($OR_{haplotype}$ =2.7; p-value= $1.6x10^{-7}$, Table S7).

We verified the imputed A*33:01 genotype by sequence-based HLA typing in 35 cases related to the main A*33:01-associated drugs (Table S8). The A*33:01 predictions were confirmed in all cases except that one methyldopa case was negative for this allele (false positive) and an additional terbinafine case was a carrier (false negative). This validation result suggests that methyldopa might not share the HLA risk factor. The validation confirmed that all the A*33:01-positive terbinafine cases carried the complete HLA A*33:01-B*14:02-C*08:02 haplotype, increasing the strength of the haplotype association in the terbinafine DILI cases ($OR_{haplotype}$=70; p-value=$8.7x10^{-13}$) and in the overall analyses ($OR_{haplotype}$=2.8; p-value=$5.1x10^{-8}$). We also typed the A*33:01 proxy SNP across DILIN cases to confirm imputed genotypes. We found only one new carrier of the minor allele, not related to the major-A*33:01 associated drugs.

Analysis by type of injury and causative drugs

We further investigated the association of genotypes with particular patterns of DILI by grouping the cases into hepatocellular (HC) and cholestatic/mixed (CM) pattern. The chromosome 2 association described above was similar in the two phenotypic categories (direct comparison between CM cases vs HC cases, logistic p-value=0.5), although the effect was marginally stronger in the CM cases (Table 2). The association with rs114577328 was genome-wide significant only in the CM cases (n=323, OR=5.3, 95% CI=3.4-8.2, p-value=$4.5x10^{-14}$, Figure 2B and Figure S6) and similarly with A*33:01 (OR=5.1; 95% CI=3.3-7.9, p-value=$4.2x10^{-13}$, Figure 2B and Figure S6). Conditional analysis on the variant and HLA allele indicated only one genetic association was present in the region, as shown for the main analysis (Figure S7). There was no association between the proxy SNP or A*33:01 in the HC cases (n=474, OR for A*33:01=1.5, 95% CI=0.82-2.6, p-value=0.19, Table 2). The A*33:01-B*14:02-C*08:02 haplotype showed an $OR_{haplotype}$ = 5.6; p-value = $2.5x10^{-13}$ in CM cases.

The CM only terbinafine-specific OR increased two fold compared with the value for all terbinafine cases (OR=88.1, 95% CI = 19.3-402.4, p-value = $7.5x10^{-9}$) since all the A*33:01 carriers belonged to this injury type. Following the injury correlation pattern established for terbinafine, A*33:01 appeared to be a stronger risk factor for CM injury than for HC injury also for fenofibrate, ticlopidine, enalapril and erythromycin-related DILI. This was not the case for injury due to sertraline and methyldopa. These top seven drugs account for 51% (n=21) of all A*33:01 positive cases (Table S9). Sixteen other drugs account for the rest of the carriers showing slight enrichment in CM phenotypes, which showed a marginal association with A*33:01 (OR = 2.6, 95% CI = 1.4-4.9, p-value = 0.003, Table S9 and Table S10).

We detected a new HC-specific genome-wide significant signal on chromosome 4 (Figure 2B). The signal lies within the *LRBA* (LPS-responsive vesicle trafficking, beach and anchor containing) gene with the imputed variant rs28521457, located in an intronic region, the most significant SNP (OR=2.1, 95% CI=1.6-2.7, p-value=$4.8x10^{-9}$)(Table 2 and Figure 2B). The allele frequency for this SNP in the CM cases (0.04) was comparable to that in controls with no evidence of association with this phenotype. The risk allele was carried by more than 4% of the HC cases in cases due to a total of 45 drugs but in general, there were no drug-specific signals (Table S11).

We also investigated associations with particular causative drugs or specific therapeutic classes where a group including more than 40 samples was available. Detail on the groups studied is summarized in Table S12. Genome-wide significance was detected only for one group examined, the statins, with no significant signals for the other drug classes (Figure S8 and Table S13). In the case of the statins, rs116561224, a common intergenic SNP on chromosome 18, was genome-wide significant (OR=5.4, 95% CI=3.0-9.5, p-value=7.1×10^{-9}, Figure 2C and Figure S9) with the signal mainly driven by simvastatin (Table S14).

Confirmation of associations

The European cohort used to confirm the associations (n=283) had a wider range of causal drugs, mostly different from the discovery cohort (Table S1). Later in time, we had access to 15 additional cases relating specifically to the statin cohort.

The A*33:01 association was further investigated in the additional cases by directly genotyping rs114577328 in 272 cases and by direct HLA typing on 11 additional samples who developed DILI due to drugs for which we had detected an enrichment in A*33:01 alleles in the discovery cases. Overall, the rs114577328 carriers were enriched in cases from drugs previously associated with the allele (Table S15). Eight out of all 23 additional cases relating to drugs previously associated with A*33:01 were shown to carry this allele or the proxy SNP (allele frequency 0.17) compared with an expected population frequency of 0.01. We specifically confirmed the association of A*33:01/rs114577328 with terbinafine having a carrier frequency of 0.63 (5 out 8 terbinafine-related cases across both the injury types) and with sertraline at a carrier frequency of 0.75 (3 out 4 sertraline-related cases) (Table S15). Although fenofibrate had a high carriage rate for A*33:01 in the discovery cohort, none of the 7 additional cases carried this allele or the proxy SNP. Few additional cases were available for other A*33:01-related drugs to confirm the association.

Interestingly, a terbinafine DILI case from Finland was positive for A*33:05, a very rare allele in the general European population (AF = 0.0001, *USA NMDP European Caucasian* in http://www.allelefrequencies.net/, n=1,242,890) and Finnish[27] populations. An additional terbinafine DILI case of Chinese origin was positive for A*33:03. In total, 10 of the 24 additional cases (23 European cases and one single Chinese case) were carriers of an A*33 allele, in line with expectations based on the effects observed in the discovery sample.

We further genotyped rs72631567 and rs28521457 in 272 additional European cases. The rs72631567 and rs28521457 variants were found at AFs comparable to those for controls ($AF_{rs72631567}$ = 0.022 and $AF_{rs28521457}$ in HC only = 0.025) and so the association was not confirmed. However, rs72631567 carriers were slightly enriched in ciprofloxacin, atorvastatin and mercaptopurine-induced DILI cases, as in the discovery cohort, with ORs in the same direction in both cohorts (Table S16). Similarly, rs28521457 carriers seemed to be more common in the same subgroup of causal drugs in both cohorts (Table S17). This suggested a limited replication of the signal for these drugs.

We also attempted to confirm the rs116561224 signal for statins. The number of additional cases available for this purpose was small (n=29, Table S1b) with only four simvastatin cases. None of the statin cases were positive for rs116561224 so the signal could not be confirmed.

Discussion

Our previous studies have been successful in identifying genetic risk factors for both flucloxacillin and amoxicillin-clavulanate DILI.[4, 6] However, our most recent GWAS did not identify any risk factors that were common for DILI in general or specific genetic risk factors for DILI due to individual drugs which accounted for a smaller number of cases of DILI.[12] The current study included 451 additional cases of DILI due to a wide variety of causative drugs, including at least 10 DILI cases relating to each of 22 different drugs. This increase in numbers and the exclusion of the amoxicillin-clavulanate and flucloxacillin cases together with use of improved imputation methods has enabled the detection and confirmation of a novel genome-wide significant signal relating to a relatively rare HLA class I allele A*33:01. Though three other interesting signals were detected in the course of the study, an intergenic signal on chromosome 2, an intronic SNP in *LRBA* in HC cases only and a signal on chromosome 18 for statins, the failure to confirm these signals is a limitation. There are some indications that, as observed for HLA-A*33:01, the chromosome 2 and LRBA signals are shared across multiple unrelated drugs instead of being non-drug-specific risk variants. As supporting evidence, the chromosome 2 signal has been consistently associated in both replication and discovery cohorts with DILI due to ciprofloxacin, atorvastatin and mercaptopurine. There remains a possibility that replication could be achieved in a larger study involving a different mix of causative drugs but the degree of heterogeneity in drugs originally associated with the signals also increases the risk that these were chance observations.

Interestingly, unlike previously recognized HLA associations for DILI, A*33:01 also appears to be a risk factor for DILI due to several, structurally unrelated drugs. Our results also suggest that a haplotype comprising A*33:01, B*14:02, and C*08:02 may participate in concert to confer risk for DILI, as opposed to A*33:01 alone. However, because these alleles are so highly correlated, our current sample size does not allow us to distinguish between these possible explanations by genetic association evidence alone. This conceivable hypothesis could be further verified in a larger study, or by experiments with recombinant HLA proteins.[28]

In the case of terbinafine where the A*33:01 association showed genome-wide significance for cases relating to this drug only, information on the underlying mechanism for hepatotoxicity is limited, *N*-dealkylation leads to the formation of an aldehyde metabolite, TBF-A, and this metabolite shows reactivity with glutathione.[29] It has been proposed that the GSH-adduct is transported across the canalicular membrane and concentrated in the bile where it may cause damage to biliary epithelial cells. There is limited data from the various case reports on an underlying inflammatory mechanism but it has been demonstrated that treatment of monocytes with terbinafine results in the release of the proinflammatory cytokines IL-8 and TNF-alpha.[30] Metabolism of terbinafine is complex involving several different cytochromes P450.[31] However, there was no evidence from the GWAS for a role for either CYP genes or innate immunity genes in the terbinafine DILI cases studied.

The other drugs showing the most convincing associations with A*33:01 were fenofibrate, ticlopidine and sertraline. Failure to see individual genome-wide significant associations with these drugs is likely to be due to fewer cases being available than for terbinafine. The A*33:01 association was seen for 3 of 7 cases due to fenofibrate, all with CM DILI. The literature on fenofibrate DILI is quite limited, but it appears that this drug is extensively metabolized, mainly by CYP3A4,[32] and there is a report of a drug interaction resulting in cholestatic injury[33] together with other isolated reports of idiosyncratic cholestatic DILI.[34]

Ticlopidine-related DILI has been well studied previously, including two studies investigating genetic risk factors in Japanese individuals. Cholestatic liver injury also predominates in this form of DILI.[35, 36] Ticlopidine is subject to extensive metabolism by several cytochrome P450 isoforms and carboxyesterase.[37] A study in rats suggests that adducts are formed following metabolism by cytochrome P450 with evidence for toxicity after biliary excretion of glutathione-conjugated metabolites via MRP2-facilitated transport.[38] In previous studies, 22 Japanese patients with ticlopidine DILI showed an association with an HLA haplotype including A*33:03 (odds ratio 13).[39] In line with current observations, the association was strongest with cholestatic cases with 12 out of 14 cases positive for A*33:03. It should be noted that A*33:03 is relatively common in Japan with approximately 10 to 15% of individuals carrying this allele.

The observations on the HLA association for Japanese ticlopidine DILI cases were followed up by a report that those carrying a -2320T>C polymorphism in *CYP2B6* were more susceptible to ticlopidine DILI due to high CYP2B6 expression (OR 2, p-value=0.04).[40] The *CYP2B6* polymorphism (rs7254579)[41] is less frequent in Europeans than in Asians (MAF_{cau} = 0.29, MAF_{asian} = 0.45) and its low effect size limited our ability to replicate the association in this small European ticlopidine DILI population, but the non-significant effect is in the same direction as the previous study (OR=2.8, 95% CI=0.7-10.18, p-value=0.11).

In contrast with the three drug examples above, sertraline is associated predominantly with HC DILI.[42-44] In line with this phenotypic association, there is evidence that sertraline can cause mitochondrial damage[28] and induce endoplasmic reticulum stress[45] in liver cells. There are parallels with a previous example of a HLA risk factor (DRB1*15:01) which is associated with predominantly CM DILI with amoxicillin-clavulanate but HC DILI with lumiracoxib.[9, 10]

In line with the Japanese report of a role for A*33:03 in ticlopidine DILI[39] and a case report showing an association between A*33 and tiopronin-induced cholestasis in a Chinese patient,[46] we found two DILI cases positive for A*33:03 after direct HLA typing. One of these was a Chinese terbinafine DILI case and the second a European-American with erythromycin DILI. In our imputed GWAS dataset, *A*33:03* was carried by eight DILI cases due to a range of drugs in our cohort and showed an apparent risk effect for CM DILI but this was not genome-wide significant (OR=2.1, 95% CI=1.02-4.6, p-value=0.04). Another A*33 allele, A*33:05, was also represented in a terbinafine case from Finland. There is very strong homology between these three HLA-A*33 alleles at the protein sequence level with *A*33:03* differing at only two positions from A*33:01 (Tyr instead of His at position 171 and Lys instead of Arg at position 186) and A*33:05 differing at only one position (Arg at 54 in place of Gln). In particular, all three alleles conserve the key residues for specific peptide binding within the B and F pockets.[47] This is in contrast to a related HLA allele A*31:01, which is associated with carbamazepine-induced skin rash,[48] but does not appear to be a risk factor for DILI, where the B pocket sequence, though homologous, is not conserved.[47]

As mentioned above, the association of a common HLA allele with DILI due to chemically-unrelated compounds had been observed previously for DRB1*15:01[10] with amoxicillin-clavulanate and lumiracoxib and for DRB1*07:01[49] with DILI from lapatinib and ximelagatran. The association of A*33:01 with DILI in general and secondary to a number of structurally dissimilar compounds is consistent with these observations. Together with recent findings from *in vitro* studies on T-cell responses to flucloxacillin and amoxicillin-clavulanate,[50, 51] these observations support the hypothesis that either the parent drug or metabolites bind covalently to cellular or circulating proteins to form adducts in a mechanism that is probably slightly different to the direct drug effect seen with hypersensitivity reactions to abacavir.[52] Adduct formation may then allow binding to the peptide binding groove of HLA molecules leading to activation and differentiation of T-cells with a consequent adaptive immune response-mediated liver injury. Evidence that the majority of the drugs showing the A*33:01 association undergo hepatic metabolism and biliary excretion may explain the stronger association of A*33:01 with CM DILI and could indicate that, unlike in the case of flucloxacillin and amoxicillin-clavulanate, metabolites contribute to the toxicity mechanism. Further investigation of potential interaction of both the various drugs and their metabolites with the A*33:01 gene product by molecular modelling and *in vitro* studies on T cells as previously undertaken for flucloxacillin[50] would be of interest.

The novel association of HC DILI with *LRBA* is interesting because this gene is a biologically plausible DILI candidate. LRBA deficiency due to rare mutations is associated with primary immunodeficiency of variable severity with a particular feature of decreased regulatory T cell (Treg) levels, other immunodeficiencies and inherited autoimmune disease.[53-56] Patients with mutations in LRBA leading to immunodeficiency have been demonstrated to show loss of cytotoxic T lymphocyte antigen-4 (CTLA4).[57] Studies in a mouse model suggest that low CTLA4 is a risk factor for DILI.[58] Unlike the HLA-A*33:01 association, no genome-wide significant associations for single drugs were detected with the *LRBA* SNP and there were no obvious features in common between cases positive for the variant other than the HC phenotype. It remains possible that this association could be replicated if a larger cohort were available.

The intergenic signal on chromosome 2 is from a region 800 kb upstream from *SOX11*, is independent of *A*33:01* and associated with an almost two-fold risk of DILI with the top SNP showing a frequency of 0.02 in Europeans. This risk factor seems to be shared across unrelated drugs among which ciprofloxacin showed the strongest association. The ENCODE project suggests there are no regulatory elements in this region so the basis for the signal is unclear. Neither rs72631567 nor any of its LD SNPs (r^2>0.5) are known eQTL variants (http://www.gtexportal.org/). The failure to confirm this association and the absence of any apparent biological basis suggests the observed significance could have been a chance finding.

Most data for individual drug classes that were comparatively well represented in our cohort were entirely negative but the finding of a signal for statins which was driven by several class members was entirely novel. Similar to the more general signal seen on chromosome 2, the chromosome 18 is intergenic with the closest known gene, cadherin 19 located approx. 300000 bp downstream. Although functionally such a protein could be of relevance to the liver injury process,[59] any biological significance seems tenuous. The failure to confirm the signal in additional cases could be due to the availability of only a small cohort of additional cases which reflects the rarity of this form of DILI.[60]

In conclusion, this study has detected a novel HLA association (HLA-A*33:01*)* in cases of DILI due to a number of different drugs, together with several novel non-HLA signals. Overall sensitivity and specificity of the A*33:01 allele as a predictor of DILI is low but our findings may be important for future drug treatment in cases of

DILI due to one of the drugs for which the A*33:01 association is relevant. Follow-up studies are required to further explore the intergenic signal on chromosome 2, the biologically interesting signal in *LRBA* and the rs116561224 signal for statins in larger cohorts.

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Figure legends

Figure 1 Manhattan plots displaying the association results of (A) the overall analysis (n=864); (B) terbinafine only cases (n=14 cases). SNPs in green have a significance level less than $5x10^{-6}$ and red have a significance level less than $5x10^{-8}$.

Figure 2 Manhattan plot displaying the association results for (A) Cholestatic/Mixed only cases (n=323); (B) Hepatocellular only cases (n=474 cases); (C) Statin cases (n=59). SNPs in green have a significance level less than $5x10^{-6}$ and red have a significance level less than $5x10^{-8}$.

Tables

Table 1. Clinical details of the DILI cases included in the GWAS

CHARACTERISTICS	**Phase 1** (n=411)	**Phase 2** (n=451)	**Combined** (n=862)
Clinical information			
Age (mean years)	51	54	53
% Female	63.0%	60.5%	61.8%
ALT (mean IU/L)	895.1	757.7	822.2
ALP (mean IU/L)	388.2	282.5	330.6
Latency (mean days)	201.7	177.4	188.2
Injury type			
Cholestatic	76	87	163
Hepatocellular	202	272	474
Mixed	69	91	160
Not available*	64	1	65
Genotype chip			
Illumina 1 M	261		261
Illumina 1M/Illumina Infinium HumanCoreExome BeadChip	150		150
Illumina Infinium HumanCoreExome BeadChip		447	447
Illumina HumanOmniExpress BeadChip		4	4
Country of birth			
USA	274	112	386
UK	71	79	150
Spain	16	95	111
Sweden		81	81
France	30	7	37
Germany		20	20
Italy	16	1	17
Others	4	56	60

*Because of the retrospective nature of the phase I study, minimal clinical information needed to establish the type of injury were not available for a subset of initial NSAID DILI cases from DILIGEN because of missing ALP and upper limit of normal values.

Table 2. Association effect size of rs72631567, A*33:01 and rs28521457 across different liver injury patterns

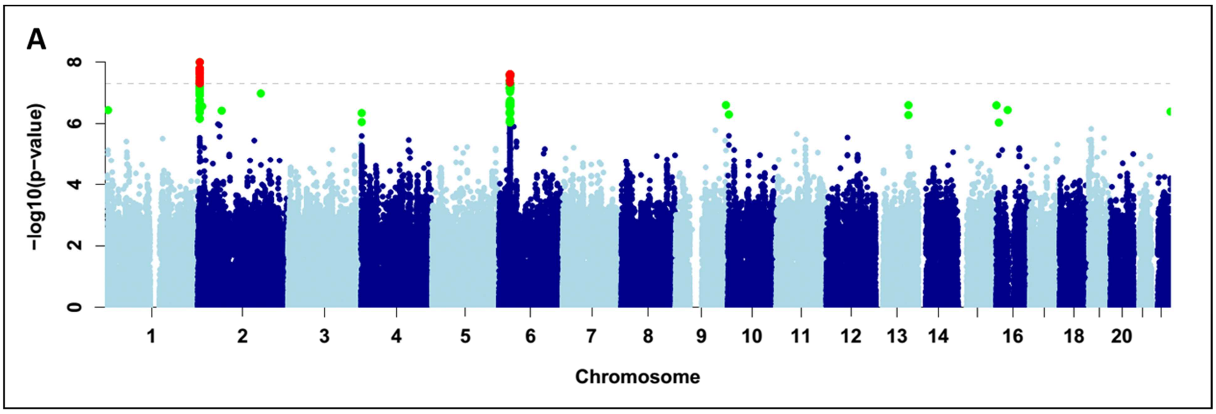
COHORTs	Variant	OR	95% CI	P	AF Cases	AF Controls
Entire DILI cohort	**rs72631567**	2.0	1.6-2.5	$9.7x10^{-9}$	0.05	0.03
	A*33:01	2.6	1.8-3.7	$7.0x10^{-8}$	0.02	0.01
	rs28521457	1.5	1.3-1.9	$7.0x10^{-8}$	0.06	0.04
Cholestatic and Mixed DILI cohort	**rs72631567**	2.4	1.7-3.4	$9.5x10^{-7}$	0.06	0.03
	A*33:01	5.0	3.3-7.9	$4.2x10^{-13}$	0.04	0.01
	rs28521457	1.0	0.7-1.5	0.9	0.04	0.04
Hepatocellular DILI cohort	**rs72631567**	1.6	1.2-2.3	$2.5x10^{-3}$	0.04	0.03
	A*33:01	1.5	0.8-2.6	0.19	0.01	0.011
	rs28521457	2.1	1.6-2.7	$4.8x10^{-9}$	0.08	0.040

COHORTs = type of comparison; Variant = associated variant; OR=Odds Ratio; 95% CI = 95% Confidence Interval of the odds ratio; P=logistic p-value; AF = allele frequency

Table 3. Association effect size of A*33:01 signal for the causal drugs enriched in A*33:01 positive carriers

DRUGs	**Number of cases tested**	**Number of A*33:01 alleles in cases**	**OR**	**95% CI**	**P**	**CF**
TICLOPIDINE	5	4	163.1	16.2-1642	0.00002	0.8
METHYLDOPA	4	2	97.8	12.8-743.8	0.00001	0.5
FENOFIBRATE	7	4	58.7	12.3-279.8	$3.2*10^{-7}$	0.43
TERBINAFINE	14	6	40.5	12.5-131.4	$6.7*10^{-10}$	0.43
ENALAPRIL	4	2	34.8	3.9-302.9	0.001	0.5
SERTRALINE	5	2	29	4-207.2	0.0008	0.4
ERYTHROMYCIN	10	2	10.2	2-51.7	0.005	0.2

DRUGs=Causal drug involved; OR=Odds Ratio; 95% CI = 95% Confidence Interval of the odds ratio; P=logistic p-value; CF= Carrier Frequency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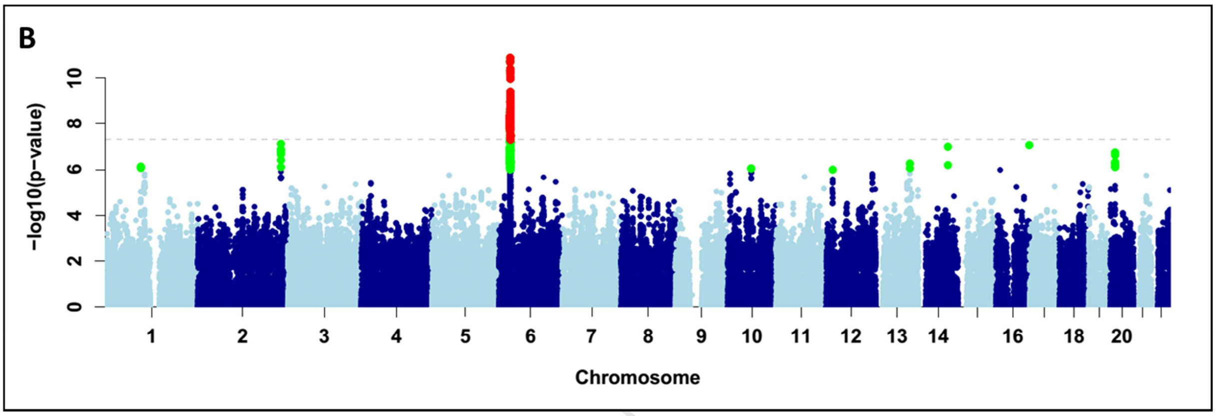
Chromosome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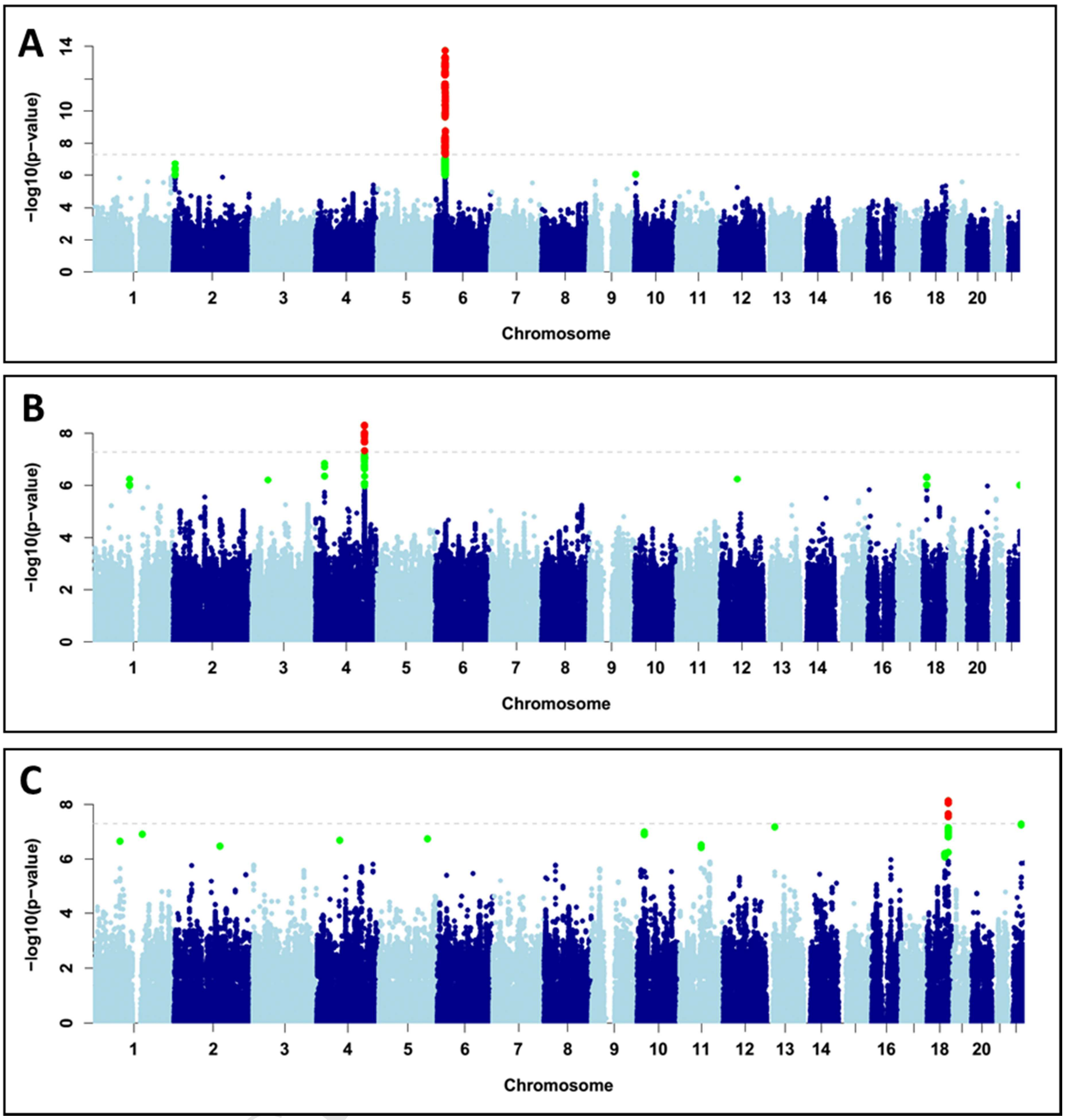
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Supplementary Appendix

Supplement to:

Association of Liver Injury From Specific Drugs, or Groups of Drugs, With Polymorphisms in HLA and Other Genes in a Genome-wide Association Study

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Supplementary Methods

Imputation

The imputation was performed in batches dividing the cohorts according to genotyping platforms. For each batch, we first phased the data by SHAPEIT (version v2.r727),[1-2]. Then, imputation was carried out using IMPUTE2 (version 3) with 1000 Genomes Project (release v3[3]) ethnically mixed dataset as the reference panel.[4] [5] We retained imputed genotypes with: (a) posterior probability > 0.9 in each genotyping batch, (b) no significant difference in missingness between cases and controls ($\chi 2$ test, p-value > 0.0001), (c) no significant deviation from Hardy-Weinberg expectations (p-value > 0.0001), (d) no variants missing greater than 5% of genotypes in any single genotyping batch and (e) info score greater than 0.8 in each genotyping batch, (f) MAF in the 1000 Genomes Project ≥ 0.01. Batch effects for imputed SNPs were corrected by testing for association between ethnically-matched controls typed by different platforms (using logistic regression). SNPs with association p-values less than 0.005 were excluded from the analysis. For each cohort, four digit HLA alleles were also inferred using HIBAG[6] with the reference predictor panels specific for each genotyped chip.[7]

Genome-wide association study QC for each cohort

QC 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: (i) genotype call rate in the batch of subjects greater than 95%, (ii) missing genotype rate greater than 5%, (ii) p-value for Hardy-Weinberg equilibrium greater than 10^{-7} in controls (if applicable). Any subject that did not pass the following criteria was excluded from analysis: (i) missing genotype rate < 0.05 among the SNPs that passed QC; (ii) not a sample duplicate or closely related based on estimated identity-by-descent (IBD) using PLINK v 1.07

Quality controls on the A*33:01 association

To assess whether the A*33:01 signal was an artefact of population structure, we tested population-specific association. Although the allele is rarer in Northern Europeans, with no difference between Sweden and the UK (Allele Frequency $(AF)_{uk} = 0.004$; $AF_{sw} = 0.003$), the OR was comparable across the three major clusters (Table S3). The heterogeneity test cannot reject the null hypothesis ($\chi 2$ method p-value of 0.06). We also confirmed that the association was not due to synthetic differences in imputation performance relating to the genotype chips. The AF was comparable across the three control groups genotyped by different platforms ($AF_{1M} = 0.01$, $AF_{HEC} = 0.018$, $AF_{OE} = 0.013$). Logistic regression to test for differences between genotype platforms among control samples showed no difference in the Spanish (p-value=0.44) or Italian (p-value=0.9) subsets.

Validation of the predicted genotypes

We validated the predictions of the most associated SNPs within the discovery samples by matching the predicted and the typed genotypes. We calculated the concordance rate as the percentage of accurately predicted genotypes over the total number of samples typed in the validation based on specimen’s availability. In particular, the rs72631567 genotypes were validated in 564 discovery cases (386 DILIN cases and 178 iDILIC) with respectively 99.7% and 100% concordance. Both the rs114577328 and rs28458792 genotypes were fully validated in 386 DILIN cases with 100% concordance. The two most associated SNPs in the statin comparison, rs116561224 and rs28458792, were typed in 25 iDILIC statin cases and rs116561224 only was typed in 378 DILIN samples across multiple causal drugs. The concordance was 100% for iDILIC cases and 97.6% for DILIN cases. In both cohorts this genotyping was performed by TaqMan® predesigned and custom SNP genotyping assays (ThermoFisher Scientific, Waltham, MA) in accordance with the manufacturer's recommendations.

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6. Zheng X, Shen J, Cox C, et al. HIBAG--HLA genotype imputation with attribute bagging. Pharmacogenomics J 2014;14:192-200.
7. Browning SR, Browning BL. Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. Am J Hum Genet 2007;81:1084-97.

Supplementary Figures

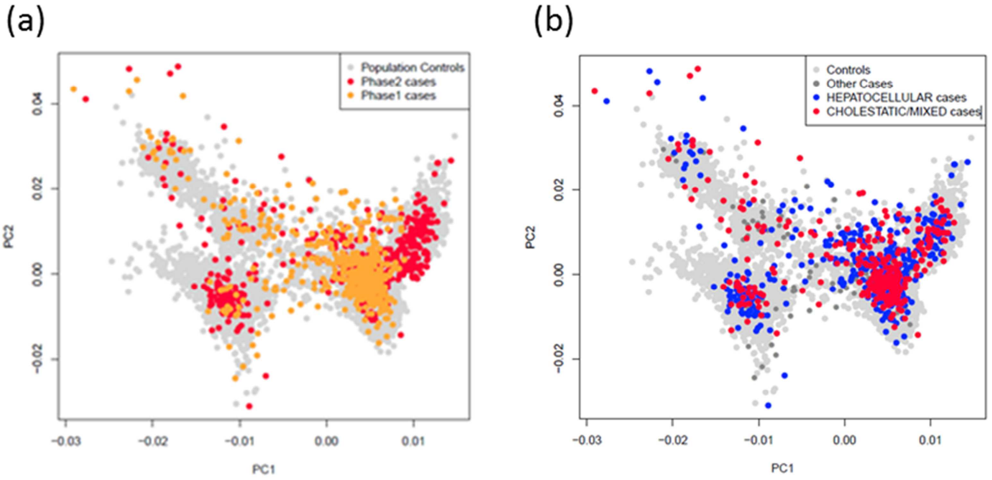

Figure S1. Scatterplots representing the first two principal components of the current study cohort. The homogenous distribution between cases and controls across the three major European clusters is shown. In panel (a) cases from phase II are highlighted in red and the cases from phase I in orange. In panel (b) the cholestatic/mixed cases are highlighted in red and the hepatocellular cases in blue.

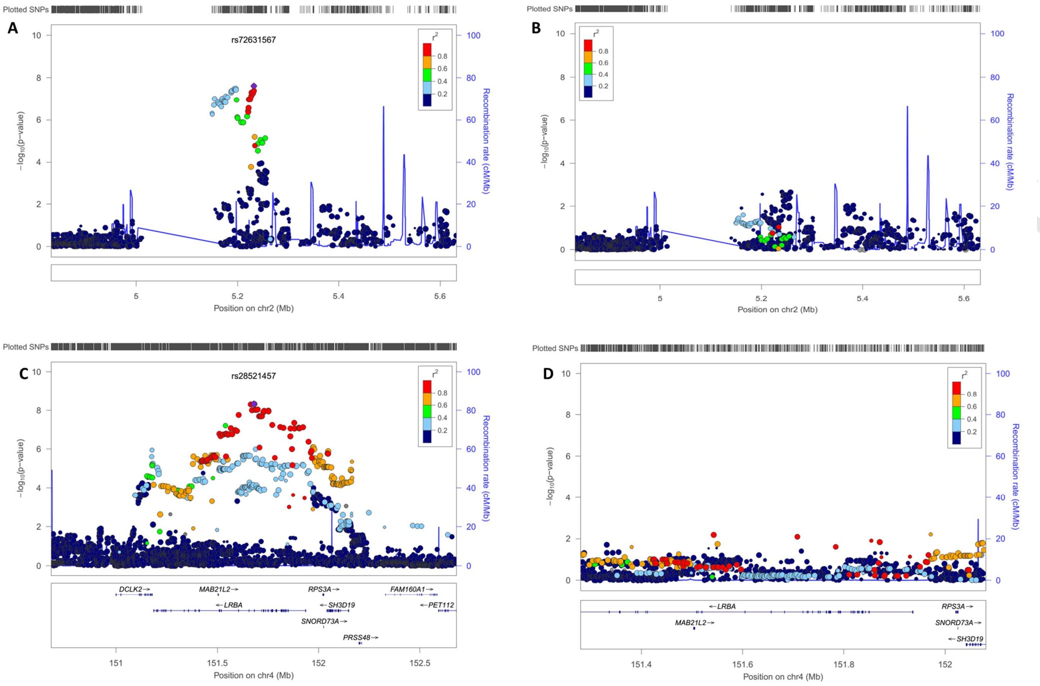

Figure S2. Regional Manhattan plots for chromosome 2 and 4 in the region of the rs72631567 and rs28521457 signals. (A) Chromosome 2 for the overall cohort, (B) Chromosome 2 for the overall cohort conditioned on rs72631567. (C) Chromosome 4 for the HC only cohort, (D) Chromosome 4 for the HC only cohort conditioned on rs28521457.

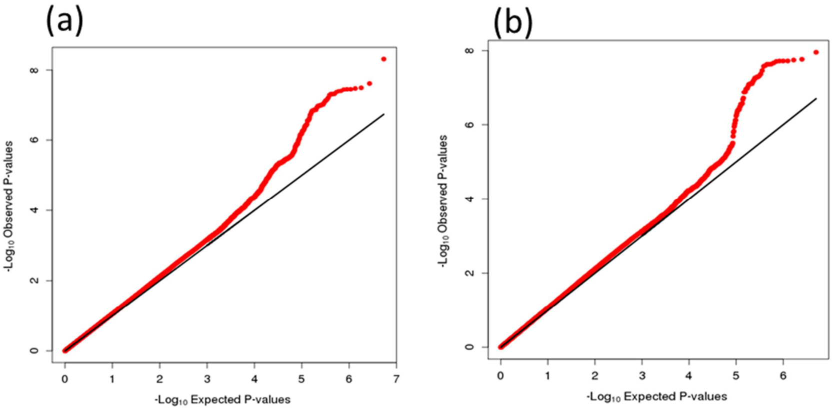

Figure S3. QQ plots. (a) for the overall original analysis (b) after eliminating variants in MHC region. The QQ plot in the (b) panel highlights the signal on chromosome 2. The inflation factor is 1.05 after correction and 4.65 before correction.

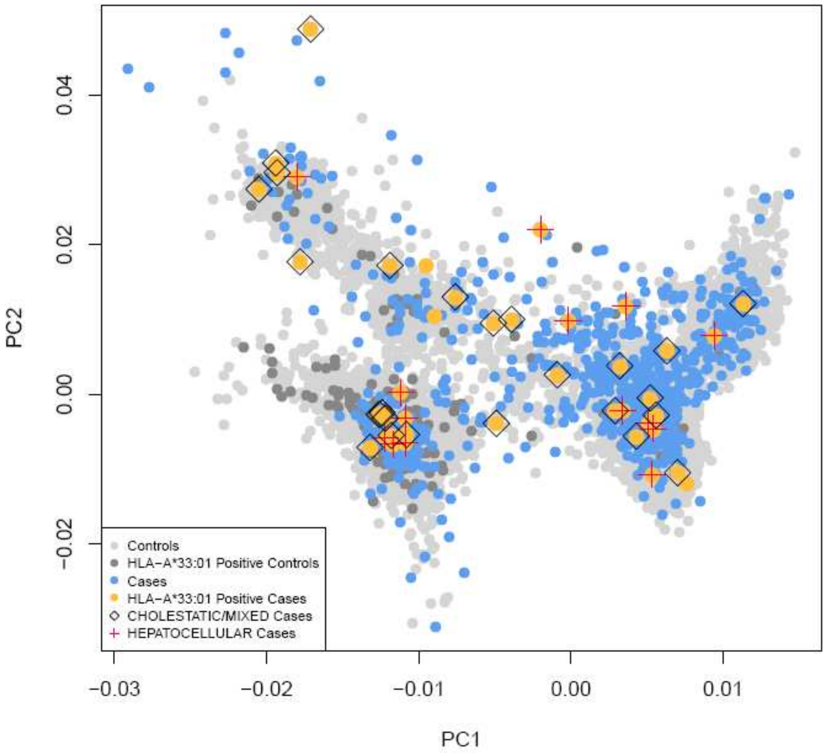

Figure S4. Scatterplot representing the first two principal components of the current study cohort. The A*33:01-positive cases and their injury type are highlighted. A*33:01-positive cases are homogenously distributed in all the major population clusters.

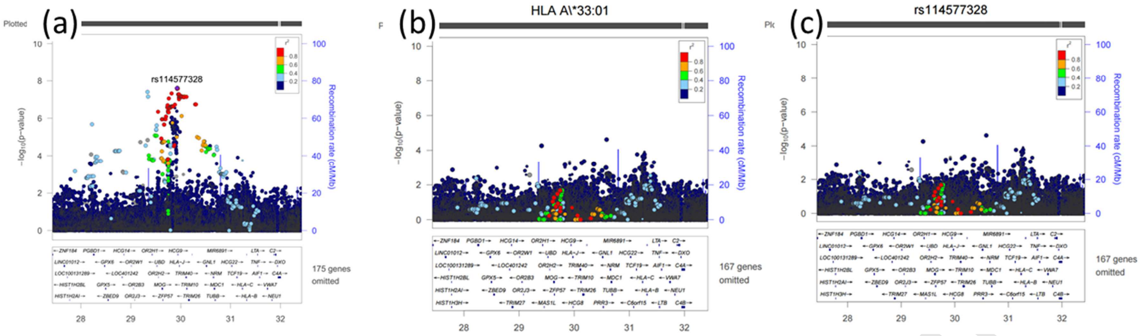

Figure S5. MHC Regional Manhattan plots for (a) the overall cohort (b) the same cohort conditioned on A*33:01 (purple dot) and (c) the same cohort conditioned on rs114577328 (purple dot).

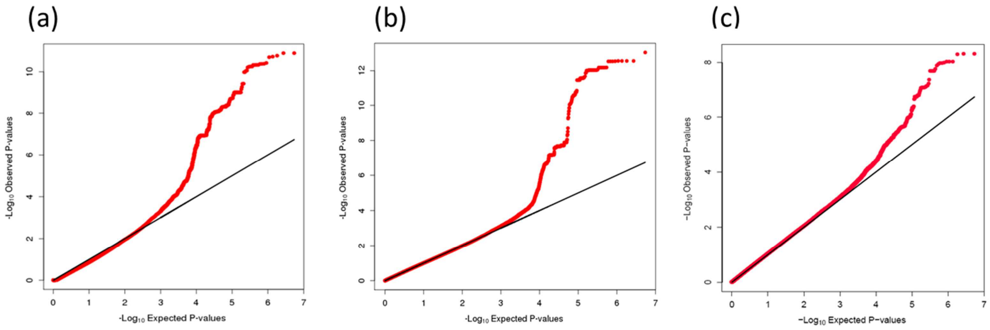

Figure S6. QQ plot for (a) Terbinafine cases only, (b) Cholestatic/Mixed cases only, (c) Hepatocellular cases only.

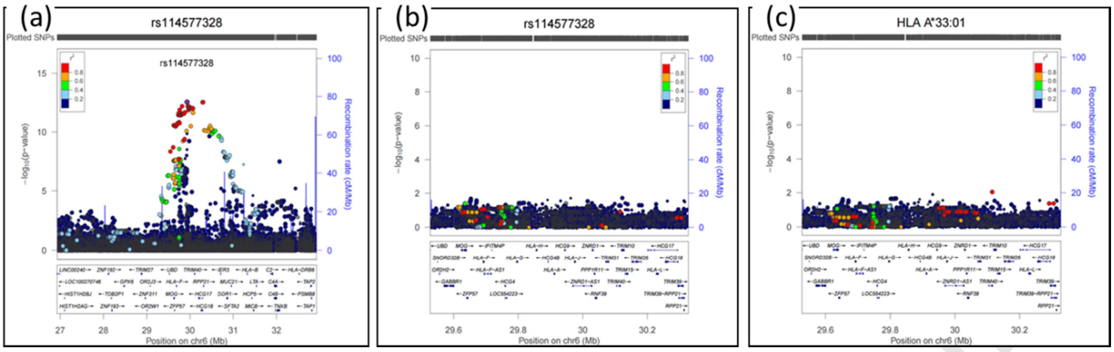

Figure S7. MHC Region Manhattan plots for (a) all cholestatic/mixed cases (b) the same cases conditioned on rs114577328 (purple dot), the top SNP (c) the same cases conditioned on A*33:01 (purple dot), the top HLA allele

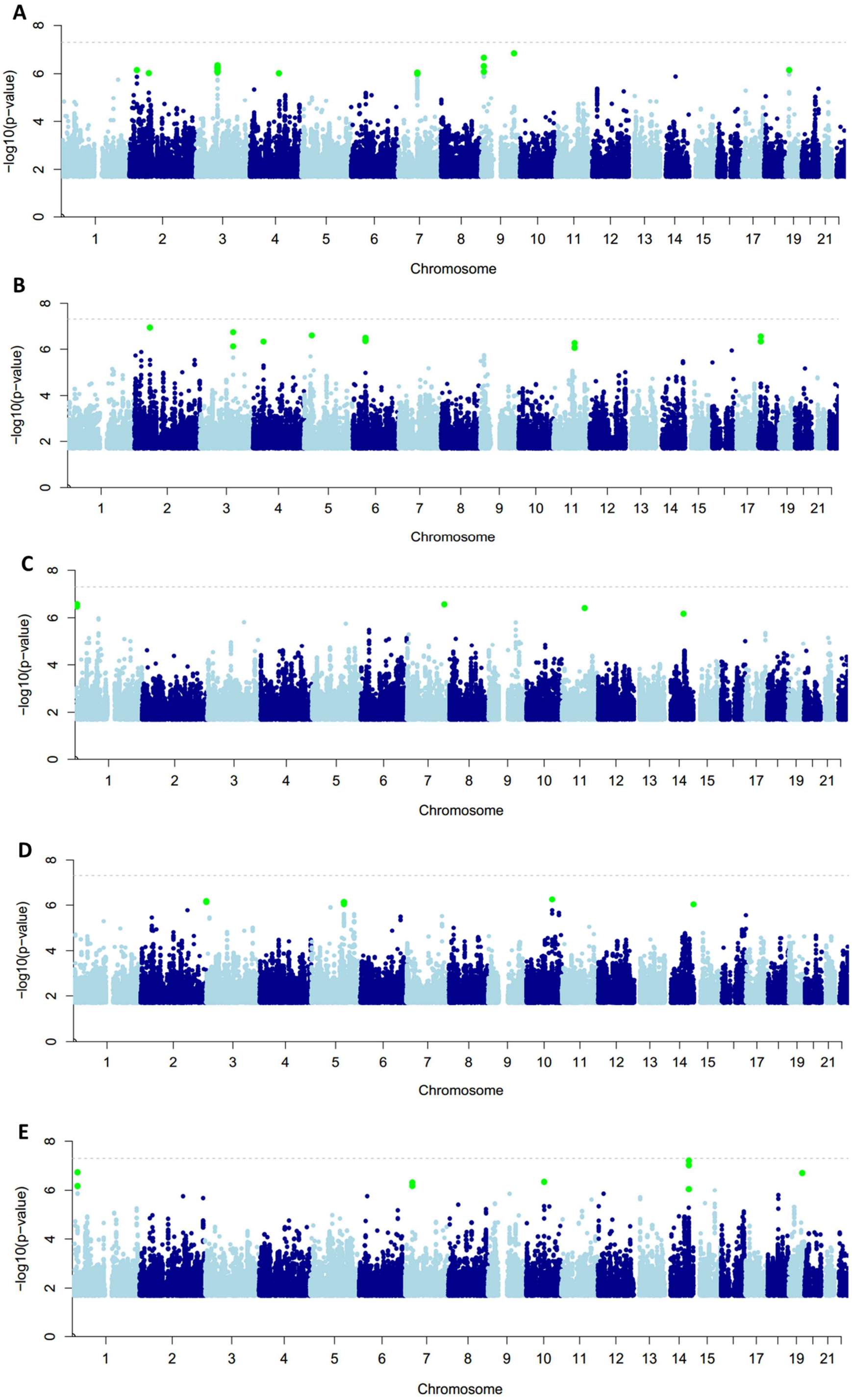

Figure S8. Manhattan plots for DILI due to (A) Anti-TB drugs, (B) Fluoroquinolones, (C) NSAIDs, (D) Diclofenac (E) Nitrofurantoin.

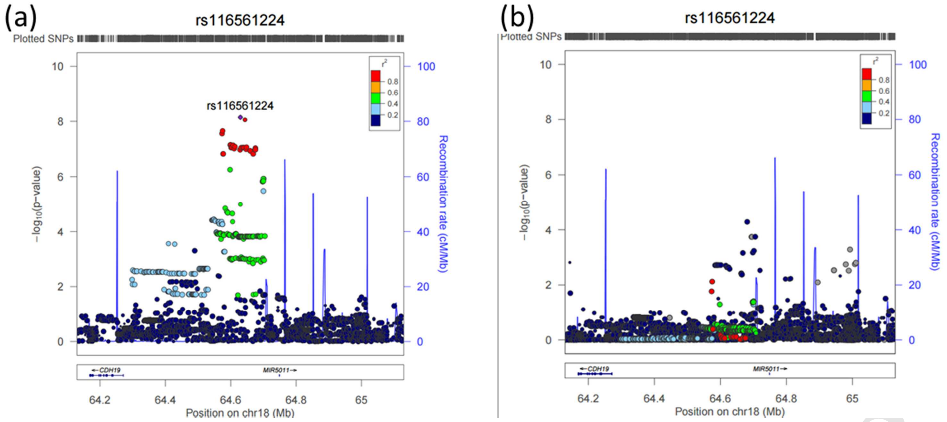

Figure S9. Regional Manhattan plots for chromosome 18 in the area of the rs116561224 signal for (a) statin-induced DILI cases and (b) the same cases with conditioning on rs116561224

Table S1. Causal drugs in the replication cohort.

A

DRUG	PD	SNP typing	HLA typing	TOT
Sulfamethoxazol	yes	17		17
Nitrofurantoin	yes	15		15
Isoniazid	yes	11		11
Ciprofloxacin	yes	9		9
Azathioprine	yes	8		8
Atorvastatin	yes	8		8
Minocycline	yes	7		7
Infliximab	yes	7		7
Cefazolin	yes	6		6
Terbinafine	yes	6	2	8
Levofloxacin	yes	6		6
Azithromycin	yes	5		5
Carbamazepine	yes	5		5
Mercaptopurine	yes	4		4
Fenofibrate	yes	4	3	7
Oxaliplatin		4		4
Lisinopril		4		4
Methylprednisolone		3		3
Exemestane		3		3
Flavocoxid		3		3
Metformin		3		3
Pravastatin	yes	3		3
Sulfasalazine		3		3
Vancomycin		3		3
Sertraline	yes	1	3	4
Methyldopa	yes	1	2	3
Erytromycin	yes	-	1	1
Others (98 drugs)		123		123
TOTAL		272	11	283

B

DRUG	PD	DILIN	iDILIC	TOT
Atorvastatin	yes	8	8	16
Pravastatin	yes	3	3	6
Fluvastatin	yes		1	1
Rosuvastatin	yes	1		
Lovastatin	yes	1		
Simvastatin	yes	1	3	4
Total		**14**	**15**	**27**

DRUG = causal drug; PD=presence of the drug in the discovery cohort; TOT = total number of cases. The list in A panel includes only drugs for which at least 3 cases were available. The category "other" includes a total of 98 different drugs. The drugs highlighted in bold are the drugs found to be associated with A*33:01.

Panel A shows the breakdown of causal drugs for the replication cohort and the methods utilized to replicate the main results. 272 DILIN samples underwent to direct SNPs typing while 12 samples whose DILI was due to A*33:01-associated drugs underwent HLA typing. Panel B shows the breakdown of causal drugs for the statin-specific replication cohort: besides the 14 samples previously collected within 272 DILIN samples we add extra 15 iDILIC statin cases.

Table S2. Genotyping details for the DILI control cohorts

COHORT	#SAMPLEs	CHIP
Welcome Trust Case Control Consortium (WTCCC)	4824	Illumina 1M BeadChip
Spanish Cohort (phs000346.v1)	2077	Illumina 1M BeadChip
Hypergenes	901	Illumina 1M BeadChip
Swedish Twin Registry	1499	Illumina HumanOmniExpress BeadChip
National Spanish DNA Bank	209	Illumina 1M Duo/Illumina Infinium HumanCoreExome BeadChip
	173	Illumina Infinium HumanCoreExome BeadChip
iSAEC Italian Penicillin Tolerant Controls	147	Illumina HumanOmniExpress BeadChip
PGX40001	103	Illumina 1M Duo BeadChip
POPulation REference Sample (POPRES)	655	Illumina 1M Duo BeadChip

Table S3. Causative drugs across the overall DILI cohort

DRUGs	Phase1	Phase2	Combined	P
DICLOFENAC	29	38	67	0.37
NITROFURANTOIN	28	36	64	0.44
ISONIAZID	16	20	36	0.61
AZATHIOPRINE	6	21	27	<0.01
MINOCYCLINE	10	17	27	0.24
SULFAMETHOXAZOLE/TRIMETHOPRIM	12	14	26	0.84
ATORVASTATIN	4	19	23	<0.01
CIPROFLOXACIN	5	16	21	0.03
NIMESULIDE	12	8	20	0.37
VALPROICACID	14	4	18	0.02
SIMVASTATIN	5	12	17	0.14
ISONIAZID/PYRAZINAMIDE/RIFAMPIN	6	9	15	0.80
IBUPROFEN	4	10	14	0.18
TERBINAFINE	4	10	14	0.18
AZITHROMYCIN	3	10	13	0.09
CEFAZOLIN	7	6	13	0.79
ISONIAZID/PYRAZINAMIDE/RIFAMPIN/ETHAMBUTOL	6	6	12	1.00
LEVOFLOXACIN	5	6	11	1.00
PHENYTOIN	9	2	11	<0.01
ERYTHROMYCIN	2	8	10	0.11
IMATINIB	2	8	10	0.11
CELECOXIB	9		9	<0.01
MERCAPTOPURINE	5	4	9	0.75
NAPROXEN	2	7	9	0.18
METHIMAZOLE	2	6	8	0.29
ANABOLIC STEROID	7		7	<0.01
CARBAMAZEPINE	2	5	7	0.28
DULOXETINE	7		7	<0.01
ESTRADIOL/LEVONORGESTREL	2	5	7	0.45
FENOFIBRATE	4	3	7	0.72
FLUVASTATIN	2	5	7	0.45
METHOTREXATE	3	4	7	1.00
MOXIFLOXACIN	2	5	7	0.45
ROFECOXIB	4	3	7	0.72
TELITHROMYCIN	7		7	<0.01
DISULFIRAM	1	5	6	0.22
FLUPIRTIN		6	6	0.03
INFLIXIMAB	1	5	6	0.22
LAMOTRIGINE	5	1	6	0.12
DOXYCYCLINE	2	3	5	1.00
OMEPRAZOLE	2	3	5	1.00
PIROXICAM	3	2	5	0.68
ROSUVASTATIN	1	4	5	0.37
SERTRALINE		5	5	0.06
TICLOPIDINE	1	4	5	0.37
ENALAPRIL	1	3	4	0.62
METHYLDOPA	4		4	0.06
MONTELUKAST	1	3	4	0.62
NICOTINICACID	4		4	0.06
PRAVASTATIN	2	2	4	1.00
SEVOFLURANE	1	3	4	0.62
VENLAFAXINE		4	4	0.12
OTHER	135	71	204	<0.01
TOTAL	**411**	**451**	**862**	-

Phase 1 = number of cases extracted from previous DILI study (Urban TJ et al. Pharmacogenet Genomics 2012;22:784-95 (reference 12 in main text); Phase 2 = number of new cases; Combined = total number of cases; P = Fisher's Exact test p-value to test the disproportions between the two cohorts.

The list includes only drugs for which at least 4 cases were available. The category "other" includes a total of 140 different drugs.

Table S4. Effect of the A*33:01, rs72631567 and rs28521457 signals across populations and recruitment phases

Marker	COHORTs	PHASE	OR	95% CI	P	AF Cases	AF Controls
A*33:01	European DILI cohort	I	2.29	1.31-4	0.003	0.02	0.01
		II	2.96	1.94-4.52	$4.7*10^{-7}$	0.03	-
	Cholestatic and Mixed DILI cohort	I	4.73	2.38-9.38	$8.4*10^{-6}$	0.03	-
		II	5.76	3.36-9.88	$1.8*10^{-10}$	0.04	-
	North European Cohort	I +II	2.64	1.48-4.69	0.001	0.01	0.00
	Spanish Cohort	I +II	1.96	1.02-3.75	0.04	0.05	0.03
	Italian Cohort	I +II	5.69	2.43-13.33	$6.1*10^{-5}$	0.07	0.01
rs72631567	European DILI cohort	I	2.02	1.45-2.82	$3.6*10^{-5}$	0.05	0.03
		II	1.99	1.45-2.71	$1.1*10^{-5}$	0.05	-
	North European Cohort	I +II	1.65	1.215-2.236	0.001	0.04	0.03
	Spanish Cohort	I +II	2.12	1.21-3.75	0.009	0.06	0.03
	Italian Cohort	I +II	3.21	1.57-6.52	0.001	0.09	0.03
rs28521457	European DILI cohort	I +II	1.56	1.26-1.94	$5.0*10^{-5}$	0.06	0.04
	Hepatocellular DILI cohort	I	2.48	1.74-3.54	$4.6*10^{-7}$	0.09	-
		II	1.93	1.32-2.65	0.0003	0.07	-
	North European Hepatocellular DILI	I +II	1.91	1.41-2.56	$2.0*10^{-5}$	0.07	0.04
	Spanish Hepatocellular DILI	I +II	2.25	1.2-4.23	0.01	0.08	0.02
	Italian Hepatocellular DILI	I +II	1.93	0.44-8.46	0.38	0.08	0.04

PHASE=recruitment phase; OR=Odds ratio; 95%CI=95% Confidence Interval; P=logistic p-value; AF=Allele Frequency

Table S5. Missing genotypes rate for the most associated SNPs within case and control groups in the comparisons where SNPs were significant

SNP	**CHR**	***missing rate in cases***	***missing rate in controls***	***P***
rs114577328	6	0	0.0002	1.0
rs72631567	2	0.007	0.005	0.3
rs28521457	4	0	0.0009	1.0

P= X^2 p-value of missing genotype rate between cases and controls

Table S6. Causative drugs across the rs72631567 signal on chromosome 2

DRUGS	No. Cases Tested	OR	95% CI	P	AF
FLUVASTATIN	7	10.15	2.58-39.85	0.0009	0.21
FENOFIBRATE	7	8.85	1.80-43.63	0.0074	0.17
LAMOTRIGINE	6	9.40	1.53-57.61	0.02	0.17
CIPROFLOXACIN	21	7.41	3.16-17.36	$4.0\text{x}10^{-6}$	0.14
ISONIAZID/PYRAZIN	12	5.80	1.70-19.75	0.005	0.14
AZITHROMYCIN	13	4.92	1.39-17.39	0.01	0.12
MERCAPTOPURINE	9	4.16	0.51-34.08	0.2	0.11
ATORVASTATIN	23	3.33	1.16-9.55	0.02	0.09
ISONIAZID	36	3.42	1.45-8.07	0.005	0.08
NITROFURANTOIN	64	2.33	1.08-5.0	0.03	0.06
CONTROL	10588	-	-	-	0.03

OR=Odds Ratio; 95%CI=95% confidence interval; P=logistic p-value; AF=Minor Allele Frequency in cases;

Table S7. Summary of A*33:01-B*14:02-C*08:02 haplotype specific analysis across study cohorts

DRUGs	***OR***	***P***	***HF CAs***	***HF CTLs***
OVERALL ANALYSIS	2.7	$1.8x10^{-7}$	0.02	0.009
CHOLESTATIC-MIXED ANALYSIS	5.7	$3.9x10^{-13}$	0.04	
TERBINAFINE	49.2	$9.5x10^{-11}$	0.21	
TICLOPIDINE	201.0	$7.2x10^{-6}$	0.40	
FENOFIBRATE	68.5	$1.1x10^{-7}$	0.29	
ERITHROMYCIN	13.1	0.002	0.10	
ENALAPRIL	11.4	0.1	0.13	
METHILDOPA	41.5	0.002	0.13	
SERTRALINE	11.6	0.04	0.10	

HF CAs=Haplotype Frequency in cases; HF CTLs=Haplotype Frequency in controls; OR=Odds Ratio; P=logistic p-value

Table S8. Summary of the validation by direct HLA typing

	Predictions	Validations		
COHORT	**#subjects (#CARRIERs)**	**# subjects**	**Not-CARRIERs (FN)**	**CARRIERs (FP)**
TERBINAFINE	14 (6)	13	6 (1)	6 (0)
FENOFIBRATE	7 (3)	6	3 (0)	3 (0)
METHYLDOPA	4 (2)	3	2 (0)	0 (1)
SERTRALINE	5 (2)	3	3 (0)	-
ENALAPRIL	4 (2)	2	1 (0)	1 (0)
ERYTHROMYCIN	10 (2)	6	6 (0)	-
TICLOPIDINE	5 (4)	2	-	2 (0)

FN=False Negative; FP=False Positive

Table S9. The A*33:01 signal across the main six A*33:01-associated drugs by type of injury

DRUG	**TI**	**No. Cases Tested**	**OR**	**95% CI**	**P**
TICLOPIDINE	CM*	3	93.5	18.78-465.9	2.37E-05
	HC	2	36.5	1.77-750.9	0.02
METHYLDOPA	CM	-	-	-	-
	HC	3	54.4	4.7-635.7	0.001
FENOFIBRATE	CM	7	58.7	12.3-279.8	$3.2*10^{-7}$
	HC	0	-	-	-
TERBINAFINE	CM	9	88.1	19.28-402.4	7.57E-09
	HC	5	-	-	-
ENALAPRIL	CM*	3	46.8	8.52-256.6	1.65E-03
	HP	1	-	-	-
SERTRALINE	CM	1	-	-	-
	HC	4	40.1	4.8-335.9	0.0006
ERYTHROMYCIN	CM	4	24.1	2.2-264	0.009
	HC	5	9.2	0.9-91.1	0.06
OTHERS	ALL	815	1.4	0.9-2.2	0.17
	CM	279	2.6	1.4-4.9	0.003
	HC	438	1.0	0.5-2	0.9

#DRUG=causal drug; TI = type of injury; OR=Odds Ratio; 95% CI = 95% confidence interval; P=logistic p-value

*For groups with 3 subjects the association has been tested by Fisher's Exact test.

Table S10. List of all causal drugs where at least one case carries a A*33:01 allele

DRUGs	**# Carriers**
DRONEDARONE	1
PIPERACILLINSODIUM/TAZOBACTAM	1
LAMOTRIGINE	1
MOXIFLOXACIN	1
METHIMAZOLE	1
CELECOXIB	1
IBUPROFEN	1
CEFAZOLIN	1
ISONIAZID	2
GENERIC COMBINATIONS OF NUTRIENTS	1
VALPROIC ACID	1
DICLOFENAC	3
ATORVASTATIN	1
AZATHIOPRINE	1
SULFAMETHOXAZOLE/TRIMETHOPRIM	1
NITROFURANTOIN	2

#Carriers = total number of A*33:01 positive carriers

Table S11. The most represented causative drugs across the rs28521457 signal on chromosome 4

DRUGS	**No. Cases Tested**	**OR**	**95% CI**	**P**	**AF**
CELECOXIB	2	50.56	3.378-756.8	0.004	0.50
EBROTIDINE	3	11.42	2.098-62.18	0.005	0.33
NIMESULIDE	4	8.51	1.73-41.82	0.008	0.25
ISONIAZID/PYRAZINAMIDE/RIFAMPIN/ETHAMBUTOL	9	7.39	2.406-22.7	0.0005	0.22
MERCAPTOPURINE	7	6.65	1.835-24.1	0.004	0.21
TELITHROMYCIN	5	6.22	1.36-28.43	0.02	0.20
IMATINIB	10	4.59	1.308-16.08	0.02	0.15
DICLOFENAC	35	2.40	1.024-5.604	0.04	0.09
SIMVASTATIN	12	2.19	0.5164-9.264	0.29	0.08
MINOCYCLINE	19	2.17	0.6713-7.028	0.20	0.08
ISONIAZID	32	2.15	0.8605-5.351	0.10	0.08
OTHERS	119	-	-	-	0.16
CONTROLS	10588	-	-	-	0.04

OR=Odds Ratio; 95%CI=95% confidence interval; P=logistic p-value; AF=Allele Frequency in cases

The table shows the causal drugs with more than two positive carriers only.

Table S12. Summary of drug and class comparisons with more than 40 samples

DRUG/CLASS	#CASES
NSAIDs	144
ANTI TUBERCULOSIS DRUGs	67
DICLOFENAC	67
NITROFURANTOIN	64
STATINs	59
FLUOROQUINOLONEs	43

Table S13. The most associated variants for each drug in the class-specific analysis

COMPARISON	SNP	CHR	BP	OR	95% CI	P
NITROFURANTOIN	rs72696020	14	88571907	7.18	3.52-14.66	6.16E-08
NITROFURANTOIN	rs72696089	14	88588493	6.98	3.42-14.24	9.48E-08
NITROFURANTOIN	rs6694270	1	19120377	2.59	1.81-3.71	1.85E-07
NITROFURANTOIN	rs10404821	19	51161088	2.56	1.80-3.65	1.98E-07
NITROFURANTOIN	rs61858823	10	66855253	5.63	2.88-11.02	4.52E-07
DICLOFENAC	rs114811931	5	160684731	6.59	3.54-12.28	2.76E-09
DICLOFENAC	rs113206698	10	94577904	3.79	2.25-6.39	5.61E-07
DICLOFENAC	rs115266745	3	821213	6.80	3.19-14.48	6.59E-07
DICLOFENAC	rs149014830	5	120876337	6.04	2.96-12.3	7.24E-07
DICLOFENAC	rs116316305	5	120853119	6.03	2.96-12.28	7.4E-07
ANTI TUBERCULOSIS DRUGs	rs117491755	9	119643656	3.92	2.35-6.51	1.43E-07
ANTI TUBERCULOSIS DRUGs	rs143575776	9	9593742	4.96	2.70-9.09	2.17E-07
ANTI TUBERCULOSIS DRUGs	rs73122578	3	79359633	2.42	1.71-3.40	4.48E-07
ANTI TUBERCULOSIS DRUGs	rs78671883	9	9558149	4.65	2.55-8.47	4.84E-07
ANTI TUBERCULOSIS DRUGs	rs73124503	3	79368237	2.42	1.71-3.41	5.12E-07
FLUOROQUINOLONEs	rs186920977	2	56649930	7.22	3.47-15.01	1.17E-07
FLUOROQUINOLONEs	rs144941777	2	56672204	7.22	3.47-15.01	1.17E-07
FLUOROQUINOLONEs	rs191153876	3	123637182	7.33	3.46-15.5	1.85E-07
FLUOROQUINOLONEs	rs116606120	5	28665952	8.60	3.79-19.5	2.54E-07
FLUOROQUINOLONEs	rs112655218	18	9841515	4.54	2.55-8.09	2.8E-07
NSAIDs	rs185305928	1	6905711	4.55	2.55-8.11	2.66E-07
NSAIDs	rs2240395	7	139718147	1.84	1.45-2.31	2.72E-07
NSAIDs	rs113607154	1	7004613	4.42	2.47-7.82	3.37E-07
NSAIDs	rs597480	11	85436868	1.84	1.45-2.32	3.9E-07
NSAIDs	rs2025009	14	68843605	1.81	1.43-2.29	6.77E-07

COMPARISON = causal drug/class; CHR=chromosome; BP=base-pair position; OR=Odds Ratio; 95%CI=95% confidence interval; P=logistic p-value

Table S14. Causative drugs associated with rs116561224 signal in the statin cohort

DRUG	**No. Cases Tested**	**AF**
SIMVASTATIN	17	0.21
ROSUVASTATIN	5	0.20
PRAVASTATIN	4	0.13
ATORVASTATIN	22	0.09
FLUVASTATIN	7	0.07
LOVASTATIN	3	0.00

DRUG = Causal drug; AF = Allele frequency

Table S15. Causative drugs associated with the rs114577328/A*33:01 signal in the additional case set

DRUG	PD	DC	SNP #TOT (#CARRIERs)	HLA #TOT (#CARRIERs)	TOT	CF
Terbinafine	yes	yes	6 (5)	2 (0)	8	0.63
Azathioprine	yes	yes	8 (2)	-	8	0.25
Exemestane			3 (2)	-	3	0.67
Amiodarone	yes		2 (1)	-	2	0.50
Daptomycin			2 (1)	-	2	0.50
Fenofibrate	yes	yes	4 (0)	3 (0)	7	0
Erytromycin	yes	yes		1 (0)	1	0
Ticlopidine	-	-	-	-	-	-
Methildopa	yes	yes	1 (0)	2 (0)	3	0
Enalapril	-	-	-	-	-	-
Sertraline	yes	yes	1 (1)	3 (2)	4	0.75

DRUG = causal drug; PD=presence of the drug in the discovery cohort; DC = Drug with at least one positive carrier in the discovery cohort; SNP #TOT (#CARRIERs) = total number of cases (total number of rs114577328/A*33:01 carriers) among the samples who underwent SNP typing; HLA #TOT (#CARRIERs) = total number of cases (total number of rs114577328/A*33:01 carriers) among the samples who underwent HLA typing; TOT= total number of samples that underwent SNP and HLA typing; CF = carriage frequency.

Table S16. Causative drugs associated with the chromosome 2 rs72631567 signal in the additional case set

DRUG	PD	DC	No. Cases Tested	AF
Cefotetan			1	0.50
Amiodarone	yes		2	0.25
Escitalopram	yes		2	0.25
Ethinylestradiol/Norgestimate	yes	yes	2	0.25
Mercaptopurine	yes	yes	4	0.13
Cefazolin	yes	yes	6	0.08
Minocycline	yes	yes	7	0.07
Azathioprine	yes	yes	8	0.06
Atorvastatin	yes	yes	8	0.06
Ciprofloxacin	yes	yes	9	0.06
Isoniazid	yes	yes	11	0.05
Sulfamethoxazole/Trimethoprim	yes	yes	17	0.03

DRUG = causal drug; PD=presence of the drug in the discovery cohort; DC = Drug with at least one positive carrier in the discovery cohort; TOT = total number of cases; AF = Minor allele frequency. Drugs also associated with an increased AF in the discovery cohort are indicated in bold.

Table S17. Causative drugs associated with the chromosome 4 rs28521457 signal in the additional case set

DRUG	PD	DC	No. Cases Tested	AF
Minocycline	yes	yes	7	0.07
Clindamycin	yes		1	0.50
Dronedarone	yes	yes	2	0.25
Ketoconazole			1	0.50
Methylprednisolone			3	0.17
Nefazodone			1	0.50
Nitrofurantoin	yes	yes	11	0.05

DRUG = causal drug; PD=presence of the drug in the discovery cohort; DC = Drug with at least one positive carrier in the discovery cohort; TOT = total number of cases; AF = Minor allele frequency.