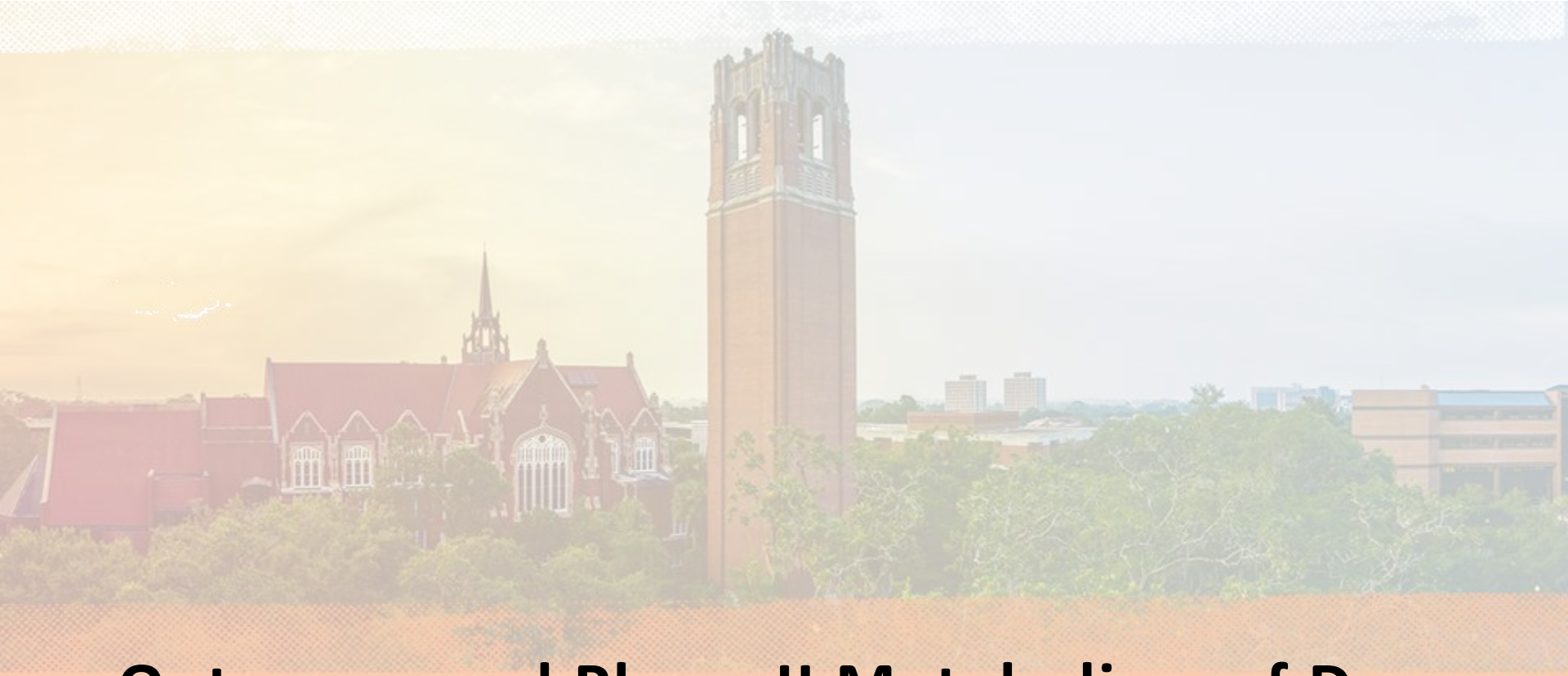

Ontogeny and Phase II Metabolism of Drugs

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University of Florida

Disclaimer

- I am a consultant to pharmaceutical industry
- I like applied & interdisciplinary research
- I am presenting on behalf of an interinstitutional and interdisciplinary research team

Thank You To The Research Team

Neil Parrott
Roche

PBPK modelling expertise
Project leadership

Stephan Schmidt
University of Florida

Pharmacometrics, Clinical pharmacology expertise
Academic supervisor

Stephen Fowler
Roche

Enzymology expertise,
in vitro work supervision

Abby Collier
University of British Columbia

DME ontogeny and UGT experimental expertise, pediatic liver sample provision

Ryan Takahashi
Genentech

Proteomics expertise / data provision

William Forrest
Genentech

Bioinformatics & Computational Biology

Saskia de Wildt
Radbound University, NL

DME ontogeny expertise,
pediatic liver sample provision

Roche Postdoc Fellowship funded project (2017/2019)

Knowledge Gaps

Phase II metabolism: Conjugation reactions (**glucuronidation**, methylation, sulphation, acetylation, gluthathione conjugation, glycine conjugation)

- UGT1A and 2B isoforms = **key determinants of pharmacokinetics, efficacy and safety** of many pediatric drugs
- Rapid and continuous differentiation and maturation of metabolic functions → **Limited knowledge**

? **Ontogeny pattern of hepatic UGTs using multiple probe substrates**

? **Differences in maturation of activity between UGT isoforms**

? **Marked age-related differences in activity across UGT isoforms**

? **Between-subject variability in UGT activity**

? **Age-independent factors affecting UGT activity efficiency**

Goals For This Presentation

1. Outline **experimental challenges** of automated **UGT** phenotyping **assays**
2. Discuss **UGT ontogeny** patterns of major UGT isoforms
3. Discuss impact of **age, sex, and ethnicity** on **UGT activity**
4. Provide a case example for the dynamic interplay between **phase I** and **II metabolism**, **gene-drug interactions**, and **drug-drug interactions**

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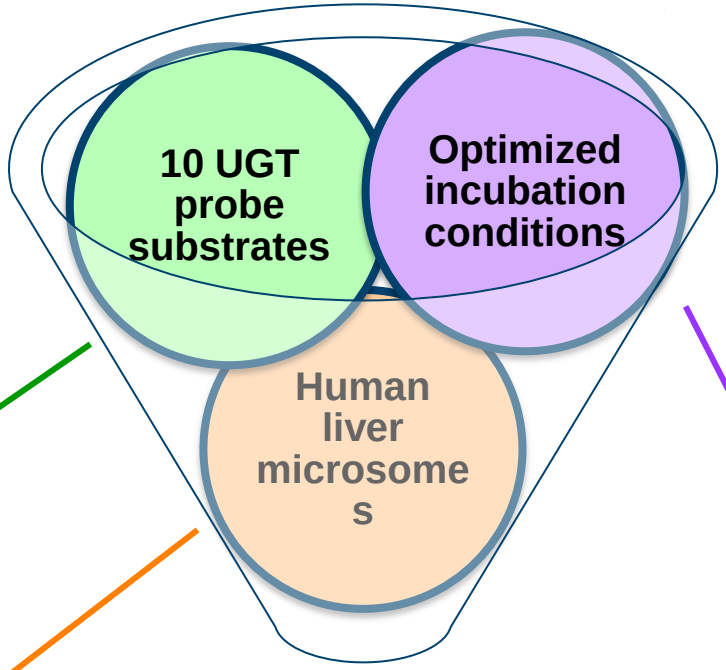
Challenges of UGT Phenotyping Assays

- **Lack of standardized experimental conditions** of UGT assays between laboratories, which hinders the comparison of UGT activity across studies
  - Pre-treatment of human liver microsomes (HLM) with detergents / pore-forming peptides (alamethicin)
  - Buffer components (i.e., $MgCl_2$)
  - Co-substrates (i.e., UDPGA, saccharolactone)
  - Bovine serum albumin (BSA) supplementation
- **Limited or not available UGT-isoform inhibitors**
- **Small number of positive control compounds** as functional markers of UGT activity
  - **Good enzyme selectivity**: β-estradiol (UGT1A1) trifluoperazine, (UGT1A4), 5-hydroxytryptophol (UGT1A6), propofol (UGT1A9) and zidovudine (UGT2B7)
  - **Less selective compounds**: gemfibrozil (UGT2B4/2B7), oxazepam (UGT2B15 (*S*), and 1A9, 2B7 (*R*)) and chenodeoxycholic acid (UGT1A3> 1A1, 2B7)

Badée *et al.*, 2019, Drug Metabolism and Disposition, 47:124-34

Optimization of UGT Profiling Assay Conditions

- UGT1A1: β-Estradiol (3-gluc)
- UGT1A3: Chenodeoxycholic acid
- UGT1A4: Trifluoperazine
- UGT1A6: 5-Hydroxtryptophol
- UGT1A9: Propofol
- UGT2B4/2B7: Gemfibrozil
- UGT2B7: Zidovudine
- UGT2B10: Amitriptyline
- UGT2B15: Oxazepam (mixture)
- UGT2B17: Testosterone



- **Incubation buffer**
  - Potassium phosphate
  - Tris-HCl
- Buffer component
  - **$MgCl_2$** (0-10 mM)
- Co-substrate
  - **UDPGA** (1-25 mM)
- **BSA** (0-2 % w/v)

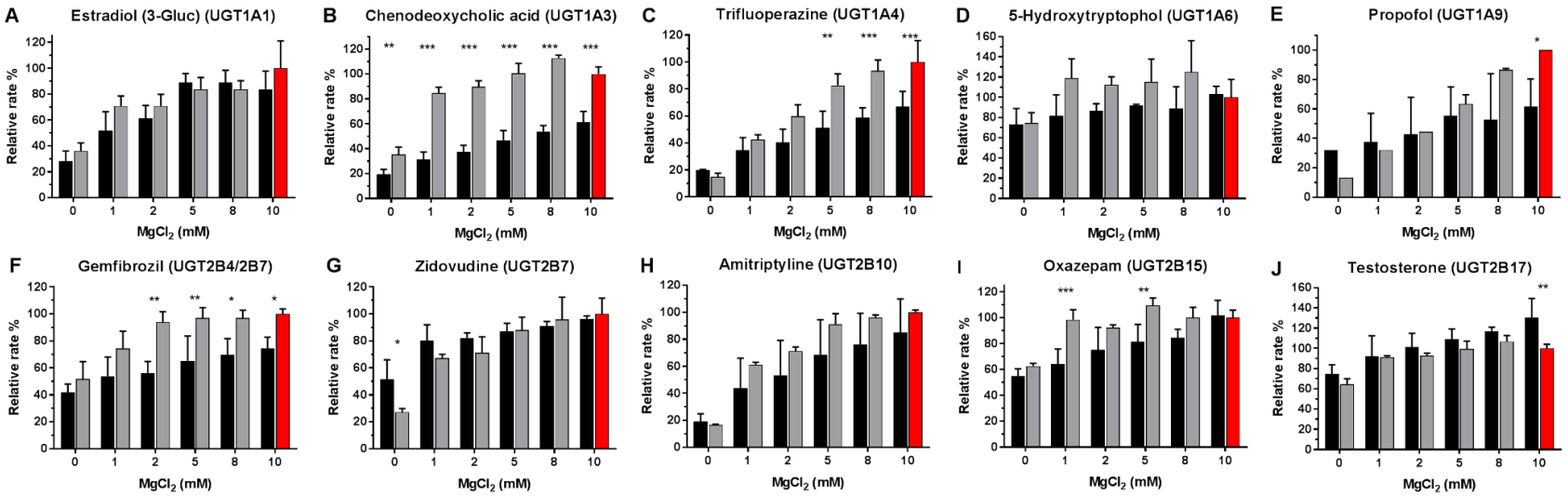
- **150-donor pooled HLM**
  - 1 mg/mL
  - Mixed gender (75 male/75 female)

Optimal experimental conditions to simultaneously characterize the hepatic UGT activity in HLM

Badée *et al.*, 2019, Drug Metabolism and Disposition, 47:124-34

Incubation Buffer Composition

- $MgCl_2$: 0, 1, 2, 5, 8 and 10 mM
- Potassium phosphate (black bar) *vs.* Tris-HCl buffer (grey bar) 0.1 M, pH 7.4
- Rate obtained with 10 mM $MgCl_2$ and Tris-HCl buffer defined as 100% (red bar)



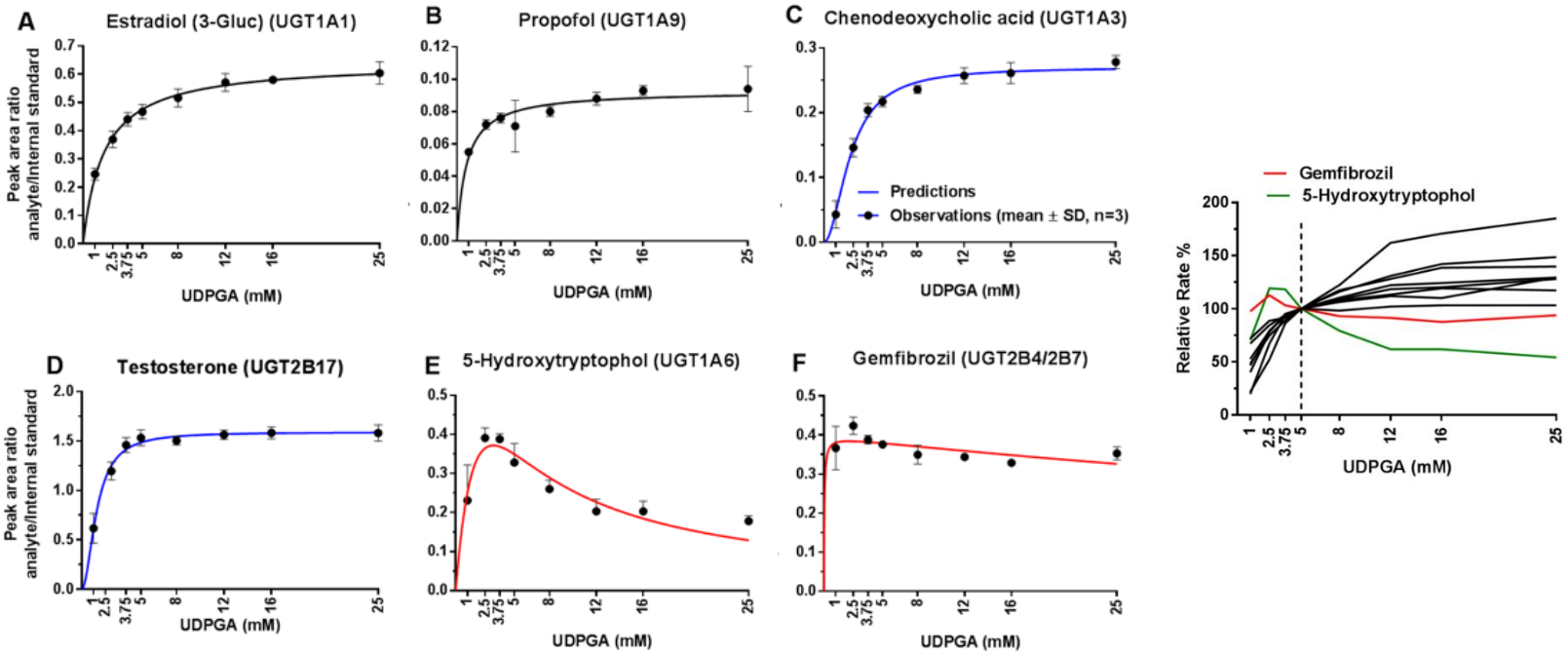
* $p < 0.05$, ** $p < 0.01$, $p < 0.001$

- Enhanced activity of UGT1A3, 1A4, 1A9 and 2B4/7 by 50 to 87% with **Tris-HCl buffer** and **10 mM $MgCl_2$**
- **Better reproducibility** using Tris-HCl (89% of CV<20%) vs Phosphate buffer (>50% of CV% <20%)

Badée *et al.*, 2019, Drug Metabolism and Disposition, 47:124-34

Co-Substrate Dependency

- UDPGA: 1, 2.5, 3.75, 5, 8, 12 and 25 mM

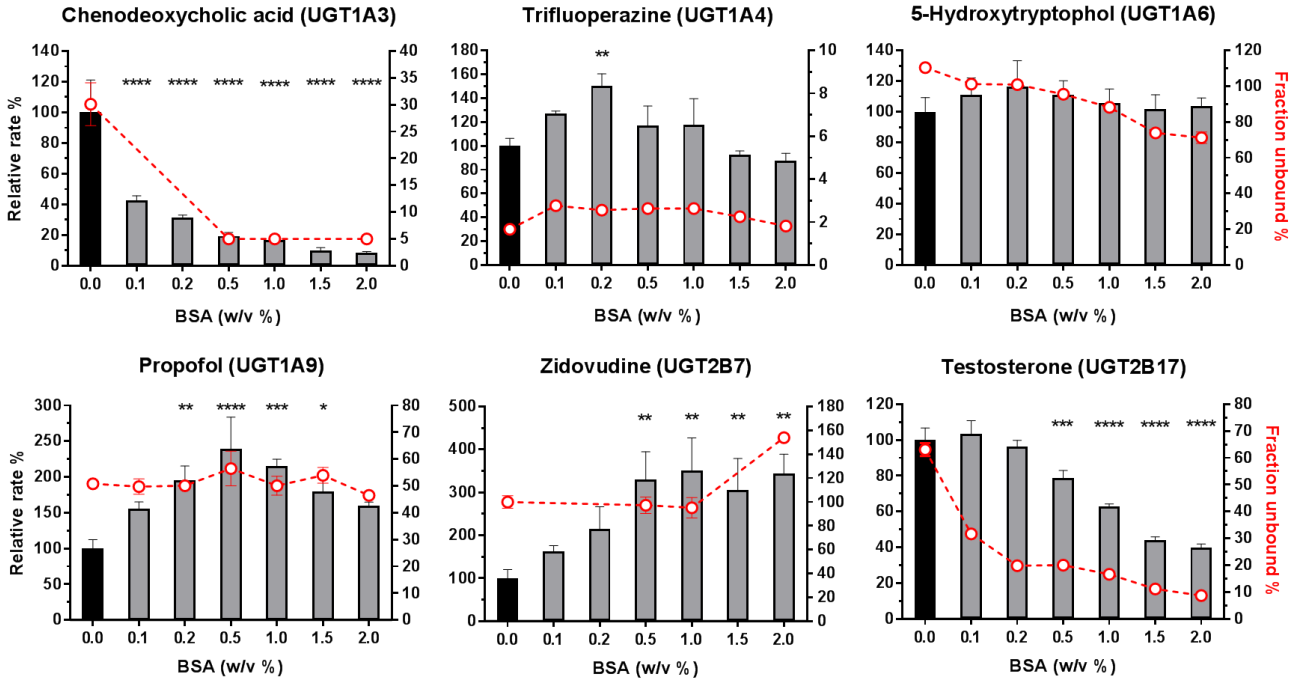


- **Hyperbolic** or **sigmoidal Michaelis-Menten kinetics:** >60% of maximal activity at 5 mM UDPGA
- **Substrate inhibition kinetic** → Decreased glucuronide formation rate above 5 mM UDPGA
- **Optimal** UDPGA concentration of **5 mM**

Badée *et al.*, 2019, Drug Metabolism and Disposition, 47:124-34

Substrate- and Enzyme-Specific Effects of BSA

- BSA: 0, 0.1, 0.2, 0.5, 1, 1.5 and 2 % w/v
- Total rate obtained in the absence of BSA defined as 100% (black bar)
- Protein binding measured via high-throughput equilibrium dialysis (red circle)



* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, *** $p < 0.0001$

- **Total activity:** a more suitable tool to characterize metabolically stable new drug candidates, when the **effect of BSA binding and the identity of UGTs had not been determined**

Badée *et al.*, 2019, Drug Metabolism and Disposition, 47:124-34

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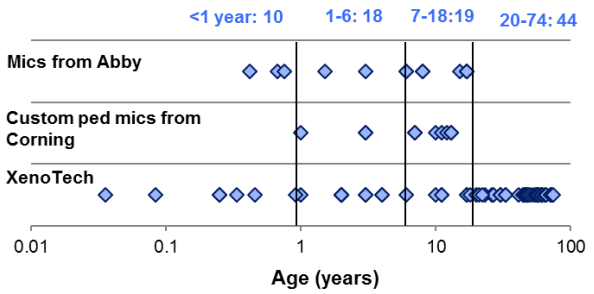
Characterization of Hepatic UGT Ontogeny

1. Define the **ontogeny** profile of major human **hepatic UGT isoforms** based on microsomal glucuronidation activity using multiple selective substrates and matched HLM samples
2. Establish **UGT protein expression - activity correlation** using matched HLM samples

HLMs (13 days-74 years)

- Adult (n=44),
- Pediatric (n=47)
- 150-donor pooled HLMs



Automated UGT assay (Roche)

- Alamethicin-treated HLMs (50 µg/mg)
- HLM concentration (0.1 or 0.5 mg/mL)
- **19 UGT probe substrates** selected
  - **In vitro** probe substrates
  - **Clinically used** drug substrates
- Single concentration (3, 5, 10 or 100 µM)
- Incubation time: (5 or 10 min)
- **Optimized incubation conditions**

UGT proteomics (Genentech)

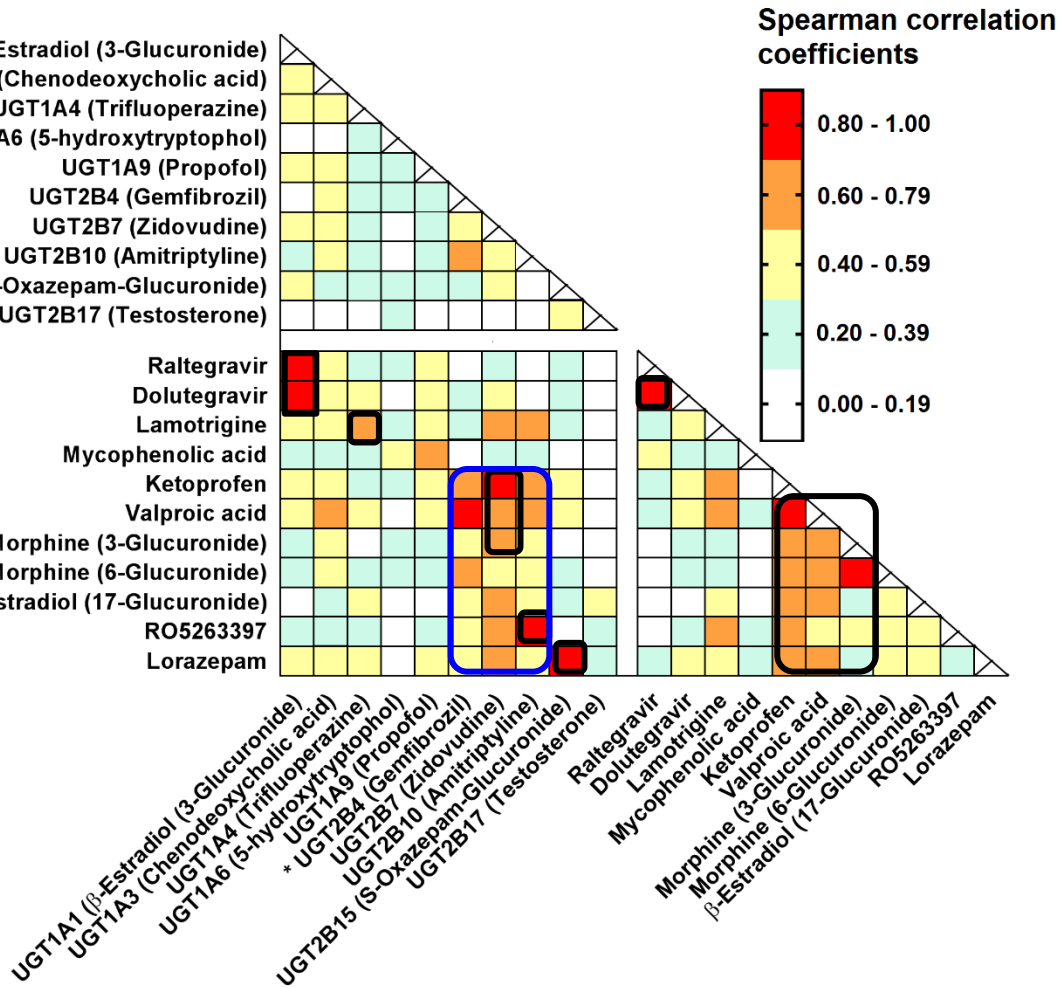
- Quantitative LC-MS/MS MRM-based method
- Optimization of digestion conditions
- Selection of suitable surrogate peptides to avoid interactions with expression measurements
- Protein expression - activity correlations for UGTs and CYPs
- **Manuscript in preparation**

Manuscript under review with DMD

Co-Regulation Between UGT Isoforms

In vitro **probe substrates**

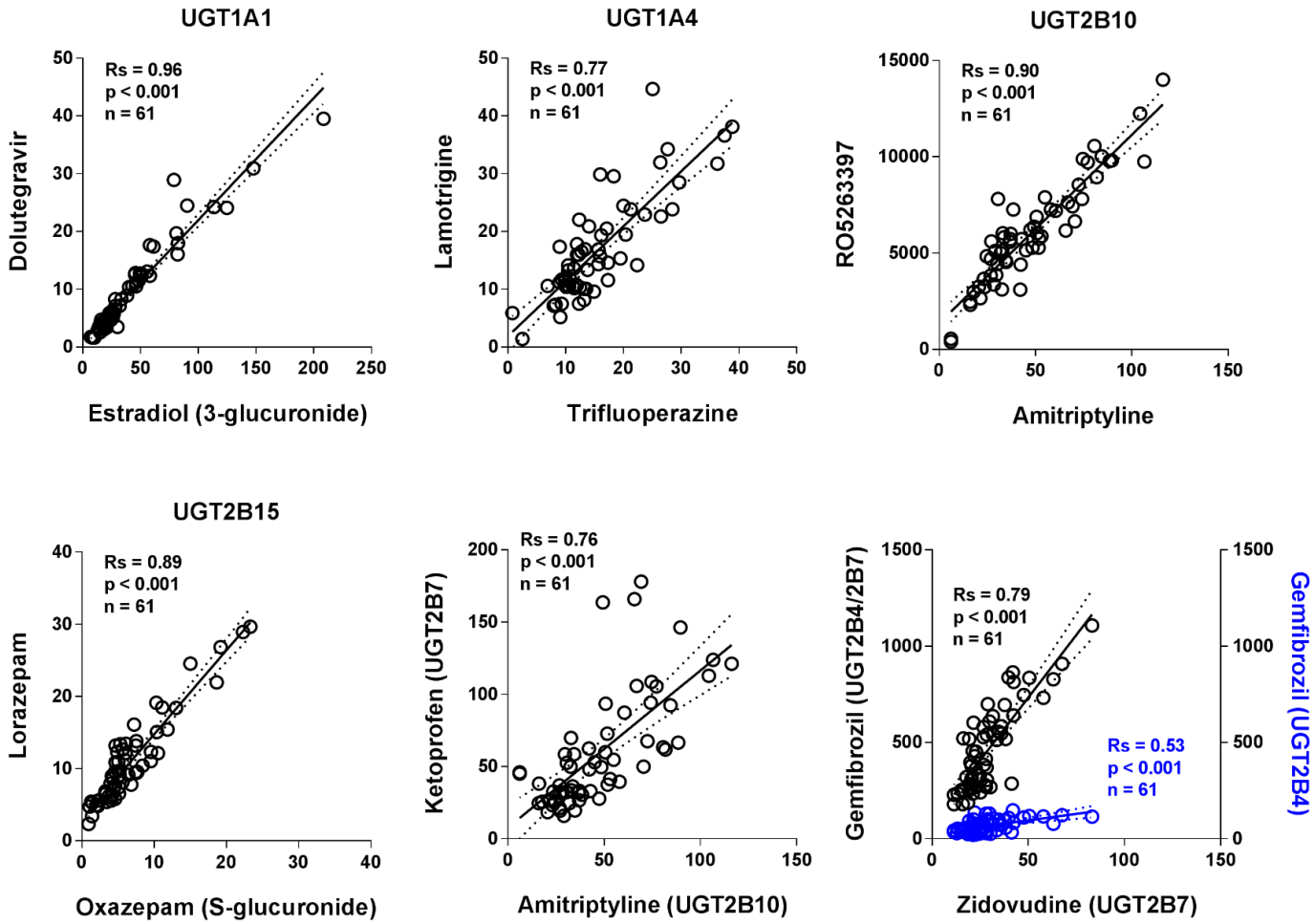
Additional probe compounds



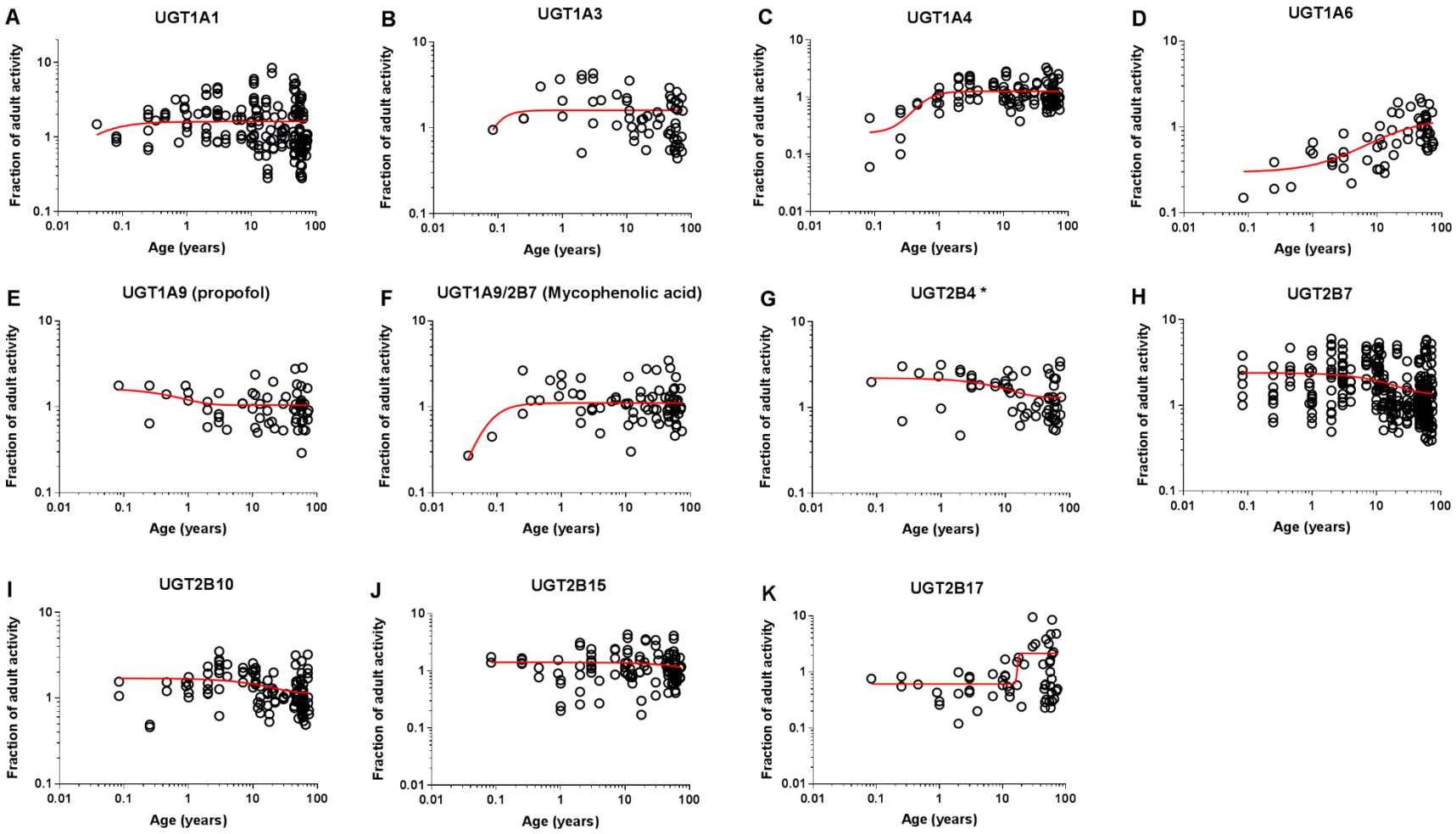
Strong activity - activity correlations across UGT isoforms → Co-regulation

* Gemfibrozil incubated with 60 µM atractylenolide I, used as an UGT2B7 inhibitor

Ontogeny of UGT1A1, 1A4, 2B7, 2B10, and 2B15 Established Using Multiple Selective Substrates



Rapid Ontogeny of UGT Isoforms



Badée *et al.,* 2019, Clin Pharmackinet, 58(2):189-211

Badée *et al.,* under review with Drug Metabolism and Disposition

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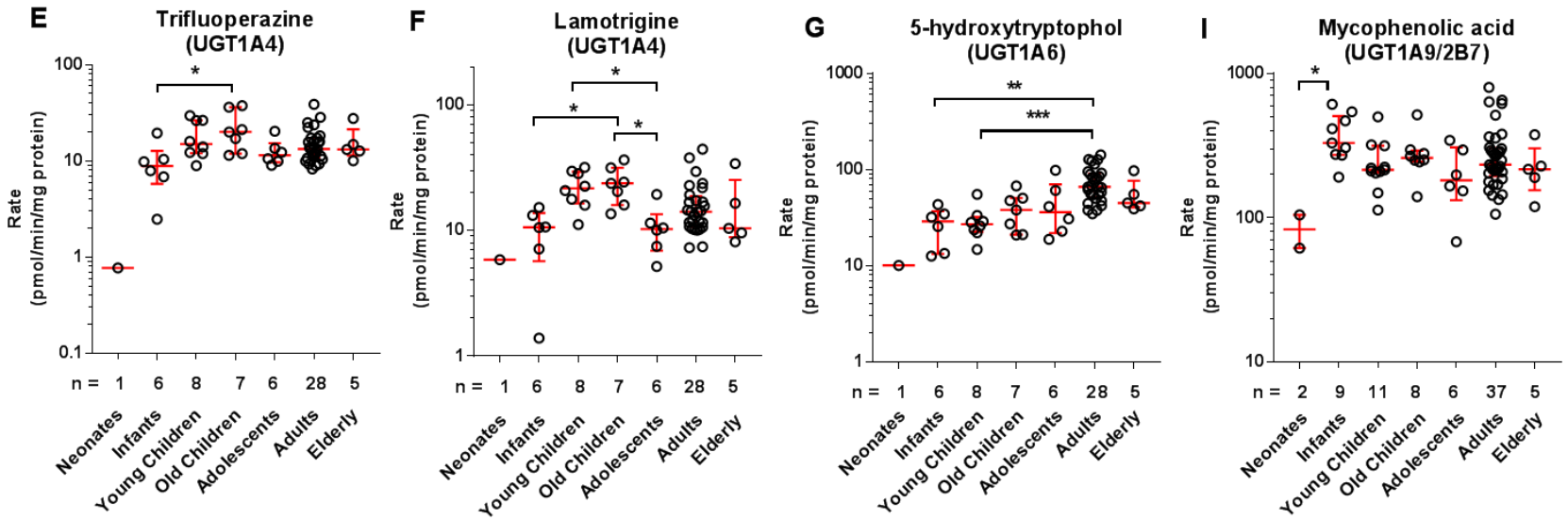
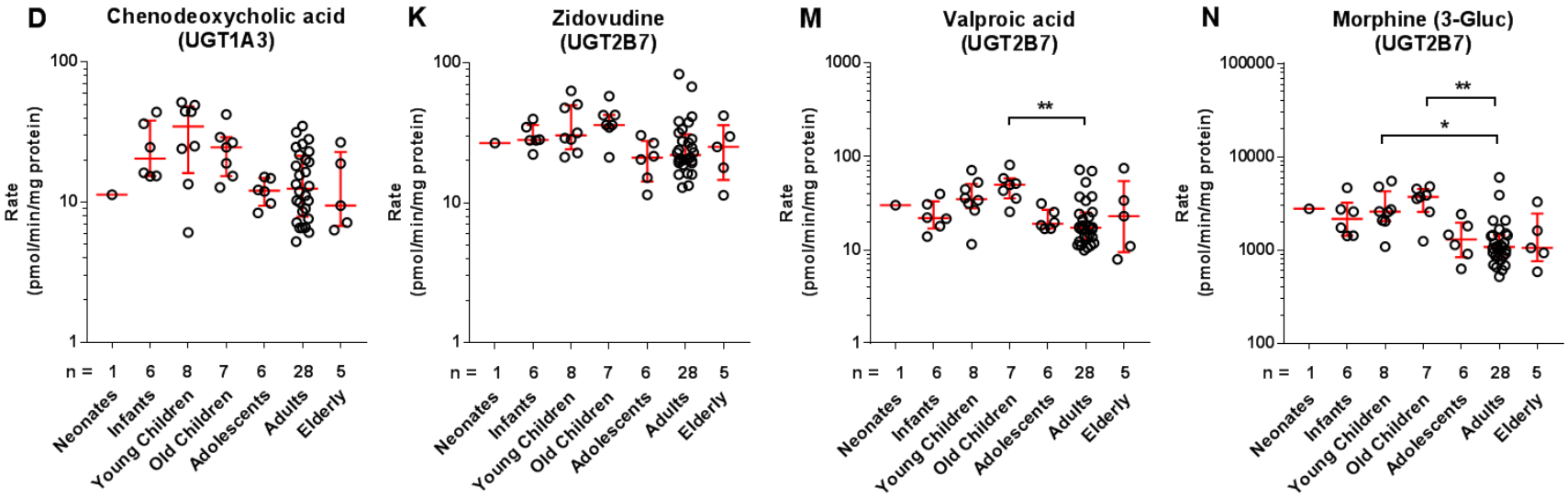
Evidence of Increased Activity with Age For UGT1A4, 1A6,1A9/2B7



* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

- **2.5-fold** change in **UGT1A4** activity (older children-infants)
- **2.7-fold** change in **UGT1A6** activity (adults-infants)
- **4.6-fold** change in **UGT1A9/2B7** activity (neonates-infants)

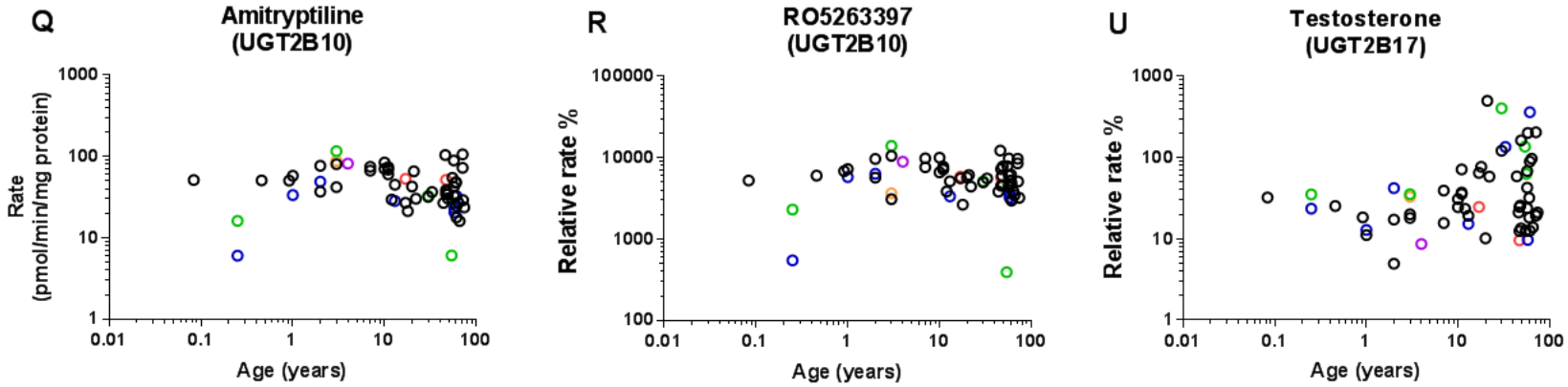
But Not For UGT1A3 and 2B7

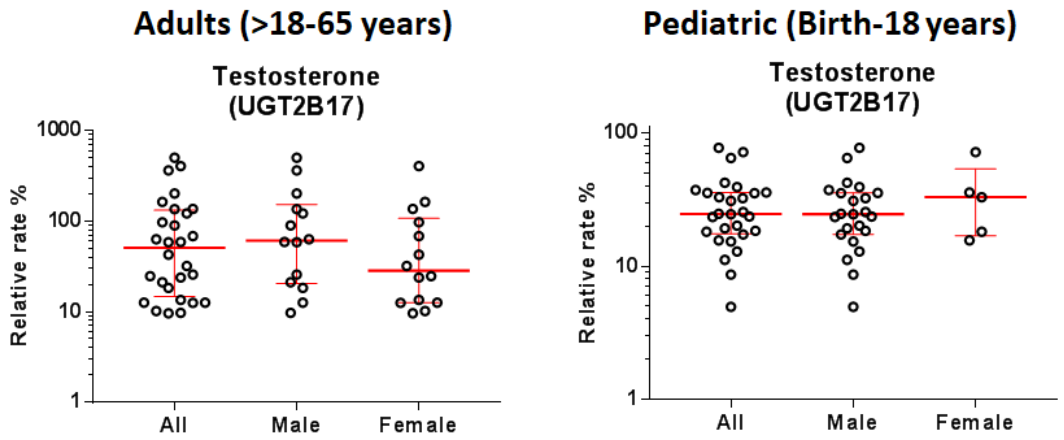


* $p < 0.05$, ** $p < 0.01$

- **Maximum activity reached in children**
- **Decreased activity in adults and elderly**

No Sex or Ethnicity-Related Effects





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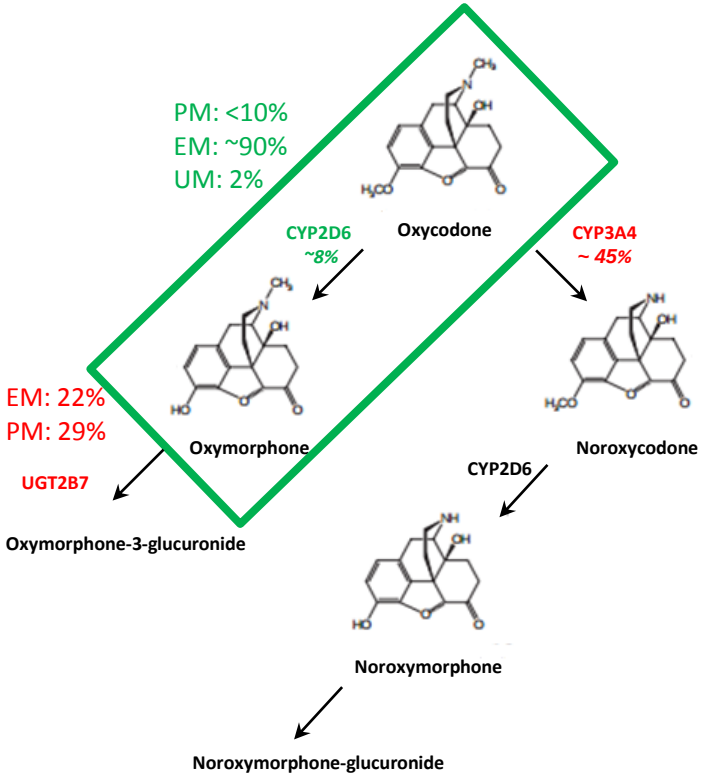
Interplay Between Phase I and Phase II Metabolism: Oxycodone Case Example

Getting closer:

Oxycodone —**CYP2D6** (*Formation*)→ **Oxymorphone** —**UGT2B7** (*Elimination*)→

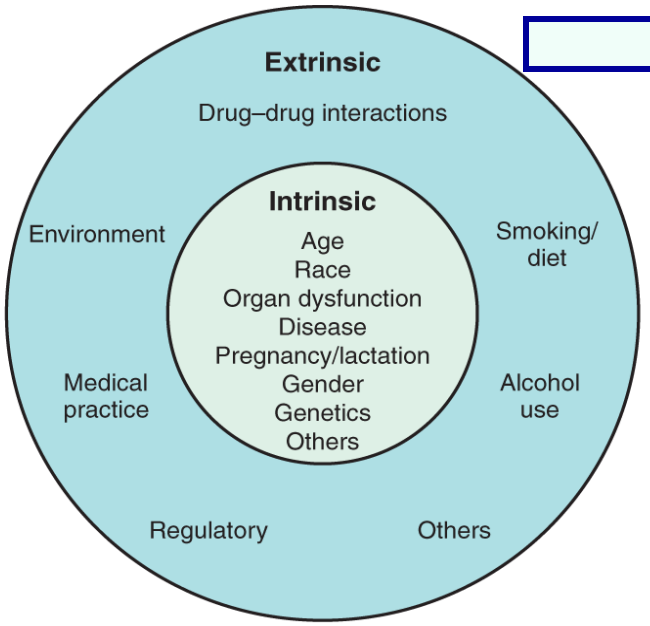
Reality: Metabolic network

- Oxycodone is primarily metabolized by CYP2D6 (~8%) and CYP3A4 (45%)
- CYP2D6 is polymorphic
- Oxycodone and Oxymorphone are considered pharmacologically active (MOR affinity: Oxymorphone >>> Oxycodone)
- Oxymorphone is further metabolized by UGT2B7
- UGT2B7 is also polymorphic



Let's Integrate To Predict – What If?

Intrinsic/extrinsic Factors



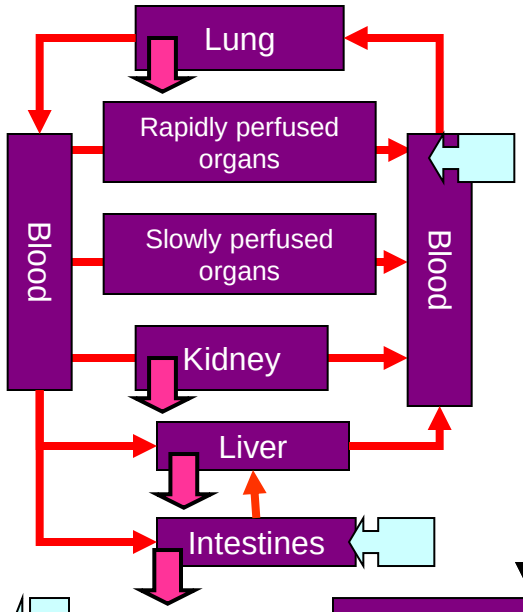
Huang and Temple, 2008

Individual or combined effects on human physiology

PBPK Model components

System component (drug-independent)

Drug-dependent component



ADME, PK, PD and MOA

Metabolism
Active transport
Passive diffusion
Protein binding
Drug-drug interactions
Receptor binding

Dosing

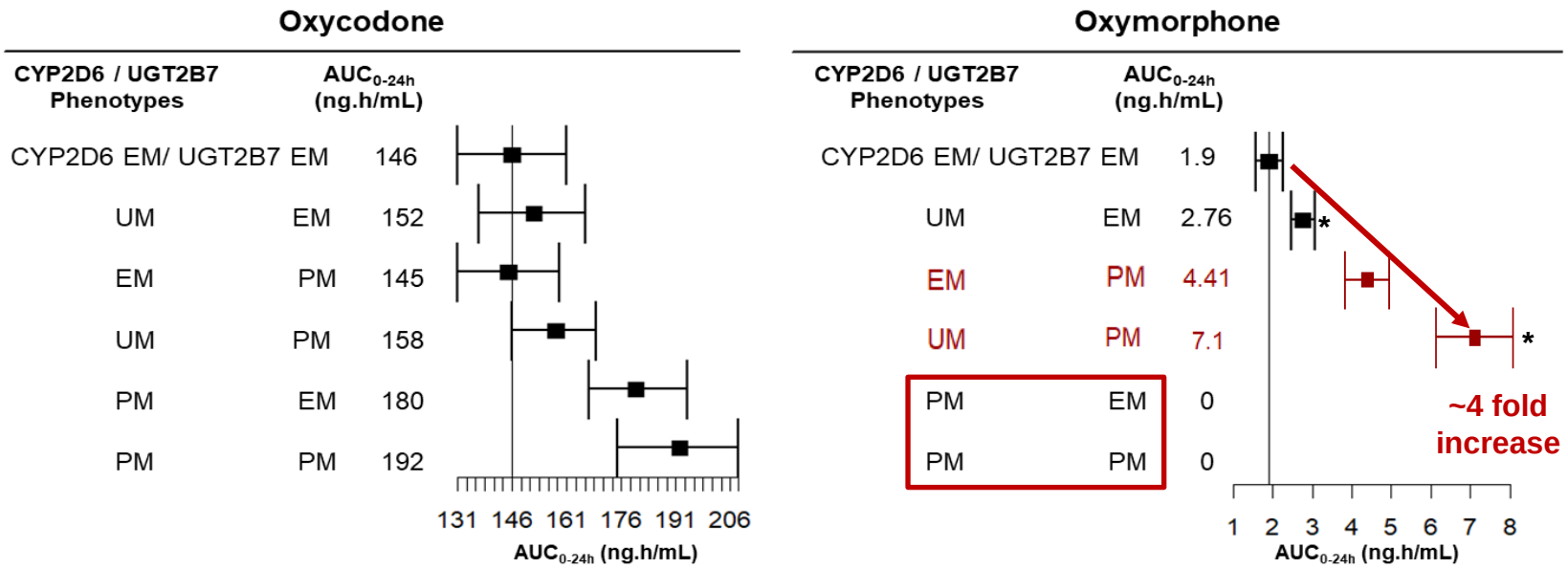
Elimination

PBPK Model

Predict, Learn, Confirm, Apply

Zhao et al. (2011) Clin Pharmacol Ther ***89****: 259-67*

What If - We Have GDIs?



- **CYP2D6 PMs** convert little to no Oxycodone to **Oxymorphone.**
- **CYP2D6 EMs** and **UMs** show no difference in Oxycodone- but in **Oxymorphone exposure.**
- The **extent** of the difference in **oxymorphone** exposure is primarily driven by the **UGT2B7 genotype.** It is largest (~4-fold) for **CYP2D6 UMs UGT2B7 PMs**.

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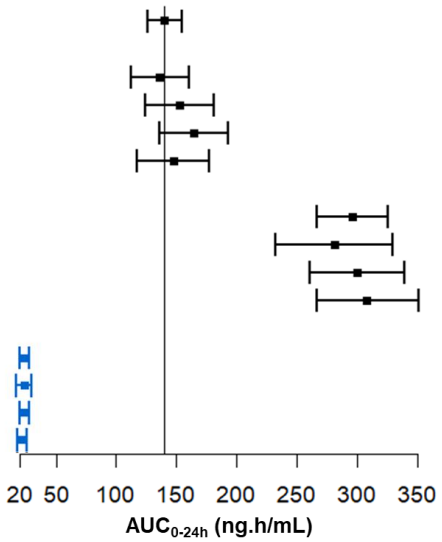
What If – We Have GDIs & DDIs?

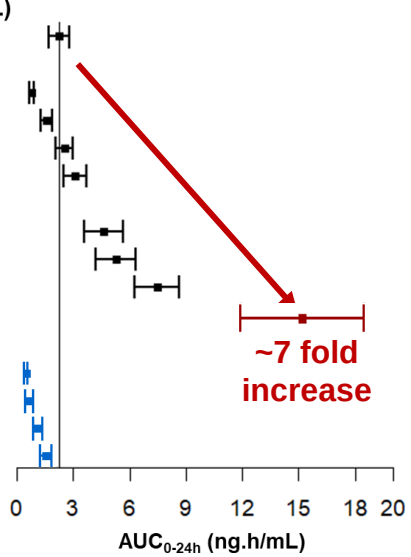
Oxycodone AUC for DDI and CYP2D6 / UGT2B7 phenotypes

CYP2D6 / UGT2B7 Phenotype + Perpetrator		AUC$_{0\text{-}24h}$ (ng.h/mL)
No DDI CYP2D6 EM UGT2B7 EM		140
Oxycodone + Quinidine		
DDI CYP2D6 EM/ UGT2B7 EM		136
UM	EM	153
EM	PM	164
UM	PM	148
Oxycodone + Ketoconazole		
DDI CYP2D6 EM/ UGT2B7 EM		296
UM	EM	281
EM	PM	300
UM	PM	308
Oxycodone + Rifampin		
DDI CYP2D6 EM/ UGT2B7 EM		23.6
UM	EM	23.6
EM	PM	23.6
UM	PM	22.3



Oxymorphone AUC for DDI and CYP2D6 / UGT2B7 phenotypes

CYP2D6 / UGT2B7 Phenotypes + Perpetrator		AUC$_{0\text{-}24h}$ (ng.h/mL)
No DDI CYP2D6 EM UGT2B7 EM		2.25
Oxycodone + Quinidine		
DDI CYP2D6 EM/ UGT2B7 EM		0.812
UM	EM	1.6
EM	PM	2.53
UM	PM	3.12
Oxycodone + Ketoconazole		
DDI CYP2D6 EM/ UGT2B7 EM		4.65
UM	EM	5.27
EM	PM	7.46
UM	PM	15.2
Oxycodone + Rifampin		
DDI CYP2D6 EM/ UGT2B7 EM		0.4
UM	EM	0.59
EM	PM	1.03
UM	PM	1.47



- **CYP3A4**-mediated DDIs have the biggest impact on Oxycodone and Oxymorphone exposure.
- **CYP3A4 inhibition** by strong CYP3A4 inhibitors (e.g. Ketoconazole) results in increased oxycodone and oxymorphone exposure. The **increase** in **oxymorphone** exposure is largest (~7-fold) for **CYP2D6 UMs UGT2B7 PMs** when co-administered with **Ketoconazole**.
- **CYP3A4 induction** (by e.g. Rifampin) results in decreased oxycodone and oxymorphone exposure (~6-fold).

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Case Study Highlights

What is already known?

CYP2D6 is an important enzyme for the biotransformation of oxycodone.

What this research adds?

- **CYP2D6**, **CYP3A4**, and **UGT2B7** are important for oxycodone and oxymorphone metabolism.
- **CYP2D6 PMs** will have **little to no oxymorphone** exposure.
- **CYP2D6** phenotypes determine the **type of interaction**, while its **extent** is determined by **UGT2B7 polymorphisms** and **CYP3A4 activity**.
- **CYP2D6 UMs UGT2B7 PMs** (rare in Caucasians) using **CYP3A4 inhibitors** will have the **highest oxymorphone** exposure → unlikely to be a problem.

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