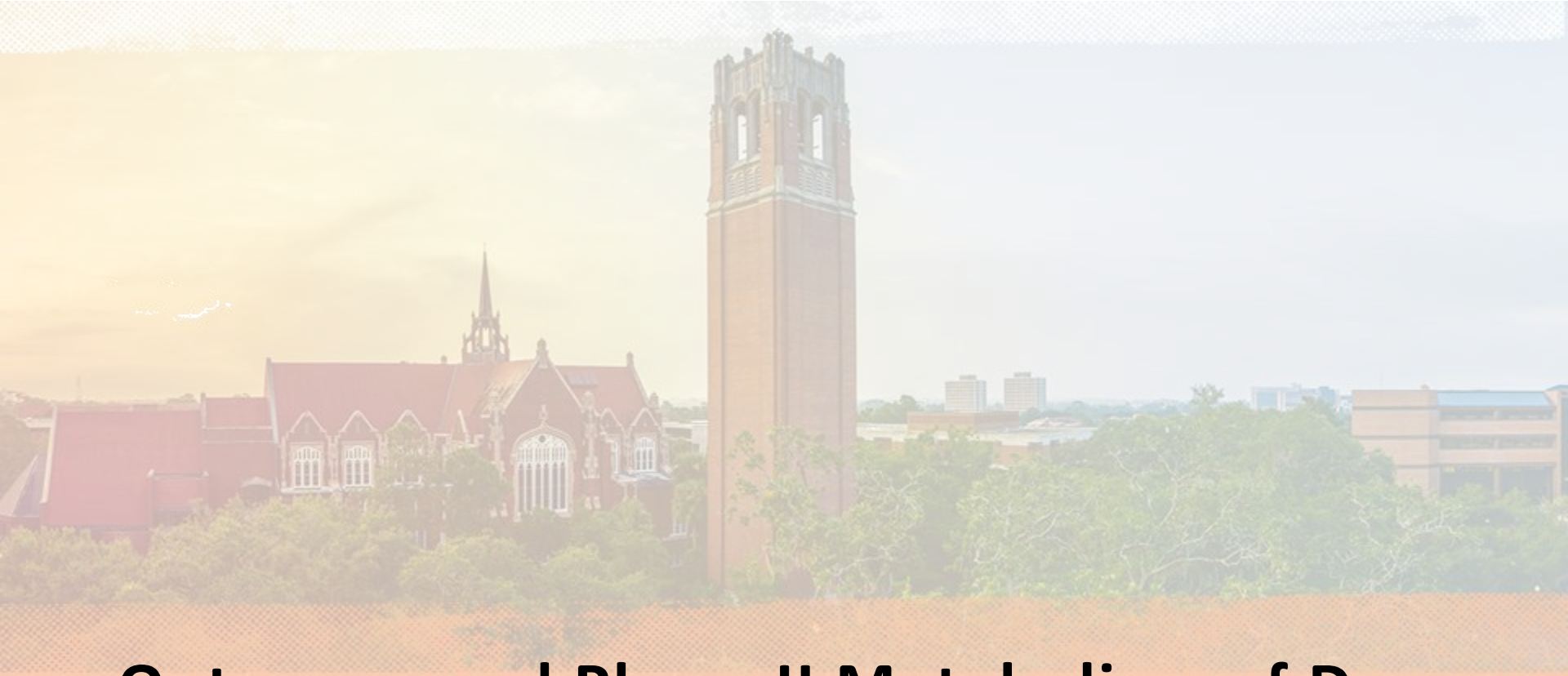

# Ontogeny and Phase II Metabolism of Drugs

**Stephan Schmidt, BPharm, PhD, FCP**
Certara Professor
Associate Professor & Associate Director CPSP
Department of Pharmaceutics
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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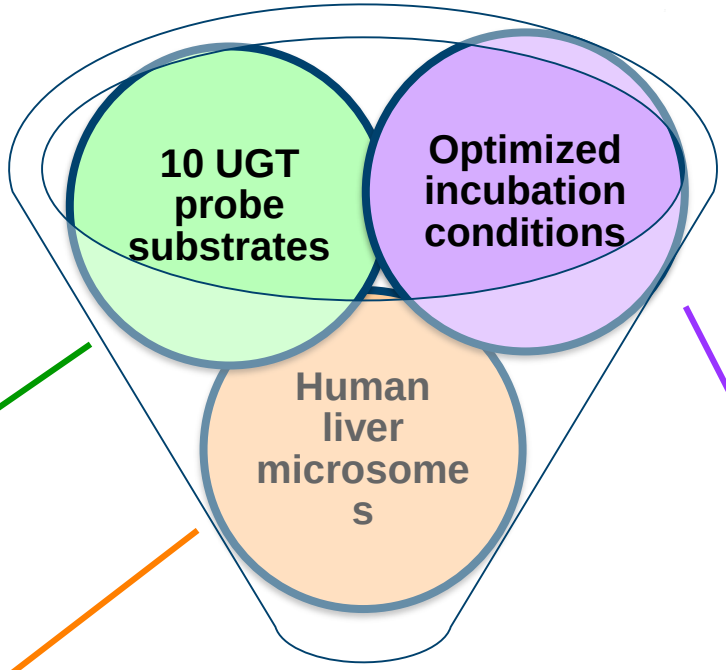
# Challenges of UGT Phenotyping Assays

- **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)

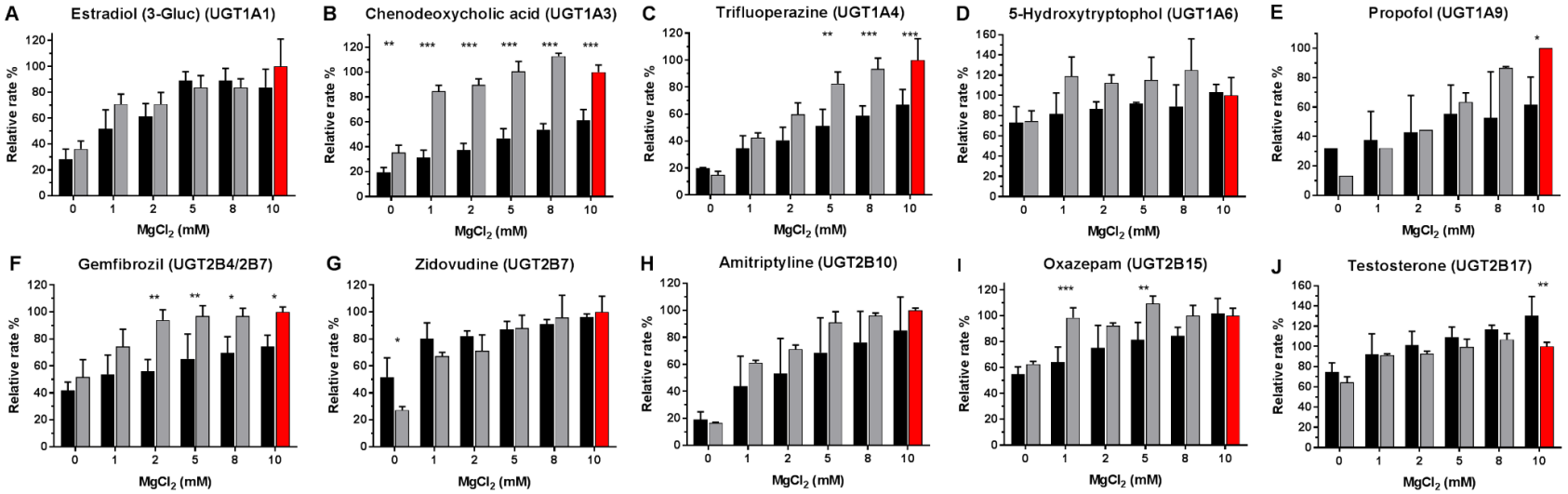
- **150-donor pooled HLM**
  - 1 mg/mL
  - Mixed gender (75 male/75 female)

**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)



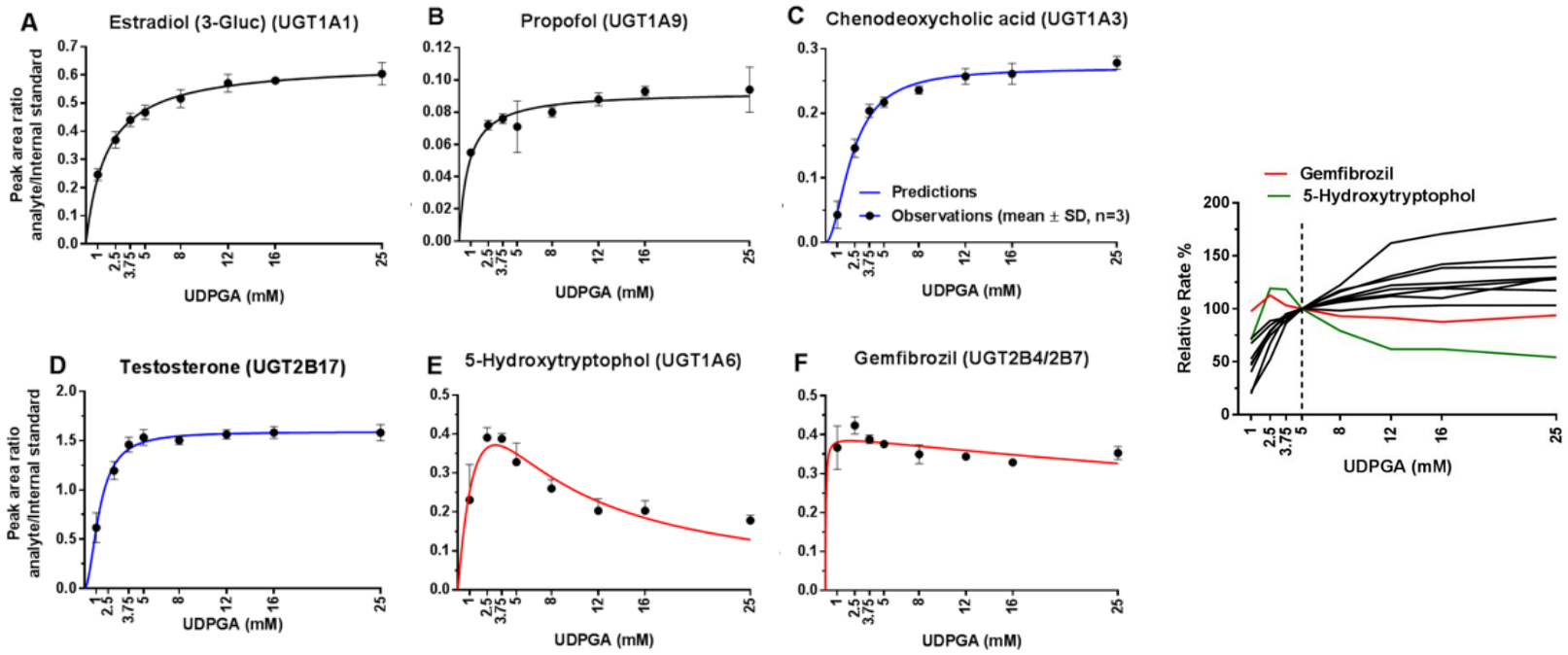

* $p < 0.05$, ** $p < 0.01$, $p < 0.001$

- 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

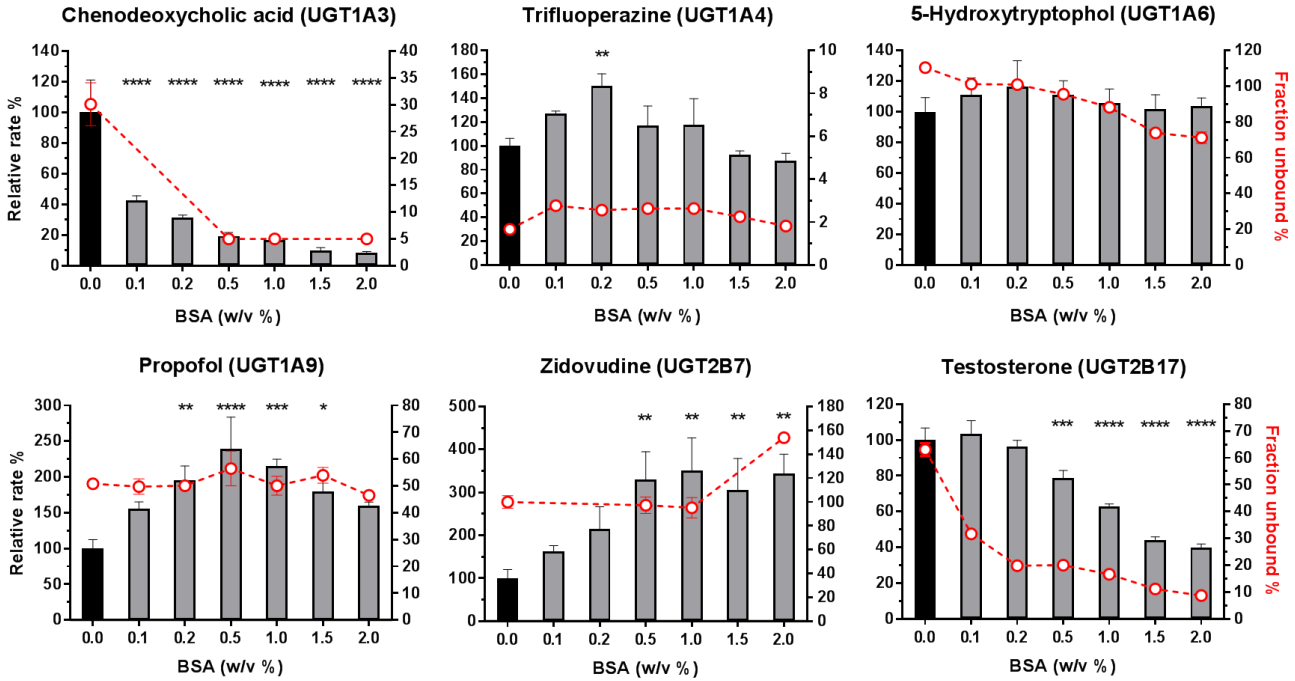



- **Hyperbolic** or **sigmoidal Michaelis-Menten kinetics:** >60% of maximal activity at 5 mM UDPGA
- **Substrate inhibition kinetic** → Decreased glucuronide formation rate above 5 mM UDPGA
- **Optimal** UDPGA concentration of **5 mM**

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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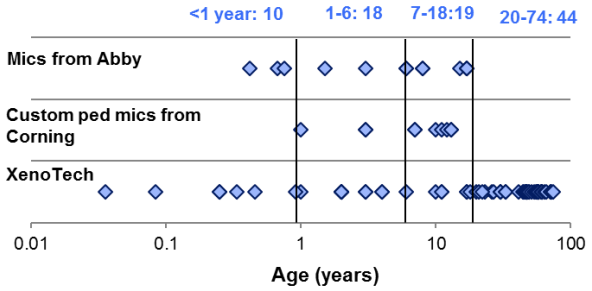
# Characterization of Hepatic UGT Ontogeny

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)

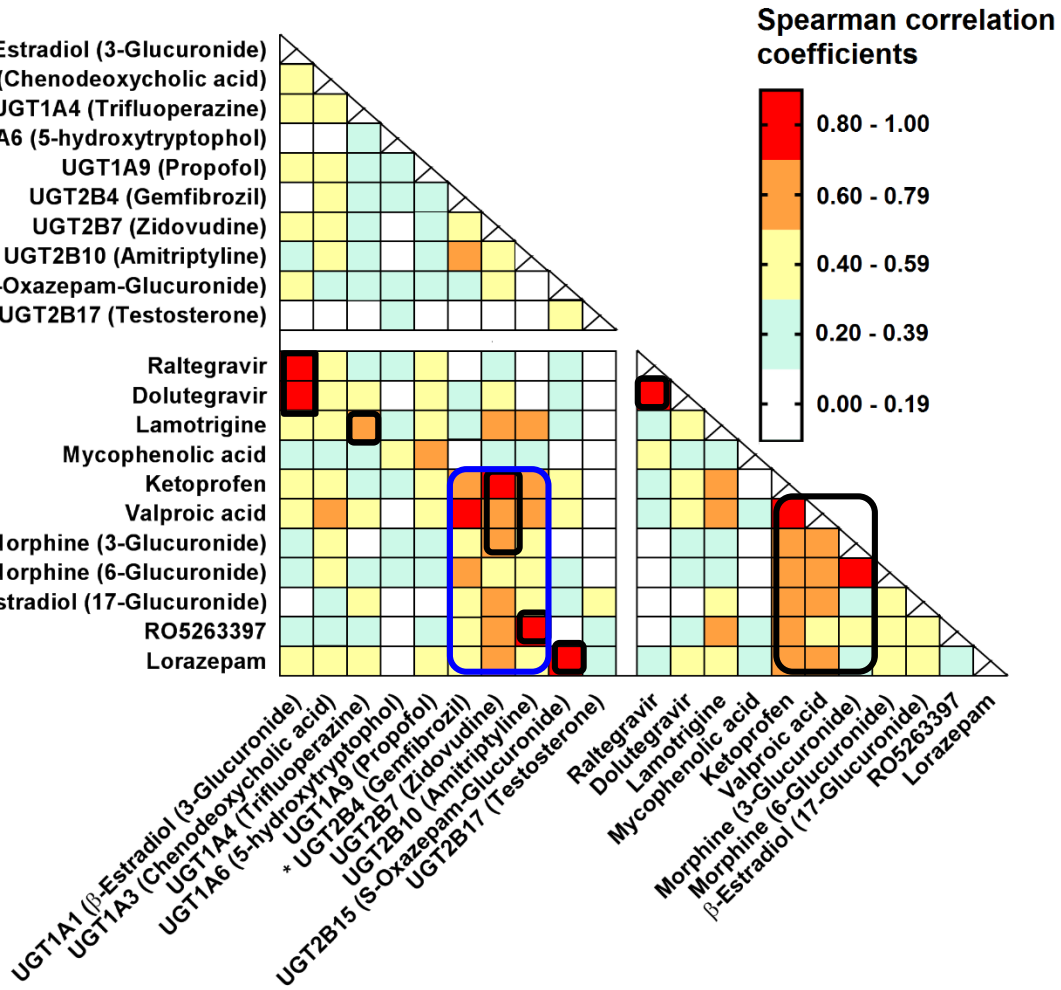
- Quantitative LC-MS/MS MRM-based method
- Optimization of digestion conditions
- Selection of suitable surrogate peptides to avoid interactions with expression measurements
- Protein expression - activity correlations for UGTs and CYPs
- **Manuscript in preparation**

**Manuscript under review with DMD**

# Co-Regulation Between UGT Isoforms

***In vitro*** **probe substrates**

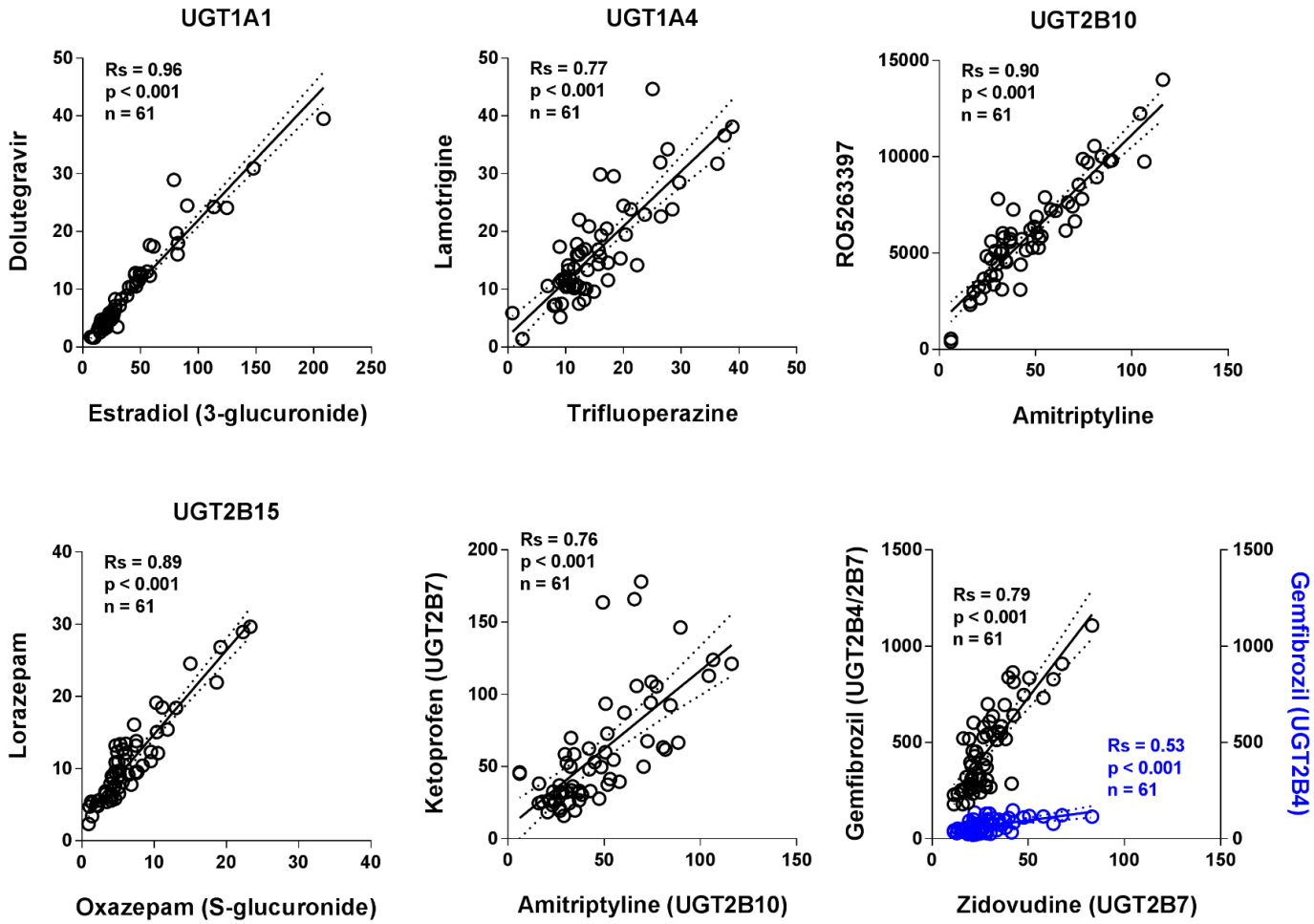
**Additional probe compounds**



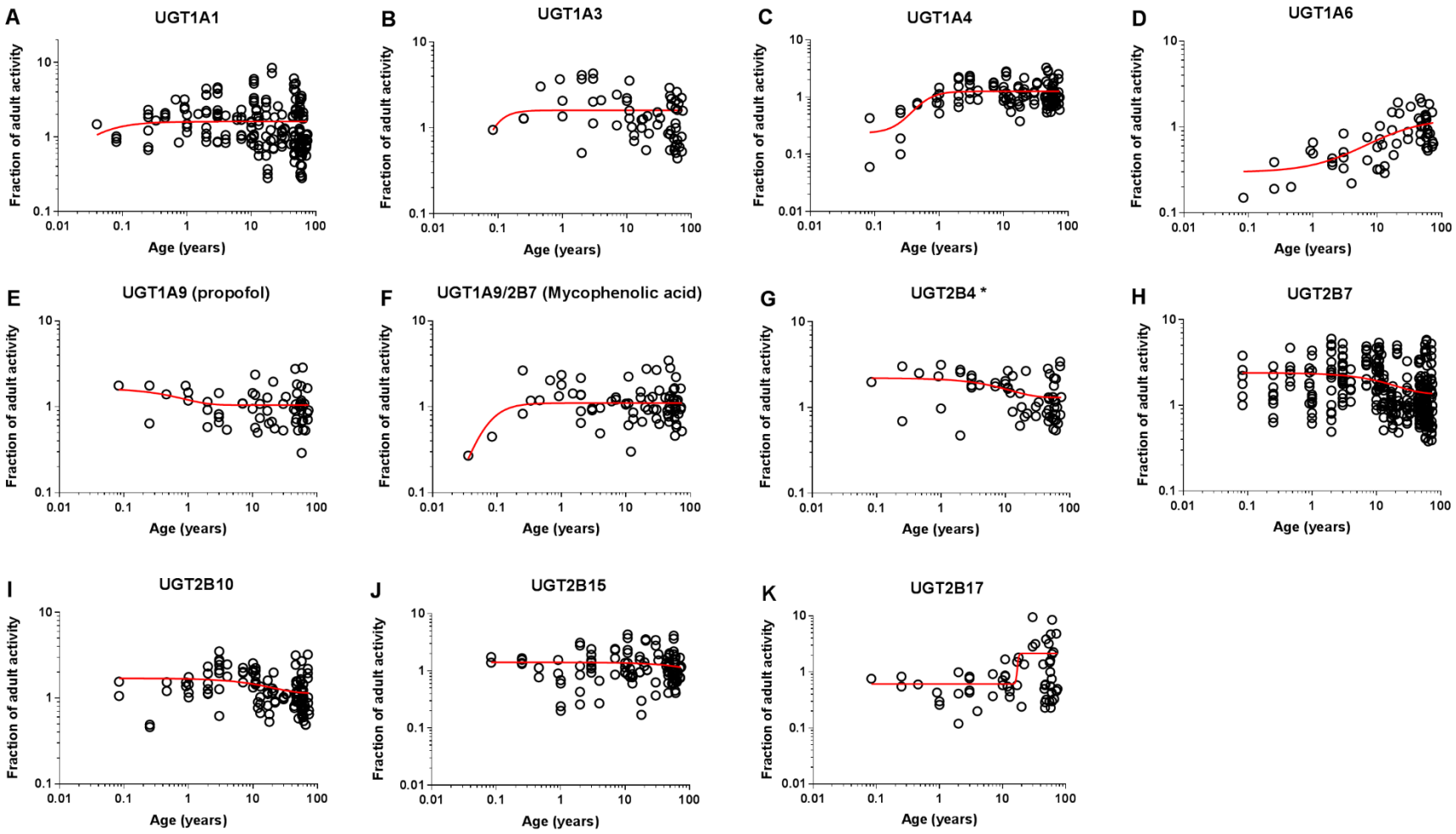

Strong activity - activity correlations across UGT isoforms → Co-regulation

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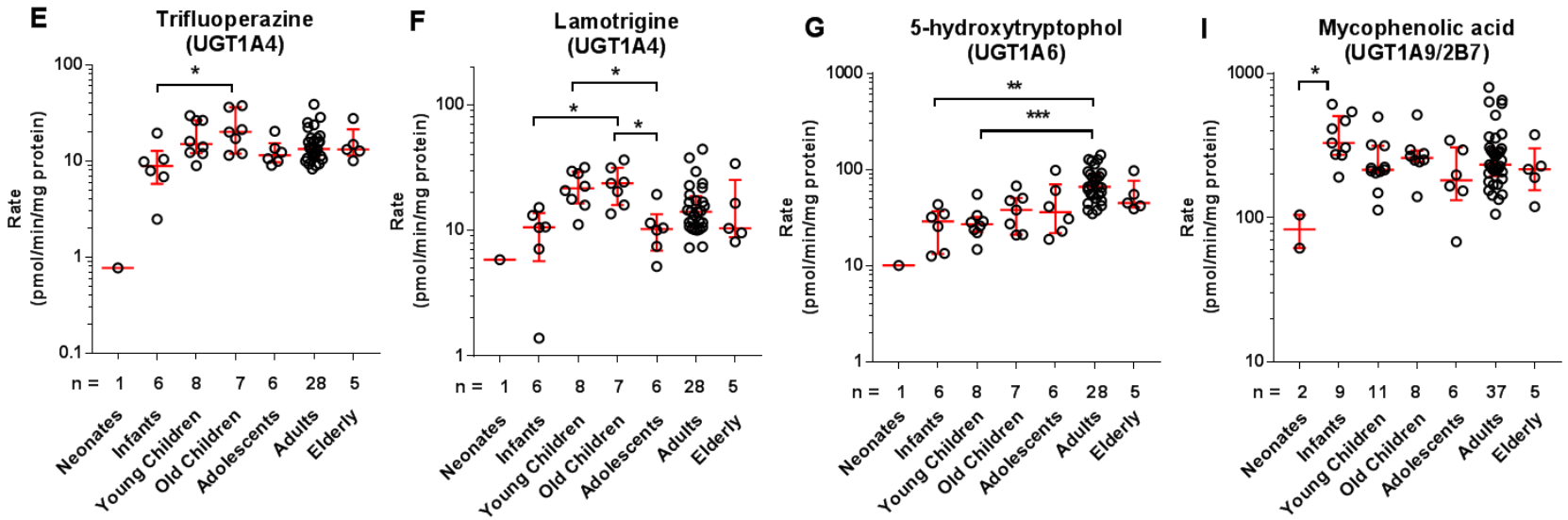
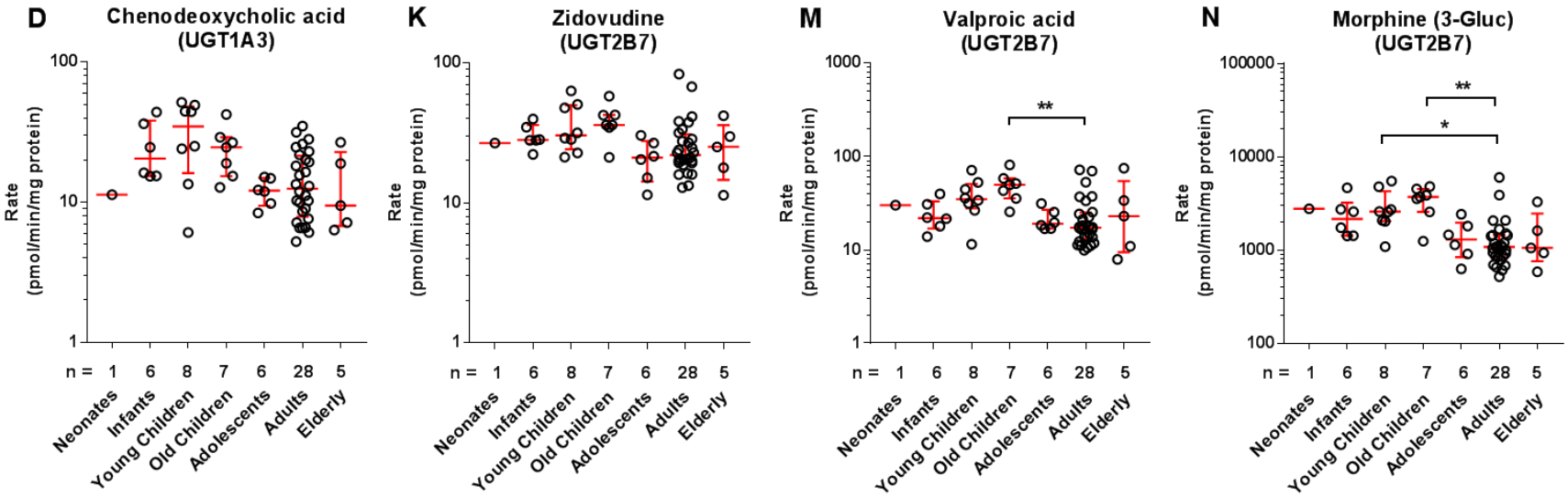
# Evidence of Increased Activity with Age For UGT1A4, 1A6,1A9/2B7




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

- **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)

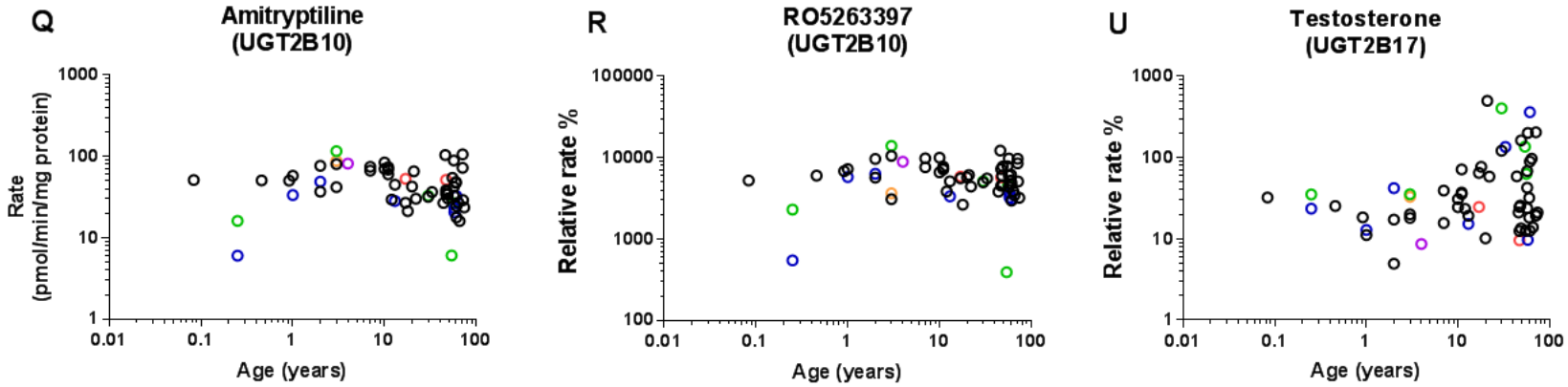
# But Not For UGT1A3 and 2B7

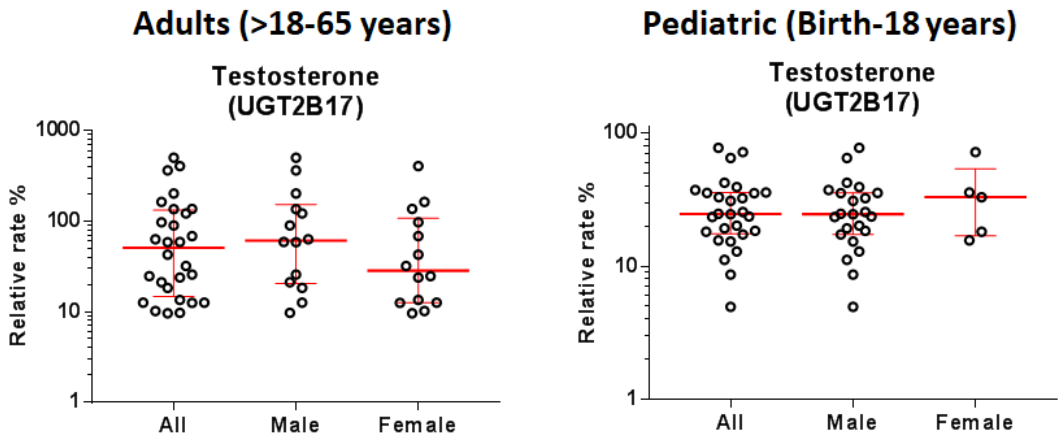



* $p < 0.05$, ** $p < 0.01$

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

# No Sex or Ethnicity-Related Effects



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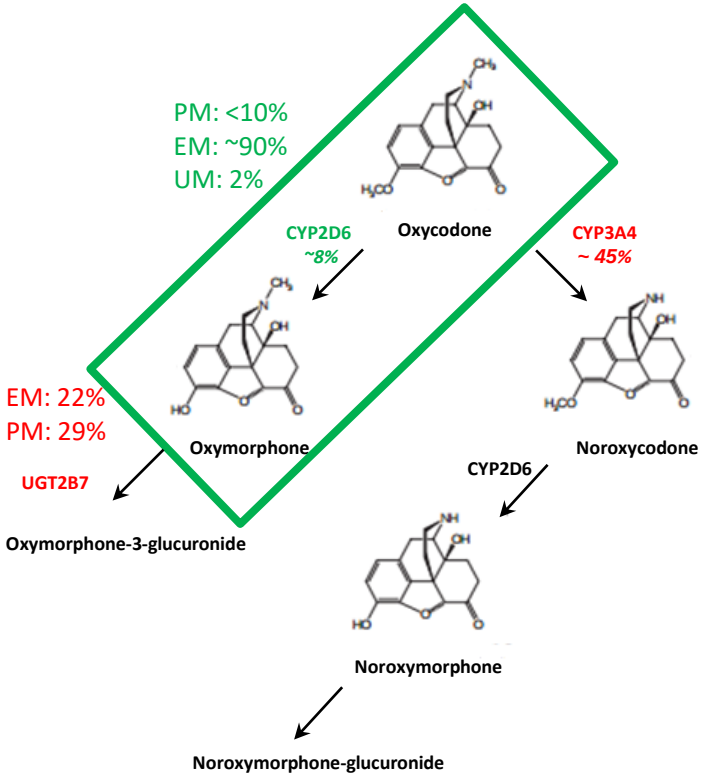
# Interplay Between Phase I and Phase II Metabolism: Oxycodone Case Example

**Getting closer:**

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

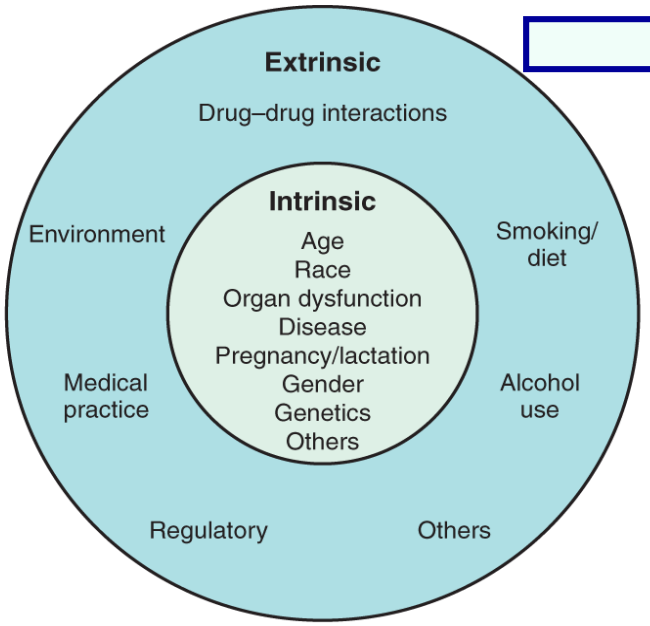
## Reality: Metabolic network

- 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



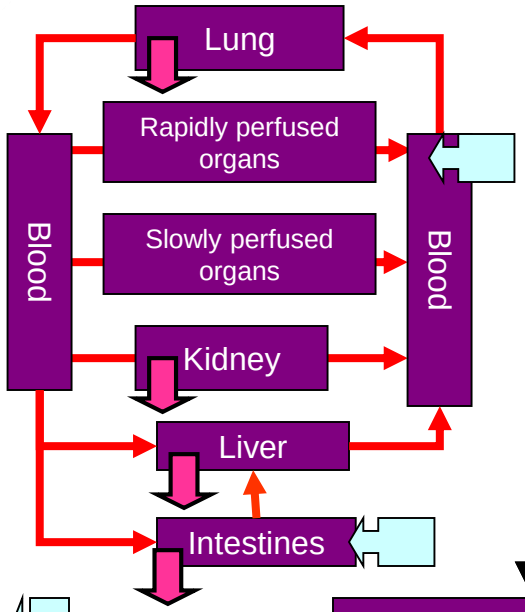

*Huang and Temple, 2008*

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

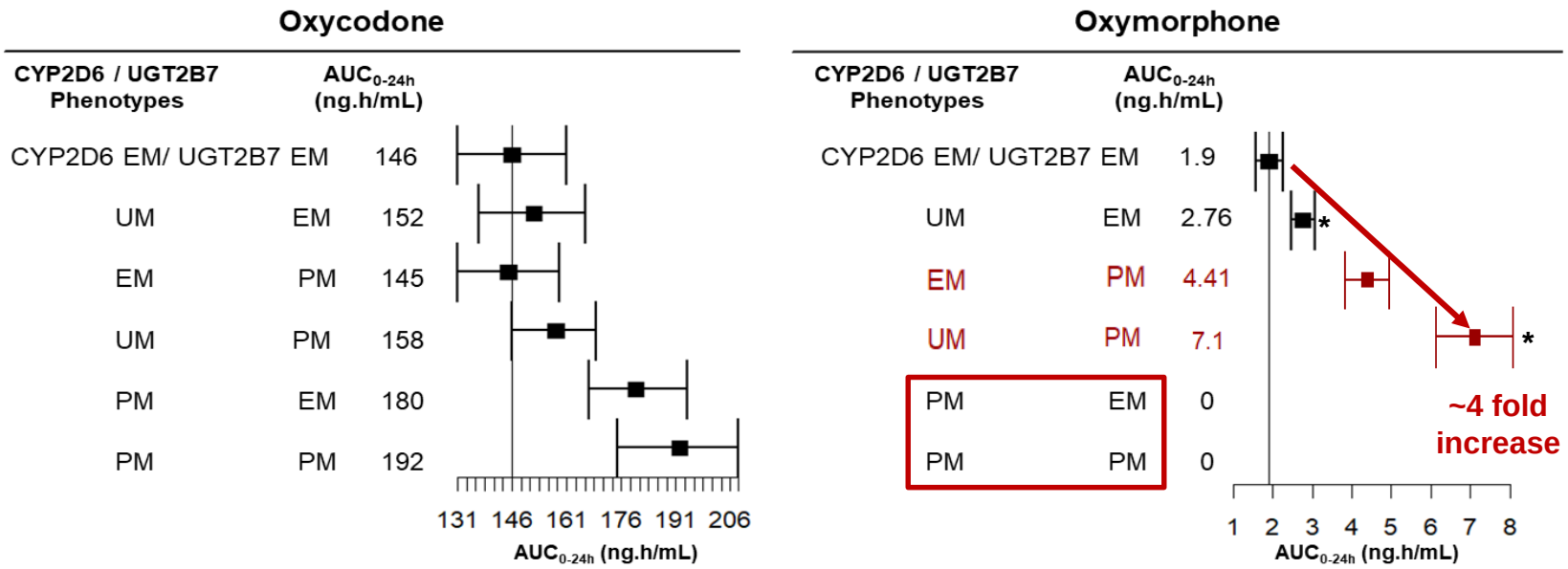
Elimination

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**.

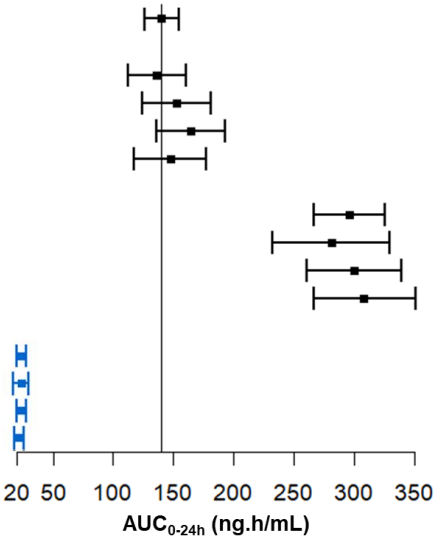
*de Miranda Silva et al. manuscript in preparation*

***Sponsored by the Florida High Tech Council***

# What If – We Have GDIs & DDIs?

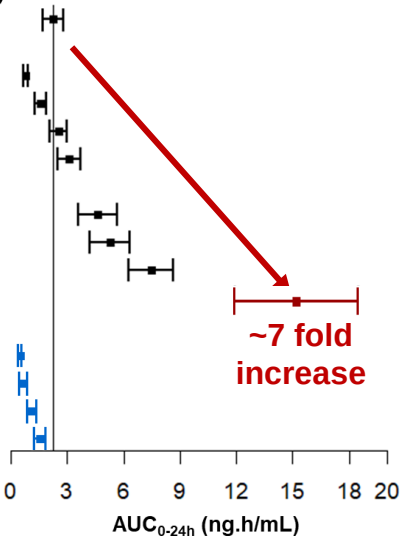
**Oxycodone AUC for DDI and CYP2D6 / UGT2B7 phenotypes**

| 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).

*de Miranda Silva et al. manuscript in preparation*

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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.

*de Miranda Silva et al. manuscript in preparation*

***Sponsored by the Florida High Tech Council***

# Acknowledgements


## University of Florida, FL, USA

Justine Badee

Carolina de Miranda Silva

Naveen Mangal

Lawrence J Lesko

Jacques Turgeon

Veronique Michaud

Valvanera Vozmediano

## University of British Columbia, Ca

Abby C. Collier

## Radmoud University , Nl

Saskia N. de Wildt

## F. Hoffmann-La Roche, Basel, Sw

Neil Parrott

Stephen Fowler

Nahong Qiu

## Genentech, SF, USA

Ryan H. Takahashi

William F. Forrest

## Funding

Roche Postdoc Fellowship Program

Florida High Tech Council


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