Interindividual variability in human drug glucuronidation

What we know and what we need to know

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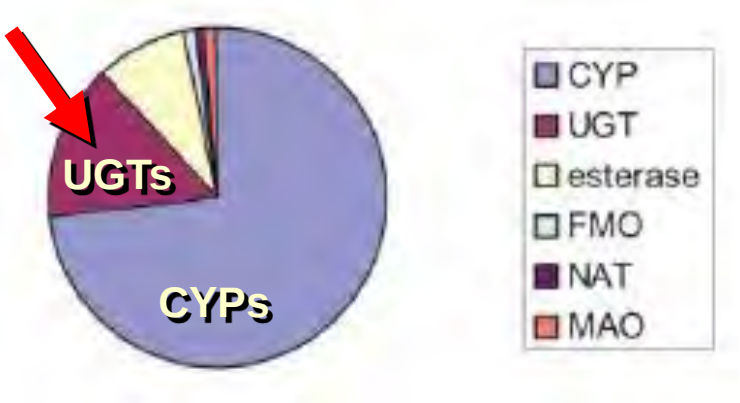
Why study drug glucuronidation?

- Main identified metabolic clearance pathway (after CYP) for top 200 prescribed drugs in the USA.
- May be more frequently encountered since selecting drug candidates for CYP stability

Enzymes known to be involved in the metabolism of the top 200 prescribed drugs in USA



(Williams *et al*: DMD, 32:1201-1208,2004.)

Advantages of glucuronidation over oxidation?

- Glucuronides are “stable” = non-reactive
  - Except some acylglucuronides
- Less potential for DDI
  - Typically low affinity (high Km) enzymes
  - Broad, overlapping substrate specificities
- Less interindividual variability in activity??

Objectives

- Brief overview of the UGTs
- Characteristics of the Tufts human liver bank
- What is the extent of interindividual variability in glucuronidation?
  - For different UGTs? Versus CYPs?
- What inherent factors determine UGT variability?
  - Gender; age; genetics; epigenetics
- What external factors influence UGT variability?
  - Smoking; alcohol; other drugs; disease

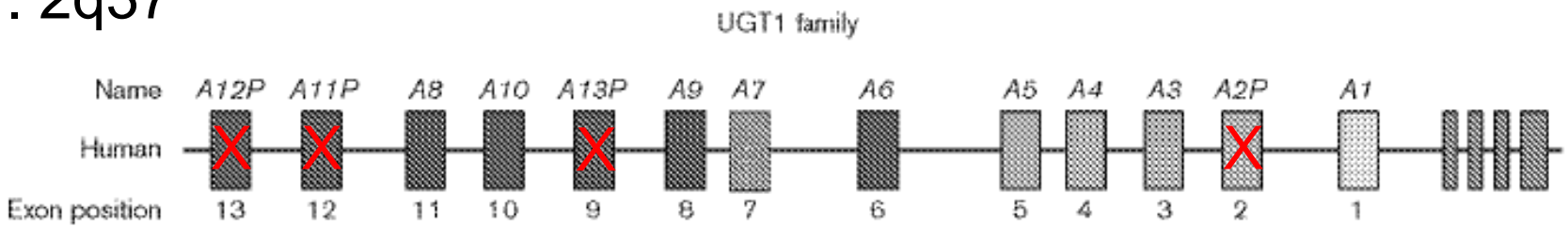
UDP-glucuronosyltransferases

Drug + UDP-glucuronic acid → Drug-glucuronide + UDP

- Substrates
  - Drug or Phase I metabolite (also hormones, toxins, etc)
  - -OH; -COOH; amino; (rarely -SH; -CH)
- Glucuronides generally inactive except:
  - Morphine-6-glucuronide
  - Acyl-glucuronides (esp. NSAIDs)
- 10 genes expressing 19 unique UGTs
  - Subfamilies UGT1A, 2A, 2B involved in drug metabolism
  - [Subfamily UGT3A - bile acid UDP-N-acetylglucosaminyltransferase]

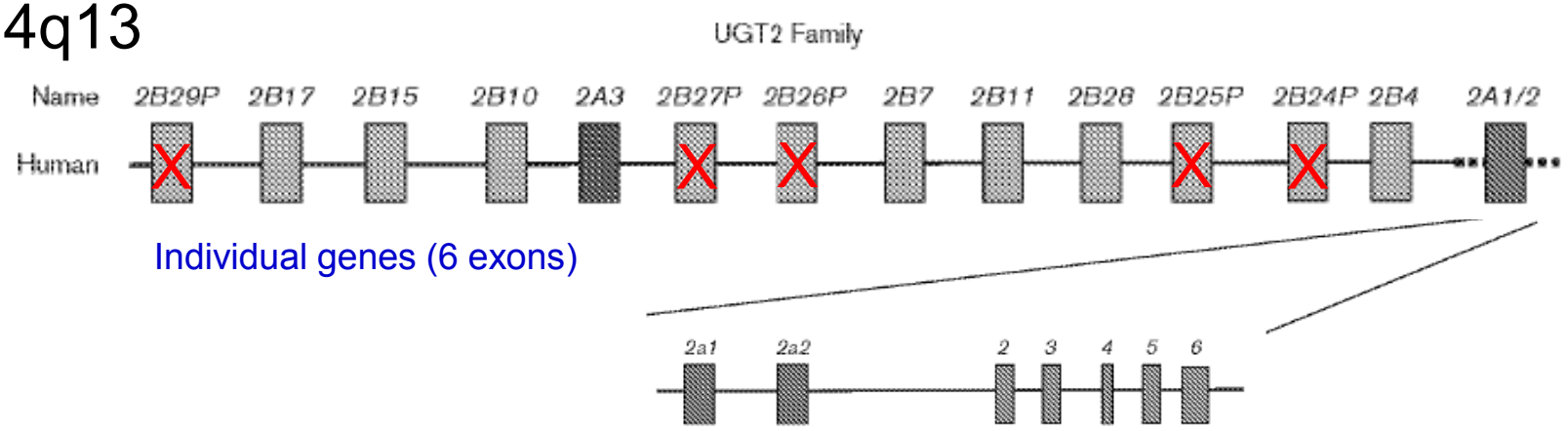
UGT gene structures

Chr. 2q37



Differential splicing of unique exon 1 to shared exons 2-5

Chr. 4q13

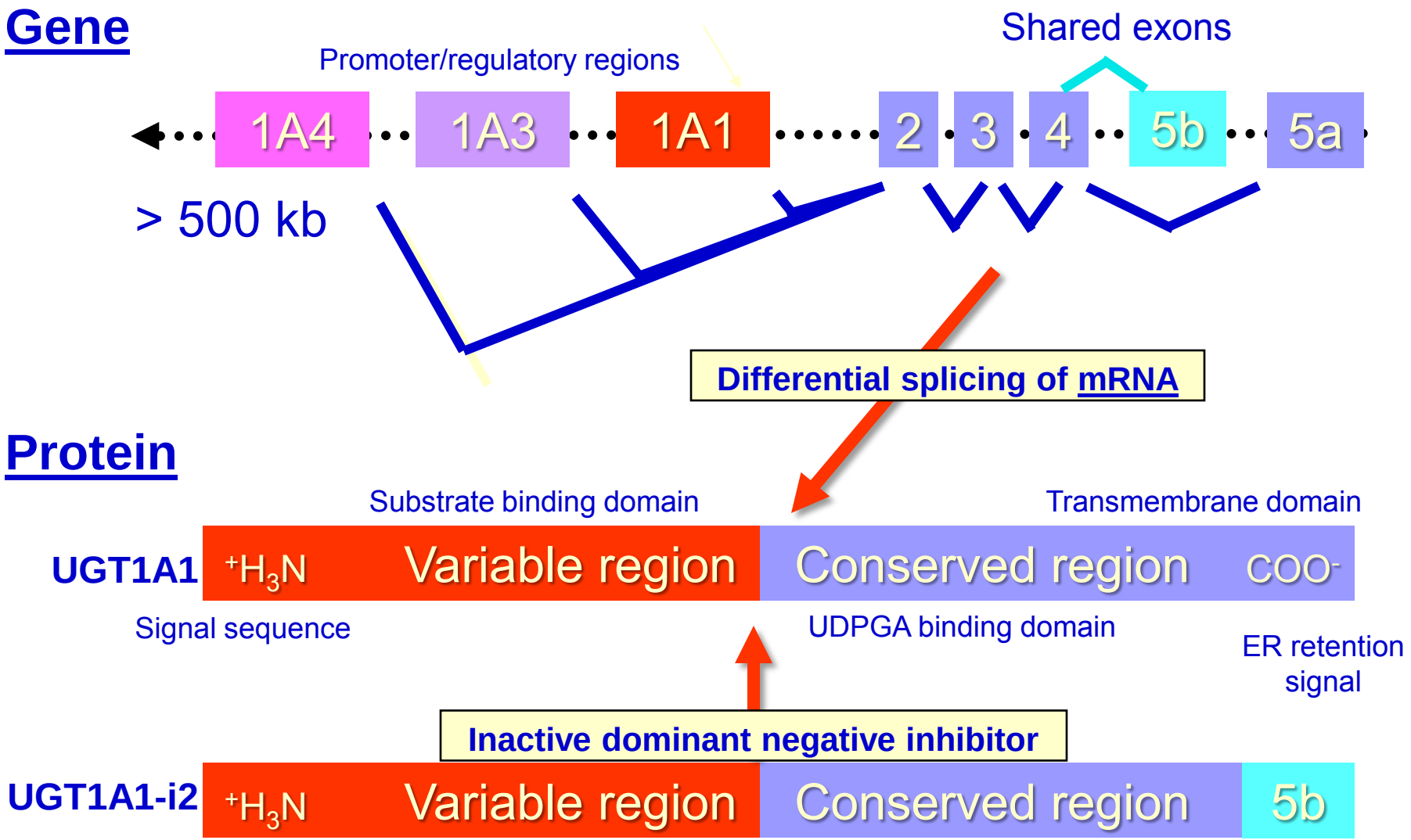


Individual genes (6 exons)

Unique exon 1 – shared exons 2-6

(Mackenzie *et al, PGEN J 2005)*

UGT1A gene – diversity through splicing



(Guillemette, Bellemare *et al, University of Laval, QC)*

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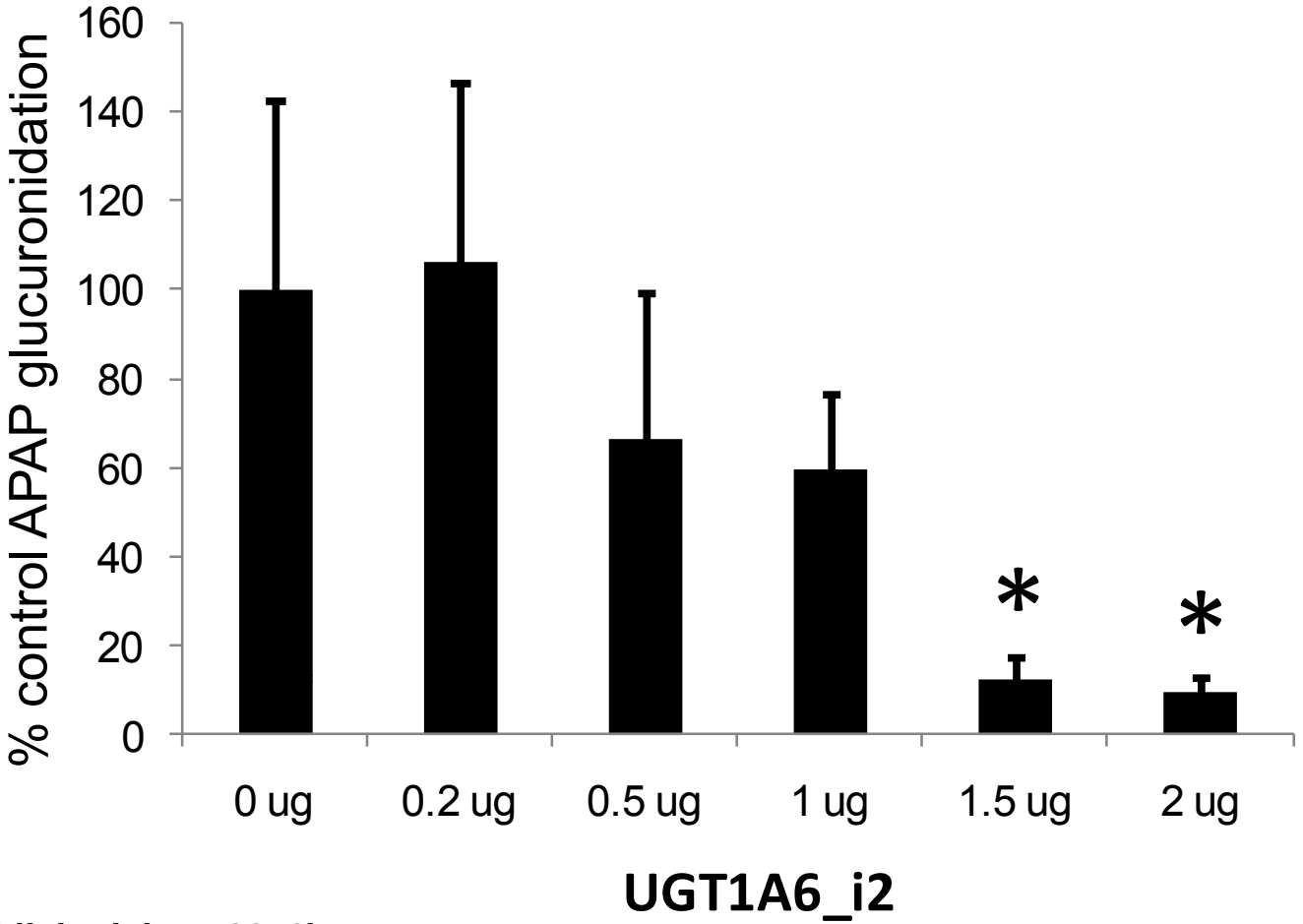
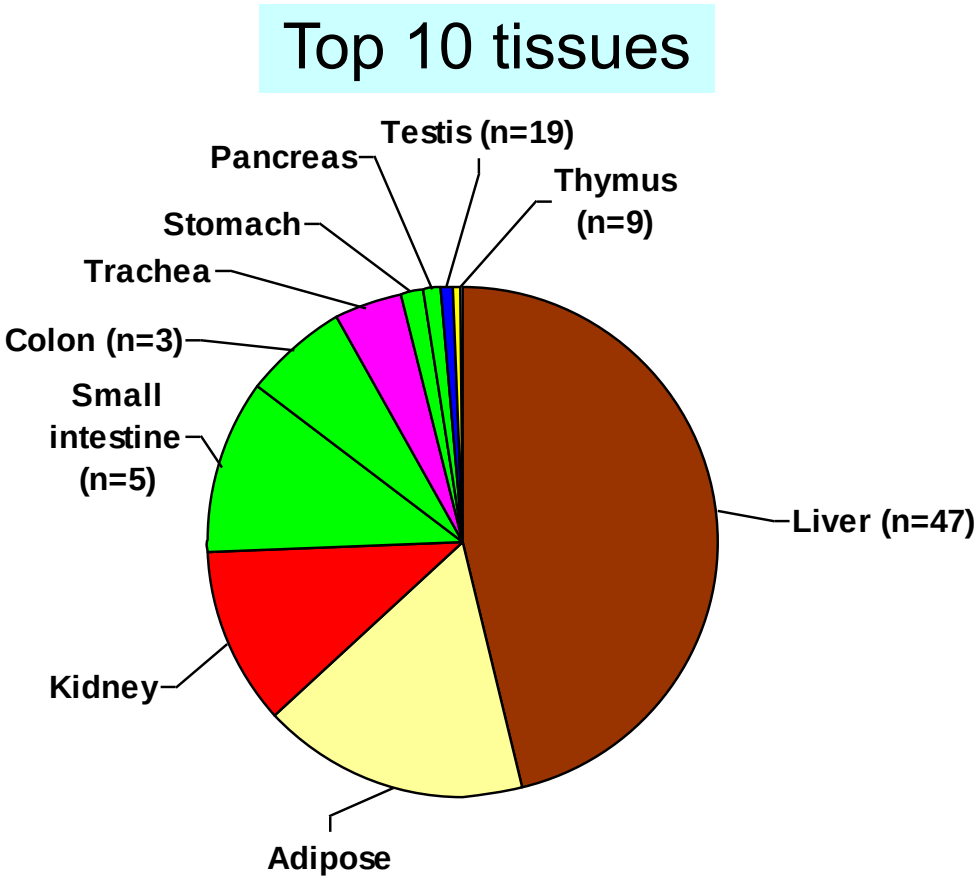
Effect of UGT1A6i2 on UGT1A6 activity



(Court, unpublished data, 2010)

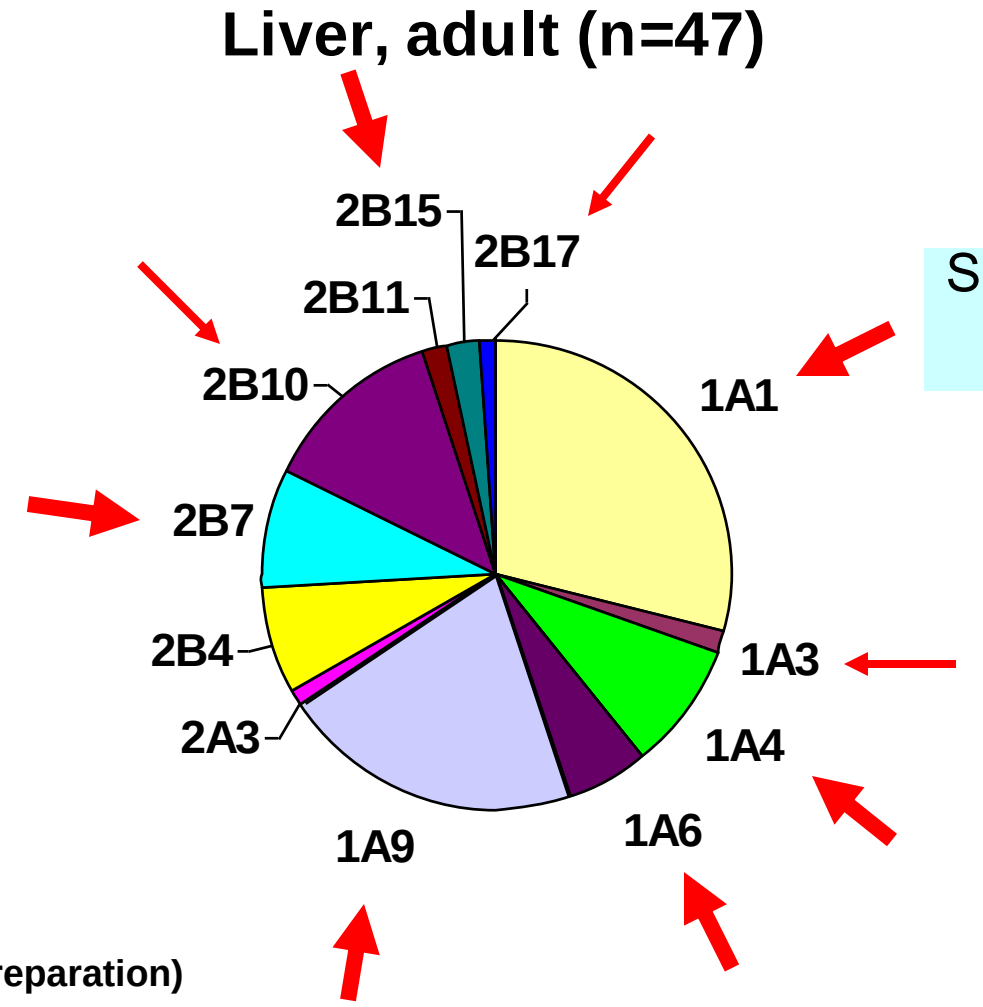
UGT mRNA expression in human tissues by QPCR

- Panel of 30 human tissues
- QPCR using UGT isoform-specific primer sets
  - All except UGT3A1, 3A2
  - Absolute quantitation using standard curve
- Summed all UGTs for each tissue and ranked top 10
  - Liver
  - Kidney
  - Adipose
  - GI: stomach, intestines, pancrea
  - Airway
  - Testis
  - Thymus



(Court *et al,* in preparation)

Most UGTs expressed in adult human liver glucuronidate drugs



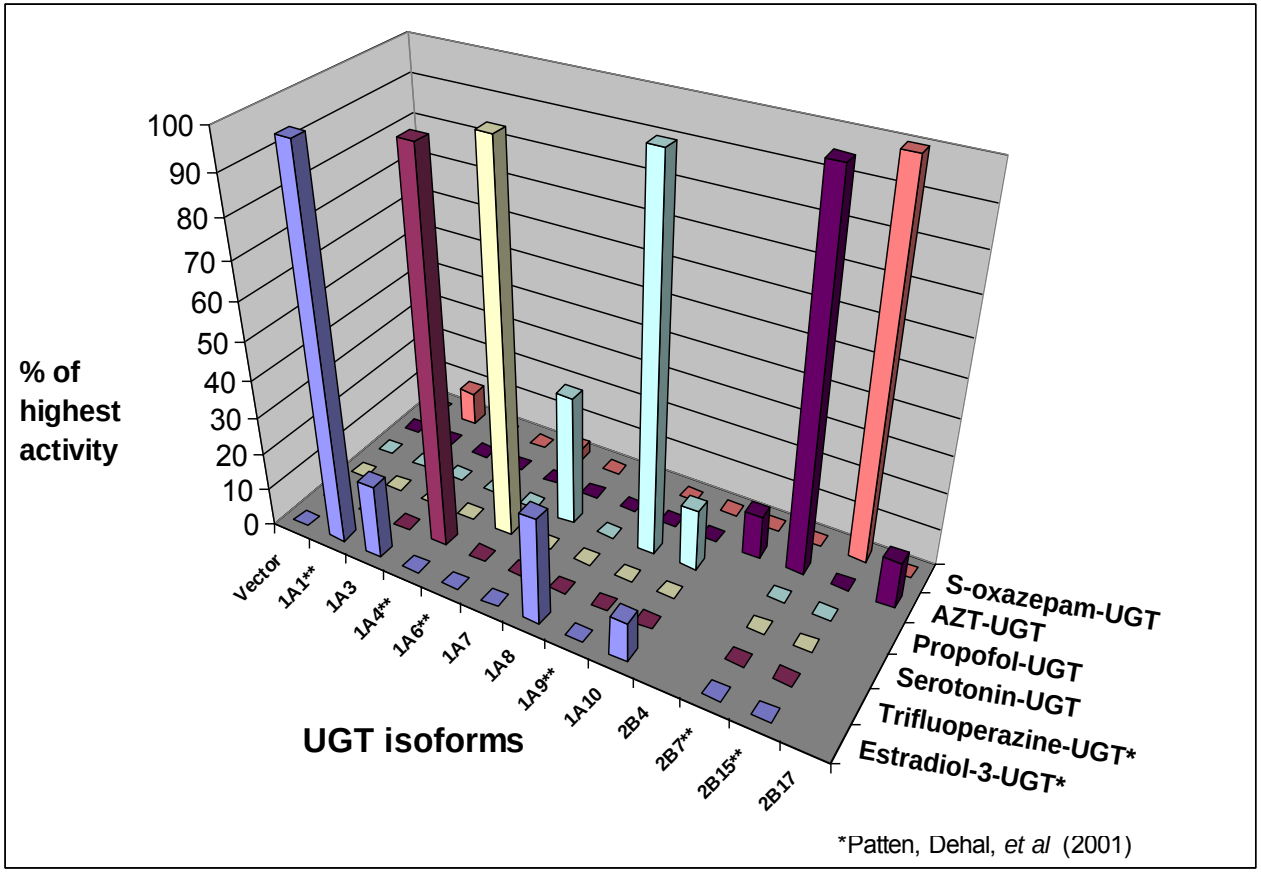
Significant activity for drugs

(Court *et al,* in preparation)

Major drug metabolizing UGTs in human liver

- UGT1A1
  - Bilirubin, estradiol, EE
  - Irinotecan -> SN38
  - “CYP2D6” of the UGTs
  - Gilbert’s, Crigler-Najjar
- UGT1A4
  - Quaternary N-glucuronides
    - Unique to primates and rabbits (?)
  - Antihistamines, tricyclic antidepressants, antipsychotics
- UGT1A6
  - Small, planar aromatics
  - Acetaminophen,valproate
  - Serotonin (5HT) –endogenous role?
- UGT1A9
  - Bulky phenols
  - Propofol, flavopiridol, salicylates, mycophenolic acid
- UGT2B7
  - “CYP3A4” of the UGTs
  - Retinoids, fatty acids, steroids
  - AZT, morphine, opioids, NSAIDS
- UGT2B15
  - Oxazepam, lorazepam, 4OH-tamoxifen, 5OH-rofecoxib
  - Androgens, bisphenol A

Identification of isoform-selective probes for measurement of glucuronidation by major UGTs



(Court: Methods Enzymol. 400:104-16, 2005)

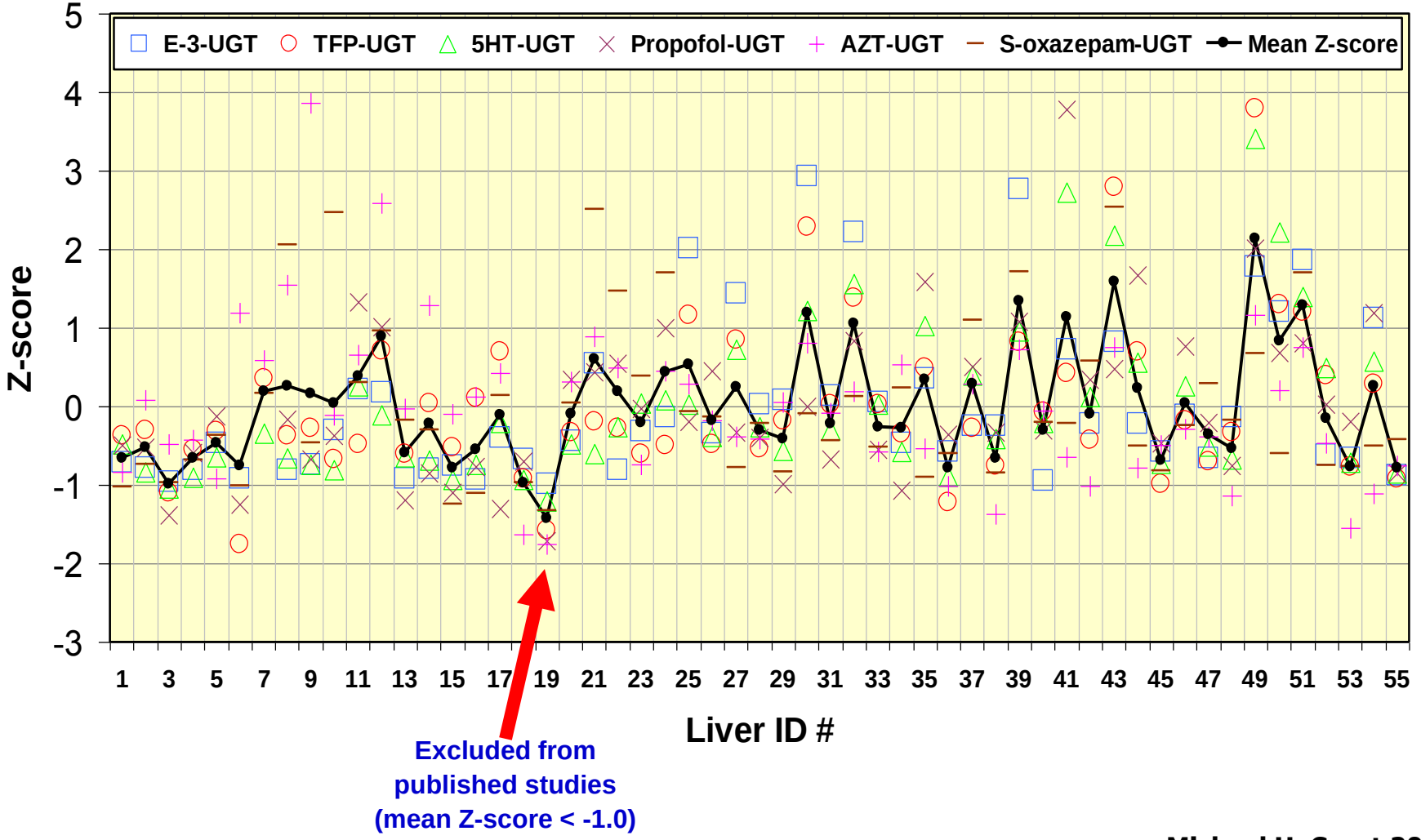
Tufts human liver bank (n=55)

- LTCDS (Liver Tissue Cell Distribution Service at U. Minnesota)
  - 50 transplant quality livers
  - Failed to match or leftover from pediatric transplant
  - Head trauma/gunshot/stroke
- NDRI (National Disease Research Interchange)
  - 5 adjacent healthy tissue in surgical patients
  - Liver cancer/metastases
- 1 gram to 500 grams
  - Microsomes on all; RNA and DNA on most
- 49 White non-Hispanic; 4 African-Americans; 2 White Hispanic
- 38 males; 17 females
- 2 – 80 years old; median 40 years
- Smoking/alcohol/prescription drug use

UGT activity trends for individual livers

Activities normalized by Z-score (SD units; 0 = mean)

(Court: Drug Metabolism Reviews, 2010)



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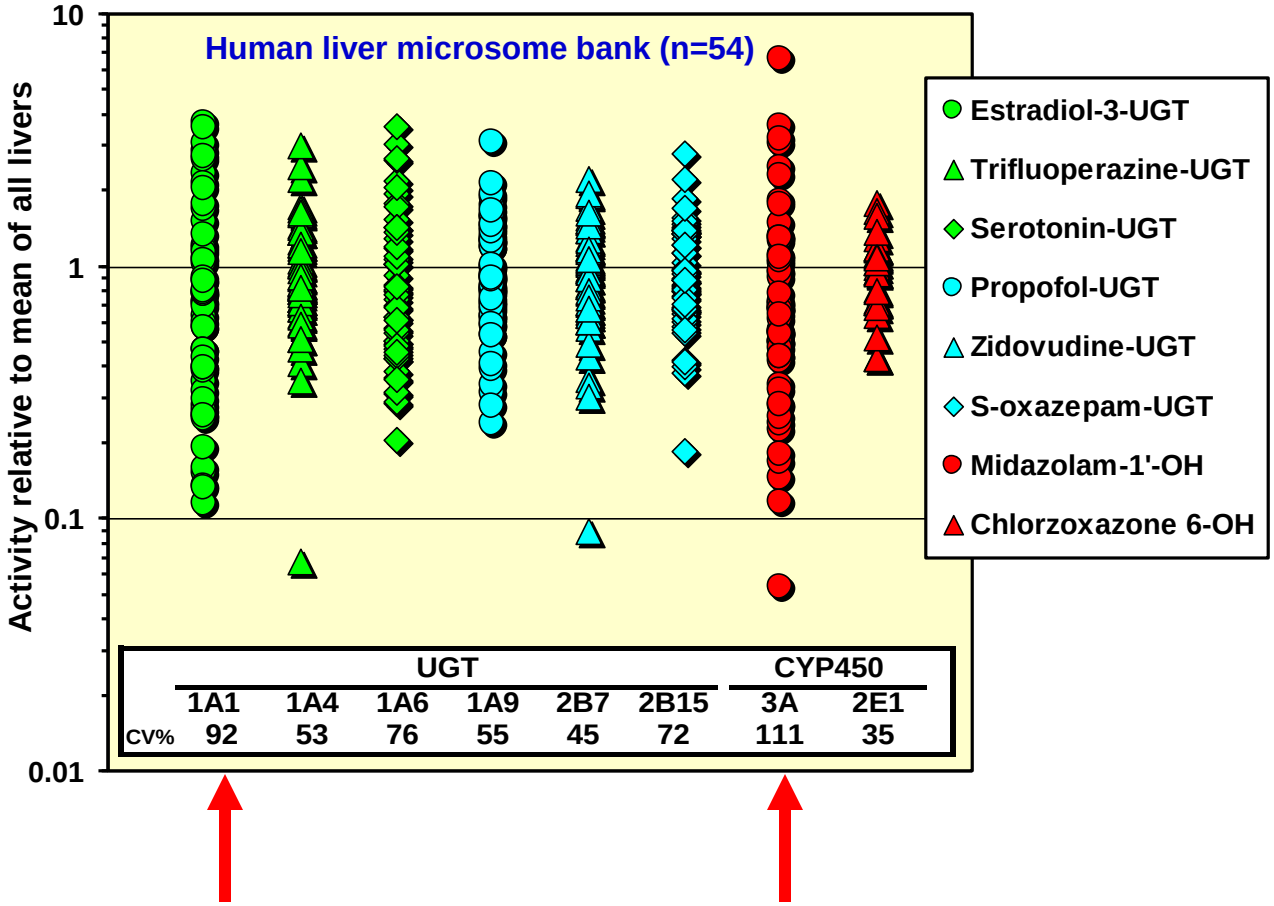
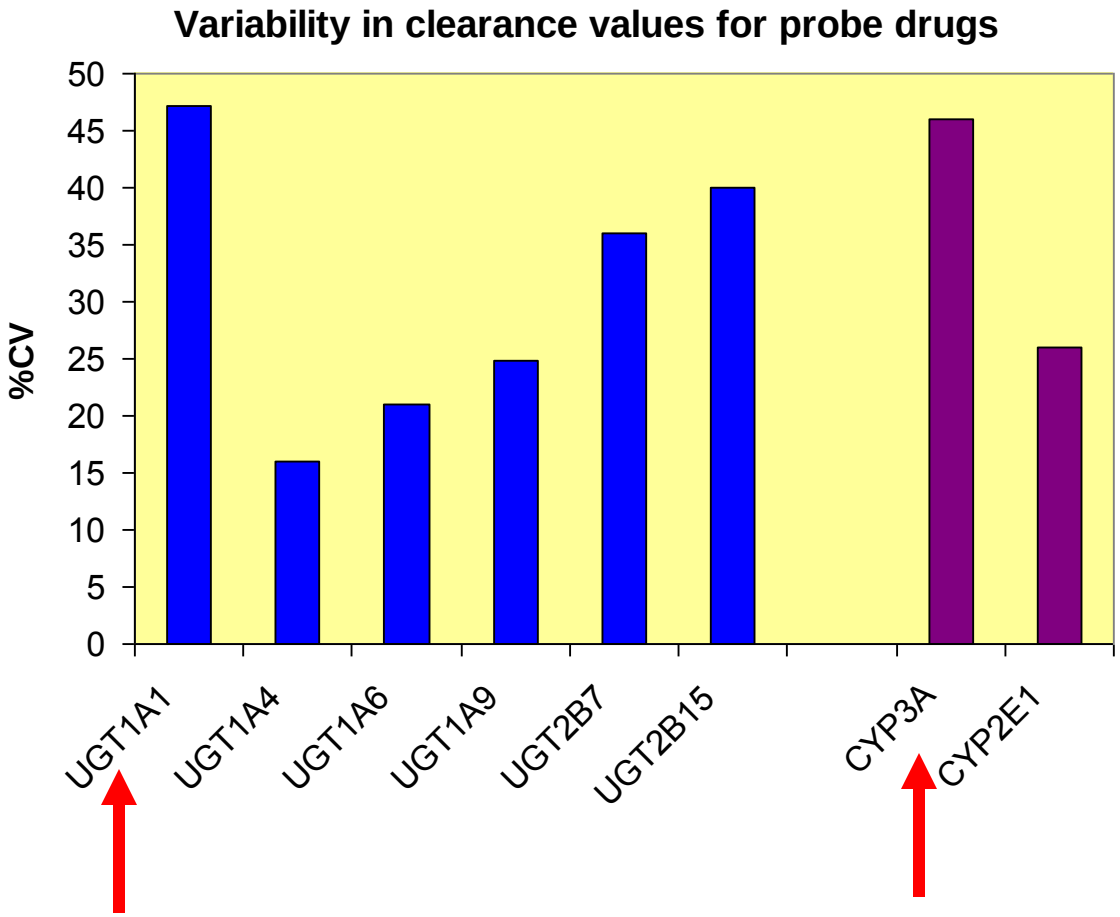
UGT1A1 shows highest UGT variability

Rivals CYP3A variability



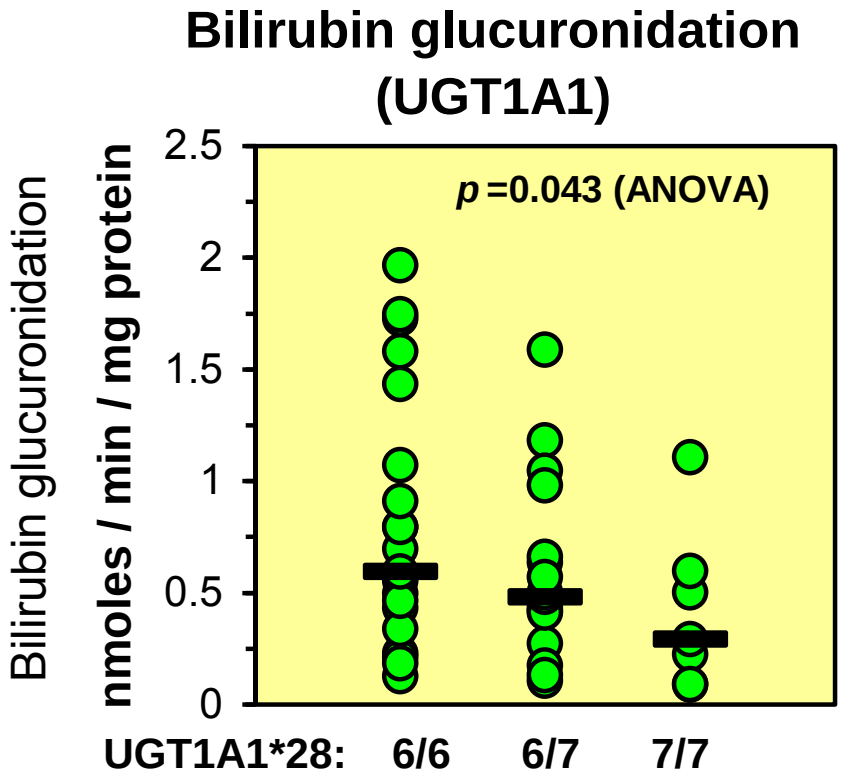
(Court: Drug Metabolism Reviews, 2010)

UGT1A1 and CYP3A show highest variability *in vivo*



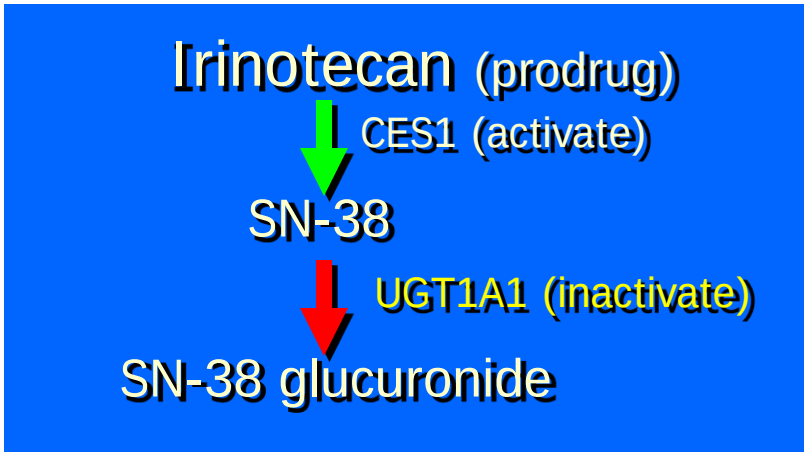
(Dorne, Walton, and Renwick, FoodChemTox, 2001, 2003, 2005 and FDA drug insert information)

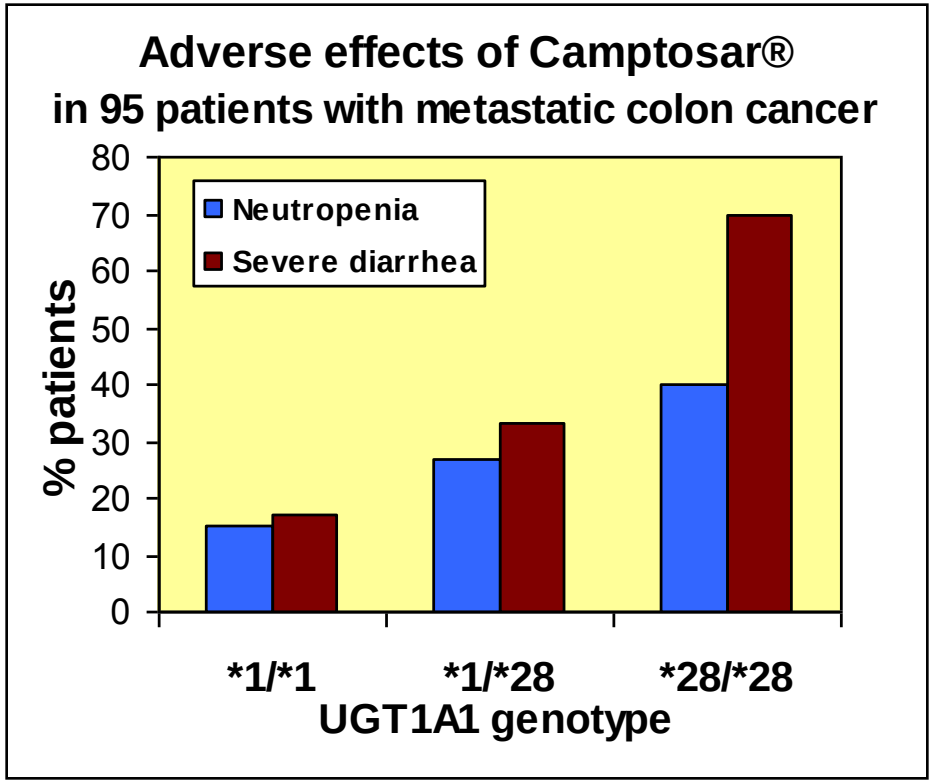
UGT1A1*28 is a major determinant of UGT1A1 variability



(Court *et al*, unpublished)

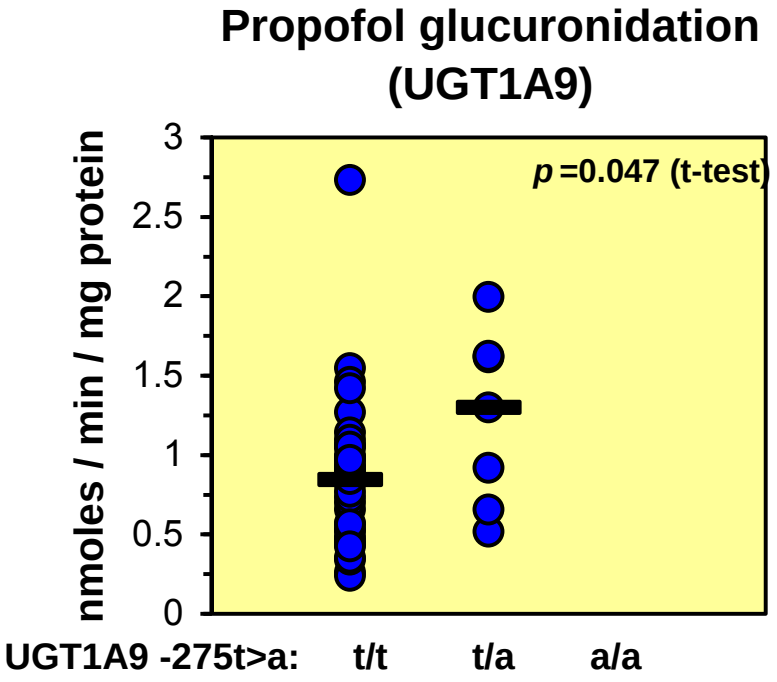
UGT1A1*28 predicts adverse effects of irinotecan





(Marcuello et al. *British J Cancer*, 2004)

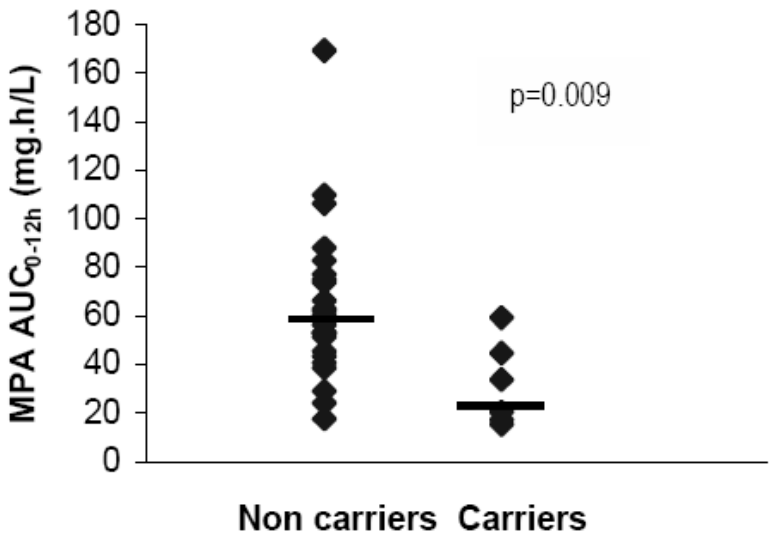
UGT1A9 -275t>a is associated with increased glucuronidation



(Girard *et al:* PGENJ, 2006)

UGT1A9 -275t>a is associated with lower MPA exposure and increased risk of renal transplant rejection

Lower mycophenolic acid levels



(Kuypers *et al:* CPT, 2005)

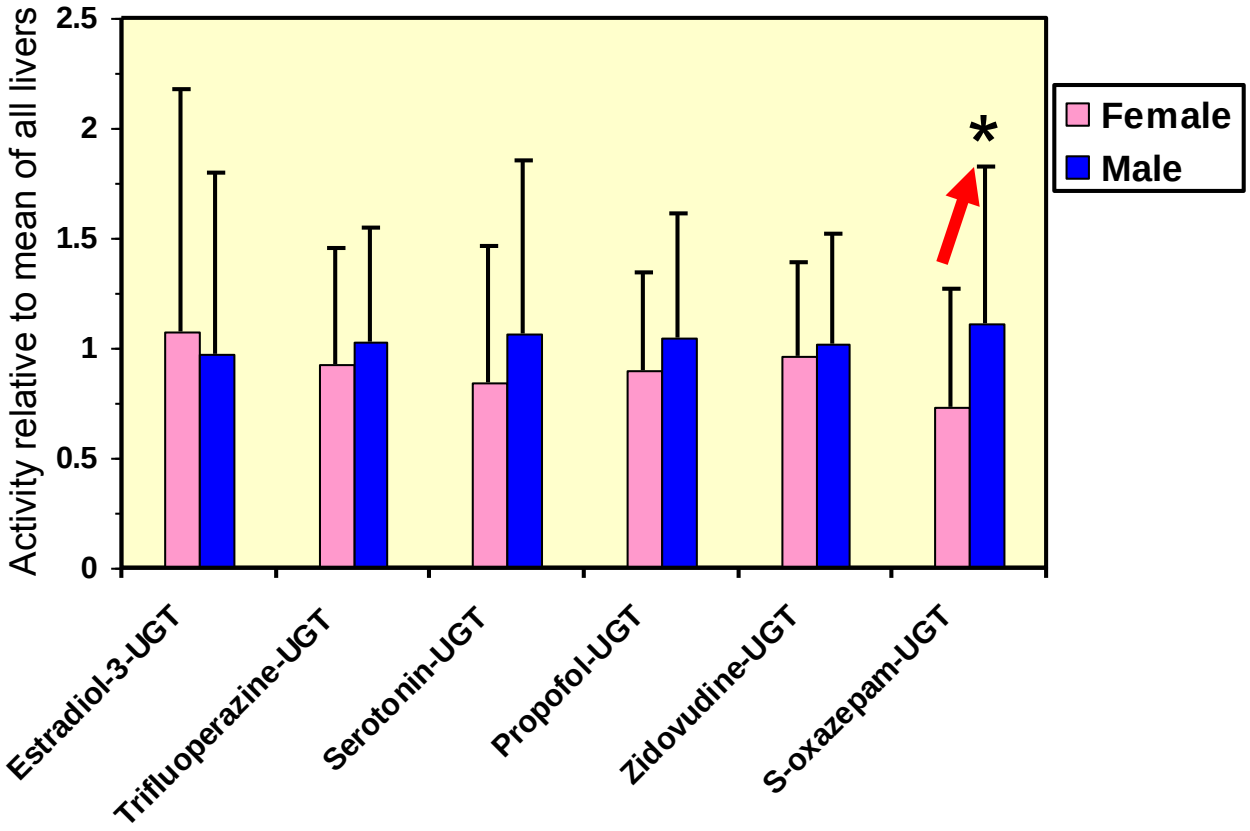
Higher transplant rejection risk

	95% Confidence interval			
	OR	Lower	Higher	*P* value
UGT1A9 -275T>A and/or -2152C>T carrier	13.337	1.096	162.279	0.042*
Number of transplantations	1.572	0.290	8.521	0.600
CYP3A5 expresser	1.152	0.175	7.576	0.883
Total number of HLA mismatches	1.135	0.725	1.778	0.579
Age	1.029	0.972	1.089	0.326
Tacrolimus trough levels	0.919	0.804	1.050	0.216
Female	0.466	0.117	1.816	0.280
Received induction therapy	0.387	0.088	1.695	0.207
Living donor	0.265	0.057	1.246	0.093
PRA <10%	0.000	0.000		0.999

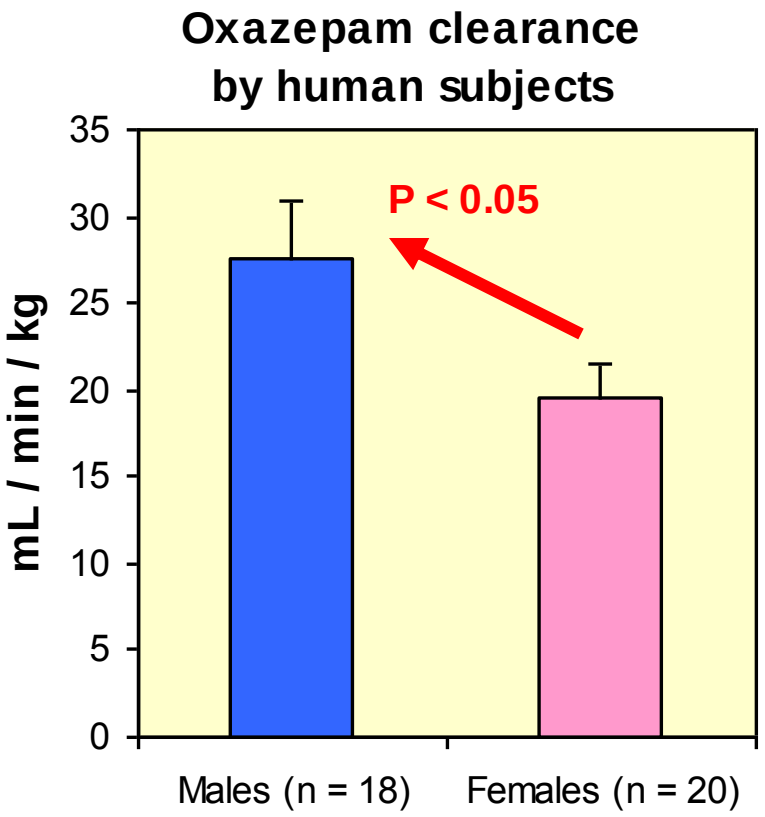
(van Schaik *et al:* CPT, 2009)

Effect of sex on UGTs

- Higher UGT2B15 activity in males
- No differences for all other isoforms



Oxazepam clearance is ~30% higher

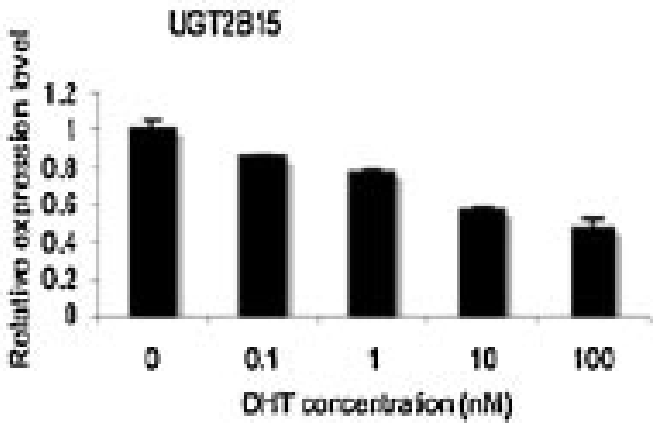


(Greenblatt *et al*: JPET, 1980)

UGT2B15 is regulated by sex steroids in cell lines

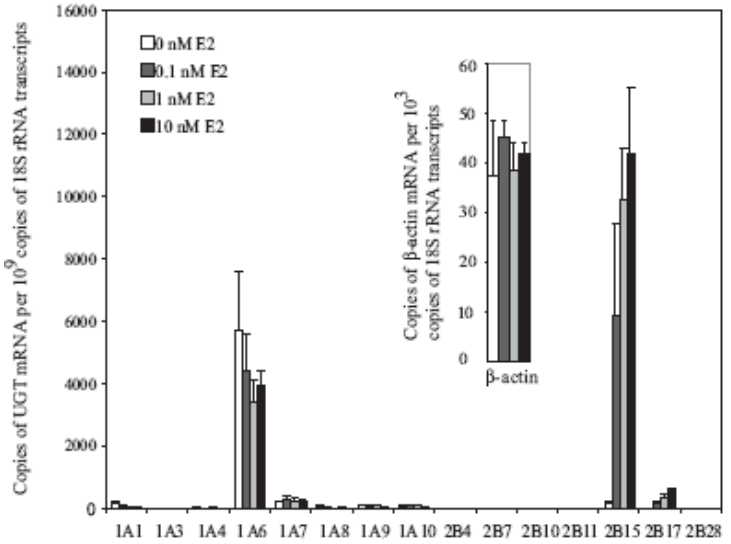
But effect is opposite to expected

LNCAP prostate cancer - ↓ by DHT?



(Bao et al: The Prostate, 2008)

MCF 7 breast cancer - ↑ by estradiol?



(Hu and Mackenzie: MolPharm, 2009)

UGT2B17 mRNA is higher in male livers

UGT2B15 – males?; UGT2B4 - females?

Gene	mRNA Expression Men (n=62) ($2^{-\Delta CT}$ Mean ± SE)*	mRNA Expression Women (n=41) ($2^{-\Delta CT}$ Mean ± SE)*	P-value**
?? UGT2B4	5.14 ± 0.24	9.90 ± 2.14	0.074
UGT2B7	1.16 ± 0.05	1.14 ± 0.07	0.866
UGT2B10	2.28 ± 0.11	2.68 ± 0.26	0.573
UGT2B11	0.014± 0.001	0.037 ± 0.021	0.339
?? UGT2B15	3.71 ± 0.55	3.35 ± 0.39	0.178
UGT2B17	0.424 ± 0.057	0.119± 0.029	**0.007**
UGT2B28	0.006 ± 0.001	0.126 ± 0.119	0.856

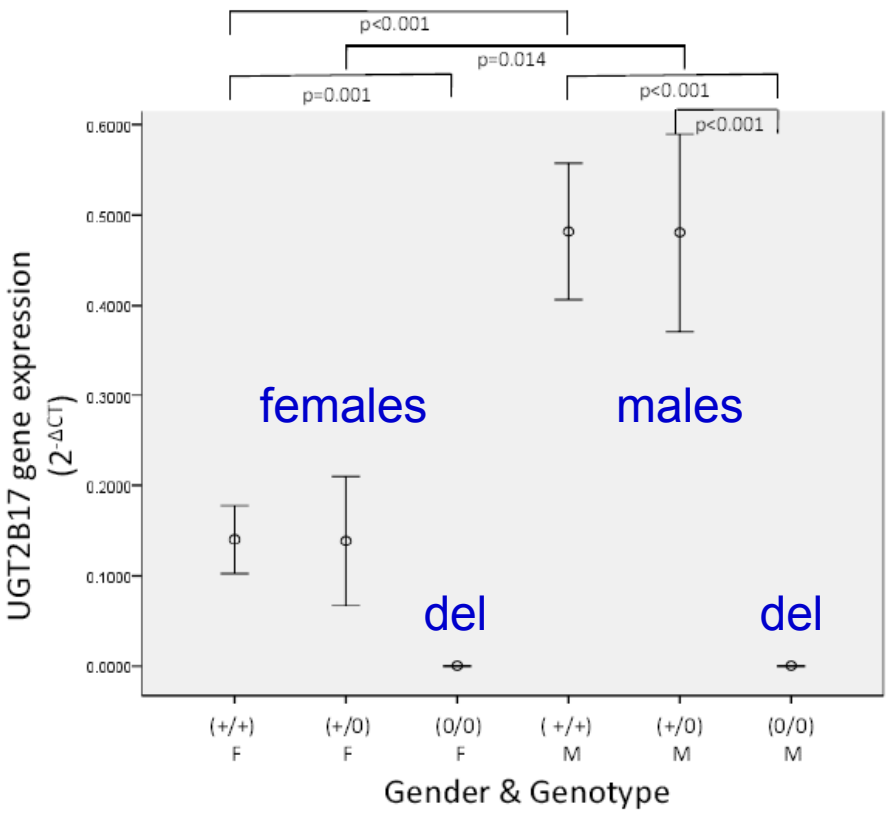
* Gene expression values are presented as each UGT mRNA relative to PPIA mRNA as $2^{-\Delta CT}$.

** Mann-Whitney test was used to determine statistical significance of expression of UGT2B genes between sexes.

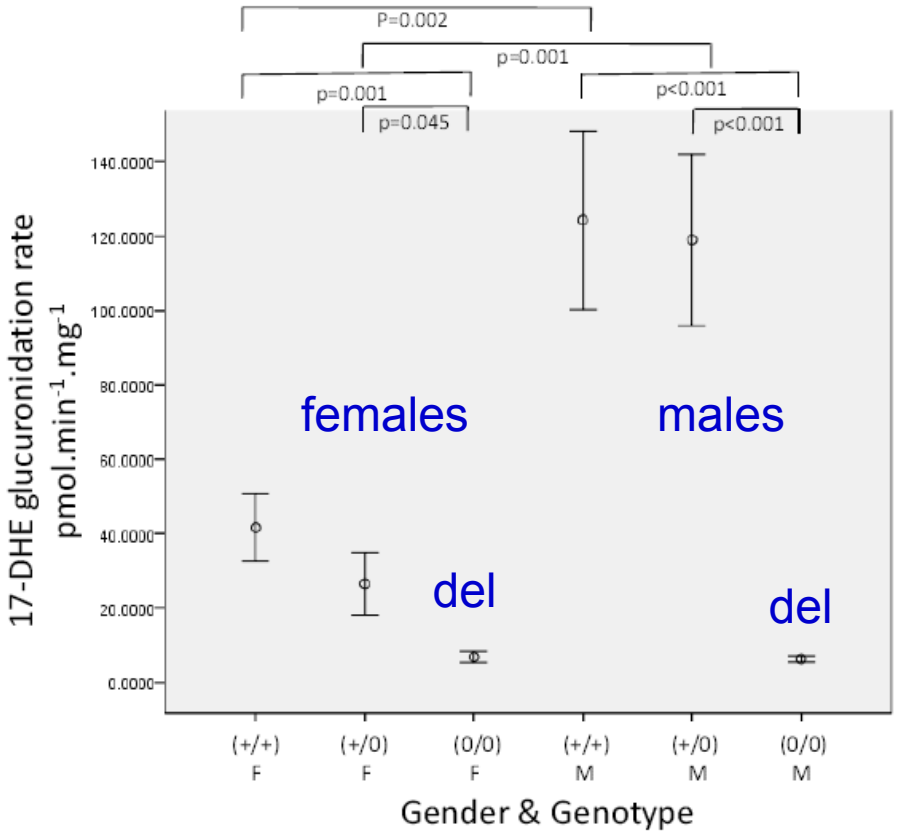
(Gallagher et al: DMD, 2010)

Effect of sex is independent of UGT2B17 deletion

UGT2B17 mRNA

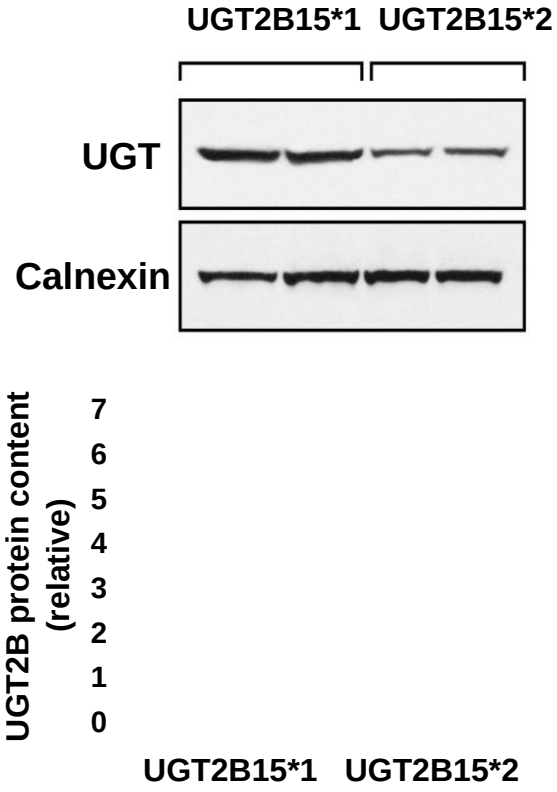


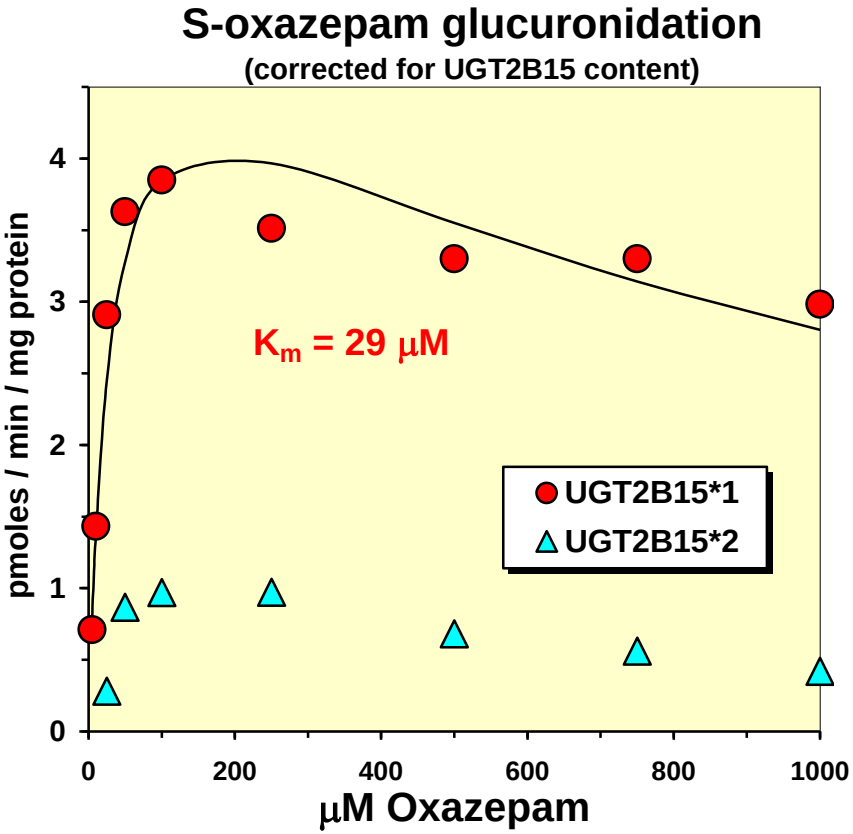
17-dihydroexemestane glucuronidation



(Gallagher et al: DMD, 2010)

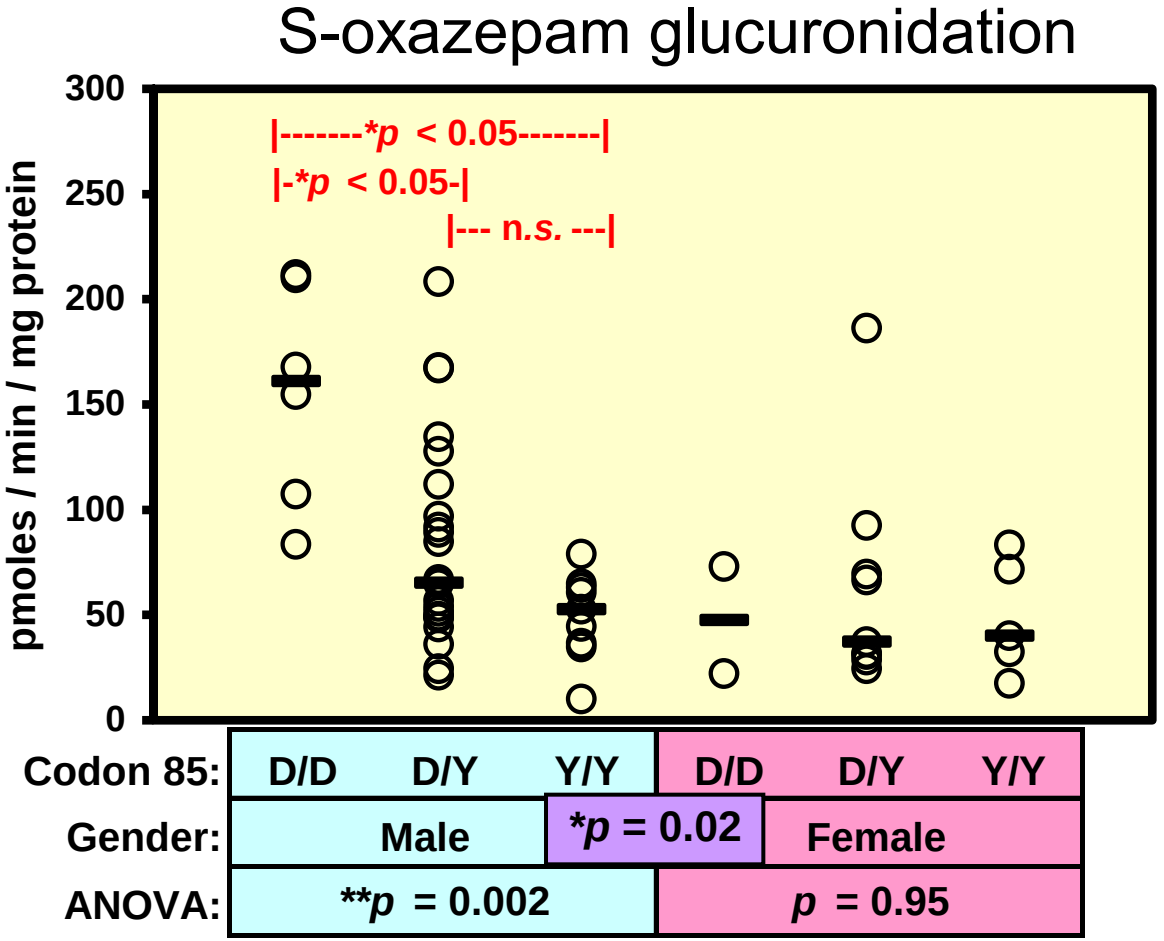
The common UGT2B15 variant (*2, D85Y) variant has lower oxazepam glucuronosyltransferase turnover





(Court *et al*: DMD, 2002)

Both sex and UGT2B15 D85Y genotype are major determinants of variability in oxazepam glucuronidation



(Court *et al*: JPET, 2004.)

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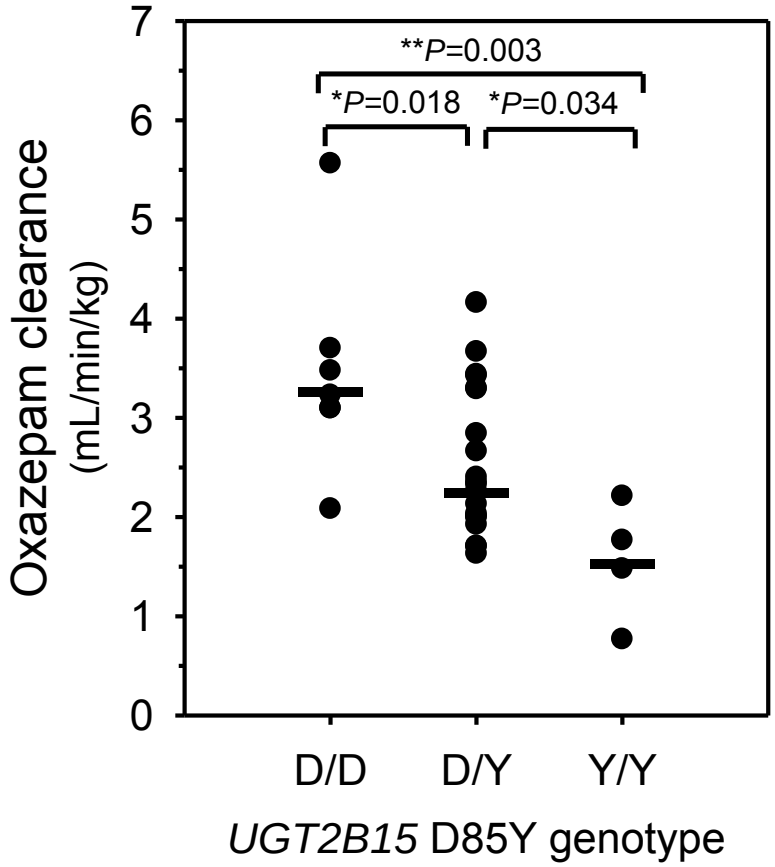


males received 0 mg
orally

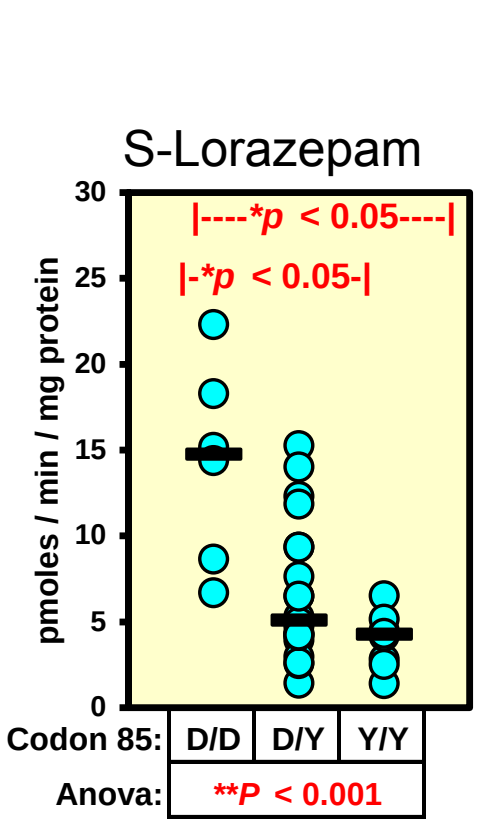
l for UGT2B15 D85Y
B17 deletion

ease with 85YY

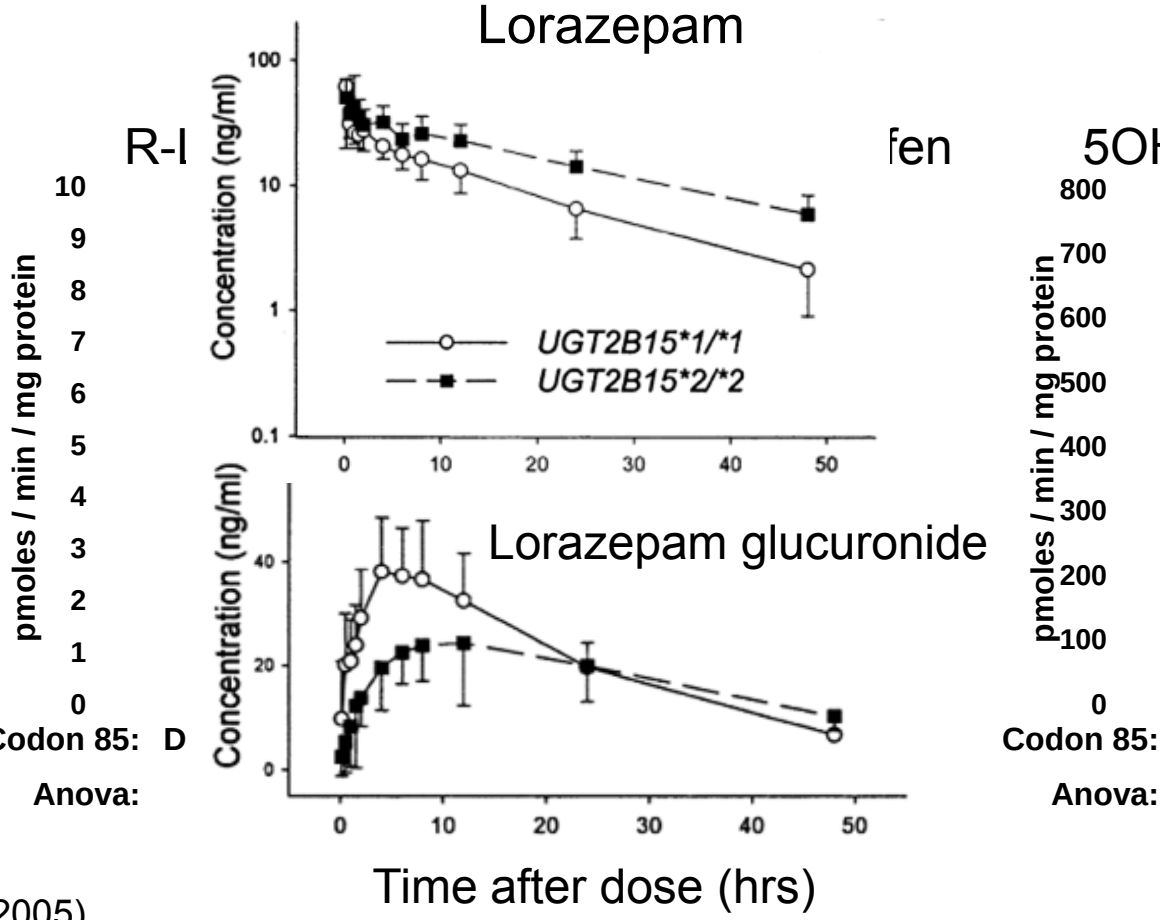
f UGT2B17 deletion

(He *et al*: BrJClinPharm, 2009)

Lorazepam glucuronidation is also decreased by D85Y *in vitro* and *in vivo*



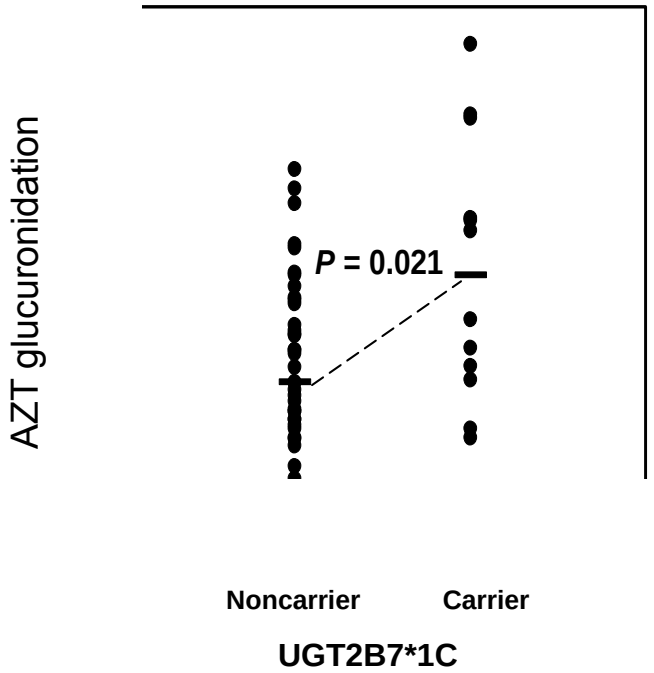
(Court: Methods Enzymol. 400:104-16, 2005)



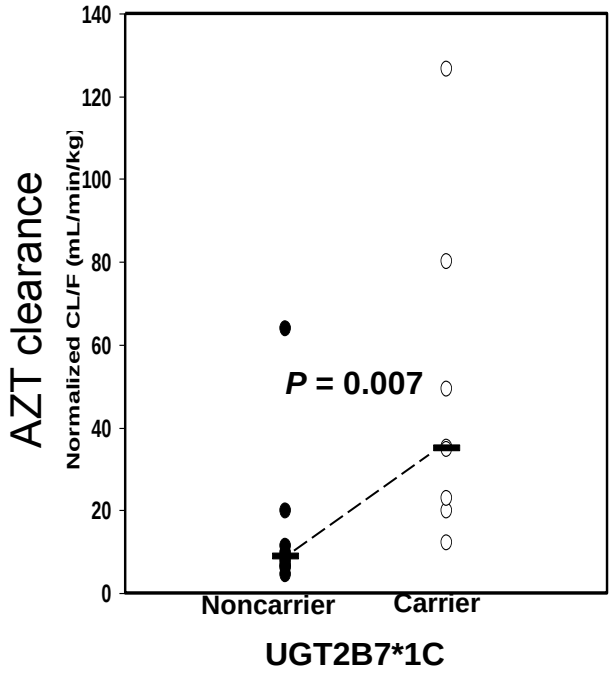
(Chung *et al*: CPT, 2005)

A common UGT2B7 haplotype (*1C) is associated with higher AZT glucuronidation and clearance

Human liver bank



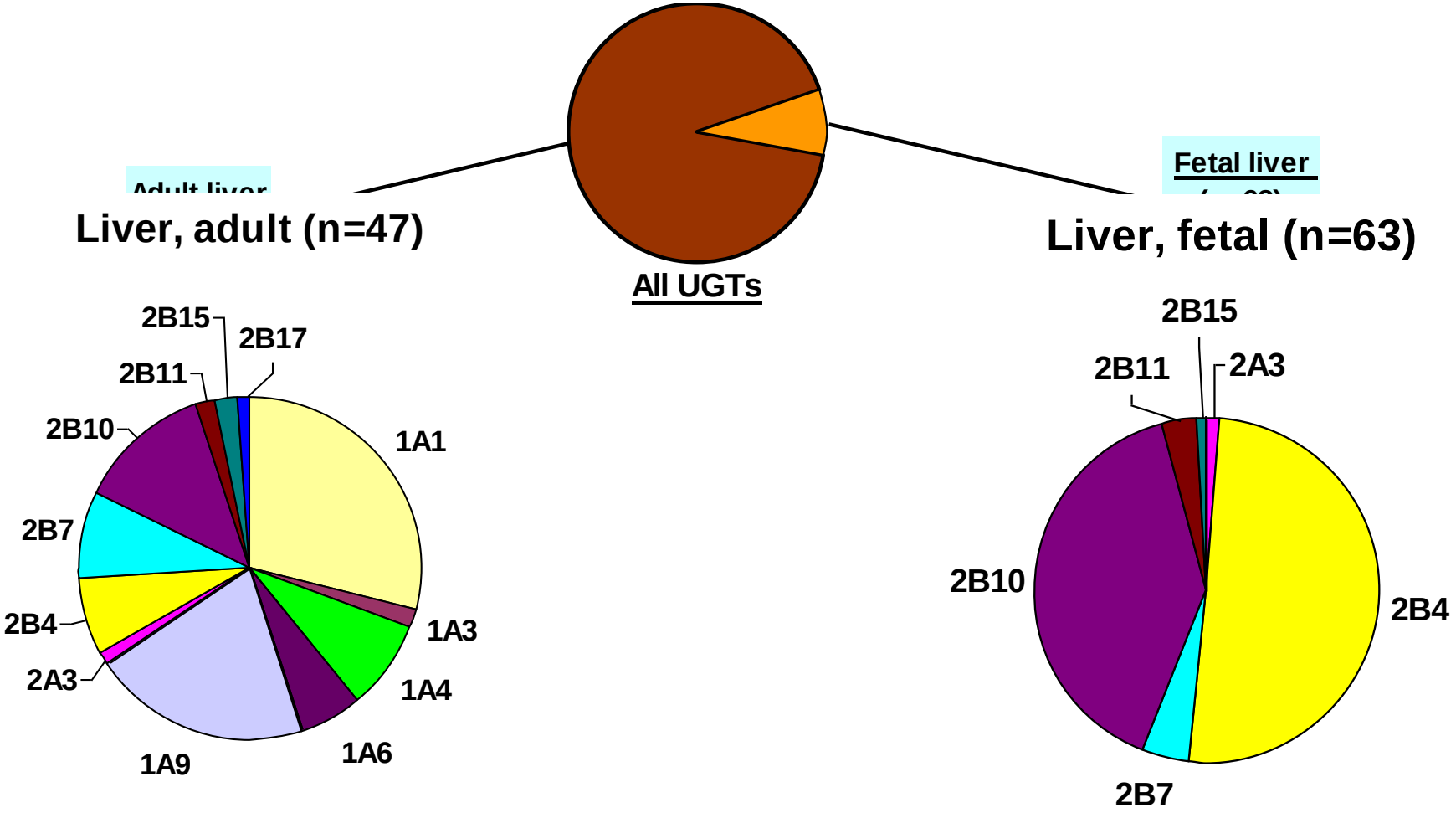
PK study



(Kwara et al: JClinPharm, 2009)

Effect of age on UGT expression

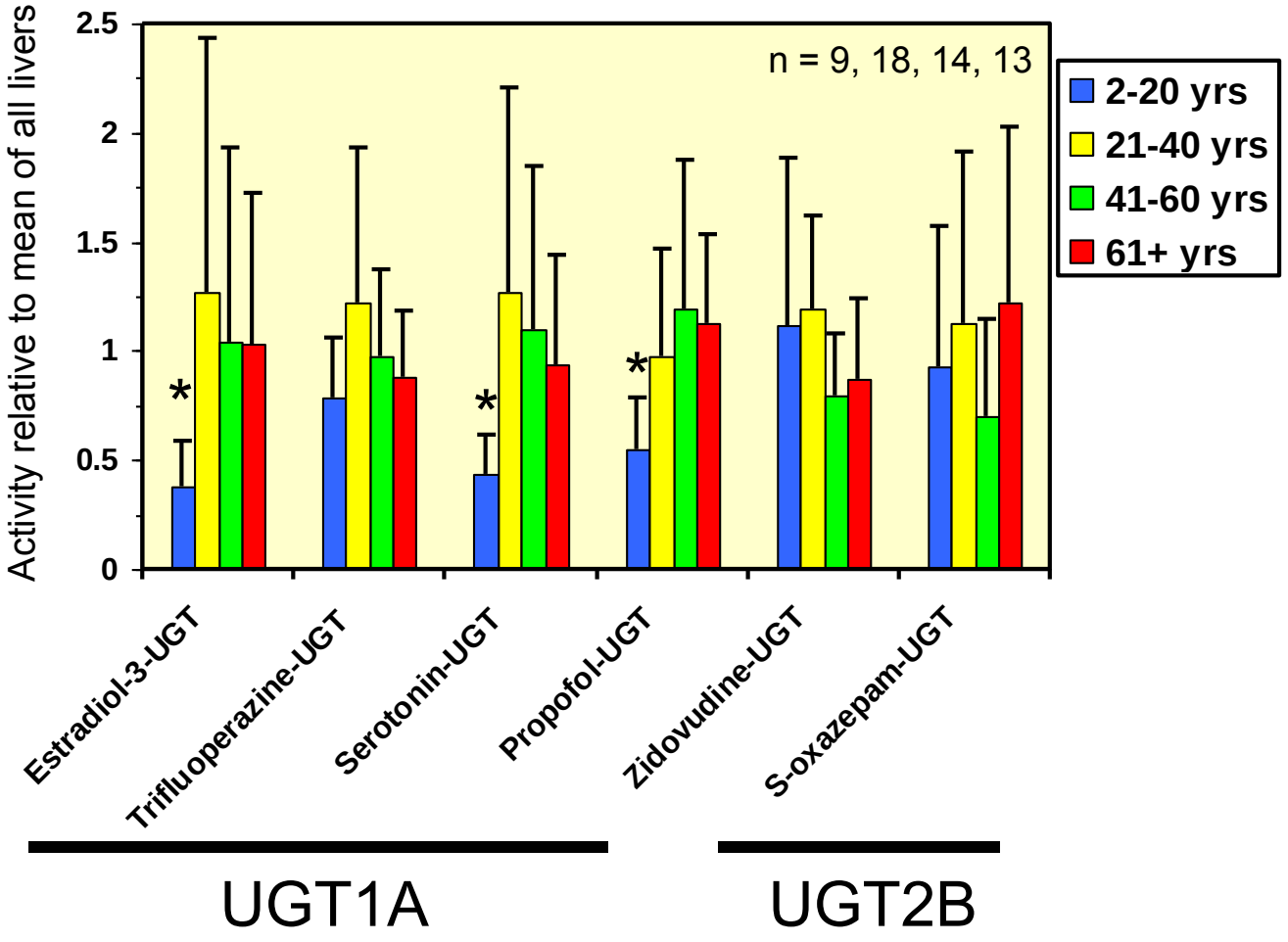
Fetal livers do not express any UGT1As



(Court *et al*, unpublished)

Effect of age on UGT activities

- Lower UGT1A activity in children/teens (<21 years)
- No effect of old age (>60 years)



(Court: Drug Metabolism Reviews, 2010)

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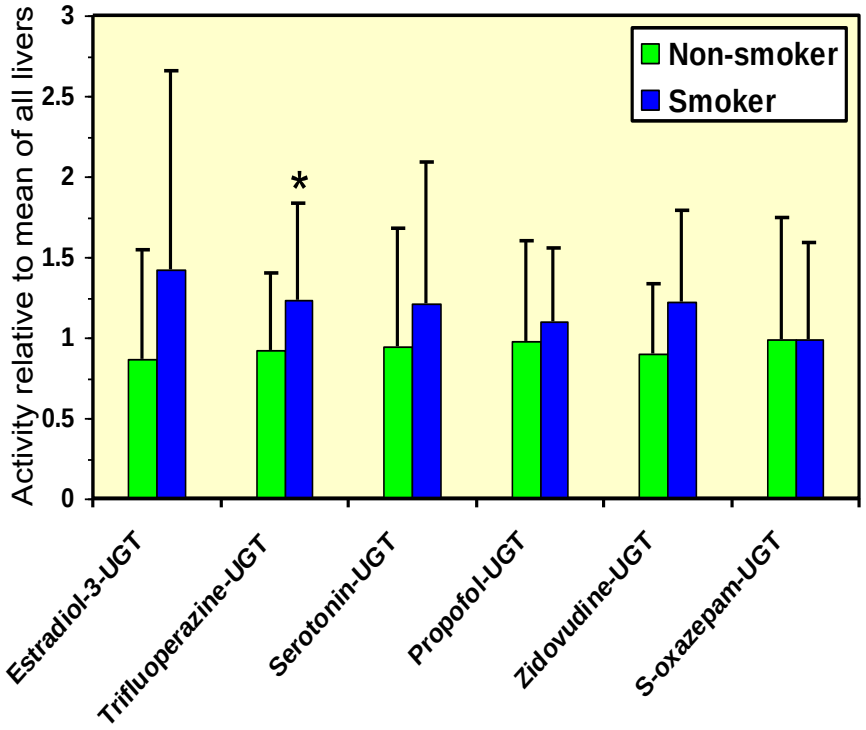
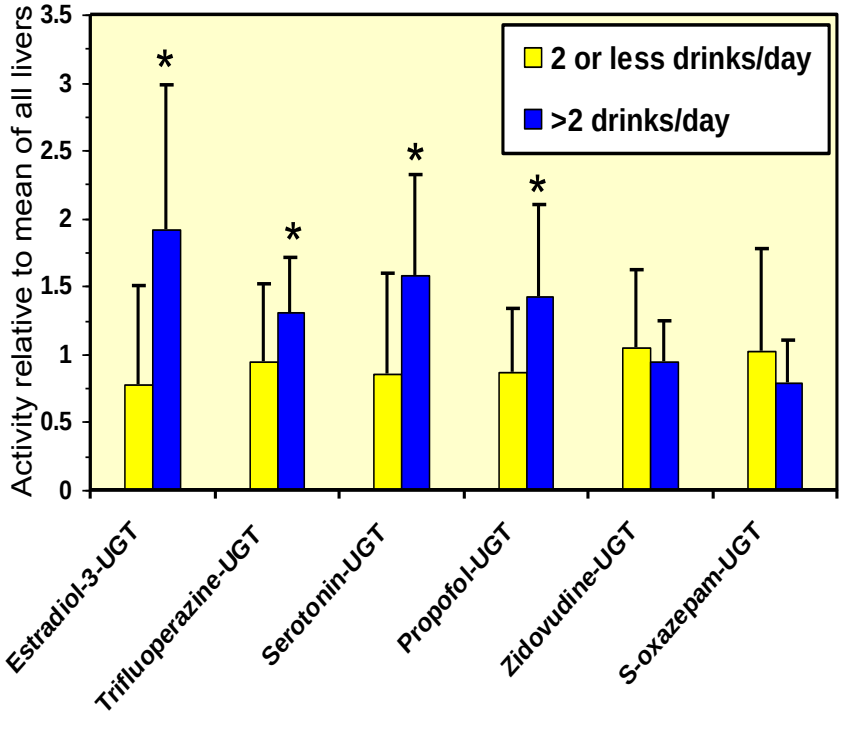
Smoking/alcohol history and UGT activities





(Court: Drug Metabolism Reviews, 2010)

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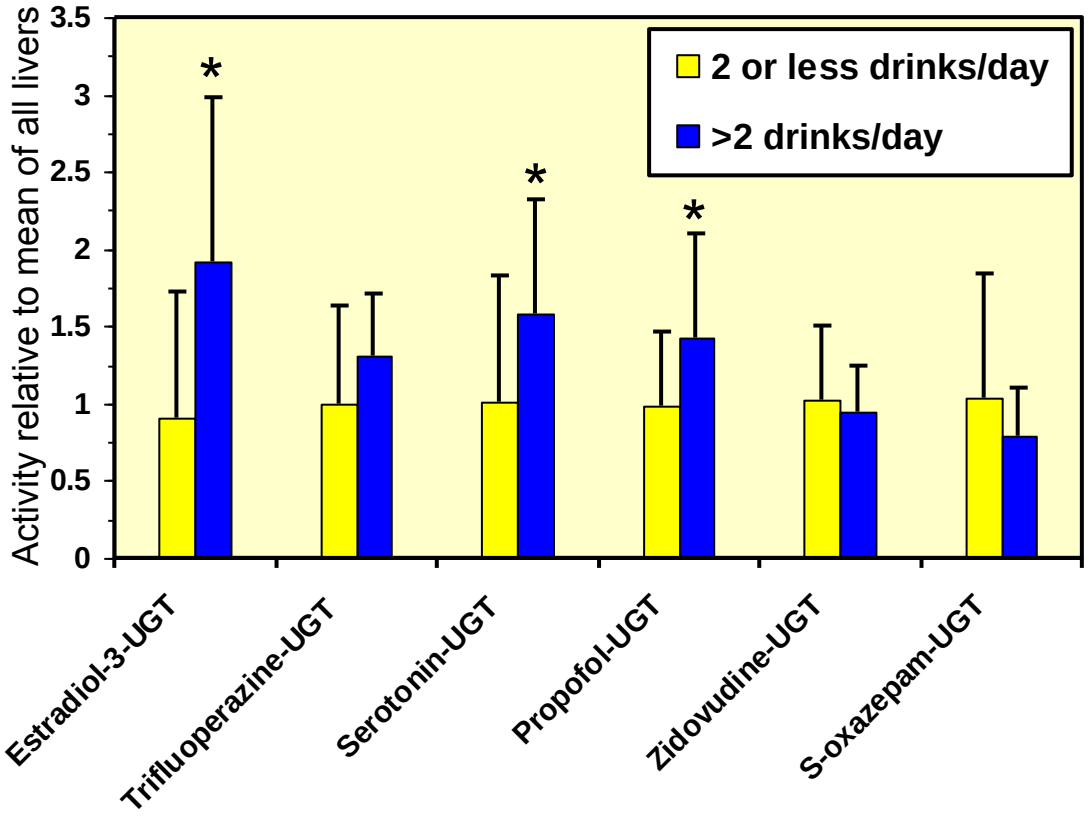
Alcohol history and UGT activities

Subjects 21 years and over



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Conclusions

- The human liver bank is a useful tool for characterizing interindividual variability in drug glucuronidation
- Interindividual variability in drug glucuronidation is comparable to CYP mediated drug metabolism
  - BUT variability is dependent on UGT isoform
  - UGT1A1 and UGT2B15 have highest variability
- Genetics, sex and age affect drug glucuronidation
  - UGT1A1 and UGT2B15 - genetic polymorphism
  - Male>female for UGT2B15/17 – sex steroids?
  - Lower UGT1A glucuronidation in infants/children – epigenetics?
  - Alcohol effect on UGT1A – transcription factor??
  - Role for regulation via protein-protein interaction (UGT-i2)??

Acknowledgements

- NIH grant R01-GM61834 (2000 – 2011)
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- Chantal Guillemette (Laval University, Quebec)
- Court lab - past and present personnel
  - Soundar Krishnaswamy
  - Qin Hao
  - Su Duan
  - Su Hazarika

"The FDA now requires that we have an actor show you what kinds of side effects you might experience."