Published in final edited form as:

Drug Metab Rev. 2010 February ; 42(1): 209–224. doi:10.3109/03602530903209288.

Interindividual variability in hepatic drug glucuronidation: Studies into the role of age, sex, enzyme inducers, and genetic polymorphism using the human liver bank as a model system.

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Abstract

The human liver bank has provided an invaluable model system for the study of interindividual variability in expression and activity of the major hepatic UGTs, including UGT1A1, 1A4, 1A6, 1A9, 2B7, and 2B15. Based on studies using UGT isoform selective probes, the rank order of activity variability is UGT 1A1 > 1A6 > 2B15 > 1A4 = 1A9 > 2B7 with coefficient of variation values ranging from 92% to 45%. Liver donor age, sex, enzyme inducers, and genetic polymorphism are factors that have been implicated as sources of this variability in UGT activity. The expression of UGTs prior to and immediately following birth is quite limited, explaining the susceptibility of neonates to certain drug toxicities. Old age appears to have minimal effect on UGT function. Sex differences in UGT activity are relatively small and are confined to several UGTs, including UGT2B15, which shows higher activity in males compared with females. Enzyme inducers, including co-administered drugs, smoking, and alcohol may increase hepatic UGT levels. Human liver bank phenotype-genotype studies using UGT isoform-selective probes have identified common genetic polymorphisms that are predictive of glucuronidation activity *in vitro* that were subsequently verified as predictors of probe drug clearance by glucuronidation *in vivo*.

Keywords

Liver; glucuronidation; variability; pharmacogenetics; sex; age

1. Introduction

Glucuronidation, catalyzed by the UDP-glucuronosyltransferases is a major metabolic pathway that promotes the elimination of drugs and other potentially toxic exogenous and endogenous compounds from the body. Substantial advances in rationale drug design with the aim of enhancing stability against cytochrome P450 mediated oxidation have lead to an

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Declaration of interest: The author reports no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

increasing number of drugs being developed that are cleared through alternate pathways, including glucuronidation. Interindividual variability in drug glucuronidation can be substantial and lead to either ineffective drug levels (with rapid metabolism) or drug toxicity (with slow metabolism). Consequently, understanding the mechanisms of variability in drug glucuronidation by the different UGT isoforms expressed in human liver, the main organ responsible for drug glucuronidation, will greatly enhance our ability to predict the risks for drug inefficacy and/or toxicity and tailor the use of these drugs in individual patients.

Over the past 15 years, our laboratory (Court, 2005; Court et al., 2002; Court et al., 2001; Court et al., 2004; Court et al., 2003; Girard et al., 2004; Girard et al., 2005; Girard et al., 2006; Krishnaswamy et al., 2003a; Krishnaswamy et al., 2003b; Krishnaswamy et al., 2005a, 2005b; Krishnaswamy et al., 2004) and others (Chen et al., 2007a; Chen et al., 2008; Innocenti et al., 2008) have made substantial use of human liver banks to characterize the extent of variability in drug glucuronidation by specific UGT isoforms and also to explore the underlying mechanisms for this variability. This review summarizes the findings of this research.

2. Human liver banks as models of drug glucuronidation

2.1. Sources of liver bank material

To date, the majority of liver banks have been established at various academic institutions primarily through various nonprofit institutions that distribute tissues as they become available from liver transplant facilities and hospitals performing partial hepatectomies. More recently, it has been possible to obtain liver (and various fractions) from banks established by commercial entities. Originally much of the liver tissues available for this purpose were donor livers that had failed to match with an appropriate recipient and so were subsequently made available for research purposes. However, the development of more efficacious immunosuppressant drugs has led to the transplant of livers that are less ideal tissue matches, with a resultant decrease in availability of this type of tissue for research purposes. Consequently, much of the healthy liver tissue currently available for research purposes are from surgical resections of liver lesions (such as cancer) where adjacent normal tissue is dissected away from abnormal tissue.

2.2. Advantages and limitations of liver bank studies

Compared with clinical studies of drug metabolism there are a number of advantages of using a liver bank. Perhaps the most important advantage is the ability to prepare microsomes, RNA and DNA from the same specimen. Consequently, it is possible to perform correlative studies assaying for enzyme activity, isoform specific protein expression, mRNA expression, and DNA genotyping in the same individual. Other advantages include lower study cost, more rapid investigation of a novel hypothesis, and the possibility of performing repeated studies in the same set of individuals allowing extensive characterization and correlations between different metabolic pathways. However, rather than replace clinical studies, liver bank studies should be used as a mechanism to complement clinical studies through either generation of hypotheses that can be confirmed

through clinical study, or providing a method to understand the mechanism underlying a phenomenon observed in a clinical study.

There are a number of limitations to liver bank studies that should be kept in mind when interpreting data generated from these studies. The quality of the liver specimen can vary as a result of circumstances surrounding the time of collection. For transplant livers, the donor frequently has a history of traumatic injury (such as gunshot, motor vehicle accident, head trauma, or cerebrovascular accident) which could potentially affect liver quality. Frequently there is also a significant but variable duration between the time of death and tissue preservation (snap freezing). Various drugs and cryopreservative agents are also commonly administered during the perimortem period. Although basic information of the liver donor (age, ethnicity, gender, cause of death and infectious disease status) are usually provided, histories of recent prescription drug, alcohol and tobacco use that might affect enzyme expression and interpretation are not always available and can be inaccurate. Perhaps in part because of these aforementioned factors, estimates of variability of enzyme activities in liver banks tend to exceed variability estimates of drug clearance from volunteer pharmacokinetic studies (see below).

It is important to have a mechanism for assessing individual liver quality in place to identify those liver specimens that may have been adversely affected by suboptimal tissue harvesting or storage conditions. This is particularly important for instances in which there has been partial inactivation of multiple enzymes (say through inadvertent tissue thawing) which would result in consistently low (but measurable) enzyme activities for that particular liver sample. As shown in Figure 1, in our laboratory we have used a statistical approach to identify such low activity livers and exclude them from the final results.

3. UGTs expressed in human liver

3.1. What are the major UGTs expressed in human liver?

While the liver appears to be the major site for drug glucuronidation, not all UGTs are expressed in human liver (i.e. there are extrahepatic isoforms that are exclusively expressed outside of the liver) and the extent of expression may vary widely between isoforms. To date, four different studies have attempted to systematically identify and/or quantitate UGT mRNA from at least 6 different isoforms expressed in human liver (Congiu et al., 2002; Nakamura et al., 2008; Nishimura and Naito, 2006; Ohno and Nakajin, 2009). Table 1 summarizes the results of these studies with individual UGT isoforms ranked from most abundant to least abundant. Based on these studies, UGTs 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B15 and 2B17 are consistently expressed in human liver. All of these isoforms have been identified as being capable of glucuronidating various drugs with significant activity. Of note is that UGT2B4 is consistently identified as the most abundant of all the isoforms analyzed (a prominent RT-PCR band was noted in the study by Nakamura et al, 2008). Such high expression may reflect the role of this isoform in detoxifying potentially hepatotoxic bile acids through glucuronidation (Pillot et al., 1993). UGT2B4 along with UGT2B7 has also been shown to glucuronidate various drugs including zidovudine, morphine and codeine (Court et al., 2003). Apart from UGT2B4, UGT2B7, UGT2B15 and UGT1A4 were consistently identified as having high abundance across the different studies,

possibly reflecting the diverse array of drugs that are substrates for these enzymes when all three are considered together.

On the other hand various UGTs were either expressed in human liver inconsistently (UGT2B11), at low levels (UGT1A5, 1A7, 1A10, and 2B28), or were not detected in liver (UGT1A8) (Table 1). Apart from UGT2B11, these most likely represent the extrahepatic UGT isoforms. Of multiple tissues analyzed, UGTs 1A5, 1A7, 1A10 appear to be most highly expressed in the gastrointestinal tract (Ohno and Nakajin, 2009), while UGT2B28 is highly expressed in bladder (Nakamura et al., 2008) and breast (Levesque et al., 2001). It should be noted that all 4 studies presented in Table 1 represent only a small number of individual livers (single livers in two studies, 9 livers in another study and 12 – 18 in the other study) and so these results will need to be confirmed through study of a larger number of individual livers.

Although none of the UGT2A isoforms were assayed in those studies, recent work suggests that UGT2A3 mRNA is highly abundant in human liver (Court et al., 2008), while UGT2A1 and UGT2A2 appear to be poorly expressed in liver, if at all (Sneitz *et al*, manuscript under review).

3.2. Relative abundance of the major UGTs expressed in human liver

A question that is frequently raised when attempting to identify the main isoform responsible for glucuronidation of a particular drug in human liver (referred to as “reaction phenotyping” (Court, 2004; Court, 2005; Miners et al., 2006)) is “what is the relative abundance of the different UGTs in human liver”. This information can be used to “calibrate” glucuronidation activity data obtained for a particular substrate from different recombinant UGTs to account for underestimation of the contribution of UGTs that are highly expressed in human liver and overestimation of the contribution of UGTs poorly expressed in human liver. Although mRNA expression data provides some evidence for relative expression of different isoforms between different tissues and within a particular tissue, use of this data as a surrogate for enzyme levels and/or activities is limited by differences between UGT isoforms in post-transcriptional processing including translational efficiency, mRNA splicing, and post-translational modifications, such as phosphorylation, glycosylation and mechanisms influencing protein stability. For example, Turgeon et al (Turgeon et al., 2001) demonstrated substantial differences in translation efficiency and protein stability between UGT2B4, 2B7, 2B15 and 2B17, with UGT2B17 notably showing relatively low protein stability compared with other UGT2B isoforms. Consequently UGT2B17 mRNA levels may over-predict UGT2B17 enzyme levels. Work in our laboratory using our liver bank samples (Court et al., 2003)) showed excellent correlation between UGT2B7 dependent zidovudine glucuronidation and immunoquantified UGT2B7 protein content (Spearman correlation coefficient [R_s] = 0.77, $P < 0.001$), but no correlation between UGT2B7 mRNA and protein content (R_s = -0.20, P = 0.2) (Kwara et al, Journal of Clinical Pharmacology, in press, 2009). In contrast, results of another study in our laboratory showed excellent correlations between UGT1A6 mediated serotonin glucuronidation, UGT1A6 protein content, and UGT1A6 mRNA content (R_s values from 0.47 to 0.76, $P < 0.001$) (Krishnaswamy et al., 2005a). The poor correlation between UGT2B7 mRNA and protein

levels may be the result of alternate mRNA splicing of the UGT2B7 gene resulting in some mRNA forms that lack exons 1, 3, 4, 5, and 6 and do not code for full length enzymatically functional enzyme (Innocenti et al., 2008). Consequently quantitation of UGT2B7 mRNA using probes or real-time PCR primers within exon 2 will result in measurement of mRNA forms encoding both full length and truncated protein.

Absolute quantitation of UGT enzyme protein by immunochemical techniques (quantitative blotting or ELISA) using recombinant enzyme standards or by proteomic techniques including quantitative mass spectrometry (see Phil Smith's chapter) will likely be needed to derive useful estimates of the relative abundance of different UGT isoforms in human liver.

4. Interindividual variability in activity of the major UGT isoforms expressed in liver

Over the past 10–15 years various UGT isoform-selective substrate probes (“phenotyping probes”) have been identified that can be used to study the major UGTs expressed in human liver. A comprehensive listing and evaluation of these probes is provided elsewhere in this volume (see John Miner's chapter). In addition, antibodies for several UGTs have been developed for commercial purposes (UGT1A1, 1A6, and 2B7) or by research laboratories (UGT1A4, UGT1A9) that appear to be reasonably isoform-specific and when used for semiquantitative immunoblotting can provide further supporting evidence for the UGT substrate probe data.

Figure 2 shows a compilation of published data from our human liver bank (n=54) demonstrating the variability in glucuronidation of estradiol (3-hydroxy), trifluoperazine-glucuronidation, serotonin, propofol, zidovudine, and s-oxazepam as probes for UGTs 1A1, 1A4, 1A6, 1A9, 2B7, and 2B15, respectively based on evidence provided in (Court, 2005). Also shown in Figure 2 for comparison (and measured in the same microsomal samples) are midazolam 1'-hydroxylation (He et al., 2005) and chlorzoxazone 6-hydroxylation (Court et al., 1997), which are cytochrome P450 (CYP450) probe activities for CYP3A and CYP2E1, respectively. CYP3A is well known to demonstrate high interindividual variability (He et al., 2005), while CYP2E1 shows relatively low interindividual variability (Ernstgard et al., 2007). The coefficient of variation (CV), which is the sample standard deviation divided by the mean expressed as a percentage, is also given in Figure 2 as an index of variability. As expected, midazolam 1'-hydroxylation showed the highest variability (117% CV), while chlorzoxazone 6-hydroxylation had the lowest variability (35% CV). All UGT activity variabilities ranged between these values (from 92% to 45% CV) with a rank order of UGT 1A1 > 1A6 > 2B15 > 1A4 = 1A9 > 2B7.

Possible factors influencing UGT activity variability may include age (young and old), sex, genetic polymorphism, and enzyme inducers (as discussed below and also in an excellent review by Miners and Mackenzie (Miners and Mackenzie, 1991)). Demographic factors including age, gender, race/ethnicity, cause of death and histories of smoking, alcohol use and prescription drugs for our liver bank donors are provided in Table 1. Apart from genetic variation, race/ethnicity could also have an additional influence on enzyme activity,

although most human liver banks (including ours) tend to include few (if any) livers from individuals other than those of white European descent.

4.1 Liver donor age

4.1.1 UGT ontogeny—The ontogeny (development up to adulthood) of hepatic drug glucuronidation has been the subject of several reviews (Burchell et al., 1989; de Wildt et al., 1999; McCarver and Hines, 2002), most recently by Hines (Hines, 2008) based upon a relatively small number of studies. Prior to and immediately following birth, the liver appears to have a limited ability to glucuronidate drugs. Initial studies using phenolic substrates (such as 1-naphthol and 4-nitrophenol) which are glucuronidated by UGT1A isoforms demonstrated very low to undetectable activities in fetal human livers (Rane et al., 1973; Rollins et al., 1979). On the other hand morphine glucuronidation (by UGT2B isoforms) was low but still about 10% of adult liver activities (Pacifici and Rane, 1982; Pacifici et al., 1982). A broader range of substrates was evaluated by Leakey et al (Leakey et al., 1987) using human fetal livers (16–25 weeks gestation). They demonstrate very low, although detectable activities for a range of substrates now known to be glucuronidated by UGT1A1 (bilirubin), UGT2B7 (androsterone), UGT2B15/17 (testosterone), UGT1A6/9 (1-naphthol and 4-nitrophenol). The only exception was glucuronidation of 5-hydroxytryptamine (serotonin) which was present at activity levels similar to adult liver. This latter finding suggests that UGT1A6, which selectively glucuronidates serotonin (Krishnaswamy et al., 2003a), may be expressed to a significant extent in fetal liver. However, arguing against this contention is that 1-naphthol and 4-nitrophenol were glucuronidated poorly in fetal liver and both these substrates are also glucuronidated extensively by UGT1A6. Furthermore, in a previous study, glucuronidation of acetaminophen (another UGT1A6 substrate) could not be detected in liver cells isolated from 19 and 22 week gestation human fetuses, whereas acetaminophen sulfation and oxidation were readily measurable, demonstrating cell viability (Rollins et al., 1979). A later study by Coughtrie et al (Coughtrie et al., 1988) demonstrated that glucuronidation activities for bilirubin, testosterone, and 1-naphthol are low in the fetus and neonate but rapidly rise within the first month following birth. Furthermore immunoblotting of liver microsomal proteins using several nonspecific UGT antibodies demonstrated a reduced number of UGT isoforms of differing molecular size and overall low abundance of those UGTs expressed in fetal liver compared with adult liver.

More recently, Strassburg et al (Strassburg et al., 2002), utilized (semi-quantitative) RT-PCR, immunoblotting, and glucuronidation activity assays to study UGT expression in fetal (5 months gestation; n=2), pediatric (6–24 months postnatal; n=16) and adult (n=12) human livers. None of the UGTs assayed by RT-PCR could be detected in the fetal liver examined. However, by 9 months of age, all UGTs expressed in adult liver (UGTs 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7 and 2B15) could be readily detected by RT-PCR. Assay of selected UGTs by semiquantitative RT-PCR suggested that UGT1A9 and UGT2B4 were expressed at lower levels than adult at 6 months of age but increased over the next 18 months, while UGT1A1 and UGT2B7 had already achieved adult levels by 6 months of age. Immunoblotting with UGT1A1, UGT1A6, and UGT2B7 substantiated that protein levels of these isoforms had already reached adult levels by 7 months of age. On the other hand, enzyme activity measures, showed a different trend in that the majority of glucuronidation

activities evaluated consistently showed from 3- to 10-fold lower glucuronidation activities compared with adult livers. Included in the assessment were multiple substrates glucuronidated by UGT 1A6 (1-naphthol and 4-nitrophenol), UGT1A4 (amitriptyline and desipramine) and UGT2B7 (buprenorphine, hyodeoxycholic acid), all of which were glucuronidated much more slowly in pediatric than adult liver. Although other possibilities exist, such discrepancies between enzyme activity and protein levels might be a reflection of differences in post-translational modification such as phosphorylation, which has been identified as essential for UGT enzymatic activity (Basu et al., 2003). However, it is also important to point out that the pediatric livers studied were explants (i.e. the original liver that was removed prior to transplant) from patients with biliary atresia (i.e. a blocked or absent bile duct) and so expression and function of the UGTs in these livers may have been affected by the biliary obstructive disease.

Several more recent studies have focused on individual UGT isoforms. A study by Zaya *et al* (Zaya et al., 2006) that included a set of 28 neonatal and pediatric livers spanning 0 to 21 years of age examined the glucuronidation of epirubicin, a probe for UGT2B7 and also UGT2B7 protein levels by immunoblotting. As seen in Figure 3 there was an obvious relationship between age and epirubicin glucuronidation rates (panel A) and UGT2B7 protein content (panel B). At 0 to 1 year of age, activity levels were approximately 10% of adult levels, at 1 to 11 years of age, they were about 25% of adult levels, and at 12 to 17 years of age they were about 50% of adult levels. Activities and UGT2B7 protein content were also reasonably well correlated ($r^2 = 0.74$, $P < 0.001$). However, despite the lower expression and activity of UGT2B7 in pediatric livers versus adults, the effect on pharmacokinetic clearance of UGT2B7 substrates (such as morphine) is much less. For example, morphine clearance (normalized to body-weight) while low in the neonate achieve an adult rate between 6 and 30 months of age (Choonara et al., 1989). This discrepancy between *in vitro* and *in vivo* results is (in part) explained by the higher mass of liver tissue (relative to body size) in children as compared with adults (Murry et al., 1995). One empirical method to correct for this is to “scale” activities using the “three-quarter power rule” whereby the data are adjusted by dividing by the liver donor’s body-weight (actual or predicted from standardized charts) raised to the power of 0.75. Using this adjustment, Zaya *et al* showed that scaled epirubicin glucuronidation activities were essentially unchanged with age in the pediatric livers (Zaya et al., 2006).

Most recently, the ontogeny of hepatic UGT1A4 was studied by examining changes in trifluoperazine glucuronidation (an index reaction for UGT1A4) in pediatric human liver microsomes (Miyagi and Collier, 2007). The results showed only minimal change in trifluoperazine glucuronidation over the age range examined (approximately 1 month to 20 years old).

The results from our human liver bank studies (Figure 4) indicate that mean probe activities for UGT1A1, UGT1A6, and UGT1A9 are more than 50% lower ($P < 0.05$, Kruskal-Wallis ANOVA on ranks with Dunn’s test adjusted for multiple comparisons) in livers from children (2 – 20 years old; $n = 9$) compared livers from young adults (21–40 years; $n = 18$), middle age adults (41 – 60 years; $n = 14$) and older individuals (61 years and older; $n = 13$), which is in agreement with the results of the study by Strassburg et al (Strassburg et al.,

2002) mentioned above. However, probe activities for UGT1A4, UGT2B7 and UGT2B15 were unaffected by donor age in our liver bank ($P>0.05$, Kruskal-Wallis ANOVA on ranks), which contrasts with the results of Strassburg et al which showed lower activity of UGT1A4 and UGT2B7 substrates, while the effect of age on UGT2B15-mediated glucuronidation in human liver has not been reported.

As yet there is no evidence for a fetal-specific UGT isoform as there has been for other drug metabolizing enzymes such as CYP3A7 (Hines, 2008).

The mechanisms underlying enhanced expression of the UGTs in liver following birth are not understood at present, but may involve regulation by transcription factors as well as epigenomic changes. There is some evidence that expression of the constitutive androstane receptor (CAR; NR1I3) is involved in the regulation of UGT1A1 and several other UGTs, and low expression of CAR was associated with neonatal jaundice (unconjugated hyperbilirubinemia) (Huang et al., 2003). However, it was not clear from those studies whether CAR is a limiting factor for UGT1A1 expression.

4.1.2 Old age—Old age (aging) is thought to have only a modest effect (if any) on the hepatic glucuronidation of drugs (Greenblatt et al., 1980; Greenblatt et al., 1991; Klotz, 2009). Using 4-methylumbelliferone as a probe, which is glucuronidated by multiple UGT1A forms, Parkinson *et al* (Parkinson et al., 2004) showed no difference in glucuronidation activities between livers from elderly (60 years and older; $n = 16$) versus younger livers ($n = 48$). Similarly, valproate glucuronidation (again by multiple UGT isoforms) was not different in livers from older donors (65 years and older; $n = 18$) compared with younger donors (2 – 56 years; $n = 18$) (Argikar and Remmel, 2009). Results from our human liver bank (Figure 4) are consistent with these findings in that glucuronidation activities for 6 different probes are not different in livers from elderly donors (over 60 years old) compared with younger donors (60 years and less).

4.2 Sex

While sexual dimorphism is quite apparent in rodent models of drug metabolism, sex differences in hepatic drug metabolism in people is rarely encountered and generally results in relatively small effects (Waxman and Holloway, 2009). For example, a recent meta-analysis of the effect of sex on weight-normalized clearance of CYP3A metabolized drugs from 38 data sets showed an average 26% higher clearance of parenterally administered drugs and 17% higher clearance of orally administered drugs in women versus men (Greenblatt and von Moltke, 2008). With regards to hepatic drug glucuronidation, 65% higher median S-oxazepam glucuronidation were reported for livers from male ($n = 16$) versus female ($n = 38$) donors (Court et al., 2004). These data are confirmed by results from a previous pharmacokinetic study that showed 40% higher mean oxazepam clearance in healthy men ($n = 18$) versus women ($n = 20$) (Greenblatt et al., 1980). Presumably this difference results from higher UGT2B15 expression in the livers of men versus women, although this has not yet been tested or the underlying mechanism explored. Almost 50% higher median acetaminophen glucuronidation were also reported for livers from male versus female donors ($P = 0.047$, Mann-Whitney rank sum test), although the sex difference

in UGT1A6 protein levels measured in a subset of the same livers did not achieve statistical significance ($P = 0.076$). Acetaminophen clearance has also been reported to be 22% higher in men compared with women (Miners et al., 1983). In the study by Parkinson *et al* (Parkinson et al., 2004) mentioned above, there were no sex differences in 4-methylumbelliferone glucuronidation in either liver microsomal samples (over 40 samples of each sex) or primary human hepatocytes (over 30 samples of each sex). The UGT probe activities measured in our human liver bank (Figure 5) show no sex-related differences in glucuronidation other than higher S-oxazepam glucuronidation in livers from male donors compared with female donors ($P < 0.05$, Mann-Whitney rank sum test) as was previously reported using the same data (Court et al., 2004).

4.3 Enzyme inducers

Smoking, as well as consumption of certain prescription drugs, herbal medicines, and/or alcohol by liver donors within 2 weeks prior to tissue collection has the potential to increase drug metabolism activity through enzyme induction. Unfortunately, such detailed patient history is not always available or reliable and so may confound liver bank study results. Patient demographic information for our liver bank donors regarding prescription drug usage, alcohol consumption and smoking history is given in Table 1. Two subjects were identified with a history of consuming medications that are known CAR inducers including LV29 (phenobarbital and carbamazepine), and LV32 (phenytoin), while two subjects had a history of consuming a known glucocorticoid receptor (GR) inducer (dexamethasone) including LV15 and LV36. LV32 had also received prednisone, another GR inducer. Despite these positive histories, there was no evidence for increased glucuronidation activities in any of these subjects (see Figure 1) with the possible exception of LV32 which had Z-scores of greater than +1 (more than 1 SD above the mean for all liver bank samples) for estradiol-3-glucuronidation, trifluoperazine glucuronidation, and serotonin glucuronidation.

Positive histories for smoking (19 of 48 donors smoking at least half a pack per day) and drinking alcohol (12 of 47 donors consuming an average of more than 2 drinks per day) were relatively commonly reported in our liver bank donors. As shown in Figure 6 only trifluoperazine glucuronidation (by UGT1A4) showed significantly higher glucuronidation activity ($P = 0.047$, Mann-Whitney rank sum test) in smokers compared with non-smokers. Previous published studies also show variable, small, and in some cases inconsistent effects of smoking on drug glucuronidation, which may be dependent on UGT isoform evaluated and smoking intensity. Fleischman *et al* showed higher 1-naphthol glucuronidation (presumably by UGT1A6) but no effect on morphine (UGT2B7) in liver biopsies from smokers versus nonsmokers (Fleischmann et al., 1986). Dragacci *et al* showed no effect of smoking on bilirubin (UGT1A1) or clofibrate (UGT2B7) glucuronidation in microsomes from liver biopsies (Dragacci et al., 1987). Results from several *in vivo* studies suggest that the clearance of acetaminophen (Bock et al., 1994; Bock et al., 1987), oxazepam (Ochs et al., 1981), and codeine (Yue et al., 1989; Yue et al., 1994) is higher in cigarette smokers, although the extent of the difference was small and probably clinically unimportant. In contrast, results of several other studies show no effects of smoking on the clearance by glucuronidation of acetaminophen (Miners et al., 1984; Scavone et al., 1990), diflunisal (Macdonald et al., 1990), and lorazepam (Ochs et al., 1985).

A positive drinking history was consistently associated with higher glucuronidation activity in our liver bank for all assayed UGT1A probes, but did not influence UGT2B7 or UGT2B15 activities (Figure 7). Few studies have investigated the effects of chronic drinking on drug glucuronidation in humans. Dragacci *et al* reported no effect of drinking history on bilirubin (UGT1A1) or clofibrate (UGT2B7) glucuronidation in microsomes from liver biopsies (Dragacci et al., 1987). In one pharmacokinetic study, acetaminophen clearance was not different in alcoholics versus non-alcoholics (Villeneuve et al., 1983), while in another somewhat larger study, faster clearance (by about 20%) and shorter elimination half-life (by 30%) was reported for chronic alcoholics versus abstinent subjects (Girre et al., 1993). Several animal model studies have shown increased bilirubin and morphine glucuronidation and increased UGT1A1 expression in animals chronically exposed to alcohol (Kardon et al., 2000; Li et al., 2000; Narayan et al., 1991). Although the mechanism for this effect is unknown, one study suggested that it was dependent on the presence of hepatic Kupfer cells (Kardon et al., 2002). On the other hand another study that involved adding 10% alcohol to the drinking water of rats showed no effect on hepatic bilirubin, nitrophenol or testosterone glucuronidation (Mori et al., 2002).

4.4 Genetic polymorphism

The human liver bank has provided a useful model system for the study of genetic polymorphism and drug glucuronidation. DNA, RNA, protein and enzyme activity measurements are performed in the same individual for multiple studies of multiple UGT isoforms. DNA is used to identify liver genotypes within the UGT encoding gene of interest, which can then be correlated with UGT isoform probe activities. RNA and UGT protein levels are used to determine whether the polymorphism might influence gene expression through effects on gene transcription or translation. This hypothesis is then tested by mechanistic studies using appropriate cell based models (promoter-reporter plasmid constructs for example). RNA derived from liver bank tissues also allows investigations of mRNA splicing and effects of polymorphisms on splicing through alteration of splice donor, acceptor, or regulatory sites. As with other pharmacogenetic studies, the size of the study population is a limiting factor in being able to detect a difference between genotype groups and so for typical sized liver banks of 50 to 100 donors statistically significant differences can only be determined for relatively common polymorphisms, generally over 10% minor allele frequency for dominantly expressed traits.

Although similar studies could be conducted in healthy volunteers through collection of liver biopsy specimens with the advantage of additionally being able to perform *in vivo* pharmacokinetic studies in the same subjects, there are significant limitations including the small amount of tissue collected, as well as ethical concerns related to the risk of the biopsy procedure.

Table 2 summarizes results of UGT genotype-phenotype association studies that have utilized human liver banks. Although most studies were conducted using validated UGT isoform selective probes, several studies used non-selective substrates including NNAL that was used to evaluate UGT1A4 (N-glucuronidation), as well as UGT2B7 and UGT2B17 (o-glucuronidation) variants, SN-38 that was used to evaluate UGT1A1 (primary isoform) and

UGT1A9 (minor isoform), and 4-nitrophenol that was used to evaluate UGT1A6. Also shown in Table 2 are the results of other *in vitro* studies that were conducted to provide mechanistic validation of the effects of the polymorphism and involve either promoter-reporter plasmid constructs transfected into mammalian cell lines (UGT1A1*28) or assay of activity of cDNA constructs (reference and amino acid variants) transfected into mammalian cell lines (UGT1A4*2 and *3, UGT1A6*2, UGT2B7*2, UGT2B10*2 and UGT2B15*2). The other UGT polymorphisms evaluated were located in the intron (UGT1A9*1q) or in the exon coding region but were synonymous (UGT2B7*1c) and the underlying mechanism is as yet unclear. Finally, Table 2 shows results of pharmacokinetic studies in both healthy subjects and treated patients, providing *in vivo* confirmation (or refutation) of *in vitro* results.

In most instances, genotype-phenotype association results from human liver bank studies were replicated by *in vivo* pharmacokinetic studies especially when isoform-selective *in vitro* and *in vivo* probes (respectively) were used. For example, UGT2B15*2 was associated with decreased oxazepam glucuronidation in a human liver bank and decreased oxazepam oral clearance *in vivo* (Figure 8a and 8b), while UGT2B7*1c was associated with increased AZT and morphine glucuronidation in several human liver banks and higher AZT oral clearance *in vivo* (Figure 8c and 8d). On the other hand UGT2B7*2 was not associated with altered AZT or morphine glucuronidation in human liver bank studies, nor with altered AZT or morphine pharmacokinetics *in vivo*. In some instances for the same variant (for example UGT1A6*2) there were inconsistent findings between liver bank studies, as well as between recombinant UGT studies, and also between *in vivo* pharmacokinetic studies. The reasons for these inconsistencies are not immediately apparent. In other instances, including evaluation of the UGT1A4*2 and *3, UGT2B10*2 and UGT2B17*2 polymorphisms, definitive studies have not yet been conducted using UGT selective *in vivo* probes (or any *in vivo* probe in the case of UGT2B10*2 and UGT2B17*2).

5. Conclusions

The human liver bank has provided an invaluable model system for studies of interindividual variability in expression and activity of the major hepatic UGTs, including UGT1A1, 1A4, 1A6, 1A9, 2B7, and 2B15. Of these UGTs, UGT1A1 show the highest variability, while UGT2B7 shows the lowest variability. Several other isoforms expressed in human liver, including UGT1A3, UGT2B4, and UGT2B17 have yet to be evaluated using isoform selective probe substrates. UGT2B4 is of interest since it appears to be the most abundant hepatic UGT, at least based on mRNA expression levels. Several patient factors have been identified that likely explain in part the high variability in UGT activity, including age, sex, enzyme inducers, and genetic polymorphism. The expression of UGTs prior to and immediately following birth is quite limited, explaining the susceptibility of neonates to certain drug toxicities. Old age appears to have minimal effect on UGT function. Sex differences in UGT activity are relatively small and are confined to several UGTs, including UGT2B15 and possibly UGT1A6, both of which show higher activity levels in males compared with females. Enzyme inducers, including co-administered drugs, smoking, and alcohol may increase hepatic UGT levels. Although a positive alcohol use history was associated with higher UGT1A isoform activities in our liver bank, the mechanism for this

association is as yet unclear. Human liver bank phenotype-genotype studies using UGT isoform-selective probes can identify common polymorphisms that are predictive of glucuronidation activity *in vitro* and drug clearance by glucuronidation *in vivo*.

Acknowledgments

Dr Court was supported by grant GM-061834 from the National Institute of General Medical Sciences (NIGMS), National Institutes of Health (Bethesda, MD). Drs Chantal Guillemette and Hugo Girard are gratefully acknowledged for their contributions to several collaborative projects involving UGT genotype-phenotype analyses of the Tufts human liver bank. Su Duan, Qin Hao, and Drs Leah Hesse, Ping He, Xi He, and Soundar Krishnaswamy are also acknowledged for their role in generating much of the UGT genotype-phenotype data. Drs David Greenblatt and Lisa von Moltke were also instrumental in setting up the Tufts liver bank.

References

- Argikar UA, Rimmel RP (2009). Effect of aging on glucuronidation of valproic acid in human liver microsomes and the role of UDP-glucuronosyltransferase UGT1A4, UGT1A8, and UGT1A10. *Drug Metab Dispos* 37(1):229–236. [PubMed: 18838507]
- Basu NK, Kole L, Owens IS (2003). Evidence for phosphorylation requirement for human bilirubin UDP-glucuronosyltransferase (UGT1A1) activity. *Biochem Biophys Res Commun* 303(1):98–104. [PubMed: 12646172]
- Belanger AS, Caron P, Harvey M, Zimmerman PA, Mehlotra RK, Guillemette C (2009). Glucuronidation of the antiretroviral drug efavirenz (EFV) by UGT2B7 and an *in vitro* investigation of drug-drug interaction with zidovudine (AZT). *Drug Metab Dispos*.
- Bock KW, Schrenk D, Forster A, Griese EU, Morike K, Brockmeier D, et al. (1994). The influence of environmental and genetic factors on CYP2D6, CYP1A2 and UDP-glucuronosyltransferases in man using sparteine, caffeine, and paracetamol as probes. *Pharmacogenetics* 4(4):209–218. [PubMed: 7987405]
- Bock KW, Wiltfang J, Blume R, Ullrich D, Bircher J (1987). Paracetamol as a test drug to determine glucuronide formation in man. Effects of inducers and of smoking. *Eur J Clin Pharmacol* 31(6): 677–683. [PubMed: 3556375]
- Burchell B, Coughtrie M, Jackson M, Harding D, Fournel-Gigleux S, Leakey J, et al. (1989). Development of human liver UDP-glucuronosyltransferases. *Dev Pharmacol Ther* 13(2–4):70–77. [PubMed: 2515047]
- Chen G, Blevins-Primeau AS, Dellinger RW, Muscat JE, Lazarus P (2007a). Glucuronidation of nicotine and cotinine by UGT2B10: loss of function by the UGT2B10 Codon 67 (Asp>Tyr) polymorphism. *Cancer Res* 67(19):9024–9029. [PubMed: 17909004]
- Chen G, Dellinger RW, Gallagher CJ, Sun D, Lazarus P (2008). Identification of a prevalent functional missense polymorphism in the UGT2B10 gene and its association with UGT2B10 inactivation against tobacco-specific nitrosamines. *Pharmacogenet Genomics* 18(3):181–191. [PubMed: 18300939]
- Chen Y, Kuehl GE, Bigler J, Rimorin CF, Schwarz Y, Shen DD, et al. (2007b). UGT1A6 polymorphism and salicylic acid glucuronidation following aspirin. *Pharmacogenet Genomics* 17(8):571–579. [PubMed: 17622933]
- Choonara IA, McKay P, Hain R, Rane A (1989). Morphine metabolism in children. *Br J Clin Pharmacol* 28(5):599–604. [PubMed: 2590614]
- Ciotti M, Marrone A, Potter C, Owens IS (1997). Genetic polymorphism in the human UGT1A6 (planar phenol) UDP- glucuronosyltransferase: pharmacological implications. *Pharmacogenetics* 7(6):485–495. [PubMed: 9429234]
- Congiu M, Mashford ML, Slavin JL, Desmond PV (2002). UDP glucuronosyltransferase mRNA levels in human liver disease. *Drug Metab Dispos* 30(2):129–134. [PubMed: 11792680]
- Coughtrie MW, Burchell B, Leakey JE, Hume R (1988). The inadequacy of perinatal glucuronidation: immunoblot analysis of the developmental expression of individual UDP-glucuronosyltransferase

- isoenzymes in rat and human liver microsomes. *Mol Pharmacol* 34(6):729–735. [PubMed: 3143908]
- Court MH (2004). In vitro identification of UDP-glucuronosyltransferases (UGTs) involved in drug metabolism In Yan Z, and Caldwell GW (Ed.), *Optimization in drug discovery: In vitro methods* (pp. 185–202). Totowa: Humana press, Inc.
- Court MH (2005). Isoform-selective probe substrates for in vitro studies of human UDP-glucuronosyltransferases. *Methods Enzymol* 400:104–116. [PubMed: 16399346]
- Court MH, Duan SX, Guillemette C, Journault K, Krishnaswamy S, von Moltke LL, et al. (2002). Stereoselective conjugation of oxazepam by human UDP-glucuronosyltransferases (UGTs): S-oxazepam is glucuronidated by UGT2B15, while R-oxazepam is glucuronidated by UGT2B7 and UGT1A9. *Drug Metab Dispos* 30(11):1257–1265. [PubMed: 12386133]
- Court MH, Duan SX, von Moltke LL, Greenblatt DJ, Patten CJ, Miners JO, et al. (2001). Interindividual variability in acetaminophen glucuronidation by human liver microsomes: identification of relevant acetaminophen UDP-glucuronosyltransferase isoforms. *J Pharmacol Exp Ther* 299(3):998–1006. [PubMed: 11714888]
- Court MH, Hao Q, Krishnaswamy S, Bekaii-Saab T, Al-Rohaimi A, Von Moltke LL, et al. (2004). UDP-glucuronosyltransferase (UGT) 2B15 pharmacogenetics: UGT2B15 D85Y genotype and gender are major determinants of oxazepam glucuronidation by human liver. *J Pharmacol Exp Ther* 310(2):656–665. [PubMed: 15044558]
- Court MH, Hazarika S, Krishnaswamy S, Finel M, Williams JA (2008). Novel polymorphic human UDP-glucuronosyltransferase 2A3: cloning, functional characterization of enzyme variants, comparative tissue expression, and gene induction. *Mol Pharmacol* 74(3):744–754. [PubMed: 18523138]
- Court MH, Krishnaswamy S, Hao Q, Duan SX, Patten CJ, von Moltke LL, et al. (2003). Evaluation of 3'-azido-3'-deoxythymidine, morphine, and codeine as probe substrates for UDP-glucuronosyltransferase 2B7 (UGT2B7) in human liver microsomes: specificity and influence of the UGT2B7*2 polymorphism. *Drug Metab Dispos* 31(9):1125–1133. [PubMed: 12920168]
- Court MH, von Moltke LL, Shader RI, Greenblatt DJ (1997). Biotransformation of chlorzoxazone by hepatic microsomes from humans and ten other mammalian species. *Biopharm Drug Dispos* 18(3): 213–226. [PubMed: 9113344]
- de Wildt SN, Kearns GL, Leeder JS, van den Anker JN (1999). Glucuronidation in humans. Pharmacogenetic and developmental aspects. *Clin Pharmacokinet* 36(6):439–452. [PubMed: 10427468]
- Dragacci S, Hamar-Hansen C, Fournel-Gigleux S, Lafaurie C, Magdalou J, Siest G (1987). Comparative study of clofibric acid and bilirubin glucuronidation in human liver microsomes. *Biochem Pharmacol* 36(22):3923–3927. [PubMed: 3120730]
- Duguay Y, Baar C, Skorpén F, Guillemette C (2004). A novel functional polymorphism in the uridine diphosphate-glucuronosyltransferase 2B7 promoter with significant impact on promoter activity. *Clin Pharmacol Ther* 75(3):223–233. [PubMed: 15001974]
- Ehmer U, Vogel A, Schutte JK, Krone B, Manns MP, Strassburg CP (2004). Variation of hepatic glucuronidation: Novel functional polymorphisms of the UDP-glucuronosyltransferase UGT1A4. *Hepatology* 39(4):970–977. [PubMed: 15057901]
- Ernstgard L, Johanson G, Karlsson AS, Warholm M (2007). Phenotyping of cytochrome P450 2E1 in vitro and in vivo. *Curr Drug Metab* 8(5):493–498. [PubMed: 17584020]
- Fleischmann R, Remmer H, Starz U (1986). Induction of cytochrome P-448 iso-enzymes and related glucuronosyltransferases in the human liver by cigarette smoking. *Eur J Clin Pharmacol* 30(4):475–480. [PubMed: 3743625]
- Girard H, Court MH, Bernard O, Fortier LC, Villeneuve L, Hao Q, et al. (2004). Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. *Pharmacogenetics* 14(8):501–515. [PubMed: 15284532]
- Girard H, Thibaudeau J, Court MH, Fortier LC, Villeneuve L, Caron P, et al. (2005). UGT1A1 polymorphisms are important determinants of dietary carcinogen detoxification in the liver. *Hepatology* 42(2):448–457. [PubMed: 15986396]

- Girard H, Villeneuve L, Court MH, Fortier LC, Caron P, Hao Q, et al. (2006). The novel UGT1A9 intronic I399 polymorphism appears as a predictor of 7-ethyl-10-hydroxycamptothecin glucuronidation levels in the liver. *Drug Metab Dispos* 34(7):1220–1228. [PubMed: 16595709]
- Girre C, Hispard E, Palombo S, N'Guyen C, Dally S (1993). Increased metabolism of acetaminophen in chronically alcoholic patients. *Alcohol Clin Exp Res* 17(1):170–173. [PubMed: 8452199]
- Greenblatt DJ, Divoll M, Harmatz JS, Shader RI (1980). Oxazepam kinetics: effects of age and sex. *J Pharmacol Exp Ther* 215(1):86–91. [PubMed: 7452494]
- Greenblatt DJ, Harmatz JS, Shader RI (1991). Clinical pharmacokinetics of anxiolytics and hypnotics in the elderly. Therapeutic considerations (Part I). *Clin Pharmacokinet* 21(3):165–177. [PubMed: 1684924]
- Greenblatt DJ, von Moltke LL (2008). Gender has a small but statistically significant effect on clearance of CYP3A substrate drugs. *J Clin Pharmacol* 48(11):1350–1355. [PubMed: 18757784]
- He P, Court MH, Greenblatt DJ, Von Moltke LL (2005). Genotype-phenotype associations of cytochrome P450 3A4 and 3A5 polymorphism with midazolam clearance in vivo. *Clin Pharmacol Ther* 77(5):373–387. [PubMed: 15900284]
- Hesse LM, He P, Krishnaswamy S, Hao Q, Hogan K, von Moltke LL, et al. (2004). Pharmacogenetic determinants of interindividual variability in bupropion hydroxylation by cytochrome P450 2B6 in human liver microsomes. *Pharmacogenetics* 14(4):225–238. [PubMed: 15083067]
- Hines RN (2008). The ontogeny of drug metabolism enzymes and implications for adverse drug events. *Pharmacol Ther* 118(2):250–267. [PubMed: 18406467]
- Holthe M, Rakvag TN, Klepstad P, Idle JR, Kaasa S, Krokan HE, et al. (2003). Sequence variations in the UDP-glucuronosyltransferase 2B7 (UGT2B7) gene: identification of 10 novel single nucleotide polymorphisms (SNPs) and analysis of their relevance to morphine glucuronidation in cancer patients. *Pharmacogenomics J* 3(1):17–26. [PubMed: 12629580]
- Huang W, Zhang J, Chua SS, Qatanani M, Han Y, Granata R, et al. (2003). Induction of bilirubin clearance by the constitutive androstane receptor (CAR). *Proc Natl Acad Sci U S A* 100(7):4156–4161. [PubMed: 12644704]
- Innocenti F, Liu W, Fackenthal D, Ramirez J, Chen P, Ye X, et al. (2008). Single nucleotide polymorphism discovery and functional assessment of variation in the UDP-glucuronosyltransferase 2B7 gene. *Pharmacogenet Genomics* 18(8):683–697. [PubMed: 18622261]
- Kardon T, Coffey MJ, Banhegyi G, Conley AA, Burchell B, Mandl J, et al. (2000). Transcriptional induction of bilirubin UDP-glucuronosyltransferase by ethanol in rat liver. *Alcohol* 21(3):251–257. [PubMed: 11091029]
- Kardon T, Mile V, Banhegyi G, Csala M, Burchell B, Mandl J, et al. (2002). Ethanol-dependent induction of bilirubin UDP-glucuronosyl-transferase in rat liver is mediated by Kupffer cells. *Life Sci* 70(10):1205–1212. [PubMed: 11848303]
- Klotz U (2009). Pharmacokinetics and drug metabolism in the elderly. *Drug Metab Rev* 41(2):67–76. [PubMed: 19514965]
- Krishnaswamy S, Duan SX, von Moltke LL, Greenblatt DJ, Court MH (2003a). Validation of serotonin (5-hydroxytryptamine) as an in vitro substrate probe for human UDP-glucuronosyltransferase (UGT) 1A6. *Drug Metab Dispos* 31(1):133–139. [PubMed: 12485962]
- Krishnaswamy S, Duan SX, von Moltke LL, Greenblatt DJ, Sudmeier JL, Bachovchin WW, et al. (2003b). Serotonin (5-hydroxytryptamine) glucuronidation in vitro: assay development, human liver microsome activities and species differences. *Xenobiotica* 33(2):169–180. [PubMed: 12623759]
- Krishnaswamy S, Hao Q, Al-Rohaimi A, Hesse LM, von Moltke LL, Greenblatt DJ, et al. (2005a). UDP glucuronosyltransferase (UGT) 1A6 pharmacogenetics: I. Identification of polymorphisms in the 5'-regulatory and exon 1 regions, and association with human liver UGT1A6 gene expression and glucuronidation. *J Pharmacol Exp Ther* 313(3):1331–1339. [PubMed: 15761114]
- Krishnaswamy S, Hao Q, Al-Rohaimi A, Hesse LM, von Moltke LL, Greenblatt DJ, et al. (2005b). UDP glucuronosyltransferase (UGT) 1A6 pharmacogenetics: II. Functional impact of the three most common nonsynonymous UGT1A6 polymorphisms (S7A, T181A, and R184S). *J Pharmacol Exp Ther* 313(3):1340–1346. [PubMed: 15761113]

- Krishnaswamy S, Hao Q, von Moltke LL, Greenblatt DJ, Court MH (2004). Evaluation of 5-hydroxytryptophol and other endogenous serotonin (5-hydroxytryptamine) analogs as substrates for udp-glucuronosyltransferase 1a6. *Drug Metab Dispos* 32(8):862–869. [PubMed: 15258112]
- Kwara A, Lartey M, Boamah I, Rezk NL, Oliver-Commeey J, Kenu E, et al. (2009). Interindividual variability in pharmacokinetics of generic nucleoside reverse transcriptase inhibitors in TB/HIV-coinfected Ghanaian patients: UGT2B7*1c is associated with faster zidovudine clearance and glucuronidation. *J Clin Pharmacol* 49(9):1079–1090. [PubMed: 19628728]
- Lazarus P, Zheng Y, Aaron Runkle E, Muscat JE, Wiener D (2005). Genotype-phenotype correlation between the polymorphic UGT2B17 gene deletion and NNAL glucuronidation activities in human liver microsomes. *Pharmacogenet Genomics* 15(11):769–778. [PubMed: 16220109]
- Leakey JE, Hume R, Burchell B (1987). Development of multiple activities of UDP-glucuronyltransferase in human liver. *Biochem J* 243(3):859–861. [PubMed: 3117034]
- Levesque E, Benoit-Biancamano MO, Delage R, Couture F, Guillemette C (2008). Pharmacokinetics of mycophenolate mofetil and its glucuronide metabolites in healthy volunteers. *Pharmacogenomics* 9(7):869–879. [PubMed: 18597651]
- Levesque E, Delage R, Benoit-Biancamano MO, Caron P, Bernard O, Couture F, et al. (2007). The impact of UGT1A8, UGT1A9, and UGT2B7 genetic polymorphisms on the pharmacokinetic profile of mycophenolic acid after a single oral dose in healthy volunteers. *Clin Pharmacol Ther* 81(3):392–400. [PubMed: 17339869]
- Levesque E, Turgeon D, Carrier JS, Montminy V, Beaulieu M, Belanger A (2001). Isolation and characterization of the UGT2B28 cDNA encoding a novel human steroid conjugating UDP-glucuronosyltransferase. *Biochemistry* 40(13):3869–3881. [PubMed: 11300766]
- Li YQ, Prentice DA, Howard ML, Mashford ML, Wilson JS, Desmond PV (2000). Alcohol up-regulates UDP-glucuronosyltransferase mRNA expression in rat liver and in primary rat hepatocyte culture. *Life Sci* 66(7):575–584. [PubMed: 10794513]
- Limenta LM, Jirasomprasert T, Tankanilt J, Svasti S, Wilairat P, Chantharaksri U, et al. (2008). UGT1A6 genotype-related pharmacokinetics of deferiprone (L1) in healthy volunteers. *Br J Clin Pharmacol* 65(6):908–916. [PubMed: 18318774]
- Macdonald JJ, Herman RJ, Verbeeck RK (1990). Sex-difference and the effects of smoking and oral contraceptive steroids on the kinetics of diflunisal. *Eur J Clin Pharmacol* 38(2):175–179. [PubMed: 2338115]
- McCarver DG, Hines RN (2002). The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. *J Pharmacol Exp Ther* 300(2):361–366. [PubMed: 11805192]
- Miners JO, Attwood J, Birkett DJ (1983). Influence of sex and oral contraceptive steroids on paracetamol metabolism. *Br J Clin Pharmacol* 16(5):503–509. [PubMed: 6416284]
- Miners JO, Attwood J, Birkett DJ (1984). Determinants of acetaminophen metabolism: effect of inducers and inhibitors of drug metabolism on acetaminophen's metabolic pathways. *Clin Pharmacol Ther* 35(4):480–486. [PubMed: 6705446]
- Miners JO, Knights KM, Houston JB, Mackenzie PI (2006). In vitro-in vivo correlation for drugs and other compounds eliminated by glucuronidation in humans: pitfalls and promises. *Biochem Pharmacol* 71(11):1531–1539. [PubMed: 16455060]
- Miners JO, Mackenzie PI (1991). Drug glucuronidation in humans. *Pharmacol Ther* 51(3):347–369. [PubMed: 1792239]
- Miyagi SJ, Collier AC (2007). Pediatric development of glucuronidation: the ontogeny of hepatic UGT1A4. *Drug Metab Dispos* 35(9):1587–1592. [PubMed: 17556526]
- Mori Y, Koide A, Kobayashi Y, Morimura K, Kaneko M, Fukushima S (2002). Effect of ethanol treatment on metabolic activation and detoxification of esophagus carcinogenic N-nitrosamines in rat liver. *Mutagenesis* 17(3):251–256. [PubMed: 11971997]
- Murry DJ, Crom WR, Reddick WE, Bhargava R, Evans WE (1995). Liver volume as a determinant of drug clearance in children and adolescents. *Drug Metab Dispos* 23(10):1110–1116. [PubMed: 8654200]
- Nagar S, Zalatoris JJ, Blanchard RL (2004). Human UGT1A6 pharmacogenetics: identification of a novel SNP, characterization of allele frequencies and functional analysis of recombinant allozymes

in human liver tissue and in cultured cells. *Pharmacogenetics* 14(8):487–499. [PubMed: 15284531]

- Nakamura A, Nakajima M, Yamanaka H, Fujiwara R, Yokoi T (2008). Expression of UGT1A and UGT2B mRNA in human normal tissues and various cell lines. *Drug Metab Dispos* 36(8):1461–1464. [PubMed: 18480185]
- Narayan SS, Hayton WL, Yost GS (1991). Chronic ethanol consumption causes increased glucuronidation of morphine in rabbits. *Xenobiotica* 21(4):515–524. [PubMed: 1910230]
- Nishimura M, Naito S (2006). Tissue-specific mRNA expression profiles of human phase I metabolizing enzymes except for cytochrome P450 and phase II metabolizing enzymes. *Drug Metab Pharmacokinet* 21(5):357–374. [PubMed: 17072089]
- Nozawa M, Ohnuma T, Matsubara Y, Sakai Y, Hatano T, Hanzawa R, et al. (2008). The relationship between the response of clinical symptoms and plasma olanzapine concentration, based on pharmacogenetics: Juntendo University Schizophrenia Projects (JUSP). *Ther Drug Monit* 30(1): 35–40. [PubMed: 18223460]
- Ochs HR, Greenblatt DJ, Knuchel M (1985). Kinetics of diazepam, midazolam, and lorazepam in cigarette smokers. *Chest* 87(2):223–226. [PubMed: 3155675]
- Ochs HR, Greenblatt DJ, Otten H (1981). Disposition of oxazepam in relation to age, sex, and cigarette smoking. *Klin Wochenschr* 59(16):899–903. [PubMed: 7278081]
- Ohno S, Nakajin S (2009). Determination of mRNA expression of human UDP-glucuronosyltransferases and application for localization in various human tissues by real-time reverse transcriptase-polymerase chain reaction. *Drug Metab Dispos* 37(1):32–40. [PubMed: 18838504]
- Pacifici GM, Rane A (1982). Distribution of UDP-glucuronyltransferase in different human foetal tissues. *Br J Clin Pharmacol* 13(5):732–735. [PubMed: 6805493]
- Pacifici GM, Sawe J, Kager L, Rane A (1982). Morphine glucuronidation in human fetal and adult liver. *Eur J Clin Pharmacol* 22(6):553–558. [PubMed: 6813127]
- Parkinson A, Mudra DR, Johnson C, Dwyer A, Carroll KM (2004). The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes. *Toxicol Appl Pharmacol* 199(3):193–209. [PubMed: 15364537]
- Peterkin VC, Bauman JN, Goosen TC, Menning L, Man MZ, Paulauskis JD, et al. (2007). Limited influence of UGT1A1*28 and no effect of UGT2B7*2 polymorphisms on UGT1A1 or UGT2B7 activities and protein expression in human liver microsomes. *Br J Clin Pharmacol* 64(4):458–468. [PubMed: 17555467]
- Pillot T, Ouzzine M, Fournel-Gigleux S, Lafaurie C, Radominska A, Burchell B, et al. (1993). Glucuronidation of hyodeoxycholic acid in human liver. Evidence for a selective role of UDP-glucuronosyltransferase 2B4. *J Biol Chem* 268(34):25636–25642. [PubMed: 8244999]
- Ramchandani RP, Wang Y, Booth BP, Ibrahim A, Johnson JR, Rahman A, et al. (2007). The role of SN-38 exposure, UGT1A1*28 polymorphism, and baseline bilirubin level in predicting severe irinotecan toxicity. *J Clin Pharmacol* 47(1):78–86. [PubMed: 17192505]
- Ramirez J, Liu W, Mirkov S, Desai AA, Chen P, Das S, et al. (2007). Lack of association between common polymorphisms in UGT1A9 and gene expression and activity. *Drug Metab Dispos* 35(12):2149–2153. [PubMed: 17761781]
- Rane A, Sjoqvist F, Orrenius S (1973). Drugs and fetal metabolism. *Clin Pharmacol Ther* 14(4):666–672. [PubMed: 4719974]
- Rollins DE, von Bahr C, Glaumann H, Moldeus P, Rane A (1979). Acetaminophen: potentially toxic metabolite formed by human fetal and adult liver microsomes and isolated fetal liver cells. *Science* 205(4413):1414–1416. [PubMed: 38505]
- Scavone JM, Greenblatt DJ, LeDuc BW, Blyden GT, Luna BG, Harmatz JS (1990). Differential effect of cigarette smoking on antipyrine oxidation versus acetaminophen conjugation. *Pharmacology* 40(2):77–84. [PubMed: 2345775]
- Strassburg CP, Strassburg A, Kneip S, Barut A, Tukey RH, Rodeck B, et al. (2002). Developmental aspects of human hepatic drug glucuronidation in young children and adults. *Gut* 50(2):259–265. [PubMed: 11788570]

- Tankanitlert J, Morales NP, Howard TA, Fucharoen P, Ware RE, Fucharoen S, et al. (2007). Effects of combined UDP-glucuronosyltransferase (UGT) 1A1*28 and 1A6*2 on paracetamol pharmacokinetics in beta-thalassemia/HbE. *Pharmacology* 79(2):97–103. [PubMed: 17164591]
- Turgeon D, Carrier JS, Levesque E, Hum DW, Belanger A (2001). Relative enzymatic activity, protein stability, and tissue distribution of human steroid-metabolizing UGT2B subfamily members. *Endocrinology* 142(2):778–787. [PubMed: 11159850]
- van Oijen MG, Barthelemy C, Janssen MJ, Joiris E, Peters WH, Laheij RJ, et al. (2009). Effect of genetic polymorphisms in UDP-glucuronosyltransferase 1A6 (UGT1A6) on acetylsalicylic acid metabolism in healthy female volunteers. *Pharmacology* 83(4):237–242. [PubMed: 19262071]
- Villeneuve JP, Raymond G, Bruneau J, Colpron L, Pomier-Layrargues G (1983). [Pharmacokinetics and metabolism of acetaminophen in normal, alcoholic and cirrhotic subjects]. *Gastroenterol Clin Biol* 7(11):898–902. [PubMed: 6653975]
- Waxman DJ, Holloway MG (2009). Centennial Perspective: Sex Differences in the Expression of Hepatic Drug Metabolizing Enzymes. *Mol Pharmacol*.
- Wiener D, Fang JL, Dossett N, Lazarus P (2004). Correlation between UDP-glucuronosyltransferase genotypes and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone glucuronidation phenotype in human liver microsomes. *Cancer Res* 64(3):1190–1196. [PubMed: 14871856]
- Yamanaka H, Nakajima M, Katoh M, Hara Y, Tachibana O, Yamashita J, et al. (2004). A novel polymorphism in the promoter region of human UGT1A9 gene (UGT1A9*22) and its effects on the transcriptional activity. *Pharmacogenetics* 14(5):329–332. [PubMed: 15115919]
- Yue QY, Svensson JO, Alm C, Sjoqvist F, Sawe J (1989). Interindividual and interethnic differences in the demethylation and glucuronidation of codeine. *Br J Clin Pharmacol* 28(6):629–637. [PubMed: 2611085]
- Yue QY, Tomson T, Sawe J (1994). Carbamazepine and cigarette smoking induce differentially the metabolism of codeine in man. *Pharmacogenetics* 4(4):193–198. [PubMed: 7987403]
- Zaya MJ, Hines RN, Stevens JC (2006). Epirubicin glucuronidation and UGT2B7 developmental expression. *Drug Metab Dispos* 34(12):2097–2101. [PubMed: 16985101]

Variability of UGT probe activities in a human liver bank

Figure 1.

Liver quality evaluation through assessment of systematic bias (as reflected by Z-scores) in glucuronidation activities measured in individual livers within a human liver bank (n=55). Glucuronidation activities measured in our laboratory for the various probe substrates in these livers have been previously reported (Court, 2005; Court et al., 2004; Court et al., 2003; Court et al., 1997; Girard et al., 2004; Girard et al., 2005; He et al., 2005; Krishnaswamy et al., 2005a). For each activity, a Z-score was derived by subtracting the mean value for all livers from the value for an individual liver and dividing by the standard deviation for all livers. A mean Z-score of all 6 glucuronidation activities is also shown for each liver. An arbitrary cut-off of one standard deviation unit below the mean (average Z-score less than -1) was used in the described studies to exclude livers with consistently low activities possibly as a result of poor collection or storage conditions. In this instance, liver #19 was identified as such a liver (denoted by an arrow). Note that 7 individuals also have Z-scores greater than +1 possibly resulting from exposure to enzyme inducers that enhance expression of multiple enzymes. E-3-UGT: estradiol-3-glucuronidation, TFP-UGT: trifluoperazine glucuronidation, 5HT-UGT: serotonin glucuronidation, Propofol-UGT: propofol glucuronidation, AZT-UGT: zidovudine glucuronidation, S-oxazepam-UGT: S-oxazepam glucuronidation.

Variability of UGT and CYP probe activities in a human liver bank

Figure 2.

Variability in glucuronidation of probes specific for UGT1A1, 1A4, 1A6, 1A9, 2B7 and 2B15 measured in the same bank of human liver microsomes. Also shown are activities for midazolam 1'-hydroxylation and chlorzoxazone 6-hydroxylation, as probes for CYP3A and CYP2E1. Data for each activity are given relative to the mean activity for all livers. Also shown are the coefficients of variation (CV%) for each activity. Source of data is given in the legend to Figure 1.

Figure 3.
Relationship between liver donor age and microsomal epirubicin glucuronidation activity and UGT2B7 content measured in a human liver bank. From (Zaya et al., 2006).

Figure 4.

Relationship between donor age and UGT probe activities measured in a human liver bank. Livers were grouped by age into livers from children (2 – 20 years old; $n = 9$), young adults (21–40 years; $n = 18$), middle age adults (41 – 60 years; $n = 14$) and older individuals (61 years and older; $n = 13$). Group differences were analyzed by Kruskal-Wallis ANOVA on ranks with Dunn's test adjusted for multiple comparisons. * $P < 0.05$ versus other age groups. Source of data is given in the legend to Figure 1.

Figure 5. Relationship between donor sex and UGT probe activities measured in a human liver bank. Livers were grouped by donor sex and differences were analyzed by Mann-Whitney rank sum test. * $P < 0.05$ versus other sex for same activity. Source of data is given in the legend to Figure 1.

Figure 6.

Relationship between donor history of smoking at least ½ pack of cigarettes per day and UGT probe activities measured in a human liver bank. Livers were grouped by smoking history (smoker or non-smoker) and differences were analyzed by Mann-Whitney rank sum test. * $P < 0.05$ versus other non-smoker for the same activity. Source of data is given in the legend to Figure 1.

Figure 7. Relationship between alcohol consumption history (consuming more than 2 alcoholic beverages per day) and UGT probe activities measured in a human liver bank. Livers were grouped by alcohol use history and differences were analyzed by Mann-Whitney rank sum test. * $P < 0.05$ versus individuals consuming 2 or less drinks per day for same activity. Source of data is given in the legend to Figure 1.

Figure 8. The effects of UGT polymorphism on the *in vitro* glucuronidation of probe substrates in a human liver bank can be recapitulated by *in vivo* pharmacokinetic studies in human subjects. The UGT2B15*2 (D85Y) polymorphism results in a gene dose dependent decrease in S-oxazepam glucuronidation (normalized to R-oxazepam glucuronidation) in male human liver bank samples (panel A; (Court et al., 2004)) and oxazepam clearance in a group of healthy male volunteers (panel B; He et al, in press, British Journal of Clinical Pharmacology, 2009). Similarly, UGT2B7*1c (c.735A>G) is associated with higher zidovudine (AZT) glucuronidation in human liver bank samples (panel A) and higher zidovudine apparent oral clearance in a cohort of HIV-infected patients being treated with this drug (panel B) (Kwara et al, in press, Journal of Clinical Pharmacology, 2009).

Table 1

Demographics of liver donors

Donor identity	Age	Gender	Ethnicity ^a	Cause of death	Smoking	Alcohol ^c	Other drug exposure ^d
LV 01	40	M	C	Head trauma			Dopamine
LV 02	8	F	A	Head trauma	No	No	
LV 03	5	M	C	Meningitis	No	No	Dopamine, penicillin
LV 04	24	F	C	Auto accident			Dopamine
LV 05	20	M	C	Head trauma	Yes	No	Nafcillin, mannitol, furosemide, erythromycin, nystatin, neomycin
LV 06	35	F	C	Head trauma	No	No	None
LV 07	21	M	A	Head trauma	Yes	Yes	DDAVP
LV 08	26	F	A	Head trauma	Yes	No	None
LV 09	17	M	C	Head trauma	Yes	No	None
LV 10	19	F	C	Head trauma	No	No	Dopamine
LV 11	46	F	A	Stroke	Yes	No	Diazide, dopamine
LV 12	36	M	C	Head trauma	No	No	Labetalol, morphine
LV 13	16	M	C	Head trauma	No	No	Dopamine, norepinephrine, DDAVP, methylprednisolone, mannitol
LV 14	14	M	C	Head trauma	Yes	No	Dopamine, mannitol, ranitidine, erythromycin, norcuronium, furosemide
LV 15	35	M	C	Auto accident	Yes	No	Dopamine, mannitol, insulin, dexamethasone
LV 16	45	F	H	Stroke	No	No	Dopamine
LV 17	6	M	C	Auto accident	No	No	Dopamine
LV 18	2	F	C	Cerebral anoxia	No	No	Dopamine, atropine, epinephrine
LV 20	61	M	C	Colon cancer ^b			
LV 21	74	M	C	Bladder cancer ^b	Yes		
LV 22	68	M	C	Colon cancer ^b			
LV 23	74	M	C	Colon cancer ^b			
LV 24	66	M	C	Hepatoma ^b			
LV 25	49	F	C	Stroke	Yes	No	Diazepam, furosemide
LV 26	52	F	C	Stroke	No	Yes	Nifedipine, dopamine, nitroprusside, vasopressin
LV 27	40	M	C	Head trauma	No	Yes	Dopamine, insulin
LV 28	58	M	C	Stroke	No	No	Dopamine, ranitidine, dexamethasone, mannitol, cefazolin
LV 29	22	F	H	Cardiac arrest	No	No	Phenobarbital, carbamazepine, valproate
LV 30	40	F	C	Head trauma	Yes	Yes	Mannitol, furosemide, phentolamine
LV 31	66	M	C	Stroke	No	Yes	Mannitol, cefotaxime, dopamine, cefazolin, furosemide
LV 32	45	F	C	Stroke	Yes	Yes	Prednisone, synthroid, phenytoin, naproxen, diazepam
LV 33	42	M	C	Gun shot wound	Yes	Yes	Dopamine, vasopressin, mannitol, cefazolin, methylprednisolone
LV 34	33	M	C	Stroke	No	No	Dopamine

Donor identity	Age	Gender	Ethnicity ^a	Cause of death	Smoking	Alcohol ^c	Other drug exposure ^d
LV 35	65	F	C	Stroke	No	No	Thyroid drug
LV 36	43	M	C	Stroke	No	No	Metoprolol, dexamethasone, hydrocortisol
LV 37	32	M	C	Head trauma	No	No	None
LV 38	65	F	C	Stroke	Yes	No	Diltiazem, lisinopril, dopamine, acetaminophen
LV 39	36	M	C	Head trauma	Yes	Yes	Cefazolin, norcuronium, phytonadione, enalapril, metoclopramide
LV 40	34	M	C	Gun shot wound	Yes	No	Crack cocaine, dopamine, norcuronium, fentanyl, midazolam
LV 41	43	M	C	Head trauma	No	Yes	Dopamine, furosemide, neosynephrine, acetaminophen
LV 42	35	M	C	Encephalopathy	No	No	Dopamine, norepinephrine, naloxone
LV 43	24	M	C	Blunt trauma	No	No	
LV 44	43	M	C	Head trauma	No	Yes	Diazepam, dopamine, epinephrine, pancuronium
LV 45	75	F	C	Stroke	No	No	Dopamine, vasopressin, furosemide, mannitol, vecuronium
LV 46	49	M	C	Stroke	Yes	Yes	Occasional crack cocaine and marijuana
LV 47	72	M	C	Stroke	No	No	Aspirin, amiodarone, bisoprolol, hydrochlorothiazide, glipizide, metformin
LV 48	68	F	C	Stroke	No	No	Synthroid, celecoxib, fluoxetine, atenolol, omeprazole
LV 49	21	M	C	Head trauma	Yes	No	Crack cocaine, marijuana
LV 50	37	M	C	Head trauma	Yes	Yes	Marijuana
LV 51	62	M	C	Stroke	No	No	Dopamine, norepinephrine, insulin, DDAVP, cefazolin
LV 52	53	M	C	Stroke	Yes	No	Triamterene, amlodipine, doxazosin, loratadine, doxepin
LV 53	54	M	C	Brain abscess	No	No	Simvastatin, acetaminophen, alprazolam, flurazepam, venlafaxine
LV 54	63	M	C	Stroke	No	Yes	Dopamine, norepinephrine, nitroprusside
LV 55	46	M	C	Stroke	No	No	

^aC, Caucasian; A, African-American; H, Hispanic.

^bApparently normal liver tissue obtained by surgical biopsy during exploratory laparotomy.

^cHistory of consumption of more than 14 drinks per week.

^dDDAVP, desmopressin acetate.

Table 2.

Relative abundance of UGT mRNA expression in human liver. Shown are the results of 4 systematic studies of UGT isoform-specific mRNA expression in human liver presented as relative abundance (“++++” - highest; “++” - high to medium; “+” - medium to low; “-” - lowest; “tr” - trace level at limit of assay detection, “-” - not detected) based on quantitative real-time PCR assay (Congiu et al., 2002; Nakamura et al., 2008; Nishimura and Naito, 2006) or subjective intensity of RT-PCR bands (Ohno and Nakajin, 2009). Also shown is the average abundance of each UGT isoform in liver derived from averaging results from the 4 studies.

UGT isoform	Relative abundance of UGT mRNA in human liver				Average across studies
	(Congiu et al., 2002) ^a	(Nishimura and Naito, 2006) ^b	(Nakamura et al., 2008) ^c	(Ohno and Nakajin, 2009) ^d	
UGT1A1	+	+++	++	+++	++
UGT1A3	+++		+	+	++
UGT1A4	+++		+++	++	+++
UGT1A5			tr	tr	tr
UGT1A6	++	++	+	++	++
UGT1A7			-	tr	tr/-
UGT1A8			-	-	-
UGT1A9	+		+++	+++	++
UGT1A10			tr	-	tr/-
UGT2B4	++++		++++	++++	++++
UGT2B7	++	++++	++	+++	+++
UGT2B10	++	+++	+	+++	++
UGT2B11	+++		+	-	+
UGT2B15	+++	++	++	++++	+++
UGT2B17	++	+	+	+	+
UGT2B28			tr		tr
UGT2A1					?
UGT2A2					?
UGT2A3					?

^a Liver tissue biopsies (n = 10 to 12 per UGT assayed) collected from patients at St. Vincent's Hospital, Melbourne, Australia.

^b Liver tissue (n = 9) supplied by the National Disease Research Interchange, Philadelphia, Pennsylvania, USA.

^c Liver total RNA (n = 1) supplied by Stratagene, La Jolla, California, USA.

^d Liver total RNA (n = 1) supplied by Ambion, Austin, Texas, USA. Blank - not assayed in that study. “?” - not assayed in any of the studies.

Table 3.

Results of human liver bank UGT genotype-phenotype association studies compared with cell-based *in vitro* assays and *in vivo* pharmacokinetic studies.

Enzyme	Polymorphism	Liver bank effect	Other <i>in vitro</i> effects	<i>In vivo</i> PK effect	References
UGT1A1	*28 (TA6>7)	Lower SN38 glucuronidation	Lower reporter gene expression	Higher SN-38 AUC	(Ramchandani et al., 2007) (Girard et al., 2005)
		Lower estradiol-3-glucuronidation and UGT1A1 protein content			(Girard et al., 2005)
		Lower estradiol-3-glucuronidation			(Peterkin et al., 2007)
UGT1A4	*2 (P24T)	Lower NNAL N-glucuronidation	Reduced enzyme activity	No effect on olanzapine concentrations	(Wiener et al., 2004) (Ehmer et al., 2004) (Nozawa et al., 2008)
	*3 (L48V)	No effect on trifluoperazine glucuronidation			(Benoit-Biancamano et al., in press) (Ehmer et al., 2004) (Nozawa et al., 2008)
UGT1A6	*2 (S7A, T181A, R184S)	Higher 4-nitrophenol glucuronidation	Reduced enzyme activity	No effect on deferiprone AUC	(Nagar et al., 2004) (Ciotti et al., 1997) (Limenta et al., 2008)
		No difference in serotonin glucuronidation or UGT1A6 protein			(Krishnaswamy et al., 2005a) (Nagar et al., 2004) (van Oijen et al., 2009)
			Increased enzyme activity	Lower salicylate AUC	(Krishnaswamy et al., 2005b) (Chen et al., 2007b)
				Lower acetaminophen AUC	(Tankanilt et al., 2007)
UGT1A9	*1b (T9>T10)	No effect on mycophenolate or propofol glucuronidation or UGT1A9 protein	Lower reporter gene expression	No effect on mycophenolate levels	(Girard et al., 2004) (Yamanaka et al., 2004) (Levesque et al., 2008)
		No effect on flavopiridol or mycophenolate glucuronidation			(Ramirez et al., 2007) (Girard et al., 2006)
	-2152c>t; -275t>a	Higher mycophenolate and propofol glucuronidation and UGT1A9 protein	Not determined	Lower mycophenolate AUC	(Girard et al., 2004) (Levesque et al., 2007)
		No effect on flavopiridol or mycophenolate glucuronidation			(Ramirez et al., 2007)

Enzyme	Polymorphism	Liver bank effect	Other <i>in vitro</i> effects	<i>In vivo</i> PK effect	References
UGT2B7	*2 (H268Y)	No effect on AZT or morphine glucuronidation or UGT2B7 protein	Reduced AZT glucuronidation; unchanged efavirenz glucuronidation	No effect on morphine or glucuronide AUC	(Court et al., 2003) (Belanger et al., 2009) (Holthe et al., 2003)
		No effect on AZT glucuronidation		No effect on morphine or glucuronide AUC	(Peterkin et al., 2007) (Duguay et al., 2004)
		Lower NNAL 0-glucuronidation		No effect on AZT oral clearance	(Wiener et al., 2004) (Kwara et al., 2009)
	*1c (c.735A>G)	Higher AZT glucuronidation and UGT2B7 protein	Not determined	Higher AZT oral clearance	(Kwara et al., 2009)
		Higher morphine glucuronidation			(Innocenti et al., 2008)
UGT2B10	*2 (D68Y)	Lower nicotine and cotinine glucuronidation	Lower activity enzyme	Not studied	(Chen et al., 2007a)
UGT2B15	*2 (D85Y)	Lower oxazepam glucuronidation	Minimal activity enzyme	Lower oxazepam oral clearance	(Court et al., 2004) (Court et al., 2002) (He et al, in press)
UGT2B17	*2 (del)	Lower NNAL 0-glucuronidation	Enzyme deletion assumed	Not studied	(Lazarus et al., 2005)