Pharmacogenomics in Drug Induced Liver Injury

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Abstract: Drug-induced liver injury (DILI) is a severe adverse effect. The majority of DILI cases are idiosyncratic and several mecha- nisms have been postulated to explain why some subjects develop DILI with drugs that are safe for the majority of individuals. Major mechanisms proposed for DILI are based on the production of reactive metabolites, immune-mediated hepatotoxicity, a "danger signal" hypothesis and/or alterations in mitochondrial function. These mechanisms are compatible with the hypothesis for genetic variability in drug metabolism or bioactivation and are a major determinant for DILI. In this review we summarize present knowledge on underlying mechanisms, and clinical expression as well as genetic and non-genetic factors that modulate the risk of developing DILI. With regard to DILI pharmacogenomics, we summarize current evidence on the role of polymorphisms in genes coding for the drug-metabolizing en- zymes CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, NAT2, GSTM1, GSTT1, UGT1A1, UGT1A3, UGT1A9 and UGT2B7. Conclusive evidence for association with DILI risk has been obtained for non-mutated *CYP2E1*, slow *NAT2* and slow *GSTM1* genotypes. For the rest of the genes additional pharmacogenomics and toxicogenomics studies are required. We identify potential sources of heterogeneity in studies carried out so far as well as new genetic targets which require further investigation.

Keywords: Pharmacogenomics, toxicogenomics, hepatotoxicity, intolerance, liver injury.

INTRODUCTION

Drug-induced liver injury (DILI) is a major concern for the pharmaceutical industry and regulatory agencies as it remains the single leading cause of stopping drugs for further development and drug withdrawal once on the market [1-3]. Nearly one thousand medicines used in clinical practice have been shown to induce hepatotoxicity in medical literature [4]. Two large prospective Regis- tries in Spain and the USA intended to capture DILI incidences regardless of causative agent have shown that the main compounds

incriminated in DILI were anti-bacterial agents, non-steroidal anti- inflammatory drugs (NSAIDs) and anticonvulsants [5, 6]. In both Registries, amoxicillin-clavulanate was the leading agent responsible for DILI.

The incidence of DILI remains largely undetermined in the general population. In a population-based study in France, the frequency of DILI was prospectively estimated to be about 14 per 100,000 inhabitants per year [7]. Further, in roughly 1% of medical inpatients DILI develops during the course of hospitalization [8].

An obvious 'dose effect' is the hall-mark of intrinsic "dose- dependent" adverse drug reactions. Acetaminophen (paracetamol) is, in practical terms, the only marketed drug whose capacity to produce liver injury is closely related to the dose consumed.

In contrast, *idiosyncratic* DILI, which represents the bulk of cases, is typically non-predictable. Idiosyncratic reactions cannot usually be predicted from preclinical studies. They are further classified as immunoallergic (hypersensitivity features present), or metabolic, although this is a more arbitrary division and overlap between categories can occur. Although the classical view of idiosyncrasy in DILI refers to a lack of a clear dose relationship, it has been recalled that even in the most extreme form of unpredictable adverse drug reactions (allergic), a dose threshold is required to trigger the immune response, [9]. Actually, a recent study used two comprehensive pharmaceutical databases to examine the relation- ship between daily doses of the top 200 brands and the top 200 generic medications by prescription volume in the United States during the year 2005 and reported frequency of their hepatic ad- verse events. By categorizing medications into <10 mg/day, 11-49 mg/day, and>50 mg/day groups, a statistically significant relation- ship was observed between daily dose of oral medicines and reports of liver failure, liver transplantation and death caused by DILI. In a second part of the study the authors examined serious DILI cases reported to the Swedish Adverse Drug Reactions Advisory Committee (1970-2004) and found that most of the 598 cases of idiosyncratic DILI (77%) occurred in patients taking drugs at a daily dose of at least 50 mg [10]. Additionally, it has been pointed outthat the majority of compounds that either have been withdrawn from the market, or have received a black box warning due to hepatotoxicity were prescribed at daily doses greater than 50 mg/day. These data suggest that there is some dose-response relationship in idiosyncratic DILI as well. Therefore, the classic distinction be- tween predictable and unpredictable adverse hepatic reactions to drugs is worthy of revision as the concept is overly simplified [11].

MECHANISMS

Development of DILI is a complex, multi-step process that requires the interplay

between the toxic potential of the drug, and genetic and acquired factors coupled with failure in the adaptive processes. Thus, most patients tolerate the drug without adverse liver effects or there is a background of mild, and often transient, asymptomatic liver injury (i.e. statins, isoniazid), indicating an adaptation to the drug and further tolerance. Therefore, the combi- nation of susceptibility factors coupled with drugs that (due to variation in handling between different phases of drug metabolism, detoxification, and transport) reach a threshold for exposure to drug or toxic metabolites, enhances the risk of idiosyncratic hepatotoxicity. There are nowadays several independent (yet not mutually excluding) hypotheses to explain idiosyncratic DILI. No one may satisfactory accomplish all complex circumstances in which DILI occurs.

The reactive metabolite hypothesis states that most DILI in- stances would result from the production of reactive metabolites [12-16], with capacity to initiate the damage once a critical thresh- old has been crossed. In support of this hypothesis is the common occurrence of centrilobular necrosis (the zone richest in CYPs) in severe acute DILI. This concept in fact arose from the discovery that acetaminophen (APAP) induced hepatotoxicity is caused by a reactive imidoquinone metabolite [17]. Most of this reactive metabolite is detoxified by reaction with glutathione, and significant toxicity does not occur until liver glutathione is substantially depleted. It is noteworthy that the bulk of our knowledge of DILI derives from the experimental model of APAP hepatotoxicity [18]. But whether clinical APAP liver injury is relevant to idiosyncratic hepatotoxicity has not been fully established. One study has given support to the reactive metabolite hypothesis, showing that among the 27 drugs frequently cited in adverse drug reaction studies, a significantly higher proportion (59%) were metabolized by enzymes coded for by allelic variants (“genotype”) associated with a poor metabolizer “phenotype” as compared with 22% of randomly selected drugs and 7% of the top-selling drugs [18] However, in general, substantial increases in enzyme activity reflect environ- mental rather than genetic factors [19].

Immune-mediated hepatotoxicity appears to involve the generation of reactive metabolites that undergo covalent binding with hepatocytes/carrier proteins, also known as “haptenization” [20]. The covalent binding of the reactive metabolites to “self” proteins results in the formation of neo-antigens that “mislead” the immune system into mounting an immune response against hepatocytes. However the pattern of the immune response-to-adduct is likely to vary among individuals [21, 22]. It is not clear as yet whether the association of immune response with liver injury and reactive metabolite formation is coincidental or consequential. Classic examples that seem to indicate that reactive metabolite formation can be a first step to the development of an immune response are

some kind of drug-induced immunoallergic hepatitis. Several drugs (some of them no longer on the market), including halothane, tienilic acid, dihydralazine and anticonvulsants, induce immunoallergic hepatitis associated with antibodies antihapten (neoantigen) or anti-native proteins such as CYPs. In these cases it is believed that the drug is first metabolized into a reactive metabolite which binds to the enzyme that generated it, resulting in the production of a neoantigen which, once presented to the immune system, might trigger an immune response characterized by the production of antibodies recognizing both the native and/or the modified protein. For example, most patients who develop halothane-induced hepatotoxicity have antibodies against trifluoroacetyl modified proteins
[23] and this implies that modification of protein by the reactive metabolite has led to an immune response. Halothane is administered for a brief period of time, which is insufficient in a single exposure to allow for the development of a full adaptive immune response, and the observation that hepatotoxicity almost always occurs after multiple exposures [24] also suggests a process of immune sensitization. Another example is the antihypertensive drug dihydralazine that has caused, on rare occasions, immunoallergic hepatitis characterized by the presence of anti-liver microsome (anti-LM) autoantibodies. Since these antibodies found in the serum of the patients react with CYP1A2, it is suggested that dihydralazine is biotransformed into a reactive metabolite, which covalently binds to cytochrome CYP1A2 and triggers an immunological response as a neoantigen [25].

Recent data give further support to the participation of acquired immune system (in a background of genetic predisposition) in the hepatotoxicity induced by some drugs; a genome-wide association study using 866,399 markers in 51 cases of flucloxacillin DILI and 282 controls matched for sex and ancestry showed an association peak in the major histocompatibility complex (MHC) region with the strongest association (P = 8.7 x 10(-33)) seen for rs2395029[G], and other SNPs [26] all of which are a part of an extended MHC 57.1 haplotype present in < 4% of Europeans, which is associated to hypersensitivity to abacavir [27]. Also the DILIGEN group has confirmed [31] the significant association previously reported be- tween *DRB1*1501-DRB5*0101-DQB1*0602* haplotype and cholestatic hepatitis related to amoxicillin-clavulanate [26].

A compelling hypothesis which attempts to unify several observations in DILI is the danger signal hypothesis [28]. The rational basis for this hypothesis is the observation that foreign proteins hardly induce an immune response in the absence of an adjuvant, and the primary purpose of the adjuvant is to activate antigen-presenting cells (APCs) [29]. Adapting the danger signal hypothesis to DILI would mean that if a drug is metabolized in the liver to form a reactive compound that covalently binds to proteins (haptenization),

this alone might be insufficient to trigger an immune reaction or could induce a non-pathogenic immune response [20]. The danger that primes a genetically susceptible adaptive immune system might include the background mild hepatic injury caused by the reactive metabolites [20]. Thus, the ability of a reactive metabolite to cause cell damage could be a determinant of whether a drug that forms reactive metabolites will be associated with a significant incidence of idiosyncratic DILI [9]. It appears that endogenous molecules that act as danger signals may bind to the same receptors that recognize molecules on foreign pathogens, that is, toll-like receptors [30]. Other attractive co-stimulatory signals could be infection or inflammatory conditions, such as HIV, or other viral or bacterial infections. There are specific examples where this appears to be true; however, there does not appear to be a clear and dominant pattern of factors such as infection being associated with an increased risk of idiosyncratic DILI [29]. Subsequently, hapten formation leading to major histocompatibility complex class II (MHC II) presentation of haptenized peptides by (APC) along with co-stimulation of APC by "danger" signals promote helper T-cell activation (clonal antigen-recognizing cytotoxic cell expansion) and B-cell mediated antibody production. In response to cellular stress/death signal, innate immune system leads to the production of protective and/or injurious cytokines [20]. The altered cytokine milieu of chronic viral disease can also influence susceptibility to non-allergic toxicity and helps to explain the suggested increased susceptibility of patients with HIV or chronic hepatitis B and C to isoniazid hepatotoxicity [31]. Modulating innate immune response might be crucial in determining the severity and extent of liver in- jury. Factors affecting the expression of protective cytokines (genetic polymorphisms) or an underlying disease might promote liver toxicity. The balance between protective cytokines (interleukin (IL)-10, IL-6, monocyte chemoattractant protein (MCP)-1, MCP-2) and pro-inflammatory cytokines (interferon (IFN)-y, fas ligand (FasL), tumor-necrosis factor (TNF) in the liver may determine the extent of organ damage and the mode of cell death (apoptosis and necrosis) [20].

Drug impairment of the mitochondrial function leading to de- creased fat oxidation and/or energy production which results in steatosis and cell death is the hallmark of several instances of DILI [32, 33]. Opening the mitochondrial permeability transition pore, which subsequently results in necrosis or apoptosis, either through drug-induced direct toxicity or immune reactions, is another path- way of cell damage [34]. Several drugs had been found to carry *in vivo* or *in vitro* mitochondrial hazards and recently a unifying hypothesis has been proposed to explain the susceptibility for a number of drugs that target mitochondria and cause DILI [35]. The mitochondrial hypothesis states that in the setting of underlying genetic or acquired mitochondrial abnormalities, initially silent and accumulating organelles damage

then reach a critical threshold and abruptly trigger overt liver injury. This is consistent with the fact, that the time to onset is typically delayed (by weeks or months) in idiosyncratic DILI. The heterozygous superoxide dismutase 2 gene knockout (Sod2+/-) mice provided supporting evidence for this concept showing that the intraperitoneal administration of 30 mg/kg/day of troglitazone in this animal model for twenty-eight days caused liver injury, characterized by increased serum ALT activity and hepatic necrosis [36]. However, these results were irreproducible in a repeated dose toxicity study using the same animal model treated orally with 300 mg/kg/day troglitazone for twenty- eight days, suggesting that the mitochondrial damage alone might not be the main cause of the troglitazone-induced idiosyncratic liver injury observed in humans [37]. Nevertheless, extensive genetic analyses of samples from DILI patients would be needed to provide proof-of-concept [34].

RISK FACTORS

Risk factors influencing DILI are in most instances those affecting drug disposition and metabolism. Incomplete information in case reports have precluded analyses of drug-related, environmental risk factors and clinical presentation profiles, which however can be obtained in large cohorts of patients with DILI [6, 38, 39]. Known risk factors are, nevertheless, poorly predictive of hepatotoxicity in clinical practice. Furthermore, in many instances of DILI no risk factor can be identified.

CHEMICAL HAZARDS

Chemical properties of some medicaments make them more prone to induce liver damage but this is probably critical only for selected medications. For example, ebrotidine (an H2 receptor antagonist withdrawn from the market in Spain because of severe hepatotoxicity) [40] shares with famotidine the same thiazole ring but in the side chain it bears a 4-bromo-benzene ring which could explain the differences in liver safety between both drugs [40]. Similarly, the quinolone trovafloxacin (withdrawn from the market in Europe and with black box warning in the USA because of its hepatotoxic potential) bears a unique difluorinated side chain that is not found in the other quinolones, with the exception of temafloxacin (another drug withdrawn because of unacceptable toxicity) and which renders these drugs highly lipophilic [41]. Clotiazepam and bentazepam are more structurally similar to phenothiazines than to other benzodiazepines due to the thiophene ring they bear [42] and in fact, unlike other benzodiazepines, several cases of moderate-to-severe hepatitis, and even chronic liver injury, have been reported with both drugs [43].

On the other hand, certain compounds form acyl glucuronides, which are capable of hydrolysis, intra-molecular acyl-migration and covalent binding to proteins [44]. It is worth noting that 10 out of

47 drugs (alclofenac, bendazac, benoxaprofen, fenclofenac, ibufenac, indoprofen, pirprofen, suprofen, ticrynafen and zomepirac) were removed from the US, UK and Spanish markets between 1964 and 1993 due to their poor safety profile. They are carboxylic acids and are metabolized primarily to acyl glucuronides in hu- mans. Several environmental factors exist which appear to operate in determining individual susceptibility.

Age

Elderly patients are the population most vulnerable to DILI. In particular, increased age is a susceptibility factor for developing hepatotoxicity to antituberculous drugs [45]. Old age has also been implicated in amoxicillin-clavulanate induced cholestasis [46, 47]. In contrast, valproic acid and erythromycin hepatotoxicity are more common in childhood [44]. Old age, rather than being a predisposing factor to DILI, seems to have an impact on the phenotypic expression of toxic liver damage, strongly favouring the appearance of a cholestatic pattern of injury [5, 38].

Gender

Women are believed to be more susceptible than men to most forms of DILI [7, 48-50] For instance, autoimmune hepatitis triggered by drugs is seen almost exclusively in women [24] and diclofenac hepatotoxicity has been reported more frequently in women with osteoarthritis [44].

However, new epidemiological data have challenged this traditional belief, showing a similar sex distribution in DILI cases [5, 51], with a higher prevalence of female gender only at younger ages [5]. Yet female gender is considered to be a risk factor for develop- ing fulminant liver failure [38] and the female sex accounted for 76% of patients presenting with drug-induced acute liver failure in the USA who were transplanted [52]. As with age, gender may influence the clinical presentation of DILI as women are overrepresented in the hepatocellular type of injury [5].

Chronic Alcohol Consumption

A history of alcohol intake was considered to be a general risk factor for idiosyncratic DILI, and as such scores 1 + point in the standard clinical scale for the assignation of causality developed by experts, the CIOMS (Council for International Organizations of Medical Sciences) or RUCAM (Roussel Uclaf Causality Assessment Method) scale [53]. However, for most of the drugs capable of inducing hepatotoxicity, there is no evidence for a role of alcohol in potentiating toxicity. Furthermore, in a recent report analyzing a prospective cohort in the US any alcohol use in the preceding 12 months was a negative

predictor of severe DILI (odds ratio, 0.33; 95% confidence interval, 0.15– 0.76) [6]. In practical terms, chronic alcohol intake increases the risk of developing liver fibrosis during methotrexate therapy, and enhances acetaminophen hepatotoxicity by inducing CYP2E1, (with generation of higher levels of the reactive metabolite and depletion of glutathione stores), as well as susceptibility to liver damage from isoniazid, halothane and cocaine [44].

Concomitant Drugs

Are capable of modulating the hepatotoxic potential of other drugs through CYP or hepatic transport systems induction, inhibition or substrate competition. In addition, concomitant medication with hepatotoxic potential may further increase the risk [54]. Concurrent use of anticonvulsants which induce drug metabolism greatly increases the risk of hepatotoxicity due to valproate [44]. The concurrent use of isoniazid and rifampicin, a potent microsomal inducer, enhances the risk of isoniazid hepatotoxicity [55].

Underlying Disease States

Controversy exists on whether preexisting liver disease is a susceptible factor for the development of hepatotoxicity. The presence of chronic hepatitis B, hepatitis C infection or co-infection with human immunodeficiency virus (HIV) increases the risk of isoniazid hepatotoxicity or elevated transaminases on HAART (highly active antiretroviral therapy) [56]. The increased susceptibility exhibited by HIV patients suggests a role for cytokine imbalance [31]. Rifampicin is more hepatotoxic when used for control of itching in patients with primary biliary cirrhosis [57].

Obesity, diabetes mellitus type 2 and insulin resistance are known risk factors for steatohepatitis, and have also been shown, along with psoriasis, to increase the risk of developing liver fibrosis during methotrexate therapy [58]. In the analysis of the first 300 cases of idiosyncratic hepatotoxicity included in the prospective DILIN study in the US the presence of diabetes mellitus was an independent risk factor for severe DILI (odds ratio, 2.69, 95% confidence interval, 1.14 – 6.45) [6].

CLINICAL EXPRESSION

The phenotypic expressions of DILI may present in several ways (clinical and pathological) that resemble known forms of acute and chronic liver disease; the severity ranges from sub- clinical elevations in liver enzyme concentrations to acute liver failure. On the whole, drugs tend to induce acute hepatitis, cholestasis or a mixed condition [24] (Table **1**).

Liver histology (although not very specific and, at best, “compatible with”) is still the

ideal tool for defining the pattern of liver damage. However, since a liver biopsy specimen is often not avail- able, the pattern of drug-related liver injury is, from a practical standpoint, classified according to laboratory data [59]. The classification scheme was proposed by the Council for International Organizations of Medical Sciences (CIOMS) and recently updated by the Food and Drug Administration Drug Hepatotoxicity Committee (Table **2**). Reference is made to the pattern of serum enzymes elevations to define whether the hepatic injury is "hepatocellular", "mixed" or "cholestatic", which are defined by calculation of the "R value". The R value is calculated by dividing the alanine aminotransferase (ALT) by the alkaline phosphatase (AP) using multiples of the upper limit of the normal range for both values. The values used should be from the same day and should be those from the initial blood test results. The reason is that an initial hepatocellular pattern can be converted to a cholestatic-type pattern during the follow-up, which may be related to the differences in the faster rate of improvement of ALT versus AP or glutamyltranspeptidase following drug withdrawal [60]. Calculation of the type of liver damage according to this rule correlates quite well with the underlying histological lesion, has proven prognostic value, and is essential to apply the RUCAM scale.

The acute hepatocellular (cytotoxic, cytolytic) type of liver injury is the most common expression of hepatotoxicity [6, 38] and is observed with many drugs (Table **1**). Patients with acute hepatocellular injury related to drugs are at risk of acute liver failure. The presence of combined increases in ALT and bilirubin levels in DILI reflects a substantial loss in hepatocellular function and potential for liver failure [61]. These parameters are considered a specific indicator of severe hepatotoxic potential of a drug. In patients with acute drug-induced hepatitis the presence of jaundice is the most significant predictor of mortality/liver transplantation. The observation by Hyman Zimmerman, known as "Hy's rule" [24], predicts a mean mortality (or its surrogate marker, liver transplantation) of 10% (range 5-50%) for jaundiced patients with acute toxic hepatocellular damage. Hy's rule has been validated in large case series from Spain [38], Sweden [39] and the US [6], in which analysis of pooled data showed that other variables such as old age, female gender and AST levels were independently associated with a poor outcome [38, 39]. The late Hyman Zimmerman also stated that prolonging exposure to the causative drug once hepatotoxicity is initiated may favor a fulminant outcome [24]. Interestingly, in the American Registry the median duration between first exposure to the implicated agent and DILI recognition was significantly longer in severe cases than in mild/ moderate cases although the association was not statistically significantly longer in the multivariate analysis [6]. Further analysis of the Swedish database has shown that there was also a significant linear relationship between daily dose and the outcome of death/liver

transplantation (2%, 9.4%, and 13.2% in<10, 11-49, and>50 mg/day groups, respectively, *P*= 0.03)[10].

Conversely, it has recently been suggested that eosinophilia accompanying DILI may be associated with a better short-term prognosis [62]. Actually, none of the patients included in the Spanish Registry with idiosyncratic DILI who died, evolved to liver failure, or required a liver transplantation, had eosinophilia, whereas this feature was found in the 23% of the patients with milder outcomes [38]. Of note is that the height of transaminases in acute hepatocellular DILI lacks prognosis significance. Indeed, a decrease in liver enzymes after drug withdrawal in patients with severe DILI may reflect, rather than a clinical improvement, a limited hepatic reserve associated with impending liver failure [61]. Moreover, if acute injury is superimposed on underlying liver dis- ease, monitoring liver function tests may be difficult to interpret. For example, if advanced cirrhosis is present, the severity of liver injury may be underestimated by the height of the serum ALT measurements [63]. which is predominantly centrilobular [24, 31]. In such cases director more commonly immune-mediated damage to the cholangyocite is probably caused by toxic intermediates excreted into bile.

In mixed hepatic injury, the clinical and biological picture is intermediate between the hepatocellular and the cholestatic pat- terns, and features of either type may predominate. By definition,the ALT/AP ratio is between 2 and 5 (Table **2**). When faced clini- cally with a mixed hepatitis picture, the gastroenterologist should always suspect and seek a potentially hepatotoxic medication, since this type of injury is far more characteristic of drug-induced hepatotoxicity than of viral hepatitis [24]. Almost all drugs that produce cholestatic injury are also capable of inducing a mixed pattern.Genotypic studies in patients with DILI have shown the association of some HLA alleles with cholestatic/mixed damage, which pointsto an allergic basis for this variety of hepatotoxicity [64].

Acute cholestatic injury is caused by many drugs, including cyclosporine anabolic and contraceptive steroids, which characteristically produce DILI with minimal or absent accompanying in- flammation, presumably by interfering with bilirubin and bile acids export from the canaliculi. With other medications, which include amoxicillin-clavulanate, macrolide antibiotics and phenothiazine neuroleptics, there are typically variable degrees of portal inflammation and hepatocyte necrosis, in addition to marked cholestasis Drug-induced acute cholestatic and mixed lesions are less severe in the short-term outcome than the hepatocellular type. An exception to this rule is the analysis of the American Registry in which, within 6 months of DILI diagnosis, 14.3% of patients with cholestatic injury died

(as compared with only 7.5% of patients with hepatocellular damage). However, most of the fatalities in subjects with cholestatic damage were not related to liver failure in this study [6].

The resolution of cholestatic and mixed lesions, nevertheless, are generally slower, with a higher likelihood towards chronicity in one study (9% vs 4% respectively; $p<0.031$) [60]. In contrast, in the hepatocellular pattern with jaundice at presentation, the severity of chronic lesions was greater (higher incidence of cirrhosis and chronic hepatitis) [60, 65]. Cardiovascular and central nervous sys- tem drugs are the main groups leading to chronic liver damage [60]. Of the whole population included in the Spanish Registry, 5.6% of patients had biochemical evidence of chronicity [60], a figure simi- lar to that reported in the Swedish population (6%), even though the majority of the patients included in this latter study were identified in an outpatient basis and had mild to moderate DILI [66]. A higher prevalence of chronicity (14%) has recently been reported in the US [6]. Further studies on the natural history of DILI are on-going.

PHARMACOGENOMICS IN DILI

As commented above, drug biotransformation is believed to be a relevant process in DILI. The biotransformation of drugs and xenobiotics involves several steps, including Phase I oxidation by cytochrome P450 enzymes followed by Phase II conjugations through enzymes as N-acetyltransferase or glutathione transferase. The activity of drug-metabolizing enzymes shows significant inter- individual and interethnic variation. Genetic and environmental factors are responsible for this variability. Most drug-metabolising enzymes are polymorphic, due to the presence of polymorphisms, that is, mutations which occur with a frequency higher than 1%, in genes coding for such enzymes [67, 68]. Variant genes cause abol-shed, reduced, altered or increased enzyme activity, because of complete gene deletions, single nucleotide polymorphisms that occur isolated or combined, and gene duplications. Individuals carrying enzyme-inactivating polymorphisms display higher drug plasma concentrations and lower clearance rates when treated at standard doses [67, 69]. Therefore, an increased prevalence and severity of adverse drug reactions, including DILI, could be expected among subjects carrying enzyme-inactivating mutations, when receiving drugs that are substrates of the defect enzyme. Polymorphisms which have been associated with an increased risk of drug hepatotoxicity are summarized in Table **3**.

It should be borne in mind that the biotransformation of a drug most commonly involves more than one metabolic pathway and that several enzymes may be involved in the metabolism of a single drug. As our understanding of drug metabolism increases, more and more evidence suggests that minor metabolic pathways may play a relevant role in

adverse drug reactions [70]. Therefore the analysis of the relationship between the occurrence of hepatotoxicity or adverse drug reactions and the metabolism of a drug should con- sider all the pathways involved in the metabolism of the drug.

Cytochrome P450

The most important enzymes of phase I metabolism are the cytochrome P450 system, a superfamily of isozymes that catalyze the oxidation of drugs and other xenobiotics. Multiple forms of CYP enzymes play important roles in the oxidation of structurally diverse drugs (http://medicine.iupui.edu/flockhart; http://www. imm.ki.se/CYPalleles/). CYP1, CYP2 and CYP3 families are major forms. Among these, important human drug-metabolizing enzymes are CYP1A2 (caffeine, estradiol, lidocaine, tacrine, theophylline, verapamil, R-warfarin), CYP2C8 (amodiaquine, R-ibuprofen, pacli- taxel, pioglitazone, trogitazone) CYP2C9 (diclofenac, ibuprofen, phenytoin, piroxicam, tetrahydrocannabinol, tolbutamide, warfarin), CYP2C19 (diazepam, hexobarbital, S-mephenytoin, omeprazole, pentamidine, propranolol), CYP2D6 (codeine, debrisoquine, dex- tromethorphan, encainide, haloperidol, metoprolol, mexiletine, paroxetine, phenothiazines, propranolol, risperidone, sertraline, tricyclic antidepressants, venlafaxine), CYP2E1 (chlorzoxazone, halothane, paracetamol, isoflurane, desflurane, enflurane), and fi- nally CYP3A subfamily members CYP3A4 and CYP3A5 mediate the metabolism of many very useful drugs [70-87].

With regard to CYP1A2, the enzyme shows interindividual differences due to genetic polymorphisms and to modulation with many compounds such as drugs (fluvoxamine or ciprofloxacin), foods (grapefruit juice or broccoli) and toxins (polycyclic aromatic hydrocarbons present in tobacco smoke) [88]. Several *CYP1A2* allelic variants have been reported, and some of these are related to decreased enzyme activity or decreased expression of the enzyme [89-91]. For an updated list of *CYP1A2* variant alleles see the web- site http://www.cypalleles.ki.se/cyp1a2.htm. CYP1A2 have been associated with 5-n-butyl-7-(3,4,5-trimethoxybenzoylamino) pyra- zolo[1,5-a]pyrimidine (OT-7100) hepatototoxicity [92]. The hepa- totoxicity of OT-7100, a pyrazolopyrimidine derivative with potential analgesic effects, has been studied *in vitro,* in human liver m crosomes, and could be related to the formation of a reactive metabolite produced by CYP1A2 activity. No studies have confirmed the influence of *CYP1A2* polymorphisms in DILI.

The *CYP2C* gene cluster is highly conserved. Many of the *CYP2C* genes are within the same haplotype block [93, 94]. Comparison of linkage disequilibrium between pairs of loci carried outin the frame of the HapMap project [95], indicates that the four *CYP2C* genes, namely *CYP2C8, CYP2C9, CYP2C18* and *CYP2C19*, are allocated in two clusters,

the first one including *CYP2C18*, *CYP2C19* and *CYP2C9*, and the second one including *CYP2C8* and part of *CYP2C9* flanking region [94]. Another proposed distribution includes *CYP2C18* and *CYP2C19* in one cluster and *CYP2C9* and *CYP2C8* in the other [93]. Several variant alleles have been described for both *CYP2C8* and *CYP2C9* genes. The variant alleles *CYP2C8*3*, *CYP2C9*2* and *CYP2C9*3*, are the commonest variant alleles among Caucasian individuals [70, 96]. Besides the putative implications of CYP2C enzymes in cancer risk and other diseases [70, 83, 97-100], it has been unambiguously shown that genetic variations in CYP2C enzymes cause variations in drug metabolism. In addition, changes in the expression of CYP2C enzymes may also lead to altered response, as is the case for resistance to paclitaxel [82, 86]. Regarding single nucleotide polymorphisms, the *CYP2C8*3* variant allele causes impaired bio- disposition of ibuprofen *in vivo* [80]. Other variant *CYP2C8* alleles are *CYP2C8*2*, which is related to increased Km for paclitaxel metabolism [101], *CYP2C8*4*, which causes a marginal decrease in metabolic capacity, and *CYP2C8*5*, which causes a frameshift and an early stop codon [102]. The effect of *CYP2C8* variant alleles on drug metabolism, pharmacokinetics and adverse effects remains to be investigated in detail. Because many drugs causing DILI are metabolized in part by CYP2C8, it is of particular interest to elucidate the role of *CYP2C8* variant alleles in the risk to develop DILI in patients receiving CYP2C8 substrates, and we are currently conducting such studies. With regard to CYP2C9, the enzyme encoded by the *CYP2C9*3* allele shows a reduced efficiency of paclitaxel [103] or arachidonic acid metabolism whereas it keeps full activity on amiodarone [102, 104]. Several examples of impaired drug metabolism *in vivo* related to variant alleles have been provided, either for *CYP2C9*2* or for *CYP2C9*3*, with drugs such as acenocoumarol, warfarin, several NSAIDs, hypoglycemics and angiotensin antagonists. Diverse studies have suggested a role for *CYP2C9* polymorphisms in liver toxicity caused by voriconazole, with a trend towards higher toxicity for carriers on non-mutated alleles [105], for valproic acid, with higher risk for carriers of mu- tated alleles [106], or for leflunomide with higher risk being associ- ated to mutated alleles [107]. Unfortunately no studies involving a large number of patients with DILI caused by these drugs have been conducted. The metabolism of the most NSAIDs is carried out by CYP2C9 and some them are known to be hepatotoxic. Based on this hypothesis various studies were conducted to test whether *CYP2C9* polymorphisms can be considered as risk factors for DILI[108, 109]. Aithal *et al.* investigated the influence of *CYP2C9*2* and *CYP2C9*3* in hepatotoxicity caused by diclofenac in 24 pa- tients, with negative findings [108]. The study by Pachkoria had a similar sample size (28 patients) and did not identify a positive association [109]. Although both studies point to a lack of a major association between *CYP2C9* polymorphisms and DILI, further studies are

required to rule out such an association for several rea- sons. First, the statistical power of both studies is too low to reach definitive conclusions, and second, because studies should involve patients with DILI caused by drugs that are metabolized mainly by the CYP2C9 enzyme. For instance, the study on diclofenac-induced DILI [108] should have considered other drug-metabolizing enzymes. The primary metabolism of diclofenac is carried out by acyl glucuronidation, by uridine 5'-diphosphoglucuronosyl transferase UGT2B7 and by diverse hydroxylation pathways to produce hydroxymetabolites that are usually further conjugated [110]. CYP2C9 plays a minor role in diclofenac metabolism and therefore a lack of association between *CYP2C9* polymorphisms and diclofenac-induced DILI is not surprising. Daly *et al.* analyzed the polymorphisms of several enzymes involved in the metabolism of diclofenac with relation to the risk of developing DILI and they found a positive association for *UGT2B7* and *CYP2C8* variant alleles [111]. Another factor that should be considered in further studies is that a strong linkage between the *CYP2C8*3* and the *CYP2C9*2* variant alleles has been described to occur in Caucasians [80, 81, 83, 112-114]. This fact, together with increasing evidence that support a role for the CYP2C8 enzyme in the metabolism of drugs thought to be substrates of CYP2C9 [70, 73, 80, 81, 113], indicates that, in order to be conclusive, further studies should analyze as putative risk factors genetic variations in both genes, *CYP2C8* and *CYP2C9*, simultaneously.

With regard to CYP2C19, the variant alleles *CYP2C19*2* and *CYP2C19*3* are the major variant alleles although *CYP2C19*3* is only present in Oriental individuals and is not detected in Caucasians [109, 115]. CYP2C19 deficiency activity has been implicated in alterations in the metabolism, and in toxicity events with the use of several drugs [116], including hepatotoxicity caused by Atrium (a fixed combination preparation containing phenobarbital, febarbamate and difebarbamate) [117], but no clear association of *CYP2C19* variant alleles with DILI has been identified so far. Recently a study on patients with DILI caused by several drugs has shown that the variant *CYP2C19*2* allele does not enhance the risk of DILI [109], although the study requires confirmation because of the low sample size.

Regarding the enzyme CYP2D6, *CYP2D6*3, CYP2D6*4, CYP2D6*5* (gene deletion), *CYP2D6*6* and *CYP2D6* gene duplication and amplification are the main variant alleles [74]. There are important ethnic differences in *CYP2D6* genetic polymorphisms [118]. In addition, it has been shown that interindividual differences in the processes leading to gene deletion and duplication can also occur [119]. Several studies have found that poor metabolizers were at higher risk of developing adverse effects [120-123]. CYP2D6 deficiency has been associated with perhexiline hepatotoxicity [124], probably due to mitochondrial injury which causes steatosis [125]. A recent study attempted to elucidate

whether *CYP2D6* polymorphisms has a role in anti tuberculosis drug-induced hepatitis with negative findings [126]. No other studies investigating the putative role of *CYP2D6* polymorphisms in DILI have been published.

CYP2E1 is the major enzyme involved in the metabolism of general anaesthetics [85]. Other CYP2E1 substrates are paracetamol, chlorzoxazone, and ethanol [67, 127]. CYP2E1 has been related to paracetamol hepatotoxicity in humans [128, 129]. Interestingly, Zaher *et al.* demonstrated in a mouse line that deletion of CYP2E1 and CYP1A2 prevented paracetamol toxicity [130]. Knockout mice lacking CYP2E1 were also protected against carbon tetrachloride-induced hepatotoxicity [131]. At present 13 variant *CYP2E1* alleles have been described. Among these the most com- mon is the variant *CYP2E1*5* allele, initially designated as C2 allele, which is located in the 5' flanking region and causes an in- crease in the expression of the enzyme [132]. Furthermore, there are additional SNPs where the haplotypes have not yet been deter- mined (see http://www.cypalleles.ki.se/cyp2e1.htm).

Huang *et al.* analyzed the influence of the *CYP2E1*5* variant allele in a case-control study involving 49 patients with DILI, and identified a positive association with patients lacking the *CYP2E1*5* allele and thus at increased risk of developing antituberculosis drug-induced hepatitis [133]. In agreement with these findings, another study reported association of non-mutated *CYP2E1* genotypes with isoniazid-induced hepatotoxicity [134]. Two studies published recently found no association of *CYP2E1* genotypes and antituberculosis drug-induced hepatotoxicity, although the sample size was too small (18 and 23 patients) to achieve definitive conclusions [135, 136]. Overall findings and a recent meta-analysis indicate that non-mutated *CYP2E1* alleles confer a moderate risk to develop anti-tuberculosis drug-induced liver injury [137].

Regarding CYP2E1 expression, it has been shown that the inhibition of CYP2E1 with alphahederin or oleanolic acid is able to reduce carbon tetrachloride-induced hepatotoxicity in mice [138, 139]. CYP2E1 is a highly inducible enzyme. Ethanol-induced hepatotoxicity correlated with CYP2E1 expression and the inhibition of the enzyme protects from liver damage [140]. In addition, ethanol is capable of inducing CYP2E1 [141], and it is likely that the production of toxic metabolites could be increased in alcoholics and in carriers of the *CYP2E1*5* variant allele. Isoniazid and other CYP2E1 inducers increase the extent of metabolism of sevoflurane, increasing the production of the hepatotoxic inorganic fluoride [142]. Therefore, besides gene polymorphisms, induction and inhibition of CYP2E1 are likely to play a major role in DILI. Unfortu- nately, the actual impact *in vivo* of induction of CYP2E1 for DILI remains to be analyzed.

CYP3A4 and CYP3A5 enzymes are involved in the metabolism of about half of clinically used drugs [143], both enzymes making up nearly 30% of the total CYP enzymes expressed in the human liver [144]**.** High interindividual variation in enzyme activity *in vivo* and *in vitro* has been reported. The clinical relevance of this inter- individual variability lies in the wide range of drugs, carcinogens and mutagens that are CYP3A4 and CYP3A5 substrates [143]. Although over 40 allelic variants and several SNPs where the haplotypes have not yet been determined have been described (see http://www.cypalleles.ki.se/cyp3a4.htm), the genetics basis for variability in CYP3A4 metabolism remains elusive. In contrast, the major genetic factor for the interindividual variability in CYP3A5 activity has been elucidated. Only two variant alleles, *CYP3A4*1B* and *CYP3A5*3* are common across diverse ethnic populations and have functional relevance. Present evidence indicates that *CYP3A4*1B* and *CYP3A5*3* are functional polymorphisms *in vivo.* Regarding *CYP3A4*1B*, it seems to modify the ability to metabolize some CYP3A substrates, such as quinine [145], although it does not influence the metabolism of other substrates such as midazolam or dextromethorphan [87, 146]. *CYP3A5*3* is the commonest *CYP3A5* allele and is associated with severely decreased enzyme activity [79]. For CYP3A4 interindividual variability seems to be related to CYP3A4 induction and inhibition. For instance, grape- fruit juice inhibits the enzyme and may also cause pharmacokinetic changes for CYP3A4 substrates [147]. Now it is admitted that induction and inhibition are more relevant for CYP3A4 enzyme activity than variations in the *CYP3A4* gene [148]. Since CYP3A4 can be induced by dietary components, herbal medicinal products and food supplements [149], an increased production of toxic drug metabolites may be expected in individuals consuming CYP3A4 inducers.

CYP3A4 has been implicated in the toxicity of acetaminophen, and the underlying mechanism is likely to be related to the orphan pregnane X receptor [150]. It has been shown that troglitazone and flucloxacilin are biotransformed into chemically reactive metabo- lites by CYP3A and other enzymes, but it has not been demon- strated that reactive metabolites are responsible for the troglitazone or flucoxacilin induced hepatotoxicity [151-153]. Regarding poly- morphisms, Haas *et al.* analyzed the role of *CYP3A4* and *CYP3A5* polymorphisms in the risk to develop nepiravine hepatotoxicity in 53 patients and found no association of genotypes with this risk [154]. Levin *et al.* analyzed *CYP3A4* and *CYP3A5* polymorphisms with regard to voriconazole hepatotoxicity with negative findings [105]. Although overall findings are negative, it should be stated that there is very little information on *CYP3A4* and *CYP3A5* poly- morphisms in patients with DILI, and that further studies are required to rule out an association of polymorphisms in these genes and

DILI risk, especially in patients with DILI related to drugs that are CYP3A4 or CYP3A5 substrates.

ARYLAMINE N-ACETYLTRANSFERASE 2 (NAT2)

Human arylamine N-acetyltransferase 2 (NAT2, EC 2.3.1.5) is responsible for the acetylation of numerous xenobiotics and arylamine- or hydrazine-containing drugs [155]. NAT2 is involved in the metabolism of antibiotics, such isoniazid and sulfamethoxazole, and other drugs such as procainamide, hydralazine, dapsone, phenelzine, acebutolol or cafeine. Genetic polymorphisms of *NAT2* are responsible for interindividual variation in the metabolism of these drugs. The determination of NAT2 genotype or phenotype has been proposed to predict adverse reactions in patients with tubercu- losis receiving isoniazid and prior to the concomitant administration of drug combinations such as procainamide-phenytoin or doxicicline-rifampin [156, 157]. The NAT2 enzyme is encoded by a gene located in human chromosome 8 [158]. To date 52 alleles have been registered at http:/www.louisville.edu/medschool/ pharmacology/NAT.html [159]. Most variant alleles contain combinations of seven common SNPs within the coding region of the gene, designated as G191A (rs1801279), C282T (rs1041983, rs59855457), T341C (rs1801280, rs56935242), C481T (rs1799929, rs60310310), G590A (rs1799930, rs60190029), A803G (rs1208, rs56599719, rs58999469) and G857A (rs1799931, rs58803786). Among these, polymorphisms at positions 191, 341, 590 and 857 are the most studied and characterize the major *NAT2* defect allele clusters (*NAT2*5, NAT2*6, NAT2*7* and *NAT2*14* respectively) [160]. NAT2 polymorphisms are responsible for variation in the acetyla- tion phenotype. Individuals can be classified as slow or fast acetyla- tors on the basis of their genotype, although some authors consider a third intermediate acetylator status. To infer phenotypes from genotyping data, carriers of zero, one or two functional alleles are defined as slow, intermediate and fast acetylators respectively, after reconstruction of *NAT2* haplotypes from crude genotyping data, which is a complex process, although methods to clear ambiguous diplotypes are available [161]. Interethnic and intraethnic variability of *NAT2* polymorphisms has recently been analyzed [162].

Recently, the role of N- acetyltransferases in DILI has been reviewed [163]. Isoniazid is the main drug responsible for hepatotoxicity induced by antituberculosis treatment. It is metabolized to acetylisoniazid by hepatic N-acetyltransferase [164]. In turn, ace- tylisoniazid is hydrolyzed to acetylhydrazine, which is oxidized by CYP2E1 to form hepatotoxic intermediates [165]. However, the disposal of acetylhydrazine also depends on further acetylation by NAT to form a non-toxic metabolite, diacetylhydrazine [166, 167]. A study of Huang *et al.* [168] shows that slow acetylators had a higher incidence of

antituberculosis drug-induced hepatitis and that once they had hepatotoxicity, they were prone to develop more severe hepatic injury than rapid acetylators. Additionally patients with homozygous genotype CYP2E1 c1/c1 have increased risk of hepatotoxicity (OR 2.52). Combined with the acetylation status, the risk of hepatotoxicity increased from 3.94 for patients with CYP2E1 c1/c1 and rapid acetylator status to 7.43 for patients with CYP2E1 c1/c1 with slow acetylator status [133]. Cho *et al.* reported that slow acetylation is related to antituberculosis drug-induced hepatotoxicity [135]. Other independent studies supported the hypothesis of slow acetylation as a risk factor for drug-induced hepatotoxicity [136, 169-172]. However, negative findings have also been reported [134, 173]. Overall findings indicate a positive though modest association of the *NAT2* slow acetylation genotype with DILI caused by antituberculosis drugs. A common limitation to most of the studies performed so far is the small sample size and that the genotyping procedures and the assignment of variant alleles after haplotype reconstruction differ across the different studies. This is a major source of heterogeneity. Another source of heterogeneity is due to the interethnic differences in the frequency for variant *NAT2* alleles, which are rare in Oriental individuals, and therefore studies in Oriental subjects require a high sample size to have adequate statistical power. For further studies it would be advisable to analyze at least the four SNPs that define the major *NAT2* clusters, to include a sample size adequate to the ethnic ori- gin of participants and to use a well-established method to assign phenotypes.

GLUTATHIONE TRANSFERASES (GSTS)

The GSTs are a superfamily of phase II enzymes known to play important roles in protecting against endogenous oxidative stress, as well as in the detoxification of exogenous potential toxins including carcinogens, toxic chemicals and drugs. GSTs catalyse nucleophilic attacks of glutathione on electrophilic substrates, thereby decreasing the reactivity of potential toxicants with cellular molecules [174]. Mammalian cytosolic GSTs are divided into eight classes: alpha, kappa, mu, omega, pi, sigma, theta and zeta [175]. Genetic polymorphisms of these enzymes have been demonstrated and correlated to the susceptibility of many diseases including cancer and alcoholic liver disease [176-180]. However, little is known about the genetic polymorphism of these detoxification enzymes and DILI. To date, studies carried out on DILI patients have been limited to gene deletion of *GSTT1* (pi class) and *GSTM1* (mu class) genes, designed as null alleles [173, 181-185].

It has been shown that the risk of anti-TB drugs hepatotoxicity is increased in Indian patients with *GSTM1* homozygous deletion [173]. Huang *et al.* [182] also reported that

patients with *GSTM1* homozygous deletion had a high risk of anti-tuberculosis drug-induced hepatotoxicity in a Taiwanese population. However, nosignificant increase in risk was observed in patients with *GSTT1* homozygous deletion. Recently, our group reported that carriers of double *GSTT1-GSTM1* null genotypes had an increased risk of developing DILI compared with noncarriers Caucasians patients with DILI [181]. Overall findings [173, 181-186] consistently indicate that slow glutathione-S transferase activity is associated with hepatotoxicity, and that this association is a general mechanism independent of the causative drug and of the mechanism leading to either hepatocellular or cholestatic mixed type of injury [181]. However, several issues remain to be analyzed regarding GST enzymes. First, single nucleotide polymorphisms have been reported for the *GSTT1* and *GSTM1* genes [187]. These single nucleotide polymorphisms are relevant for Africans and Hispanic individuals
[188] and have not been analyzed with regard to DILI risk. There- fore studies involving these subjects should explore the influence on these polymorphisms besides gene deletion. Even more important is the absence of extensive studies on genetic variations for other GST enzymes besides GSTM1 and GSTT1. Because many GST enzymes are expressed in the liver, it is likely that variationsin the genes coding for the rest of GST enzymes will influence DILI risk. These studies are in progress.

UDP-GLUCURONOSYLTRANSFERASES (UGTS)

UGTs are a superfamily of enzymes codified by UGT genes classified into UGT 1A, 2A and 2B subfamilies which catalyse reactions of conjugation with glucuronic acid. These enzymes are responsible for the elimination of drugs and another xenobiotics and endogenous compounds like bile acids, bilirubin hydroxyl-steroids and thyroid hormones [189].

The toxicity of some drugs like irinotecan and lamotrigine has been related to impaired UGT1A activity because patients with mutant *UGT1A1* alleles are over-represented amongst those experiencing severe toxicity [190-192]. Inhibition of UGT activity is re-lated to simvastatin-ezetimibe induced liver failure [193]. The mechanism underlying this relationship seems to be due to ezetimibe inhibition of UGT enzymes, which causes increased simvastatin exposure. In a study carried out with human liver microsomes and recombinant UGTs, Yoshigae *et al.* suggested that the liver failure induced by troglitazone is not related with the *UGT1A1* polymorphism, because other UGT1 and UGT2 enzymes may be responsible for troglitazone glucuronidation [194]. Because UGT enzymes overlap in substrate specificity, it is likely that the presence of variant alleles in one of the UGT genes would be insufficient to explain the variability in enzyme activity *in vivo*, that is, the

analysis of association of UGT genetic polymorphisms and hepatotoxicity can be extremely complex if the drug is substrate of several UGTs enzymes.

UGT2B7 has been implicated in the diclofenac- induced hepatotoxicity [111]. Diclofenac metabolism is carried out by diverse hydroxylation pathways and by acyl glucuronidation. About 50% of diclofenac is eliminated as 4-hydroxydiclofenac, a CYP2C9 product. The other metabolite in humans, 5-hydroxydiclofenac, seems to be the product of several enzymes, including CYP2C8, CYP2C18, CYP2C19 and CYP2B6 [110, 173]. A role for CYP3A4 in diclofenac metabolism has also been claimed [110, 173], although this issue has not been investigated in sufficient detail. The rest of the drug is metabolized by uridine 5'-diphosphoglucuronosyl transferases UGT2B4 and UGT2B7 [195]. Diclofenac glucuronide is further metabolized by CYP2C8 [70, 110, 196]. Daly *et al.* [111] found that the variant *UGT2B7*2* allele is more common in patients with hepatotoxicity caused by diclofenac, compared with healthy controls and with patients receiving diclofenac without developing hepatotoxicity. Defective glucuronidation activity as a result of *UGT1A9* mutated alleles, have been claimed to be the cause of two cases of COMT inhibitor –induced hepatic dysfunction [197].

FUTURE PERSPECTIVES

As mentioned above, development of DILI is a complex, multi- step process in which variability in drug metabolism may play arole, but pharmacogenomic studies are still insufficient to confirmor to discount a prominent association of genetic variability in drug metabolism and DILI risk. Variability in drug metabolism or bioactivation is compatible with all proposed mechanisms for DILI. In addition, alterations in Phase II metabolizing enzymes such as GSTs may contribute to the risk by means of general mechanisms independent of the causative drug. Several major issues related to genetic polymorphisms in drug metabolism remain insufficiently studied, for instance, the role of CYP2C8, CYP3A5, and other polymorphisms. Further studies with a sufficient sample size and an exhaustive study of all genetic polymorphisms relevant to the drug responsible for DILI are required. These studies should combine genotyping for drug-metabolising enzymes with the analysis of polymorphisms related to general detoxication mechanisms, such as those of GST enzymes (especially other enzymes besides GSTM1 and GSTT1), superoxide dismutase or glutathione peroxidase enzymes. Regarding the mechanisms that propose immune-mediated hepatotoxicity or the danger-signal hypothesis, it would be worth exploring polymorphisms related to histamine that have clinical implications [198-206], and how these histamine-related polymorphisms may modulate the effect of polymorphisms of drug- metabolising enzymes in the risk to develop immune-mediated

711 DILI.

712 Besides licensed drugs, herbal and natural supplements are recognized as causing
713 hepatotoxicity with increasing frequency as patients turn more and more to alternative
714 medicine [4]. Some com- pounds in herbal and dietary constituents are bioactivated through
715 drug-metabolising enzymes [207], and in addition, these compounds are able to modulate
716 drug-metabolizing enzymes [149, 208] and hence may modify the risk for DILI even if the
717 herbal compounds are not directly hepatotoxic. This highlights the necessity to investigate
718 in detail the hepatotoxic potential of herbal com- pounds, and the mechanisms underlying
719 hepatotoxicity related to herbal and dietary products. This is a promising field that will
720 evolve in the coming years and hopefully will provide useful in- formation to obtain a more
721 comprehensive view of the mechanisms related to DILI and to develop pharmacogenetic
722 analyses to identify at-risk patients in clinical practice.

723

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