Genes and the Environment: How they Affect Drug Metabolism and Response

A Study of CYP3A Enzymes

Ken Thummel, Department of Pharmaceutics
University of Washington

Rawls-Palmer Award Lecture – 03/10/16
1980: Age 24 and Considering Career Paths
Robert Vestal, MD

- **1981**: Introduction to clinical pharmacology and pharmacokinetics at Boise VA
- Theophylline – drug interactions

Advised me to attend graduate school – Pharmaceutical Sciences at UW
Early Training

• Graduate School (John Slattery)
  – DDIs and acetaminophen hepatotoxicity

• Post-doctoral Fellowship (John Schenkman)
  – cytochrome P450 biochemistry

Evolving research interest:
  – Mechanism(s) of inter-individual differences in CYP3A-dependent drug metabolism and drug response
Hepatic CYP3A (brown) is predominantly pericentral; inter-individual differences reflect a variable number of CYP3A (+) pericentral hepatocytes.

(Thummel et al, JPET, 1994)

CYP3A (+) expression is confined to the “absorptive” enterocytes of the mucosal villi and shows high inter-individual variability.

(Courtesy of Paul Watkins)
Sources of Interindividual Variability in P450 Expression

Adapted from E. Vesell, 1981
Identify a Selective, Sensitive Dual-Purpose *In Vitro* and *In Vivo* CYP3A Probe

- Cleared by oxidative metabolism, catalyzed exclusively by CYP3A4/5/
- Intermediate clearance (~240 mL/min), 30-45% oral bioavailability; very low renal clearance (< 1%)
- High plasma protein binding (~98%)
- 1’-OH MDZ formation dominates *in vivo*

*Thummel et al. JPET, 1994*
In Vitro to In Vivo Prediction of MDZ CL_H

\[
\frac{V_{\text{max}}(\text{liver})}{K_m} = Cl_{\text{int}}'(\text{liver}) \quad \frac{(f_u \cdot Cl_{\text{int}}') \cdot Q_H^P}{(f_u \cdot Cl_{\text{int}}') + Q_H^P}
\]

**TABLE 3**

Predicted hepatic 1'-OH MDZ formation clearance

<table>
<thead>
<tr>
<th>Subject</th>
<th>Donor Body Weight</th>
<th>(Q_H^B)</th>
<th>(Q_H^P)</th>
<th>Predicted (Cl_H^{1'-OH})</th>
<th>Observed (Cl_T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-2</td>
<td>80</td>
<td>1.73</td>
<td>1.21</td>
<td>0.28</td>
<td>0.67</td>
</tr>
<tr>
<td>R-6</td>
<td>80</td>
<td>1.73</td>
<td>1.12</td>
<td>0.21</td>
<td>0.15</td>
</tr>
<tr>
<td>R-7</td>
<td>64</td>
<td>1.38</td>
<td>0.99</td>
<td>0.44</td>
<td>0.68</td>
</tr>
<tr>
<td>R-19</td>
<td>73</td>
<td>1.58</td>
<td>1.07</td>
<td>0.58</td>
<td>0.68</td>
</tr>
<tr>
<td>R-22</td>
<td>106</td>
<td>2.29</td>
<td>1.60</td>
<td>0.58</td>
<td>0.79</td>
</tr>
</tbody>
</table>

\(a\) Calculated as the product: body weight (kg) \(\times\) 0.0216 liter/kg.

\(b\) Calculated as the product: \((1 - \text{Hct}) \times (Q_H^B)\).
Midazolam Predicts the Disposition of Alfentanil when given with CYP3A Inducers and Inhibitors

Under control conditions, the clearance of alfentanil is predicted by the clearance of midazolam \( (r^2 = 0.60) \)

Drugs were co-administered with rifampin, troleandomycin or grapefruit juice; rank order effect in different individuals is predictive.

Both midazolam and alfentanil undergo intestinal metabolism and both are CYP3A5 substrates.

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*Kharasch et al., CPT, 2004; Proc Assoc Univ Anesthesiologists, 2005*
Midazolam is a Sensitive Probe for Characterizing CYP3A Inhibition

- Modeling dose-dependent inhibition of MDZ oral clearance by itraconazole.

Ian Templeton, Nina Isoherranene et al, CPT, 2010
Classifying Inhibitors of CYP3A Based on Inhibition of Oral Midazolam Elimination

UW Drug Interactions Database
Paul Watkins, MD

- Characterization of intestinal CYP3A4
- Development of ERMBT

Advised that the most interesting CYP3A science was going to be found in the intestine.
Population Distribution of Midazolam CL/F

MDZ CL/F depends on:
- Hepatic CYP3A
- Intestinal CYP3A

Yvonne Lin et al., Pharmacogenetics, 2001
Oral Midazolam Disposition

\[ [P]_{art,ss} = \frac{\text{Dose Rate} \cdot \text{Bioavailability}}{\text{Clearance}} \]

Transporter

Enzymes

Liver

M \leftrightarrow P \leftrightarrow P

Bile

Intestine

P \leftrightarrow P

Gut Lumen

Kidney

M \leftrightarrow P

Urine

Target Cells

Heart

Drug (P)

[p]_{art}
**In Vivo Intestinal Midazolam Metabolism**

- 1 mg IV or 2 mg PO during anhepatic phase of a liver transplant operation *(Mary Paine et al., CPT, 1996)*
## Intestinal Midazolam Extraction: Anhepatic Patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Intravenous Dose E (%)</th>
<th>Intraduodenal Dose E’ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>11.1</td>
<td>40.5</td>
</tr>
<tr>
<td>4</td>
<td>25.6</td>
<td>45.1</td>
</tr>
<tr>
<td>6</td>
<td>8.5</td>
<td>57.1</td>
</tr>
<tr>
<td>7</td>
<td>-2.6</td>
<td>58.6</td>
</tr>
<tr>
<td>10</td>
<td>-1.8</td>
<td>13.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean</th>
<th>8.2</th>
<th>Mean</th>
<th>43.0*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.D.</td>
<td>11.5</td>
<td>S.D.</td>
<td>18.1</td>
</tr>
</tbody>
</table>

*Mary Paine et al., CPT, 1996*
CYP3A Substrates Suspected of Undergoing Extensive First-pass Gut Metabolism

All have an oral bioavailability of < 50%.
Identifying Sources of Variable CYP3A Expression

- Exercise
- Sex
- Age
- Pregnancy
- Smoking
- Nutrition
- Alcohol
- Drugs
- Occupational Exposure
- Hepatic Function
- Renal Function
- G.I. Function
- Cardiovascular Function
- Circadian Rhythm
- Infection
- Starvation
- Exercise
- Pregnancy
- Hormones
- Cytokines
- Paracrine Factors
- Transcription Factors
- Genetic Variability

~90%?

Adapted from E. Vesell, 1981
Erin Schuetz, PhD

• St Jude Children’s Research Hospital
  Sabbatical - 2000

Opportunity for research on the regulation of CYP3A4 by vitamin D and CYP3A gene variation
Regulation of Intestinal CYP3A4 by Vitamin D

LS180 Cells: A Model for Human Enterocytes

- LS180 cells contain relative high expression of hPXR, VDR, CYP3A4 and TRPV6, compared to Caco-2 cells (low PXR and minimal basal CYP3A4)

Very low dose $1,25(OH)_2D_3$ induces all 3 VDR gene targets.

Rifampin is a selective CYP3A4 inducer.

Vitamin D

Other Tissues
- Skin
- Immune cells
- Vasculature
- Colon

CYP27B1

[DBP] 1,25(OH)₂D₃

Intracrine
- Immune cells
  - Induces cell differentiation

Kidney
- Increases reabsorption of Ca²⁺ and Pi

Endocrine
- Small Intestine
  - Increases absorption of Ca²⁺ and Pi, and CYP3A enzyme and activity

Bone
- Mineralization and remodeling

Adapted from Deeb et al, 2007
Heterogeneous expression pattern consistent with primary site of calcium absorption.

Heterogeneous Distribution of CYP3A4 Protein in Small Intestine

A common VDR signaling pathway for both calcium transport proteins (TRPV6, calbindin D9K) and CYP3A4?
VDR Expression is Relatively Constant Along the Length of the Small Intestine

What about delivery of the ligand?
Hypothesis: Biliary Vitamin D Conjugates Regulate Intestinal CYP3A4 Expression

Major Metabolic Pathways of 25OHD$_3$

Kidney

Liver

25OHD$_3$-gluc

UGT1A4/1A3

SULT2A1/1A1

CYP27B1 → 1α,25(OH)$_2$D$_3$

CYP24A1 → 24R,25(OH)$_2$D$_3$

CYP3A4 → 4β,25(OH)$_2$D$_3$

(Zhican Wang et al, Mol Pharmacol, 2012)

(Zhican Wang et al, Endocrinol, 2014)

(Tim Wong et al, In Preparation)
\[25\text{OHD}_3\] Conjugates in Human Plasma

**Conditions**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>[25\text{OHD}_3] nM</th>
<th>24R,25(OH)\textsubscript{2}D\textsubscript{3} nM (M/P ratio)</th>
<th>[25\text{OHD}_3]-3-sulfate nM (M/P ratio)</th>
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<tr>
<td>Healthy Control (n = 21)</td>
<td>52.3 ± 25.2</td>
<td>3.8 ± 2.8 (0.11 ± 0.02)</td>
<td>46.2 ± 21.1 (1.07 ± 0.73)</td>
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<td>Liver Disease (n = 20)</td>
<td>40.6 ± 21.8</td>
<td>2.9 ± 1.9 (0.11 ± 0.03)</td>
<td>42.5 ± 30.2 (1.33 ± 0.95)</td>
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<td>Kidney Disease (n = 15)</td>
<td>26.5 ± 18.5</td>
<td>1.1 ± 0.4 (0.05 ± 0.02)</td>
<td>28.0 ± 19.0 (1.06 ± 0.39)</td>
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**Solid line:** m/z 575 & 479  
**Dash line:** m/z 581 & 485 (I.S.)

**Mean concentrations:**
- 25\text{OHD}_3\textsubscript{3}-glucuronide, ~2 nM
- 25\text{OHD}_3\textsubscript{3}-sulfate, ~40 nM

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**Graph:**
- d6-25\text{OHD}_3\textsubscript{3}-25-glu
- d6-25\text{OHD}_3\textsubscript{3}-3-glu
- d6-25\text{OHD}_3\textsubscript{3}-sulfate
- 25\text{OHD}_3\textsubscript{3}-sulfate
- 25\text{OHD}_3\textsubscript{3}-3-glu
- 25\text{OHD}_3\textsubscript{3}-25-glu

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Circulating $25\text{OHD}_3$ Conjugates Tightly Bind to Vitamin D Binding Protein (DBP)

Both conjugates undetected in urine.
25OHD Glucurononides in Human Bile

- Diluted human bile
- Hexane extraction
- Aqueous solution
  - Buffered, sodium acetate (pH 4.0)
  - Mixture
  - Dried
  - Derivatization
  - ESI-LC-MS/MS

Mass spectra of 25OHD-3-glucuronide in human bile

- A) m/z 734 -> 298 (bile extracts)
- B) m/z 734 -> 298 (3-glucuronide)
- C) m/z 734 -> 298 (25-glucuronide)

25OHD$_3$-3-O-Sulfate in Human Bile

Precursor ion Scan: 25OHD-3-S-PTAD

- similar daughter ion scans for bile extract and standard (not shown) suggest the presence of the 3-sulfate metabolite in bile.

Chunying. Gao et al, Unpublished
Identification of Hepatic Vitamin D Conjugate Transporters

**Uptake** of 25(OH)D$_3$-3-sulfate: OATP2B1

**Efflux** of 25(OH)D$_3$-3-sulfate: BCRP

**Efflux** of 25(OH)D$_3$-3-glucuronide: MRP2, MRP3

Also expressed on the apical surface of enterocytes.

*Chunying Gao*

*Qingcheng Mao*

*Unpublished Results*
Hypothesis: Biliary Vitamin D Metabolites Regulate Intestinal CYP3A4 Expression

CYP3A4

Dose

Absorption

Portal vein

Liver

Gut lumen

Bioavailability

Bile

e.g.

25OHD-3-glucuronide

25OHD-3-sulfate

1α,25(OH)₂D-25-glucuronide

Gut lumen

To faeces

Metabolism

CYP3A4

Vitamin D conjugates?

Vitamin D conjugates?
Cells were treated with 25OHD₃ or 25OHD₃-S (2 µM, 4 µM) for 24 hrs, cell lysates were collected for mRNA analysis. The culture media were collected for LC-MS/MS analysis.

Zhican Wang
Brian Chapron
Unpublished
Sources of Variable P450 Expression

Genetic Template

- Smoking
- Age
- Sex
- Pregnancy
- Exercise
- Starvation
- Infection

- Nutrition
- Alcohol
- Drugs
- Occupational Exposure
- Hepatic Function
- Renal Function
- G.I. Function
- Cardiovascular Function
- Circadian Rhythm

Variable CYP3A Gene Regulation
- Hormones
- Cytokines
- Paracrine Factors
- Transcription Factors
- Genetic Variability
  ~90%?

Adapted from E. Vesell, 1981
Human \textit{CYP3A} Gene Locus on Chromosome 7q

Adapted from: Finta & Zaphiropoulos; Gene 260:13-23, 2000

- \textit{CYP3A4} > \textit{CYP3A5} > \textit{CYP3A7} are the most important for drug metabolism in the adult

- all three \textit{CYP3A} enzymes are subject to genetic and environmental sources of variability
  - \textit{CYP3A5} exhibits the most obvious polymorphic behavior
Immunodetection of Hepatic CYP3A5

Analysis of microsomes from different human livers (A-H) indicates marked inter-individual variability in specific enzyme content

*Mary Paine* et al., JPET, 1997
CYP3A5*3 Variant Allele

CYP3A5*1 (A) → CYP3A5*3 (G)

CYP3A5 protein
(wt-CYP3A5 mRNA)

truncated, inactive protein
(SV1-CYP3A5 mRNA)

Kuehl, Yvonne Lin et al., Nature Genetics, 2001; Yvonne Lin et al., Mol Pharmacol, 2002
Tacrolimus is used to prevent grafted organ rejection (immune suppressant)
CYP3A5 is one of 2 enzymes (also CYP3A4) that metabolically clear tacrolimus from the body
CYP3A5 makes all 4 primary metabolites, but preferentially the major one (13-DMT), 12-HT and, 31-DMT
## Contribution of CYP3A5 to Hepatic Tacrolimus Metabolism

<table>
<thead>
<tr>
<th></th>
<th>13-DMT Formation</th>
<th>Human Liver Microsomes</th>
<th>Tacrolimus Disappearance (nmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP3A4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_m$ ($\mu$M)</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{\text{max}}$ (nmol/min/nmol)</td>
<td>8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{int}}$ (ml/min/nmol)</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CYP3A5</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_m$ ($\mu$M)</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{\text{max}}$ (nmol/min/nmol)</td>
<td>17.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{int}}$ (ml/min/nmol)</td>
<td>82</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The CYP3A4 content for the 10 matched microsomal preparations represented in each group was equivalent. The nominal initial tacrolimus conc was 0.2 $\mu$M; unbound conc determined after measurement of nonspecific binding.

*Yang Dai et al., DMD, 2006*
CYP3A5*1 Affects Intra-Renal Tacrolimus Accumulation

Semi-Physiological Renal Compartmental Model of Tacrolimus Disposition

Simulated renal tacrolimus exposure for CYP3A5 expressors was 53% of that for CYP3A5 nonexpressors

Ben Zheng et al, CPT, 2012
Wylie Burke, MD, PhD

- Director, Center for Genomics and Healthcare Equality, UW

Pursue genomic research for those not represented in the literature – AI/AN communities in Alaska and Montana
NWA-PGRN Principal Investigators
Ken Thummel & Wylie Burke

Collaborative Site Lead Investigators

- Allan Rettie
  University of Washington
- Bert Boyer
  Center for Alaska Native Health Research/
  Yukon-Kuskokwim Health Corporation
- Denise Dillard
  Southcentral Foundation, Anchorage
- Erica Woodahl
  University of Montana, Missoula/
  Confederated Salish-Kootenai Tribes
- Denise Boudreau
  Group Health Research Institute
Challenge of Conducting Genetic Research with Indigenous Communities

• Tribes perceive that past health research has provided little benefit to indigenous populations

• Tribes often mistrust academic research due to historical and current trauma inflicted in the name of “knowledge for the greater good”, and unequal control over the research process and data or samples
Concept of Collaborative Stewardship

• Mutual recognition among stakeholders
  – Listening to each other’s voices

• Dialogue
  – Sustained engagement
  – Accept and work through conflicts

• Negotiate accommodation
  – “What touches all should be agreed to by all” (James Tully)

Wylie A, Promise and perils of an ethic of stewardship, in Embedding Ethics, Eds Meskell & Pels 2005
The Yup’ik People of Alaska

- Communities along the Yukon-Kuskokwim Delta
- >20,000 indigenous people across 50 remote Alaskan communities
- Traditional diet is high in fish and marine animals that are rich in ω-3 polyunsaturated fatty acids and vitamin D₃
- Living at 60° 47’ N, they experience significant seasonal changes in sunlight exposure and vitamin D₃ synthesis
Recruited 1000 Yup’ik study participants

- Collect 5 ml blood: fractionate for DNA, plasma, RBC
- Distribute samples to UA Fairbanks, UW Medicinal Chemistry, UW Laboratory Medicine, UW Genome Sciences

Study Design

Measure vitamin D status (serum 25OHD$_3$)

Determine association with:

Age, gender, BMI, CYP2R1, DHCR7 and DBP genotypes, dietary $\omega3$ PUFA biomarker ($\delta^{15}$N), season of blood draw
Serum 25(OH)D$_3$ Concentrations

Alie Fohner et al, J Nutrition, 2016
Distribution of Serum \(25(OH)D_3\) Level by Age
Assessment of Dietary ω3 PUFA Intake – $^{15}$N Enrichment ($\delta^{15}$N)

- Continuous-flow isotope mass spectrometry
- Surrogate marker of ω3 PUFA consumption
- Marine environments have more $^{15}$N → reflects the length of food chain in predatory fish

$\delta^{15}$N = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \times 1000‰

- $R = \frac{^{15}\text{N}/^{14}\text{N}}$
- Standard = atmospheric $\text{N}_2$, where % abundance of $^{15}\text{N} = 0.37$

*J Clin Nutr. 2009*
Correlation of 25(OH)D₃ with log(δ¹⁵N)
Sinusoidal Model of Annual Variation

Alie Fohner et al, J Nutrition, 2016

- 33 years or older
- Younger than 33 years
- 20 ng/ml (Insufficiency by Institute of Medicine standards)
### Unrelated Subset Multiple Regression

<table>
<thead>
<tr>
<th>Covariate</th>
<th>N</th>
<th>Significance in full model</th>
<th>Variability explained (R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully adjusted model</td>
<td>526</td>
<td>p &lt; 0.001</td>
<td>(0.528)</td>
</tr>
<tr>
<td>Age (younger vs older than 33)</td>
<td></td>
<td>p &lt; 0.001</td>
<td>(0.365)</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td>p &lt; 0.001</td>
<td>(0.091)</td>
</tr>
<tr>
<td>Log₁₀(δ¹⁵N value)</td>
<td></td>
<td>p &lt; 0.001</td>
<td>(0.205)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>p = 0.007</td>
<td>(0.00)</td>
</tr>
<tr>
<td>Village location (Coastal vs Inland)</td>
<td></td>
<td>p &lt; 0.001</td>
<td>(0.063)</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>p = 0.041</td>
<td>(0.006)</td>
</tr>
<tr>
<td>CYP2R1 rs11023374</td>
<td></td>
<td>p = 0.016</td>
<td>(0.011)</td>
</tr>
<tr>
<td>GC rs4588 (TA haplotype)</td>
<td></td>
<td>p &lt; 0.001</td>
<td>(0.028)</td>
</tr>
<tr>
<td>Age and log₁₀(δ¹⁵N value)</td>
<td></td>
<td></td>
<td>(0.386)</td>
</tr>
</tbody>
</table>

- 58% of the variability explained by demographic, diet, season and genetic factors; what explains for the rest?
Demographic factors, concomitant medications, diet, season, and genetic variation in CYP3A genes and vitamin D regulatory genes are all likely to contribute to inter-individual differences in CYP3A function.

Precision Medicine testing must capture both **genomic** and **environmental** variation to fully deliver on its promise.
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Scarlett Hopkins
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