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Drug interaction with UDP-Glucuronosyltransferase (UGT) enzymes is a predictor of drug-induced liver injury

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Abstract

Background and Aims: DILI frequently contributes to the attrition of new drug candidates and is a common cause for the withdrawal of approved drugs from the market. Although some noncytochrome P450 (non-CYP) metabolism enzymes have been implicated in DILI development, their association with DILI outcomes has not been systematically evaluated.

Approach and Results: In this study, we analyzed a large data set comprising 317 drugs and their interactions *in vitro* with 42 non-CYP enzymes as substrates, inducers, and/or inhibitors retrieved from historical regulatory documents using multivariate logistic regression. We examined how these *in vitro* drug-enzyme interactions are correlated with the drugs' potential for DILI concern, as classified in the Liver Toxicity Knowledge Base database. Our study revealed that drugs that inhibit non-CYP enzymes are significantly associated with high DILI concern. Particularly, interaction with UDP-glucuronosyltransferases (UGT) enzymes is an important predictor of DILI outcomes. Further analysis indicated that only pure UGT inhibitors and dual substrate inhibitors, but not pure UGT substrates, are significantly associated with high DILI concern.

Conclusions: Drug interactions with UGT enzymes may independently predict DILI, and their combined use with the rule-of-two model further improves overall predictive performance. These findings could expand the currently available tools for assessing the potential for DILI in humans.

Abbreviations: ATC, anatomical therapeutic chemical; CYP, cytochrome P450; FDA, Food and Drug Administration; IC_{50}, inhibition constant; LTKB, Liver Toxicity Knowledge Base; MCC, Matthew Correlation Coefficient; NCTR, National Center for Toxicological Research; non-CYP, noncytochrome P450; RO2, rule-of-two; UGT, UDP-glucuronosyltransferases; WHO, World Health Organization.

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INTRODUCTION

DILI can have profound impacts on human health and finances.[1] It stands as a primary cause of acute liver failure, increasing the risk of hospitalization and death.[2–4] Moreover, it has also led to significant financial setbacks within the realm of drug development, manifesting through the failure of drug candidates in clinical trials or market withdrawal of approved drugs due to detected hepatotoxicity during postmarketing surveillance.[1,5] Consequently, there is a burgeoning interest in comprehending the mechanisms underlying DILI development and in identifying factors predisposing individuals to DILI. Numerous studies have endeavored to formulate predictive models that establish correlations between diverse physicochemical and pharmacological properties of drugs and their associated DILI risks. Among the physicochemical attributes linked to the potential for causing DILI concern are carbon bond saturation and acid/base characteristics, lipophilicity, oral dose, and the rule-of-two (RO2) criteria, which stipulates an oral dose $\geq$100 mg and log P $\geq$ 3.[6–8]

While these physicochemical properties may be predictive of certain pharmacokinetic attributes of drugs, additional pharmacological attributes directly associated with DILI concern are pivotal in decision-making concerning patient care and drug development. Some pharmacological features of drugs linked to higher DILI concern include, but are not limited to, dual inhibition of liver mitochondrial function and the bile salt export pump, formation of reactive metabolites, and certain interactions with cytochrome P450 (CYP) enzymes.[9–12]

Hepatic metabolism serves as a pathway through which certain drugs may acquire the pharmacological features that predispose them to higher DILI concerns.[13,14] CYP enzymes are a large class of liver enzymes that catalyze redox reactions in phase I hepatic metabolism.[15] In a previous study investigating correlations between CYP metabolism and DILI risk,[11] it was found that drugs inhibiting CYP enzymes exhibited a dose-dependent association with increased DILI risks, whereas substrates of CYP enzymes were associated with dose-independent higher DILI risks. Notably, there was no observed association between CYP enzyme inducers and DILI risks.

In addition to CYP enzymes, studies have indicated other metabolism enzymes, including those facilitating phase II reactions such as UDP-glucuronosyltransferases (UGT), N-acetyltransferases, and sulfotransferases, may contribute to induce DILI through the formation of toxic reactive metabolites.[16,17] While the majority of drugs undergo metabolism by CYP enzymes, the involvement of noncytochrome P450 (non-CYP) metabolism enzymes remains a significant pathway for drug metabolic clearance.[18] In the realm of drug development, there is a growing trend toward optimizing pharmacokinetic properties of drug candidates by reducing their lipophilicity. This trend may lead to decreased metabolism by CYP enzymes, thus opening avenues for non-CYP enzymes to assume an increasingly crucial role in drug disposition and metabolism.[19] However, there is still a knowledge gap in terms of systematically characterizing non-CYP enzymes to provide insights into their contribution to DILI development. In this study, we conducted comprehensive analyses to explore the associations between *in vitro* drug interactions with non-CYP enzymes and their corresponding DILI concern.

METHODS

Data collection

The drugs in the data set and their classification by DILI concern (Table 1) were obtained from the Liver Toxicity Knowledge Base (LTKB) data set developed by the US Food and Drug Administration (FDA)'s National Center for Toxicological Research (NCTR).[20] Data on the drugs' interactions with non-CYP enzymes (metabolism, inhibition, and induction data) were collected from *in vitro* drug interaction studies[21] of regulatory documents stored in the PharmaPendium database and bioactivity data from the Reaxys database. The PharmaPendium database is a product of Elsevier that contains pharmacokinetic, metabolism, efficacy, and drug safety data obtained from historically paper-based drug approval documents from FDA and EMA websites and selected scientific articles.[22] The Reaxys database, another Elsevier product, contains chemistry data and bioactivity data from journal articles and patents.[23]

Drugs were classified as inhibitors of a non-CYP enzyme if a quantified measurement was reported for any of the following in vitro parameters for that enzyme: K_i (inhibition constant), IC_{50} (half maximal inhibitory concentration), kinact (inactivation rate constant), and KI (concentration required for half-maximal inactivation).

TABLE 1 Distribution of drugs in data set by non-CYP enzyme interaction and DILI concern

	Non-CYP enzyme interaction		
	No	Yes	Total
vNo-DILI-concern	88	10	98
vLess-DILI-concern	120	8	128
vMost-DILI-concern	64	27	91
Total analyzed	272	45	317
Ambiguous DILI concern[a]	2	2	4
Unknown[a]	20	12	32

[a]Drugs excluded from analysis.
Abbreviation: non-CYP, noncytochrome P450.

Drugs were classified as substrates of a non-CYP enzyme if a quantified measurement is present for any of the following in vitro parameters for that enzyme: V_{max} (maximum reaction velocity), K_m (Michaelis Menten constant), CLint (intrinsic clearance), fm (E) vitro (fraction metabolized in vitro). Drugs were classified as inducers of a non-CYP enzyme if a quantified measurement is present for any the following in vitro parameters for that enzyme: EC_{50} (half-maximum effective concentration) and E_{max} (maximal drug effect). Drugs with a value of 0 for any parameter or which had the prefix ">" for IC_{50} values were not considered to have the stated enzyme interaction. Drug interactions were labeled as no data, inhibitor only, substrate only, inducer only, inhibitor and substrate, inhibitor and inducer, substrate and inducer or inhibitor and substrate and inducer based on the criteria stated above. Daily drug doses and logP values were retrieved from an in-house data set as described in our previous study.[6]

Data curation

The initial data set contained 475 drugs. In vitro data were found for 361 drug entries only in the Pharmapendium database, of which 36 drugs were excluded from statistical analysis since no DILI classification was found in LTKB (32 drugs), or because they were classified as ambiguous in LTKB (4 drugs). The remaining drugs were classified based on their DILI concern as vNo-DILI-concern, vLess-DILI-concern, or vMost-DILI-concern, based on the previous study.[24] Drugs in the data set were also categorized based on their interactions with 42 non-CYP enzymes as substrates, inducers, and/or inhibitors (Table 2).

TABLE 2 Associations between DILI concern and the type of drug-enzyme interaction

Non-CYP enzyme interaction	DILI concern		OR (95% CI)	*p*
	Low	High		
Substrate				
No	214	75	1.71 (0.65–4.36)	0.2620
Yes	12	16		
Inhibitor				
No	217	69	6.18 (2.58–15.87)	< 0.0001
Yes	9	22		
Inducer[a]				
No	226	88	NA	NA
Yes	0	3		

[a]Variable excluded from analysis due to the few number of enzyme inducers.
Abbreviation: NA, not available.

Seven drugs with unique LTKB IDs were found in a total of 15 drug entries, with different salt forms of the same base drug (eg, 2 entries for codeine sulfate and codeine phosphate), considered to be separate entries. However, for each drug, the different salt forms were found to have the same DILI concern and exhibit similar interactions with the non-CYP enzymes; therefore, these were merged into a single drug entry. Following data curation, the resulting data set contained 317 drug entries that were used in statistical analysis, as summarized in Table 1.

Data analysis

Associations between drug DILI concern and drug interactions with non-CYP enzymes were examined using binomial classification with the response variables: high (drugs in the vMost-DILI-concern group) versus low (drugs in the combined vNo-DILI and vLess-DILI-concern groups). Since many drugs that are widely used today have some hepatotoxic potential, this study largely focuses on identifying features that distinguish drugs with high DILI concern from drugs with lower DILI concern (vNo-DILI and vLess-DILI-concern).

Multivariate logistic regression was used to assess associations between DILI concern and the different sets of predictors, except when stated otherwise. The OR for each predictor (computed as the exponent of regression coefficient estimate), its 95% CI and the associated statistical significance (*p*-value) were obtained from the output of the logistic regression.

The performance of models was assessed using the following metrics: accuracy, sensitivity, specificity, balanced accuracy, and Matthew Correlation Coefficient (MCC). Metrics reported are the average performances of test set predictions repeated 100 times, using 75% of the data set as the training set. The metrics were computed using the following equations:

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

$$Sensitivity = \frac{TP}{TP + FN}$$

$$Specificity = \frac{TN}{TN + FP}$$

$$Balanced\ accuracy = \frac{Sensitivity + Specificity}{2}$$

$$MCC = \frac{(TP*TN) - (FP*FN)}{\sqrt{(TP + FP)*(TP + FN)*(TN + FP)*(TN + FN)}}$$

where TP = True Positive; TN = True Negative; FP = False Positive; and FN = False Negative.

R software[25] was used to perform all statistical analyses. The mltools R package[26] was used to compute MCC, while the caret R package[27] was used to calculate the other performance metrics.

RESULTS

Type of enzymatic interaction and DILI concern

We first analyzed the data set such that predictor variables were set based on whether the drugs were substrates, inhibitors, and/or inducers of a non-CYP enzyme. Forty-five of 317 drugs (14.2%) interacted with non-CYP enzymes, with 60% (27 of 45) of these drugs in the vMost-DILI-concern group (Table 1). Only 3 drugs in the data set were non-CYP enzyme inducers; all belonged to the most-DILI group (Table 2). Due to the limited number of non-CYP enzyme inducers in the data set, this logistic regression was performed using only enzyme substrates and inhibitors as predictors. Our analysis revealed that acting as a substrate of any of the non-CYP enzymes has no significant association with drug DILI concern (Table 2). In contrast, inhibiting a non-CYP enzyme is associated with the drug having high DILI concern. Twenty-two drugs of the 31 non-CYP enzyme inhibitors (71.0%) had high DILI concerns (Table 2). Thus, a non-CYP enzyme inhibitor drug is more likely to be of high DILI concern (OR: 6.18) compared to a drug that does not inhibit a non-CYP enzyme.

Another analysis that compared DILI-negative (vNo-DILI-concern) drugs with DILI-positive drugs (vLess-DILI and vMost-DILI-concern) gave similar results: A drug acting as a substrate of non-CYP enzymes has no significant association with a drug being DILI-positive (Supplemental Table S1, http://links.lww.com/HEP/I552). However, a drug that is a non-CYP enzyme inhibitor has a higher likelihood of being DILI-positive (OR: 3.64).

Enzyme superfamilies and DILI concern

We also analyzed the data set based on enzyme superfamilies (Supplemental Table S2, http://links.lww.com/HEP/I552), irrespective of the type of enzymatic interaction, to investigate whether associations exist between non-CYP enzyme superfamilies and DILI concern. From our analysis, while no enzyme family could differentiate between DILI-negative and DILI-positive groups (Supplemental Table S3, http://links.lww.com/HEP/I552), interactions with UDP-glucuronosyltransferase (UGT) enzyme superfamily showed a statistically significant differentiation between drugs with high DILI concern and those of low DILI concern (Table 3). Eleven percentage of the data set (35 of 317 drugs) interacted with the UGT enzyme superfamily, of which 23 (65.71%) drugs had high DILI concern. Thus, interacting with an enzyme in the UGT superfamily significantly increases the likelihood of a drug having high DILI concern (OR: 5.81).

We categorized drugs in the data set into 14 groups based on the first level of the World Health Organization (WHO) anatomical therapeutic chemical (ATC) classification system to analyze the utility of drug interaction with UGT as a DILI predictor within these different therapeutic groups (Figure 1). For each anatomical/pharmacological group, we prepared a 2×2 contingency matrix based on the presence of drug-UGT interaction and DILI concern. Balanced accuracy

TABLE 3 Associations between DILI concern and noncytochrome P450 enzyme families

Enzyme family	DILI concern		OR (95% CI)	*p*
	Low	High		
AKR				
No	224	88	2.82 (0.35–24.37)	0.3088
Yes	2	3		
BChE				
No	225	91	5.19E-8 (NA–2.8E70)	0.9848
Yes	1	0		
CES				
No	224	88	5.07 (0.52–111.15)	0.1896
Yes	2	3		
FMO				
No	224	89	1.41 (0.13–17.11)	0.7702
Yes	2	2		
GGT				
No	226	90	1.18E6 (0.00–NA)	0.9874
Yes	0	1		
MAO				
No	224	90	0.81 (0.03–11.55)	0.8839
Yes	2	1		
SULT				
No	223	87	0.79 (0.10–6.06)	0.8241
Yes	3	4		
UGT				
No	214	68	5.81 (2.60–13.74)	< 0.0001
Yes	12	23		

Abbreviation: NA, not available.

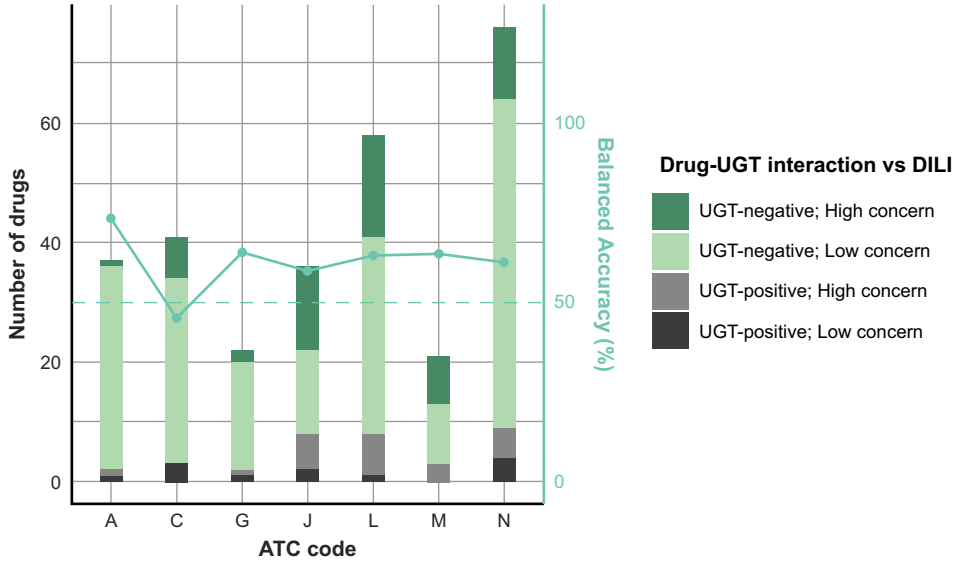

FIGURE 1 Stacked bar plot showing drug interaction with UGT applied as a predictor of DILI concern to 7 main anatomical/pharmacological groups (group size > 20) of the World Health Organization ATC classification system. The balanced accuracy of the predictor is shown as a line graph against the secondary *y*-axis, with the dotted teal line showing 50% balanced accuracy. ATC code: A, alimentary tract and metabolism; C, cardiovascular system; G, genito urinary system and sex hormones; J, anti-infectives for systemic use; L, antineoplastic and immunomodulating agents; M, musculo-skeletal system; N, nervous system. Abbreviations: ATC, anatomical therapeutic chemical; UGT, UDP-glucuronosyltransferases.

(an average of sensitivity and specificity) was used to assess the performance of the predictor.

We found that drug interaction with UGT performed above average as a DILI predictor for most groups. Because small group sizes can undermine the performance of a predictor, the performance of the predictor is reported only for groups with a minimum of 20 drugs (Figure 1). Of the 7 groups with adequate size, the balanced accuracy was $\leq$50% for one group only, C (drugs that act on the cardiovascular system).

UGT enzyme families and DILI concern

Thirty-one drugs in the data set interacted with enzymes in the UGT1 family, of which 67% (21 of 31 drugs) had high DILI concern (Supplemental Table S4, http://links.lww.com/HEP/I552). Our analysis revealed that drugs that interact with the UGT1 enzyme family are 4.36 times more likely to have high DILI concern compared to drugs that do not interact with the enzyme family. In contrast, drug interaction with the UGT2 enzyme family was not significantly associated with DILI concern (p-value = 0.11), partly due to a small number of positives.

Individual UGT enzymes and DILI concern

We further examined the association of DILI concern with individual UGT enzymes, regardless of the nature of the interactions. No drug in the data set was found to interact with UGT2B10, so the enzyme was excluded from this analysis. Based on our analysis, no individual UGT enzyme displayed a statistically significant association with DILI concern when comparing either the DILI-positive vs. DILI-negative (Supplemental Table S5A, http://links.lww.com/HEP/I552) groups or the high vs. low DILI concern groups (Supplemental Table S5B, http://links.lww.com/HEP/I552).

Type of interaction with UGT enzyme family and DILI concern

We also analyzed the data set to identify what drug-UGT interactions (as substrates, inhibitors, or inducers) have associations with DILI concern. Since there were few enzyme inducers in the data set, the UGT enzyme inducer category was not included as a predictor in the multivariate logistic regression. We discovered that neither acting as a UGT enzyme substrate nor inhibitor is statistically significant in differentiating a drug as DILI-negative or DILI positive (Supplemental Table S6, http://links.lww.com/HEP/I552). In contrast, a drug being a substrate of the UGT enzyme superfamily increases its odds of having high DILI concern, with an OR of 3.38 (Table 4). Acting as an inhibitor of the UGT superfamily also increases the chances that a drug will have high DILI concern, with an even higher OR of 4.88 (Table 4).

To further characterize the association between DILI concern and the type of drug-UGT interaction, we categorized the drugs in the data set as pure UGT substrates, pure UGT inhibitors and/or dual UGT

TABLE 4 Associations between DILI concern and the type of drug-UGT interactions

UGT enzyme interaction	DILI concern		OR (95% CI)	*p*
	Low	High		
Substrate				
No	221	77	3.38 (1.02–12.03)	0.0482
Yes	5	14		
Inhibitor				
No	218	71	4.88 (1.89–13.30)	0.0012
Yes	8	20		
Inducer[a]				
No	226	88	NA	NA
Yes	0	3		

[a]Variable excluded from analysis due to size.
Abbreviation: NA, not available.

substrate inhibitors. Pure UGT substrates refer to drugs that act only as substrates of a UGT enzyme but do not inhibit the enzyme, pure UGT inhibitors are drugs that only inhibit a UGT enzyme but are not substrates of the enzyme, while dual UGT substrate inhibitors are both substrates and inhibitors of a UGT enzyme. Based on this characterization, for example, tolcapone was considered positive for all 3 categories, since our data show that it is a pure substrate of UGT2B7 and UGT2B15; a pure inhibitor of UGT1A1, UGT1A7, and UGT1A10; and a dual substrate-inhibitor of UGT1A9. From this analysis, we observed that a drug that acts as a UGT substrate is significantly associated with high DILI concern when the drug is a dual UGT substrate-inhibitor, while a drug that acts as a pure UGT substrate is not statistically significant in predicting DILI concern (Table 5). In contrast, pure UGT inhibitors, and especially dual UGT substrate inhibitors, increase the likelihood of having high DILI concern, with ORs of 3.21 and 13.39, respectively.

We performed an ordinal logistic regression to assess the association of the types of drug-UGT interaction against increasing DILI severity levels, as described by reported clinical events.[20] From this analysis, drug-UGT interactions as dual substrate inhibitors had the only statistically significant association with higher DILI severity levels (Supplemental Table S7, http://links.lww.com/HEP/I552). With 8 of 13 dual UGT dual substrate inhibitors having the highest severity level, fatal hepatotoxicity, the OR of dual substrate inhibitors of UGT having higher DILI severity levels is 12.60. While the association DILI severity has with pure UGT substrates and pure UGT inhibitors is not statistically significant, we observed that pure UGT substrates are less likely to be associated with higher DILI severity levels.

RO2 and drug interaction with UGT enzyme family as predictors of DILI risk

We compared the performances of applying drug interaction with the UGT enzyme superfamily from our current study with the RO2 criterion that had been proposed earlier.[11] Forty-five drugs (of 317 in the data set) were excluded from this analysis because their daily dose was not specified. Multivariate logistic regression showed that both predictors, drugs being positive for either RO2 and drug-UGT interactions, are statistically significant, increasing the likelihood of having high DILI concern with ORs of 4.52 and 6.14, respectively (Supplemental Table S8, http://links.lww.com/HEP/I552), suggesting that drug-UGT interactions is a predictor for high DILI concern independent of RO2.

Since UGT substrates are not statistically significant predictors of DILI concern, we refined the definition of the UGT-based predictor to exclude drugs that do not inhibit a UGT enzyme. Thus, the UGT_i* predictor includes only pure inhibitors or dual substrate inhibitors of UGT enzymes. Following the refinement, multivariate logistic regression also showed that both predictors of

TABLE 5 Associations of DILI concern of drugs with pure substrates, pure inhibitors, and dual substrate inhibitors of UGT enzymes

UGT enzyme interaction	DILI concern		OR (95% CI)	*p*
	Low	High		
Pure substrates				
No	221	82	1.18 (0.16–5.65)	0.8472
Yes	5	9		
Dual substrate inhibitors				
No	225	79	13.39 (1.64–314.64)	0.0255
Yes	1	12		
Pure inhibitors				
No	218	73	3.21 (1.14–9.10)	0.0373
Yes	8	18		

DILI concern are statistically significant and have slightly higher odds ratios of 4.58 and 7.49 for RO2 and UGT_i*, respectively (Table 6).

We also analyzed the effect of combining both predictors in a univariate logistic regression, such that a positive prediction refers to a drug that is positive either for RO2 or for interaction with UGT (ie, UGT_i*/RO2). Forty-seven of 85 drugs were correctly predicted to have high DILI concern by the combined predictor of UGT_i*/RO2, in contrast with fewer correct predictions by UGT_i* (20 drugs) and RO2 (35 drugs) individually (Table 6). Meanwhile, UGT_i*/RO2 had fewer true negatives (158) compared with the individual predictions of UGT_i* (180) and RO2 (163).

We also performed repeated univariate logistic regression for each of the predictors to evaluate their performance as sole predictors of DILI concern. The RO2 predictor was found to have a low sensitivity of 42% and a high specificity of 87% (Table 7). In contrast, the UGT_i* predictor had an even lower sensitivity of 22% but a higher specificity of 96%. With the combined predictor, UGT_i*/RO2, sensitivity was greatly improved to 56%, while specificity suffered a slight loss to 84%. Despite this, the combined predictor improved the overall predictive performance, as shown by the balanced accuracy and MCC scores (Table 7).

DISCUSSION

In this study, we analyzed a large data set detailing in vitro interactions of 317 drugs with 42 non-CYP enzymes, acting as substrates, inducers, and/or inhibitors, to investigate correlations between these drug-enzyme interactions and their associated DILI concern. The DILI ranking employed in this study is from our previous study.[20,24] The ranking is based largely on 3 factors: the section of the drug label that reports hepatotoxicity (black box warning, warning, and precautions or adverse reactions); the severity of liver injury, and evidence of causality assessment from literature. This DILI classification contrasts with the LiverTox likelihood scale,[28,29] which is largely based on the frequency of DILI events found in literature.[30] Notwithstanding, for drugs in our data set, drugs in vLess- and vMost-DILI-concern groups have a high overlap (89% and 96%, respectively) with drugs in groups A–D of the LiverTox likelihood scale; and drugs in groups E and E* largely overlap (89%) with drugs in the vNo-DILI-concern group (Supplemental Figure S1, http://links.lww.com/HEP/I552).

TABLE 6 Drug-UGT enzyme interaction and RO2 as predictors of DILI concern

	DILI concern			
Rule	**Low**	**High**	**OR (95% CI)**	***p***
UGT_i[a]				
No	180	65	7.49 (3.03–20.50)	< 0.0001
Yes	7	20		
RO2				
No	163	50	4.58 (2.44–8.72)	< 0.0001
Yes	24	35		
UGT_i[a]/RO2				
No	158	38	6.74 (3.80–12.21)	< 0.0001
Yes	29	47		

[a]UGT_i: only inhibitors of UGT are considered.
Abbreviations: RO2, rule-of-two; UGT, UDP-glucuronosyltransferases.

The non-CYP enzymes in the data set catalyze both phase I and phase II reactions. In phase I reactions, the substrates are parent drugs, while the substrates of phase II reactions are the products of phase I metabolism. The enzymes AKR, BChE, CES, FMO, GGT, and MAO catalyze oxidation, reduction, and hydroxylation reactions as phase I reactions, while sulfotransferase and UGT facilitate conjugation reactions as either phase I or phase II reactions. Our data presents in vitro metabolism of parent drugs by these non-CYP enzymes. Our findings revealed that inhibitors of non-CYP enzymes are generally more likely to be of high DILI concern compared to non-inhibitors of these enzymes (Table 5). The 42 non-CYP enzymes we studied were grouped into 8 enzyme superfamilies of different sizes (Supplemental Table S2, http://links.lww.com/HEP/I552). Of these superfamilies, only drug interactions with the UGT enzyme superfamily were found to be associated with high DILI concern (Table 3). Variations in the genetic polymorphism and activities of UGT enzymes have been linked to increased DILI risks observed with some drugs. For instance, a strong association between UGT1A1 polymorphisms and DILI by antitubercular drugs has been reported.[17,31] Similarly, genetic polymorphisms in UGT2B7 enzymes have been associated with diclofenac-induced hepatotoxicity.[32] Our findings, as shown in Figure 1, indicate that the predictive utility of drug interaction with UGT extends beyond the pharmacological classes represented by these examples, demonstrating its value as a predictor of DILI across various pharmacological groups.

UGT enzymes catalyze the transfer of UDP-glucuronic acid to substrates with suitable functional groups such as amino, hydroxyl, and carboxylic acid groups in a conjugation reaction called glucuronidation. Generally, glucuronidation is a detoxification reaction, producing metabolites with reduced chemical and biologic reactivity and higher renal clearance.[33,34] However, for UGT substrates, the formation of toxic reactive metabolites following glucuronidation by UGT may induce DILI. This mechanism may account for the toxicity of some molecules with carboxylic acid functional groups that form acyl glucuronide metabolites, which

TABLE 7 Performance metrics of different variables as sole predictors of DILI concern

	RO2	UGT	UGT_i	UGT/RO2	UGT_i/RO2
Accuracy	0.73 ± 0.05	0.73 ± 0.05	0.74 ± 0.05	0.74 ± 0.04	0.76 ± 0.04
Sensitivity	0.42 ± 0.11	0.26 ± 0.10	0.22 ± 0.08	0.56 ± 0.09	0.56 ± 0.08
Specificity	0.87 ± 0.04	0.95 ± 0.03	0.96 ± 0.02	0.83 ± 0.05	0.84 ± 0.05
Balanced accuracy	0.64 ± 0.06	0.60 ± 0.05	0.59 ± 0.04	0.70 ± 0.05	0.70 ± 0.04
MCC	0.32 ± 0.11	0.29 ± 0.13	0.29 ± 0.12	0.41 ± 0.10	0.42 ± 0.09

Note: Drugs are positive for rule-of-two (RO2); have a drug-UGT interaction (UGT); only have inhibitory drug-UGT interaction (UGT_i); are either positive for RO2 or have an interaction with UGT (UGT/RO2); are either positive for RO2 or have an inhibitory interaction with UGT (UGT_i/RO2); data are presented as mean ± SD.
Abbreviations: MCC, Matthew Correlation Coefficient; RO2, rule-of-two; UGT, UDP-glucuronosyltransferases.

subsequently bind covalently to proteins, as observed with diclofenac.[32,35,36] Our analysis revealed that UGT metabolism had a mixed effect on DILI. Although the effects were not statistically significant, pure UGT substrates are slightly more likely to have high DILI concern (Table 5), but less likely to have higher DILI severity levels (Supplemental Table S7, http://links.lww.com/HEP/I552). Drugs that are pure UGT substrates in the data set (Supplemental Table S9, http://links.lww.com/HEP/I552) have a wide range of DILI concern: from tapentadol, a phenol-containing opioid agonist with no-DILI concern, to trovafloxacin, a carboxylic acid-containing fluoroquinolone antibiotic that was withdrawn from the European market for its hepatotoxicity.[37,38]

In addition to parent drugs and drug metabolites, UGT enzymes conjugate many endogenous molecules, including bilirubin (UGT1A1), bile acids (UGT1A3, 2B7), serotonin (UGT1A6), steroid hormones, eicosanoids, and vitamin D (UGT1A3, 1A4).[39–43] Reduced UGT enzyme activity can disrupt the normal metabolic clearance of these substances, leading to bioaccumulation and sustained biological effects. Crigler-Najjar syndrome and Gilbert syndrome are genetic disorders that lower the activity of UGT1A1, resulting in unconjugated hyperbilirubinemia.[44–46] Drugs can also inhibit UGT activity and disrupt bilirubin homeostasis in the liver. Hy's law reveals that drugs that induce elevated serum bilirubin levels together with hepatocellular injury, where other causes of liver injury are ruled out, are likely to cause severe DILI as suggested by the FDA guidance for DILI.[47] This may account for the observation made that many UGT inhibitors in our data set cause severe DILI. Similarly, genetic mutations and drugs that inhibit UGT enzyme activity have been linked with disruption of bile acid homeostasis and cholestatic liver injury.[48,49] Accordingly, our data set contains many UGT inhibitors with a pattern of liver injury that ranges from hepatocellular to mixed and cholestatic (Supplemental Table S9, http://links.lww.com/HEP/I552).

Drugs that are dual substrate inhibitors of UGT are also more likely to have high DILI concern. The OR is similar (~13) for both DILI concern and severity (Table 5 and Supplemental Table S7, http://links.lww.com/HEP/I552) and is approximately 4 times of the OR for pure UGT inhibitors. Thus, dual substrate inhibitors of UGT are even more likely to induce DILI than pure UGT inhibitors. We suspect that dual substrate inhibitors of UGT induce DILI by a similar mechanism as pure UGT inhibitors. The higher potential of dual substrate inhibitors of UGT to induce DILI may be due to autoinhibition of the drug, which prolongs the disruption in bilirubin and bile acid metabolism, leading to more extensive damage to the liver. However, further studies are needed to investigate this hypothesis.

Typically, in drug discovery and development, identifying the toxic potential of a lead compound early is beneficial. RO2 has proven to be a valuable tool to predict the hepatotoxic potential of drug candidates that is easily computed based on the drug daily dose and lipophilicity. Since the mechanism of inducing DILI is multifactorial, we consider drug-UGT interaction as an additional tool that may be used to complement RO2 as a predictor of DILI risks. Applying the presence of drug interaction with UGT as a tool to modify the previously proposed RO2 model resulted in a new predictor that had a significantly higher sensitivity but a slight decrease in specificity (Table 7). Overall, the modified rule outperformed both the RO2 and UGT rules individually, as determined by the balanced accuracy and MCC metrics. These 2 statistical metrics are widely used to assess the overall performance of binary classifiers, such as our predictors as they evaluate the model's ability to make both positive and negative predictions.[50,51]

Some caveats in this study need to be acknowledged. First, further validation from a larger data set is necessary to validate the findings on the effect of UGT on DILI. Second, the study is limited to a binomial classification that distinguishes high DILI concern (vMost-DILI-concern) from low DILI concern, which combines the vLess-DILI-concern and vNo-DILI-concern-DILI groups. However, with a larger data set, an ordinal classification of no-DILI, less-DILI and most-DILI groups could offer more insights. Third, this study is based on in vitro drug interaction studies only. Analyses based on in vivo drug interactions may be performed in subsequent studies to assess the translation of these drug-enzyme interactions for hepatotoxic drugs

in biological systems. Fourth, we employed a simple method to define drug-enzyme interaction due to the different measurement parameters reported. The method employed does not discriminate the strength of drug interaction with enzymes, eg, strong inhibitor vs weak inhibitor. Subsequent studies using a single parameter such as IC_{50}/C_{max} for inhibitory data may provide a more uniform measurement of drug interaction and assess the strength of interaction that is required to induce DILI. Additionally, the underlying mechanism for the association of dual substrate inhibitors of UGT enzymes increased DILI risk needs to be investigated further. Finally, the study is hindered by a scarcity of data for inducers of non-CYP enzymes. While this study considered the effect of non-CYP enzymes alone, further studies may investigate the combined effect of UGT enzymes along with CYP enzymes and hepatobiliary transporters on DILI outcomes. Despite these limitations, our findings enhance available tools for identifying drugs that are likely to have outcomes of high DILI concern in both drug development and clinical settings.

AUTHOR CONTRIBUTIONS

This study was conceptualized by Minjun Chen. Tsung-Jen Liao, Jinwen Zhao, Patrice Dehanne, Catherine Noban, Yeliz Angın, Olivier Barberan, and Minjun Chen collected the data used in the study. AyoOluwa O. Olubamiwa and Minjun Chen performed statistical analysis of the data. AyoOluwa O. Olubamiwa wrote the initial draft of the manuscript. All authors edited and agreed on the final version of the manuscript.

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CONFLICTS OF INTEREST

The authors have no conflicts to report.

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