

## Title Page

Title:

### **Pharmacogenomics of drug-induced liver injury (DILI): Molecular biology to clinical applications.**

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### **Author contributions:**

KK, WJHG, AD, GPA identified patients and completed clinical analysis, data acquisition, evaluation and diagnosis of the patients forming the basis of this manuscript. RMD generated and interpreted the three-dimensional protein structural model described. JIG and GPA reviewed literature, interpreted findings in context of published results and drafted the article with critical revision by all authors.

### **Key Words:**

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### **Key Points:**

- 1) Candidate gene and genome-wide association studies (GWAS) in well characterized cases have delineated molecular mechanisms underlying development of DILI.
- 2) Distinct physicochemical properties of the peptide-binding grooves of HLA molecules determine the specificity of drug-antigens presented and resultant activation of drug-specific T cells.
- 3) Carriage of single nucleotide polymorphisms (SNPs) in particular genes and Human Leucocyte Antigen (HLA) alleles can be utilized as tests to support or refute the diagnosis of DILI.
- 4) Different HLA alleles associated with DILI and autoimmune hepatitis (AIH) can be used to distinguish these two conditions with similar manifestations.
- 5) Evidence does not support treatment of DILI with corticosteroids routinely.
- 6) In cases of drug-induced AIH treated with corticosteroid therapy, withdrawal of immunosuppressive therapy does not lead to relapse of liver injury.

### **Abbreviations:**

AIH: autoimmune hepatitis; ALT: alanine transaminase; ANA antinuclear antibodies;

ASM: anti-smooth muscle antibodies; CIOMS: Council for International

Organizations of Medical Sciences; DILI: drug-induced liver injury; GWAS: genome-

wide association study; HLA: Human leukocyte antigen; H&E: Haemotoxylin and Eosin; LKM anti-liver-kidney microsomal-1 antibodies; MELD: Model for Endstage Liver Disease; MHC: Major histocompatibility complex; NAC: N-acetyl cysteine; SNP: single nucleotide polymorphism; TB: tuberculosis.

### **Conflicts of Interest:**

Guru Aithal declares associations with SHIRE, AGIOS and GLAXO SMITH KLINE, and advises Medicines and Healthcare products Regulatory Agency (MHRA), all outside the submitted work. There are no other conflicts of interest to report.

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### **Summary**

A number of drug-specific and host-related factors contribute to the development of drug-induced liver injury (DILI). Investigations focused on genetic susceptibility to DILI have advanced our understanding of the pathogenesis of this rare, yet potentially life-threatening adverse reaction. Candidate gene studies involving well-characterized patients with DILI and drug-exposed controls have identified single nucleotide polymorphisms (SNPs) affecting the metabolism and clearance of specific drugs and hence, influencing individual's susceptibility to DILI. On the other hand, a series of genome-wide association studies (GWASs) have revealed a number of

Human Leucocyte Antigen (HLA) alleles that are associated with DILI secondary to compounds with dissimilar chemical structures, highlighting the role of adaptive immune responses in the development of liver damage. These risk alleles, such as HLA-DRB1\*15:02 illustrated by the example presented in the clinical vignette, determine the physicochemical properties of the peptide-binding grooves of the HLA molecules and increase the likelihood of DILI in a susceptible individual by altering the nature or the magnitude of immune-mediated liver injury. Associations of HLA alleles with DILI secondary to specific drugs can be translated into genetic tests, and when performed selectively, can improve the accuracy of diagnosis of DILI as well as assist in identifying the correct causal agent when the event could be attributed to more than one drug.

### **Clinical vignette**

A 21-year old woman was admitted with a 2-week history of painless jaundice, fatigue and anorexia having been previously fit and well. One month prior to presentation, the patient had taken a 5-day course of amoxicillin-clavulanic acid for an infected skin cyst. She was otherwise on the oral contraceptive pill only and reported minimal alcohol intake. On examination, she was deeply jaundiced, but, alert and oriented with no asterixis. She had no stigmata of chronic liver disease, but hepatomegaly extending 3 cm from below the right subcostal margin was evident. Investigations showed a white cell count of  $13.4 \times 10^9/L$  (normal 3.6-9.3), hemoglobin was 11.8 g/dL (normal 11-15), platelet count of  $356 \times 10^9/L$  (normal 170-420), sodium was 138 mmol/L (normal 134-144), potassium was 3.5 mmol/L (normal 3.5-5.0), creatinine 32  $\mu\text{mol/L}$  (normal 40-75), albumin 30 g/L (normal 35-48), alanine transaminase (ALT) 707 IU/L (normal 15-54), alkaline phosphatase 151 IU/L (normal

30-130), bilirubin 384  $\mu\text{mol/L}$  (normal 7-31) and prothrombin time 27.2 s (normal 11.7-14). Screening for hepatitis A, B, C, E, Epstein-Barr virus, cytomegalovirus and autoimmune hepatitis was negative. Tests for anti-smooth muscle (ASM), antinuclear (ANA), and anti-liver-kidney microsomal-1 (LKM) antibodies were negative; immunoglobins levels and caeruloplasmin levels were normal. Liver ultrasonography demonstrated a liver of normal contour with no biliary dilatation, a normal spleen size and patent vessels. Liver biopsy revealed severe portal interface hepatitis with lobular inflammation and scant plasma cells.

Her clinical condition deteriorated in the following days with prothrombin time and bilirubin rising to 56.6 s and 470  $\mu\text{mol/L}$ , respectively. At follow-up after 11 days her ALT was 1931 IU/L. She developed grade 2 hepatic encephalopathy 14 days after presentation, and was listed for a super-urgent liver transplant. HLA typing was performed as a part of preparatory investigations and showed the patient carried the HLA haplotype HLA-DRB1\*15:02-DQB1\*06:01. Following orthotopic transplantation of a deceased donor graft her explant histology revealed severe ongoing hepatitis with multi-acinar necrosis (Fig. 1 A and B).

This case raised a number of important questions about the diagnosis of DILI and tools available for clinicians to make best decisions for patient care:

How is the diagnosis of DILI made?

What are the risk factors including genetic risk factors?

What are the mechanisms of liver injury?

Are there prognostic factors that can be considered?

What are the potential therapeutic options?

This grand round will try to explore these questions and describe the pathophysiology, diagnostic and prognostic biomarkers, clinical management and discuss areas of uncertainty.

### **Clinical-molecular pathological correlation**

The HLA DRB1\*15:01-DQB1\*06:02 haplotype common in European populations is a well-established risk factor for increased susceptibility to DILI on exposure to amoxicillin-clavulanic acid [1]. The patient described in the clinical vignette was of South Asian (Indian) origin and carried the HLA haplotype HLA-DRB1\*15:02-DQB1\*06:01. HLA-DRB1\*15:02 is present in only 0.7% of the Caucasian population while its prevalence is 8% in Taiwan and Japan [2, 3] and 13%-18% among Asians [4, 5].

The differences between protein products of different HLA-DRB alleles are concentrated in the peptide-binding groove of the MHC molecule. Variations in the physicochemical properties of the peptide-binding groove determine the specificity of the drug-metabolite-protein product that is presented by the HLA molecules, a key step in the immune mechanism mediating the DILI that ensues. The only difference in the 266 amino acid sequence of HLA DRB1\*15:01 and DRB1\*15:02 is at position 86 located in the peptide binding groove of the molecule (Fig. 2). Most people carry DNA sequence encoding a glycine (Gly) or Valine (Val) at this position. As these amino acids have similar physiochemical properties, this variation is unlikely to mediate major functional differences relevant to antigen presentation. Residue 86 of the HLA-DRB1\*15:01  $\beta$ -chain is integral to the formation of the P1 binding pocket for peptide antigens [6] as illustrated in Fig. 2A and C (PDB 1BX2, visualised in PyMol [7]). Comparison of a Val86Gly structural homology model representative of HLA-

DRB1\*15:02, derived from the solved structure of HLA-DRB1\*15:01 ([8], Fig. 2B and D), shows broadly equivalent electrostatic potential of pocket P1 for both alleles ([9, 10], Fig 2E and F, [6]). This suggests that most DRB P1 pockets have an overall neutral charge which is also minimally affected by Val86Gly variation. Therefore, DRB1\*15:02-DQB1\*06:01 would be predicted to have similar association with amoxicillin-clavulanic acid DILI in Asian populations as described for DRB1\*15:01-DQB1\*06:02 in Caucasians [1].

Members of the DRB1\*15 family of alleles, have been associated with immune-allergic reactions following exposure to a variety of potential toxins and xenobiotic agents including DILI due to nitrofurantoin and halothane [11, 12]. DRB1 is a HLA class II gene but genes from the closely related HLA class I family, especially HLA-B, are also relevant to adverse drug reactions. Association of HLA-B\*57:01 with serious cutaneous hypersensitivity secondary to abacavir, an anti-retroviral agent is the most well investigated example of a genetic risk factor for an adverse drug reaction with a negative predictive value of 100% and positive predictive value of 48% [13]. Therefore, HLA-B\*57:01 genotyping prior to abacavir prescription has been mandated by the Food and Drug Administration as well as the European Medicine Agency.

### **How is the diagnosis of DILI made?**

Accurate diagnosis is the key step in the management of DILI, as identifying and withdrawing the offending agent is a critical intervention; it is equally important to consider the fact that cessation of an effective medication incorrectly (by wrongly attributing DILI to a particular drug) has implications for the patient, as treatment of the underlying condition needs to be revised. In addition, attributing the clinical

manifestations to DILI inaccurately means that the correct underlying diagnosis such as autoimmune hepatitis or biliary obstruction is missed, with long-term adverse consequences to the patient.

Clinicians' awareness of the association of a particular drug with a pattern of manifestation is an important first step in the process of diagnosis. However, barring a few exceptions such as 'acute fatty liver'-associated drugs such as valproate, stavudine, zalcitabine or didanosine, the vast majority of DILI do not have sufficiently distinct 'signature' patterns that are consistently recognisable. Considering the fact that over 350 drugs have been convincingly linked to DILI [14], many clinicians access dedicated websites such as LiverTox [15] as an up to date source of information. A systematic evaluation of the circumstantial evidence that supports the diagnosis of DILI and exclusion of alternative aetiology that could lead to similar pattern of liver injury has been termed 'causality assessment'. One such method involves the use of the 'Council for International Organizations of Medical Sciences (CIOMS) Scale' which includes a weighted scoring of an event according to distinct domains related to the temporal relationship between exposure to a particular drug and the liver injury, exclusion of alternative, non-drug related aetiologies, exposure to other medications that could explain DILI, risk factors for the adverse hepatic reaction, evidence in the literature regarding DILI from drug in question and response to re-exposure to the medication. Causality methods such as the CIOMS scale provide a degree of objectivity and consistency in the process of diagnosis of DILI [16-18]. However, the tool has a number of limitations; it recommends an exhaustive list of exclusions making the diagnosis expensive and thus causality assessments are not widely used in clinical practice. So overall, the accuracy of clinical diagnosis of DILI remains low; a recent prospective study based in a



secondary care hospital setting demonstrated that only 12% of drug-related liver enzyme elevations of more than 3 times upper limit of normal were identified by the clinical team in charge of the care for the patient [19].

Over the past decade, candidate gene studies, initially, and GWAS, more recently, have identified several genetic factors associated with DILI (Table 1) (reviewed by [20-22]). While associations with single nucleotide polymorphisms (SNPs) in genes involved in drug metabolism and excretion have been drug specific, some HLA alleles have been associated with DILI from a number of drugs of varying structure. There are over 15 currently used drugs where HLA genotype or haplotype increases the susceptibility to DILI and, as demonstrated by high relative risks, some of these associations are strong [23-26]. Rarity of occurrence of DILI in relationship with a given drug means that many of these HLA alleles have a negative predictive value of >95%. Consequently, genetic tests have been used to exclude the diagnosis of DILI or identify the right aetiological agent when more than one potential medication could have caused DILI [27, 28]. In addition to the above, HLA genotyping in combination with other serological markers could strengthen the diagnosis of DILI.

There are substantial overlaps between DILI and autoimmune hepatitis (AIH), so that 9% of DILI cases are indistinguishable from AIH [29]. On the other hand, 9% of AIH occurrences are considered drug-induced, highlighting the challenge in establishing the diagnosis of both clinical scenarios [30]. In routine clinical practice, a combination of clinical features, serological, histological parameters as well as genetic tests are considered in reaching the diagnosis of idiopathic AIH as none of the individual features are pathognomonic [31]. In a recent nationwide cohort involving 1267 patients with AIH, only 65% of those meeting the original International AIH Group criteria also met a simplified International AIH score [32]. HLA genotyping

in particular has been included within the original International AIH Group criteria [33]. Therefore, when a patient suspected to have DILI also tests positive for liver specific auto-antibodies (ANA, anti-SMA, anti-LKM) or has raised immunoglobulins [34], carriage of specific HLA alleles in the patient would support the diagnosis of AIH (DRB1\*03:01 and \*04:01) or DILI (risk allele for specific drug in question).

Table 2 lists diagnostic tests useful for diagnosis of DILI and the frequencies for specific HLA genes of interest in the reference population and in cases of DILI due to common drugs. These data provide an indication of the positive yield of the genetic tests in cases of DILI compared to their frequency in the reference population. For example, if applied in clinical practice, genotyping for HLA-DRB1\*15:01 when amoxicillin-clavulanic acid DILI is suspected, HLA-B\*57:01 in suspected flucloxacillin-induced DILI and HLA-B\*35:02 in a possible minocycline DILI case would have similar performance characteristics to anti-nuclear antibody (ANA), immunoglobulin G estimation and anti-liver kidney microsomal (LKM) antibody test, respectively in a case of suspected AIH. Considering the importance of clinical decision-making, such as permanent withdrawal of an effective medication in a patient and/or initiation of long-term immunosuppressive regimen, incorporating genetic tests into diagnostic armamentarium is justified and would increase the accuracy and confidence in the diagnosis.

### **What are the risk factors including genetic risk factors?**

A number of drug-related and host factors increase an individual's susceptibility to DILI as well as reduce the likelihood of the development of clinically significant adverse reaction. Drugs with a daily dose of 50 mg or higher are more likely to be associated with DILI and there is also a significant relationship between

daily dose and reports of liver failure, liver transplantation, as well as death caused by DILI [35]. In addition, compounds with >50% hepatic metabolism are more likely to be associated with ALT >3 times the upper limit of normal, liver failure and fatal DILI [36]. Suggestion that lipophilicity of a compound, independently of other properties, may increase its hepatotoxic potential has recently been refuted [37, 38]. Drugs with dual potency as mitochondrial and bile salt export pump inhibitors are associated with severe human DILI [39].

Age may be a risk factor increasing susceptibility to DILI due to anti-tuberculosis drugs and flucloxacillin [40, 41]. Chronic hepatitis B and C are also associated with increased risk of DILI from anti-HIV and anti-tuberculosis therapies [40, 42, 43]. In different ethnic groups, significantly different medications underlie DILI [44]. Trimethoprim/sulfamethoxazole, methyldopa and phenytoin are more often the cause of DILI among African-Americans, while amoxicillin-clavulanic acid is a causative agent in a higher proportion of Caucasians. In addition, severe cutaneous reactions, rates of hospitalisation, liver transplantation or liver-related deaths are more frequent among African-Americans, compared to Caucasians, after controlling for selected covariates [44]. As discussed previously, accumulating evidence over the past decade points consistently towards genetic factors being associated with the individual's risk of developing liver injury on exposure to medications.

### **What are the mechanisms of liver injury?**

It is generally believed that low molecular weight organic chemicals, such as drugs, are not immunogenic but may become so when bound to a macromolecule, such as a protein. Medications with a high daily therapeutic dose, those which

predominantly undergo hepatic metabolism and biliary excretion are more frequently associated with severe DILI suggesting that formation and clearance of reactive metabolites, steps that are common for many compounds, are likely upstream events mediating liver injury. Consistent with this, SNPs in genes coding for drug-metabolizing enzymes and transporters involved in the excretion of drug metabolites have been associated with increased susceptibility to DILI [21]. An increasing number of drugs are shown to be 'haptens', generating sensitizing molecules that are directly protein reactive and form covalent adducts *in vivo* [45-47]. The observation that the formation of reactive metabolites and covalent adducts are associated with exposure to the drug, even in the absence of clinically significant liver injury, indicates that, while upstream events are essential steps in the pathogenesis, they are not sufficient on their own to lead to severe DILI. Rather, a subclinical event may release drug-altered peptide to be taken up by antigen presenting cells or stimulate production of 'danger signals', hence triggering a pathogenic adaptive immune response [48].

In the past 8 years, GWASs have identified several HLA alleles and haplotypes as strong risk factors associated with DILI (reviewed in [20]). A relatively small number of HLA class I and class II alleles are associated with DILI from a wide variety of structurally dissimilar drugs. In addition, examples of HLA alleles that increase susceptibility to specific drug-related liver injury while being protective of DILI from others supports the 'hapten hypothesis'. The majority of these alleles vary in the amino acid residues that determine the physicochemical properties of the peptide-binding grooves of the HLA molecules they encode. Presentation of drug- or metabolite-altered peptide in the peptide-binding groove of particular HLA molecules is a key step in the activation of drug-specific T cells and immune-mediated injury

manifesting as DILI. While HLA class I molecules can be associated with cytotoxic CD8<sup>+</sup> T cell-mediated hepatocyte injury, HLA class II molecules invoke interaction between antigen presenting cells and CD4<sup>+</sup> T cells, leading to inflammation and liver injury (Fig. 2G). With regards to flucloxacillin and amoxicillin-clavulanic acid-induced DILI, experimental evidence supports the 'hapten mechanism' where MHC class II and I restricted CD4<sup>+</sup> and CD8<sup>+</sup> clones are activated by a drug-derived antigen [49, 50].

Considering that the structure of drugs are often designed to facilitate binding to specific receptors, some compounds or their metabolites may bind directly to HLA molecules or T cell receptors. Such 'pharmacological interaction' (p-i concept) may trigger activation and proliferation of T cells leading to immune-mediated liver injury [51]. More recently, some drugs have been shown to occupy the peptide-binding groove of HLA molecules, hence altering the repertoire of peptides (altered peptide repertoire model) that can bind to a particular HLA protein [52, 53]. This permits interaction between self-peptides and HLA molecules leading to immune-mediated DILI.

### **Prognostic markers**

A number of serum/ plasma biomarkers are being investigated with a view to develop tests that can assist in identifying patients with DILI who are likely to progress to acute liver failure or develop chronicity (reviewed in [54]). These include: organ-specific markers such as microRNA-122 associated with hepatocellular injury [55]; organelle-specific markers such as glutamate dehydrogenase, reflecting mitochondrial injury; mechanistic biomarkers such as cytokeratin-18 caspase-cleaved fragment, and acetylated high mobility group box 1, reflecting apoptosis and

necrosis, respectively; macrophage colony-stimulating factor receptor 1 associated with liver inflammation, and osteopontin associated with inflammatory cell activation and with liver regeneration due to activation of hepatic stem cells [54]. None of these have been validated in a well-characterized cohort of patients with idiosyncratic DILI and hence, they are not yet ready for clinical application.

### **Areas of uncertainty**

While we apply the knowledge gained with regards to molecular mechanisms underlying the development of DILI in clinical practice, there is a compelling case of need for a technique that is capable of reflecting host genetic factors at the same time, recapitulating initial essential steps involving the metabolism and clearance of drugs as well as key events related to immune-mediated liver injury. Such a methodology could bring about a step change in investigations related to hepatotoxicity both in experimental and clinical settings.

A recent publication describes a hepatotoxicity assay using monocyte-derived hepatocyte-like cells which show several donor-specific characteristics, reflect their monocyte/macrophage origin and potential innate immune function (expressing surface marker CD14 at low levels) [56]. In addition, these cells show activities of phase I and II metabolism as well as expressing transporter proteins involved in excretion of xenobiotics. Investigators describe the method of maintaining these cells in culture for a period of 42 days and these cells as having the expression patterns of hepatic enzymes that are similar to primary human hepatocytes maintained for 28 days [57]. In a pilot study, *in vitro* testing using monocyte-derived hepatocyte-like cells derived from patients with acute liver injury was able to identify DILI with good performance characteristics. However, these cells would not reflect the key influence

of the adaptive immune system in DILI pathogenesis and, hence, the application of this methodology in investigations related to hepatotoxicity from a wide range of drugs is uncertain.

## **Management**

Early recognition of DILI and prompt withdrawal of the causal medication is crucial to minimise injury and its progression. In addition, measures such as clear documentation in the medical notes, information provided to the general practitioner, referring clinician or pharmacist and the patient to avoid inadvertent re-exposure to the causal medication are important. Although re-exposure does not always lead to recurrence of DILI, in 11% to 51% of cases when liver injury manifests (called positive rechallenge), [58] the consequences could be serious including mortality in 2% to 13% of cases [59, 60]. So re-introduction of medication that has caused DILI in an individual should only be considered in discussion with the patient and under circumstances where benefits clearly outweigh the risks [58]. For example, when providing potentially life-saving cancer chemotherapy or when alternative medications to treat underlying conditions are not accessible or affordable as in cases of anti-tuberculosis treatment [61].

Currently, treatment of DILI is limited to management of the patient's symptoms such as itching [62]. There is not yet sufficient evidence to support the treatment of DILI with corticosteroids or ursodeoxycholic acid although these have been used in clinical practice and have been thought to enhance recovery [63]. In a cohort of patients with acute liver failure, there was no statistically significant improvement in overall survival in patients with DILI who received corticosteroids. Furthermore, in contrast, steroid use was associated with diminished survival in a

subgroup of patients with a Model for Endstage Liver Disease (MELD) score of more than 40 [64]. However, initiation of corticosteroid treatment is justified in certain cases where drug-induced AIH is indistinguishable from the acute presentation of idiopathic AIH. Nevertheless, once the liver injury has resolved, as evidenced by normalization of liver enzymes, immunosuppression could be completely withdrawn with regular and close monitoring of patients. Long-term follow-up studies demonstrate that drug-induced AIH does not recur over a median follow up of 4 years [65] with idiopathic AIH relapses in 63% of cases in 1 year and 75% of cases in 5 years [66]. Therefore, this is a reasonable approach considering that treatment of relapse of AIH is identical to that of its initial presentation.

### **Potential Therapies**

With regards treatment of severe liver disease, administration of N-acetyl cysteine (NAC) significantly improved transplant-free survival in early stage non-acetaminophen acute liver failure [67]. Transplant-free survival for DILI patients specifically was 58% (95% CI=33-83%) for those receiving NAC compared to 27% (95% CI=8-46%) receiving placebo. High-volume plasma exchange has also been shown to significantly improve outcome in acute liver failure in a prospective randomised multicentre trial of 182 patients [68]. Finally, emergency liver transplantation is an established procedure for refractory fulminant hepatic failure as in the case presented here.

It is conceivable, from its crucial role in the pathogenesis, that interventions that modulate the adaptive immune response early in the course of DILI would arrest injury progression or assist in its resolution. Potential candidates include budesonide, based on its efficacy and lower rate of adverse effects in the treatment of autoimmune



hepatitis [69], and nor-ursodeoxycholic acid, for its properties of reducing expression of MHC class II molecules on macrophages and inhibiting the proliferation of CD4<sup>+</sup> T lymphocytes [70]. International consortia that have collaborated successfully in the last decade are well-placed to coordinate randomised clinical trials of treatments for DILI in the near future.

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## Figure Legends:

**Fig. 1. Liver explant histology in the DILI patient described.** (A) Reticulin stained section x10 magnification. There is panacinar cell loss with occasional multiacinar collapse of the reticulin framework and regenerative-appearing islands of residual parenchyma. (B) H&E stained section x10 magnification. There is extensive bridging parenchymal collapse with haemorrhage into cell plates and residual islands of regenerative-appearing hepatocytes. The porto-septal areas contain a mild chronic inflammatory infiltrate, predominantly lymphocytic, and with florid ductular reaction adjacent to areas of hepatocyte loss. The findings are in keeping with a severe ongoing acute hepatitis with massive cell necrosis.

**Fig 2. Structure and electrostatic properties of the peptide binding groove of heterodimeric HLA-DRB1 alleles and their role in antigen presentation and stimulation of an immune response during DILI.** (A) Structure of the HLA-DRB1\*15:01 (PDB 1BX2) peptide binding groove with valine 86 highlighted (space-filled representation in lilac): the two different subunits that form the groove are coloured gold ( $\beta$  chain, HLA-DRB1) and claret ( $\alpha$  chain, HLA-DRA) [8-10]. (B) Modelled structure of the HLA-DRB1\*15:02 peptide binding groove [8-10] with glycine 86 highlighted (space-filled representation in lime green): the two different subunits that form the groove are coloured gold and claret [8-10]. (C) Surface representation of the pocket P1 region of HLA-DRB1\*15:01 peptide binding groove with the position of valine 86 highlighted in lilac. (D) Surface representation of the pocket P1 region of HLA-DRB1\*15:02 peptide binding groove with glycine at position 86. (E) Molecular surface electrostatic potential of the HLA-DRB1\*15:01 peptide binding groove highlighting the overall neutral charge of pocket P1 [8-10]. Potentials are graded from -10kT/e, shown in blue, to +10kT/e in red, with neutral potentials (0 kT/e) coloured white. (F) Molecular surface electrostatic potential of HLA-

DRB1\*15:02 peptide binding groove highlighting the overall neutral charge of pocket P1 [8-10]. Potentials are graded from -10 kT/e, shown in blue, to +10 kT/e in red, with neutral potentials (0 kT/e) coloured white. (G) Suggested role of HLA-DRB1 in presentation of drug-derived antigens by antigen presenting cells in DILI. Circulating drug or drug metabolite arising from hepatic metabolism, 'hapten', may be taken up by antigen presenting cells and processed as an antigen. The subsequent presentation of derived fragments in the peptide groove of the MHC class II HLA-DRB1\*15:01 or DRB1\*15:02 has the potential to activate CD4<sup>+</sup> T cells, co-stimulated by other factors released from damaged hepatocytes such as cytokines and danger-signalling molecules. Thus stimulating immune responses associated with DILI. MHC II: major histocompatibility complex class II protein; APC: antigen-presenting cell; TCR: T cell receptor.

**Table 1. Genetic susceptibility loci for DILI identified in GWAS and candidate gene studies.**

Association described: HLA allele	Drug studied:	Study type & cohort population	OR
A*02:01 rs2523822 <i>TRNAI25</i>	Amoxi-clav	<b>GWAS:</b> 201 cases 532 P controls ( <i>European</i> ) [24]	2.3
A*30:02	Amoxi-clav	CGS: 75 cases 885 P controls ( <i>European</i> ) [1]	6.7
A*33	Tiopronin	CGS: 14 cases, 472 T controls ( <i>Japanese</i> ) [71]	NR
A*33:01	Multiple	<b>GWAS:</b> 862 cases (21 terbinafine; 7 fenofibrate; 5 ticlopidine cases) 10588 P controls ( <i>European</i> ) [26]	40.5; 58.7; 163.1
A*33:03 B*08	Ticlopidine Clometacin	CGS: 22 cases 85 T controls ( <i>Japanese</i> ) [72] CGS: 30 cases ( <i>European</i> ) [73]	13
B*18:01	Amoxi-clav	CGS: 75 cases, 885 P control ( <i>European</i> ) [1]	2.9
B*35:02	Minocycline	<b>GWAS:</b> 25 cases, 10588 P controls ( <i>European</i> ) [25]	29.6
B*57:01 rs2395029 <i>HCP5</i>	Flucloxacillin	CGS: 51 cases, 282 P controls ( <i>European</i> ) [23]	45
B*57:01	Pazopanib	CGS: 429 cases, 1761 T controls [74]	2.0
B*57:02	Efavirenz + Anti-TB	CGS: 46 cases, 46 controls ( <i>African</i> ) [75]	8.1
B*57:03	Efavirenz + Anti-TB	CGS: 46 cases, 46 controls ( <i>African</i> )	26.8
B*58:01	Nevirapine	CGS: 57 cases, 111 T controls ( <i>South African</i> ) [76]	
DQA1*01:02 protective	Anti-TB	CGS: 56 cases, 209 T controls ( <i>Indian</i> ) [77]	4
DRB1*15:01- DRB5*0101- DQB1*06:02; DQB1*06:02 rs9274407	Amoxi-clav	<b>GWAS:</b> 201 cases, 532 P controls ( <i>European</i> ) [24]; CGS: ( <i>European</i> ) 35 cases, 300 P controls [78]; 22 cases 134 P controls [79]; 40 cases, 140 P controls [80]; 75 cases, 885 P controls [1]	3.1
DRB1*01	Nevirapine	CGS: 21 cases 133 T control ( <i>Caucasian</i> ) [81]	NR
DRB1*01:02	Nevirapine	CGS: 57 cases 111 t controls ( <i>South African</i> ) [76]	NR
DRB1*07 <i>protective<sup>a</sup></i>	Amoxi-clav	CGS: 40 cases, 140 P controls ( <i>European</i> ) [80]	0.18
DRB1*07:	Ximelagatran	<b>GWAS:</b> 74 cases, 130 T controls ( <i>European</i> ) [82]	4.4
DRB1*07:01 (linkage with DQA1*02:01)	Lapatinib	<b>GWAS:</b> 37 cases, 286 T controls [83]; CGS: 37 cases 1071 T controls. ( <i>European</i> ) [84]; <b>GWAS:</b> 34 cases, 810 T controls [85]	NR
DRB1*13 <i>protective<sup>a</sup></i>	Diclofenac	CGS: ( <i>European</i> ) [86]	NR

DRB1*15:01	Lumiracoxib	<b>GWAS</b> 41 cases, 176 T controls ( <i>International</i> ) [87]	5
DRB1*16:01- DQB1*05:02	Flupirtine	<b>GWAS:</b> 614 cases (6 flupirtine) 10588 P controls ( <i>European</i> ) [88]	18.7
DQB1*0201	Anti-TB	CGS: 56 cases, 209 T controls ( <i>Indian</i> ) [77]; <b>GWAS:</b> 59 cases, 111 T controls, 109 P controls ( <i>Indian</i> ): association not confirmed [89]	1.9
<b>Association described:</b> drug metabolism loci	<b>Drug studied:</b>	<b>Study type &amp; cohort population</b>	<b>OR</b>
ABCC2 rs717620	Diclofenac	CGS: 24 cases, 48 T controls ( <i>European</i> ) [90]	5
CYP2B6 *6	Efavirenz	CGS: 41 cases, 160 T controls ( <i>South African</i> ) [91]	NR
CYP2B6 rs7254579	Ticlopidine	CGS: 22 cases, 92 T controls ( <i>Japanese</i> ) [92]	NR
NAT2 slow acetylator alleles	Isoniazid	CGS: 26 cases, 101 P controls ( <i>European/Asian</i> ) [93]; <b>GWAS: 24 cases - association not confirmed</b> [94]	4.25
UGT1A6/1A9	Tolcapone	CGS: 135 cases, 234 T controls ( <i>European</i> ) [95]; CGS: 2 cases ( <i>European</i> ) [96]	NR
UGT2B7*2	Diclofenac	CGS: 24 cases, 48 T controls ( <i>European</i> ) [90]; <b>GWAS: 34 cases - association partly confirmed</b> [94]	8.5
<b>Various other associations:</b>	<b>Drug studied:</b>	<b>Study type &amp; cohort population</b>	<b>OR</b>
ALG10B rs6582630	Flucloxacillin	<b>GWAS:</b> 51 cases, 282 P controls ( <i>European</i> ) [23]	2.8
C9orf82 (CAAP1) rs10812428	Flucloxacillin	<b>GWAS:</b> 51 cases, 282 P controls ( <i>European</i> ) [23]	2.9
ERN1 rs199650082	Efavirenz	<b>GWAS:</b> 21 cases, 234 T controls [97] ( <i>African</i> )	18.2
FAM65B intron rs10946737	Rifapicin	<b>GWAS:</b> 48 cases, 354 T controls [98]; CGS: 27 cases, 217 T controls ( <i>African</i> ) [98];	3.4
lincRNA rs4842407	Efavirenz + anti-TB	<b>GWAS:</b> 42 cases, 292 T controls [97] ( <i>African</i> )	5.4
MCTP2 rs4984390	Flucloxacillin	<b>GWAS:</b> 51 cases, 282 P controls ( <i>European</i> ) [23]	3.3
OR5H2 rs1497546	Flucloxacillin	<b>GWAS:</b> 51 cases, 282 P controls ( <i>European</i> ) [23]	6.6
PPARG rs17036170	Multiple (Diclofenac)	<b>GWAS:</b> 783 cases (30 diclofenac) 3001 P controls ( <i>European</i> ) [94]	11.3
ST6GAL1 rs10937275	Flucloxacillin	<b>GWAS:</b> 51 cases, 282 P controls ( <i>European</i> ) [23]	4.1

Amoxi-clav: amoxicillin-clavulanic acid; OR: odds ratio; GWAS: genome-wide association study; CGS: candidate gene study; T: treated with same drug; P: population. Anti-TB drugs: isoniazid, rifampicin, pyrazinamide.

**Table 2. Summary of tests utilized for diagnosis of DILI and distinction from AIH and prevalence of variant alleles.**

<b>Test: antibodies</b>	<b>% positive in AIH cases [34]</b>	<b>% positive in 'normal' population [99-101]</b>
ANA 1:60	68%-75%	15% (<40 ♀) - 24% (>40 ♀)
Anti-SMA	52%-59%	Up to 43%
IgG >1600 mg/dl	86%	5%
Anti-LKM	4%-20%	1%
<b>Test: HLA type</b>	<b>% positive in DILI cases</b>	<b>% positive in 'normal' population</b>
<i>DRB1*15:01</i>	57%-67% (Amoxicillin-clavulanic acid) [1]	15%-20%
<i>B*57:01</i>	84%-87% (Flucloxacillin) [23]	6%
<i>A*31:01</i>	17% (Carbamazepine)*	2%
<i>DRB1*16:01-DQB1*05:02</i>	25% (Flupirtine) [88]	1%
<i>A*33:01</i>	80% (Ticlopidine), 50% (Methyldopa), 50% (Enalapril), 43% (Fenofibrate), 43% (Terbinafine), 40% (Sertraline), 20% (Erythromycin), [26]	1%
<i>B*35:02</i>	16% (Minocycline) [25]	0.6%

DILI: drug-induced liver injury; AIH: autoimmune hepatitis; ANA: antinuclear antibodies; SMA smooth muscle antibodies; LKM: liver-kidney microsome-1 antibodies. \* Nicoletti, unpublished.

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Fig. 1.

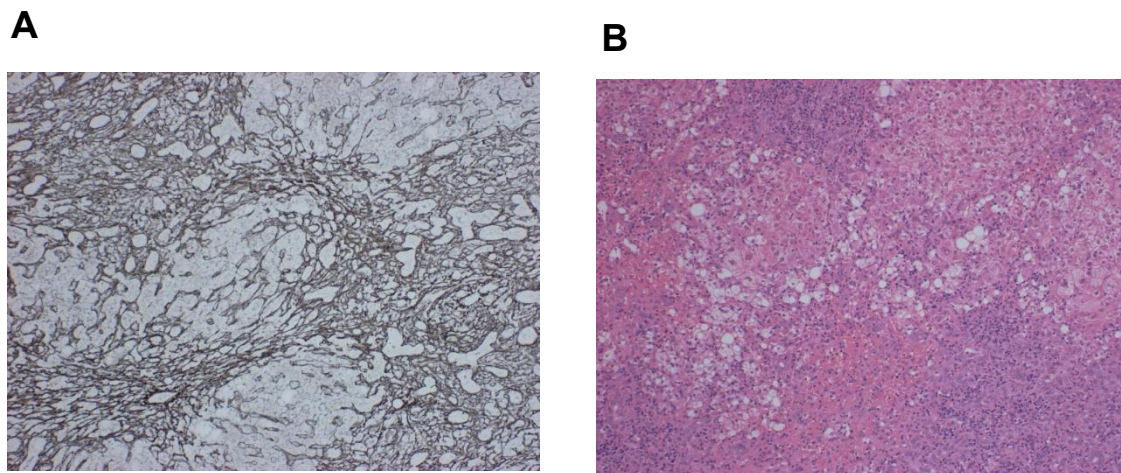


Fig. 2.



