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High daily dose and being a substrate of Cytochrome P450 enzymes are two important predictors of drug-induced liver injury

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Running title: Two important predictors for drug-induced liver injury

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List of non-standard abbreviations:

ALF, acute liver failure; CYP, cytochrome P450; CI, confidence interval; DDD, defined daily dose; DDIs, drug-drug interactions; DILI, drug-induced liver injury; DNF, data not found; FDA, Food and Drug Administration; LTKB-BD, Liver Toxicity Knowledge Base Benchmark Dataset; NDA, new drug application; NIH, National Institute of Health; NSAIDs, non-steroidal anti-inflammatory drugs; NSC, not a substrates of CYP enzymes; OR, odd ratio; RO2, Rule-of-Two; WHO, World Health Organization.

Abstract

Drug-induced liver injury (DILI) is complicated and difficult to predict. It has been observed that drugs with extensive hepatic metabolism have the higher likelihood of causing DILI. Cytochrome P450 (CYP) enzymes are primarily involved in hepatic metabolism. Identifying the associations of DILI with drugs which are CYP substrates, inhibitors, or inducers will be extremely helpful to a clinician in decision-making process when dealing with a patient suspected of having DILI. We collected the metabolism data on CYP enzymes for 254 orally administered drugs in Liver Toxicity Knowledge Base Benchmark Dataset with a known daily dose, and applied logistic regression to identify these associations. We revealed that drugs which are the substrates of CYP enzymes would have the higher likelihood of causing DILI (odds ratio (95% confidence interval) [OR (95% CI)]: 3.99 (2.07-7.67); $p < 0.0001$), which is dose-independent, and drugs which are the CYP inhibitors would have the higher likelihood of generating DILI only when they are administered at a high daily dose (OR (95% CI): 6.03 (1.32-27.5); $p = 0.0098$). However, drugs which are the CYP inducers are not observed to be associated with DILI (OR (95% CI): 1.55 (0.65-3.68); $p = 0.3246$). Our findings will be not only useful to identify suspect medication as a cause of liver injury in clinical settings where the patients are treated with comedications, but also for personalized medicines to understand the individual susceptibility of DILI.

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Introduction

Drug-induced liver injury (DILI) is one of the most frequent adverse reactions for the withdrawal of an approved drug from the market (Chen et al., 2013b). Currently more than 50% of cases of acute liver failure in the United States are attributed to DILI (Lee et al., 2008). Although DILI is complicated and difficult to predict because of its complex etiology, some simple rules can be followed to identify the drugs associated with the increased risk of liver injury. For example, the Rule-of-Two (RO2) based on combination of a daily dose (≥ 100 mg) and high lipophilicity (log*P* ≥ 3) has been proposed recently for the identification of drugs with increased risk of DILI (Chen et al., 2013a). RO2 is an effective tool in preclinical application, and it will be particularly helpful for the drug screening. However, its use in the clinical setting is limited. This is largely due to the fact that RO2 is only focused on the drug properties, without taking into account the effects that the liver has on the drug, i.e. hepatic metabolism.

The highest concentration of drugs and metabolites after oral administration is usually exposed by the liver (Jaeschke et al., 2002). Most hepatotoxic drugs are metabolized by the Cytochrome P450 (CYP) enzymes (FDA, 2009), which are primarily involved in the generation of reactive metabolites (Williams et al., 2004). The covalent binding of reactive metabolites to hepatic proteins has been considered as a key event for the occurrence of DILI in humans (Park et al., 2005; Walgren et al., 2005). However, there are some exceptions. For example, ximelagatran, although not prone to generate reactive metabolites, has been withdrawn from the market because of DILI (Keisu and Andersson, 2010). Besides being substrates of CYP enzyme, drugs can also interact with the CYP

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enzymes as either inhibitors or inducers. The exact role of these interaction modes in DILI has not been examined extensively.

It is known that dose-dependent liver toxicity is predictable, and the relationship between a daily dose of oral medications and DILI has also been observed (FDA, 2009). It is found that most drugs which are required to carry black-box warnings or which have been withdrawn from the market due to DILI are prescribed at daily doses higher than 50 mg (Uetrecht, 2007). However, DILI, which is not clearly dose-dependent, is unpredictable and hard to detect due to the differences in individual susceptibilities (FDA, 2009). In addition, polypharmacy and drug-drug interactions (DDIs) increase the risk of DILI (Stine et al., 2013). Examining the large drug data sets may allow us to fill critical gaps in current knowledge on the role of hepatic metabolism in DILI, and thus to associate individual susceptibilities with DILI in the context of hepatic metabolism in clinical application.

CYP enzymes are involved in the metabolism of approximately 75% of the marketed drugs with a wide range of structural variations (Williams et al., 2004). Often, several drugs are co-administered, which poses a challenge to a clinician who, without performing formal causality assessment, has to make a decision which drug should be stopped in case that clinical symptoms or signs of DILI are observed. The goal of our study was to find a simple rule applicable to the clinical settings, which will not only to avoid DILI risk when multiple drugs are treated to the patients, but also for personalized medicines to understand the individual susceptibility of DILI. We hypothesized that drugs which are CYP substrates, inhibitors and inducers may not have the same

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likelihood of causing DILI and that a daily dose may not be equally important for these three groups of drugs.

Materials and Methods

Collection of data on CYP-mediated metabolism

The data on the metabolism mediated by CYP enzymes for the drugs from Liver Toxicity Knowledge Base Benchmark Dataset (LTKB-BD) (http://www.fda.gov/ScienceResearch/BioinformaticsTools), (Chen et al., 2011) developed by National Center for Toxicological Research in Food and Drug Administration (FDA), were collected from SuperCYP (http://bioinformatics.charite.de/supercyp/), DrugBank (http://www.drugbank.ca/), and PubChem (http://pubchem.ncbi.nlm.nih.gov/). The obtained information was verified by searching PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and the drug metabolism data in Pharmacology Review in FDA Approved Package (FDA, 2012). In order to search PubMed, the following combinations of queries were used: '*drug name* and P450/CYP/Cytochrome/Microsome', '*drug name* and metabolism/metabolite/metabolic', '*drug name* and biotransformation', and '*drug name* and disposition/elimination/excretion'.

The drugs with known data on CYP-mediated metabolism are categorized as substrates, inhibitors and inducers. In a situation where a drug can be all three (substrate, inhibitor and inducer) via the different isoforms, the drug will be included in all three situations in statistical analysis. We did not consider the subgroups of substrates, inhibitors and

inducers based on their affinity for the CYP enzymes. The drugs classified as "not the substrates of CYP enzymes" include those drugs which are not metabolized and principally excreted in unchanged forms, those which are metabolized by other enzymes, and those for which the role of CYP enzymes on their biotransformation is currently unknown. The drugs are classified as "not inhibitors/inducers of CYP enzymes" when no available data have been found to confirm whether they are CYP inhibitors or inducers.

Data analysis

The drugs in LTKB-BD are classified as Most-DILI, Less-DILI and No-DILI concern drugs (Chen et al., 2011). We combined the Most-DILI and Less-DILI concern drugs into DILI positive drugs, while the remaining drugs were considered as DILI negatives. The drugs taken orally were classified according to their doses as drugs administered at high daily doses (≥ 100 mg) and drugs administered at low daily doses (< 100 mg). The daily dose ≥ 100 mg was considered as "a high daily dose" based on our previous findings that significantly higher proportion of medications administered orally at a daily dose ≥ 100 mg caused DILI (Chen et al., 2013a). In addition, Stepan *at al*. also consider this dose as "a high daily dose" (Stepan et al., 2011).

The conditional logistic regression was used to assess the association regarding the drugs which are CYP substrates, inhibitors or inducers and the drugs which are not, and whether the former has the higher likelihood of causing DILI compared to the latter. For each association, odds ratio (OR) with 95% confidence interval (CI) was reported. Fisher's Exact Test was used to calculate a statistical significance of the associations. Multivariate logistic regression was used to estimate the relationship between the

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potential of the drug to cause DILI and the variables such as the drug being a CYP substrate, an inhibitor, and an inducer, a high daily dose, high lipophilicity, and different combinations of the following five variables (i.e. daily dose, lipophilicity, being a CYP substrate, inhibitor, or inducer). In addition, stratified analysis by the daily dose or the lipophilicity was performed for CYP substrates, inhibitors and inducers to assess their association with DILI. The "Epicalc" R package (http://cran.r-project.org/web/packages/) was used for the statistical analysis.

Eventually, we used the statistically significant DILI predictors identified by multivariate logistic regression (i.e., a high daily dose and the drug which is a CYP substrate) to examine 5 drug pairs selected from Liver Toxicity Biomarker Study (http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/ucm079281.htm) and drugs that caused DILI in 11 cases of clinical studies retrieved from the National Institute of Health (NIH) LiverTox database (http://livertox.nlm.nih.gov/).

Results

The association of DILI with the drug which is a substrate, an inhibitor or an inducer of CYP enzymes

There are 287 drugs recorded in LTKB-BD; 26 drugs with parenteral administration and 7 drugs with an unknown daily dose were removed before the analysis. Consequently, the remaining 254 drugs used in this study included 206 DILI positive and 48 DILI negative drugs (Supplementary Table 1). There were 168 drugs which are CYP substrates, and 86 drugs which are not. Among the substrates and non-substrates, 88.7% (149/168) and

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66.3% (57/86) drugs were DILI positive drugs, respectively. As shown in Table 1, the drugs which are the substrates of CYP enzymes would have 3.99 times higher likelihood of causing DILI compared to the drugs which are not metabolized by CYP enzymes (OR (95% CI): 3.99 (2.07-7.67); $p < 0.0001$).

It was found that 42.9% (109/254) of the drugs were CYP inhibitors; 85.3% (93/109) were DILI positive drugs and 14.7% (16/109) were DILI negative drugs. Among all DILI positive drugs, there were 45.1% (93/206) CYP inhibitors. The drugs-CYP inhibitors had no significantly higher likelihood of causing DILI compared to the drugs-non CYP inhibitors (OR (95% CI): 1.65 (0.85-3.18); $p = 0.1372$). Fifty drugs (19.7%) were CYP inducers; 86% (43/50) DILI positive drugs and 14% (7/50) DILI negative drugs. Among all DILI positive drugs, only 20.9% (43/206) were CYP inducers. The drugs-CYP inducers had no significantly higher likelihood of causing DILI compared to the drugs-non CYP inducers (OR (95% CI): 1.55 (0.65-3.68); $p =0.3246$).

The role of an individual CYP enzyme on DILI risk

Since the drug which is a substrate of CYP enzyme was closely associated with DILI risk, we further analyzed the association of the individual CYP enzyme with DILI risk. Most of these enzymes exhibited a high degree of inter-individual variations (Tanaka, 1999; Meibohm et al., 2002; Wolbold et al., 2003; Parkinson et al., 2004; Zhou et al., 2009), which could be relevant to the clinical application of DILI assessment. We found that drugs metabolized by CYP1A2, 2C8/9, and 3A5 may have the higher likelihood of causing DILI (Table 2).

A combined assessment of the daily dose, lipophilicity and drug-CYP interaction modes in DILI

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As shown in Table 3, the drugs with the high daily doses had 4.98 times higher likelihood of causing DILI compared to the drugs with the low daily doses (OR (95% CI): 4.98 (2.55-9.76); $p < 0.0001$). The drugs with high lipophilicity (log$P \geq 3$) would not have the higher likelihood of causing DILI compared to the drugs with low lipophilicity (OR (95% CI): 1.44 (0.75-2.77); $p = 0.3312$). For drugs with the high daily dose, CYP inhibitors would have 6.03 times higher likelihood of causing DILI than those were not CYP inhibitors (OR (95% CI): 6.03 (1.32-27.5); $p = 0.0098$). On the contrary, CYP inducers were not observed to be closely associated with DILI even when they were administered at a high daily dose. DILI caused by CYP substrates was both dose- and lipophilicity-independent.

Identifying significant drug-specific predictors for DILI

Multivariate logistic regression revealed that among multiple drug-specific variables (a high daily dose, high lipophilicity, the drug being a CYP substrate, an inhibitor, and an inducer), a high daily dose (OR (95% CI): 7.10 (3.36-15.0); $p < 0.0001$) and the drug being a substrate of CYP enzymes (OR (95% CI): 5.04 (2.34-10.9); $p < 0.0001$) were two significant predictors for DILI (Table 4). Multivariate logistic regression on all the interaction terms of these 5 variables also demonstrated that only the combination of a high daily dose and being a CYP substrate would have the higher likelihood of causing DILI (OR (95% CI): 6.36 (1.38-29.2); $p = 0.0175$) (Data not shown).

A high daily dose and the drug being a CYP substrate in examining drug pairs

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We used these two significant predictors of DILI (i.e., a high daily dose and the drug being a CYP substrate) to examine five drug pairs identified by Liver Toxicity Biomarker Study (http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/ucm079281.htm). Each drug pair consists of a "clean" compound and a "toxic" compound. Both are similar in chemical structure and have identical primary target activity with exhibiting no liver toxicity in the preclinical study. When applying to humans, the "toxic" compound exhibits the more severe liver injury than the "clean" compound. Anxiolytic **alpidem**, antiparkinsonian **tolcapone**, and **trovofloxacin** which was used in the treatment of chronic granuloma inguinale, marked as "toxic" in three drug pairs were administered at high daily doses and at the same time they are CYP substrates. All non-steroidal anti-inflammatory drugs (NSAIDs), i.e., **benoxaprofen**, **ibuprofen**, **bromfenac**, and **diclofenac**, were predicted to be hepatotoxic based on our two predictors (Table 5).

A high daily dose and the drug being a CYP substrate in identifying drugs causing liver injury in clinical studies

A high daily dose and the drug being a substrate of CYP enzymes were also applied to identify drugs that might cause liver injury in 11 clinical cases from NIH LiverTox database (http://livertox.nlm.nih.gov/), where patients were treated with comedications. In each case, causality assessment was confirmed by physicians or health care professionals. In these case studies, drugs that caused liver injury, i.e. cyclophosphamide, valproic acid, isoniazid, efavirenz, mercaptopurine, fluconazole, diclofenac, ketoconazole, fenofibrate, disulfiram, and ticlopidine, were identified by the two predictors (Table 6).

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Discussion

In this study, we analyzed how 254 orally administered drugs (i.e. CYP substrates, inhibitors or inducers) from LTKB-BD were associated with DILI. We discovered that drugs-substrates for CYP enzymes showed statistically significant, but dose-independent association with DILI; drugs-inhibitors for CYP enzymes would have the higher likelihood of causing DILI when they were administered at a high daily dose (≥ 100 mg). On the contrary, drugs-inducers for CYP enzymes were not observed to be significantly associated with DILI. These findings will be helpful to identify the drug with the high likelihood of causing DILI in clinical settings.

We determined that a high daily dose and the drug being a substrate of CYP enzymes, which had the significantly higher likelihood of causing DILI, were two important predictors of DILI (Table 4). In addition, we also revealed that only the integration of a high daily dose and being a CYP substrate had a higher likelihood of causing DILI. A list of structural alerts associated with the formation of toxic reactive metabolites has been established (Kalgutkar et al., 2005). However, the mere presence of a structural alert in a drug will not necessarily be associated with DILI (Stepan et al., 2011). Some drugs with the same structural alerts may have different risk of DILI. For example, troglitazone, rosiglitazone, and pioglitazone contain the same thiazolidinedione scaffold, which is the structural alert to generate the toxic reactive metabolites. Troglitazone has been withdrawn because of DILI, while rosiglitazone and pioglitazone are now still in use. Their daily dose may be a key difference, i.e., troglitazone (400 mg/day) versus rosiglizatone (6 mg/day) and pioglitazone (30 mg/day). On the contrary, the key difference to explain the different risk of DILI for ibuprofen (1200 mg/day) and ibufenac

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(2400 mg/day) is the formation of toxic reactive metabolite. Therefore, although high daily dose and being the CYP substrate are two independent factors associated with DILI, both of them should be considered to identify the DILI drugs.

The drug pairs have similar chemical structures and are usually expected to have similar efficacy and safety. The drugs marked as "toxic", which were withdrawn from the market in US or elsewhere due to DILI, i.e., **alpidem, tolcapone**, **trovofloxacin, benoxaprofen,** and **bromfenac** (Table 5), were identified as positives by our two predictors of DILI. **Ibuprofen** and **diclofenac** were also identified as DILI positive drugs, and their DILI potential was confirmed by the case reports of acute liver failure (ALF) (Helfgott et al., 1990; Banks et al., 1995; Javier Rodriguez-Gonzalez et al., 2002). Because of the DILI potential of these two drugs, they are annotated as Less-DILI and Most-DILI concern drugs in LTKB, respectively (Chen et al., 2011). Thus, our two predictors can identify the "toxic" compound in drug pair. Besides that, these two predictors of DILI could identify the drugs that caused DILI among multiple medications used by patients in clinical studies (Table 6). These results confirmed that two identified predictors of DILI (a high daily dose and the drug being a CYP substrate) could have useful clinical applications, particularly for a clinician in decision-making process when dealing with a patient suspected of having DILI. Nowadays, drug developers are encouraged to provide metabolism data on CYP enzymes for new drug applications (NDA) (FDA, 2012), which will facilitate a practical use of the identified two predictors of DILI in future.

It has been reported that almost all of the drugs known to be associated with DILI cause clinically significant DDIs (Li, 2002). Many DDIs can be explained by the inhibition or induction of the expression of CYP enzymes in the liver (Tanaka, 1998). Our results

suggest that the clinically observed DILI may happen when the CYP inhibitors are administered at a high daily dose (Table 3). Based on the mechanisms of enzyme inhibition, the CYP inhibitor needs to bind to the CYP enzymes and then decreases their activity. Therefore, the inhibitory effect is expected to be increased with the increasing dose of the inhibitor. Other authors also find that the inhibition of CYP enzymes is dose-dependent (Lin et al., 2000; Hollenberg, 2002). The inhibition of CYP enzymes may increase liver exposure of the parent drug, which has the potential to cause DILI. For example, clinically dose-dependent hepatotoxicity of fluconazole, which is a potent inhibitor of CYP2C19, and a moderate inhibitor of CYP2C9 and 3A, has been observed (Wells and Lever, 1992). Phenytoin (300 mg/day) is an inhibitor of CYP2C9 with a narrow therapeutic index. It is also a substrate of CYP2C9. Therefore, the increased liver exposure of phenytoin by the inhibition of CYP2C9 might increase the risk of DILI. Phenytoin has been considered as one of the most common drugs other than acetaminophen reported to cause ALF in the United States (Russo et al., 2004), which confirmed our findings that the high dose of CYP inhibitors would have the higher likelihood of causing DILI. Inhibitory effects usually occur immediately; however, induction of CYP enzymes is usually both time- and dose-dependent (Markowitz et al., 2003), which will lead to a delay before enzyme activity increases depending on duration of exposure and half-life of the inducer in the liver. Consequently, induction of hepatic CYP enzymes rarely leads to the altered metabolism and toxicity in the patient, which typically occurs only if the induction is very extensive (Mohutsky et al., 2010). For example, three drugs were recorded by FDA as the potent CYP inducers, i.e., carbamazepine, phenytoin, and rifampin

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(http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm). All these drugs are considered as the DILI positive drugs in LTKB-BD.

Due to the differences in individual susceptibility to hepatotoxic drugs, our findings will be helpful for dose adjustment which is important in order to prevent DILI. For example, females are more susceptible to DILI than males (Russo et al., 2004). That could be partly explained by twofold higher activity of CYP3A4 in the liver samples of females compared to the male samples (Wolbold et al., 2003). CYP3A4 has the highest abundance in the human liver (~40%) and is the most prevalent enzyme involved in metabolism of DILI drugs (Li, 2002). As indicated in this study, CYP2C would have the higher likelihood of metabolizing the DILI drugs. Therefore, Hispanics with about twice the activity of CYP2C over Caucasians and African Americans (Parkinson et al., 2004), may have a higher risk to liver injury caused by drugs, such as NSAIDs, leflunomide, troglitazone and valproic acid, which are mainly metabolized by CYP2C (Kirchheiner and Brockmoller, 2005). NSAIDs were reported as the most frequently used drugs causing DILI in Spain (Ibanez et al., 2002).

This study has several limitations. Firstly, we do not have a confirmation which one among the CYP enzymes involved in the metabolism of the drugs participates in the formation of toxic reactive metabolites. Secondly, other enzymes rather than CYP isoforms can also mediate the generation of toxic reactive metabolites for some drugs, and their roles are not considered in this study. Thirdly, our results are based on *in vitro* data on CYP-mediated metabolism and their extrapolation to *in vivo* studies is uncertain. Fourthly, the data on CYP-mediated metabolism for some drugs and the data on

inhibition or induction effects of some drugs on CYP enzymes are currently unavailable. Since FDA is encouraging the industry to submit the metabolism data and safety data on drug metabolites for NDA, we can expect to summarize the more solid conclusions using the analysis we carried out in this study when those data are available in future. In addition, the authors should be encouraged by the journals to publish negative findings, as in that case it will be certain that drugs classified as “not the substrates of CYP enzymes” are not metabolized by particular CYP enzymes.

In summary, besides a high daily dose, our study indicates that the drug being a substrate of CYP enzymes is another important predictor of DILI. In addition, we have also found out that CYP inhibitors have a higher likelihood of causing DILI only when they are administered at a high daily dose, and CYP inducers are not observed to be closely associated with DILI. Only the integration of a high daily dose and being a CYP substrate has the higher likelihood of causing DILI. Our findings can serve as a simple clinical algorithm for the identification of the drug with a high likelihood of causing DILI in clinical settings.

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Authorship Contributions

Participated in research design: Weida Tong, and Katarina Ilic.

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Wrote or contributed to the writing of the manuscript: Ke Yu, and Katarina Ilic.

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Footnotes

Both authors (K.Y. and X.G.) contribute equally to this work.

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Tables

TABLE 1

Associations of DILI with drugs as CYP substrates, inhibitors and inducers

Classifications	DILI positives	DILI negatives	OR (95%CI)	*p* value
CYP Substrates	149	19	3.99 (2.07-7.67)	< 0.0001
No	57	29		
CYP Inhibitors	93	16	1.65 (0.85-3.18)	0.1372
No	113	32		
CYP Inducers	43	7	1.55 (0.65-3.68)	0.3246
No	163	41		

The analysis is based on the data from LTKB-BD shown in supplementary table 1.
DILI positives-drugs associated with DILI; DILI negatives-drugs not associated with DILI.
OR (95%CI) - odds ratio with 95% confidence interval.

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TABLE 2

Association of DILI risk with the drug being a substrate of individual CYP enzyme

CYP enzymes	Substrates	DILI positives	DILI negatives	OR (95%CI)	*p* value
CYP1A2	Yes	42	1	12.0 (1.61-89.8)	0.0010
	No	164	47		
CYP2B6	Yes	15	2	1.81 (0.40-8.18)	0.7476
	No	191	46		
CYP2C8	Yes	29	1	7.70 (1.02-58.0)	0.0226
	No	177	47		
CYP2C9	Yes	44	2	6.24 (1.46-26.7)	0.0054
	No	162	46		
CYP2C19	Yes	36	3	3.18 (0.94-10.8)	0.0728
	No	170	45		
CYP2D6	Yes	39	3	3.50 (1.03-11.9)	0.0320
	No	167	45		
CYP2E1	Yes	11	3	0.85 (0.23-3.16)	0.7322
	No	195	45		
CYP3A4	Yes	84	11	2.32 (1.12-4.80)	0.0213
	No	122	37		
CYP3A5	Yes	30	1	8.01 (1.06-60.3)	0.0137
	No	176	47		

The analysis is based on the data from LTKB-BD shown in supplementary table 1.
DILI positives-drugs associated with DILI; DILI negatives-drugs not associated with DILI.OR (95%CI) - odds ratio with 95% confidence interval.

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TABLE 3

Stratified analysis on the daily dose and lipophilicity for the association of DILI with drugs being CYP substrates, inhibitors or inducers

Classifications	DILI positives	DILI negatives	OR (95%CI)	*p* value
High daily dose (≥ 100 mg)				
CYP Substrates	101	5	**4.83 (1.59-14.7)**	**0.0029**
No	46	11		
CYP Inhibitors	68	2	**6.03 (1.32-27.5)**	**0.0098**
No	79	14		
CYP Inducers	31	3	1.16 (0.31-4.32)	0.8275
No	116	13		
Total	147	16	**4.98 (2.55-9.76)**	**0.0001**
Low daily dose (< 100 mg)				
CYP Substrates	48	14	**5.61 (2.15-14.6)**	**0.0003**
No	11	18		
CYP Inhibitors	25	14	0.95 (0.40-2.25)	0.8997
No	34	18		
CYP Inducers	12	4	1.79 (0.53-6.08)	0.3509
No	47	28		
Total	59	32		
High lipophilicity (logP≥3.0)				
CYP Substrates	73	9	**3.60 (1.22-10.6)**	**0.0274**
No	18	8		
CYP Inhibitors	47	8	1.20 (0.43-3.39)	0.7954
No	44	9		
CYP Inducers	21	1	4.80 (0.60-38.4)	0.1863
No	70	16		
Total	91	17	1.44 (0.75-2.77)	0.3312
Low lipophilicity (logP<3.0)				
CYP Substrates	76	10	**4.09 (1.76-9.54)**	**0.0009**
No	39	21		
CYP Inhibitors	46	8	1.92 (0.79-4.65)	0.2081
	69	23		
CYP Inducers	21	1	6.70 (0.86-51.9)	0.0459
No	94	30		
Total	115	31		

The analysis is based on the data from LTKB-BD shown in supplementary table 1.
DILI positives-drugs associated with DILI; DILI negatives-drugs not associated with DILI.
OR (95%CI) - odds ratio with 95% confidence interval.
The high daily dose and high lipophilicity were defined by the paper (Chen et al., 2013a)

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TABLE 4

Multivariate logistic analysis of the daily dose, lipophilicity and being a CYP substrate, inhibitor and inducer with DILI

Variable	Adjusted OR (95% CI)	*p* value
High daily dose (≥ 100 mg)	**7.10 (3.36-15.0)**	**<0.0001**
Being a CYP substrate	**5.04 (2.34-10.9)**	**<0.0001**
Being a CYP inhibitor	1.11(0.49-2.51)	0.7943
Being a CYP inducer	1.21 (0.43-3.38)	0.7143
High lipophilicity (log*P*≥3)	1.22 (0.58-2.58)	0.5927

The analysis is based on the data from LTKB-BD shown in supplementary table 1.
Daily dose, being a CYP substrate, inhibitor, and inducer as well as lipophilicity were included in the model.
OR (95%CI) - odds ratio with 95% confidence interval.

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TABLE 5

A high daily dose and the drug being a CYP substrate as two important predictors for identifying the "toxic" drugs in drug pairs

Drug pairs	Hepatotoxic potential from clinical trials[§]	Daily dose (mg)	CYP substrates[*]	Predicted result	LTKB annotation[‡]
Alpidem	**Toxic**	**150**	**3A4**	**+**	**Most-DILI concern**
Zolpidem	Clean	10	1A2, 2C9, 2C1, 2D6, 3A4	-	Less-DILI concern
Tolcapone	**Toxic**	**450**	**1A2, 2A6, 2E1, 3A4**	**+**	**Most-DILI concern**
Entacapone	Clean	1000	NSC	-	No-DILI concern
Trovafloxacin	**Toxic**	**200**	**1A2**	**+**	**Most-DILI concern**
Moxifloxacin	Clean	400	NSC	-	NA
Benoxaprofen	**Toxic**	**600**	**Unspecified CYP enzymes[†]**	**+**	**Most-DILI concern**
Ibuprofen	Clean	1200	2C8, 2C9, 2C19	+	Less-DILI concern
Bromfenac	**Toxic**	**150**	**Unspecified CYP enzymes**	**+**	**Most-DILI concern**
Diclofenac	Clean	100	1A1, 1A2, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 3A4	+	Most-DILI concern

High daily dose and being a CYP substrate are identified by multivariate logistic regression on the data shown in supplementary table 1.

[§]Toxic: hepatotoxicity can be detected in the clinical trials; Clean: hepatotoxicity can not be detected in the clinical trials.

[*]Data on CYP enzymes are from Pharmapendium, PubChem, PubMed, DrugBank and SuperCYP.

[†]The drug is metabolized by the unspecified CYP enzymes. [‡]Data from the LTKB database.(Chen et al., 2011)

NSC-not a substrates of CYP enzymes. + DILI positive drug. - DILI negative drug.

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TABLE 6

A high daily dose and the drug being a CYP substrate as predictors for identifying drugs causing liver injury in clinical studies

Case study	Co-medications	Daily dose (mg)	CYP substrates*	Predicted result	Causality†
Case 1	**Cyclophosphamide**	**100**	**2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 3A4, 3A7**	+	**Yes**
	Prednisone	15	3A4	-	No
Case 2	**Valproic Acid**	**750**	**2A6, 2B6, 2C9, 2C19, 3A5**	+	**Yes**
	Azithromycin	500	NSC	-	No
Case 3	**Isoniazid**	**300**	**2E1**	+	**Yes**
	Pyridoxine	50	NSC	-	No
Case 4	**Efavirenz**	**600**	**2B6, 3A4**	+	**Yes**
	Tenofovir	300	NSC	-	No
	Emtricitabine	200	NSC	-	No
Case 5	**Mercaptopurine**	**200**	**Unspecified CYP enzymes**	+	**Yes**
	Prednisolone	50	3A4, 3A5	-	No
	Furosemide	80	2E1	-	No
Case 6	**Fluconazole**	**400**	**2C9**	+	**Yes**
	Prochlorperazine	30	2D6, 3A4	-	No
	Acetazolamide	1000	NSC	-	No
	Cimetidine	900	NSC	-	No
Case 7	**Diclofenac**	**150**	**1A1, 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, 46A**	+	**Yes**
	Famotidine	40	2C19	-	No
	Prednisone	10	3A4	-	No
	Furosemide	40	2E1	-	No
Case 8	**Ketoconazole**	**200**	**3A4, 3A5**	+	**Yes**
	Levothyroxine	0.15	3A4	-	No
Case 9	**Fenofibrate**	**300**	**3A4**	+	**Yes**
	Dipyridamole	400	NSC	-	No
	Aspirin	3000	NSC	-	No
	Glibenclamide	10	2C9, 2C19, 3A4	-	No
	Metformin	2000	NSC	-	No
	Pravastatin	30	2C9, 3A4, 3A5	-	No
	Nifedipine	30	2D6, 3A4, 3A5, 3A7	-	No
Case 10	**Disulfiram**	**100**	**1A2, 2A6, 2B6, 2D6, 2E1, 3A4, 3A5**	+	**Yes**
	Lisinopril	20	NSC	-	No
	Metformin	2000	NSC	-	No
Case 11	**Ticlopidine**	**500**	**3A4**	+	**Yes**
	Aspirin	3000	NSC	-	No
	Prednisone	10	3A4	-	No

High daily dose and being a CYP substrate are identified by multivariate logistic regression on the data shown in supplementary table 1.

*The information of CYP enzymes is from Pharmapendium, PubChem, PubMed, DrugBank and SuperCYP.

†Causality is assessed by physicians and health care professionals (http://livertox.nih.gov/index.html).

+ DILI positive drug, - DILI negative drug. NSC-not a substrate of CYP enzymes.