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

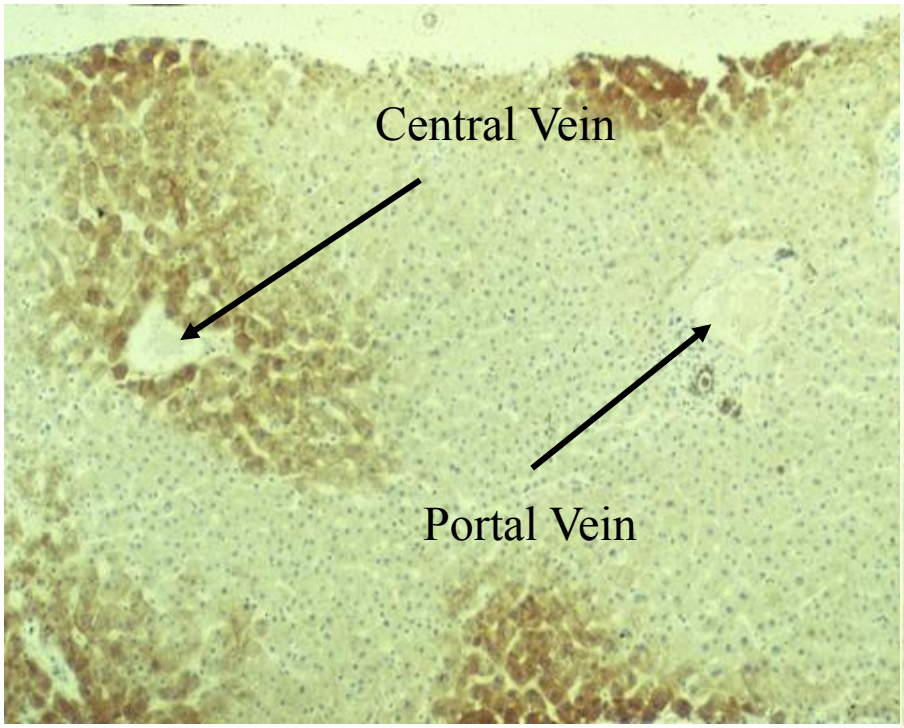
Early Training

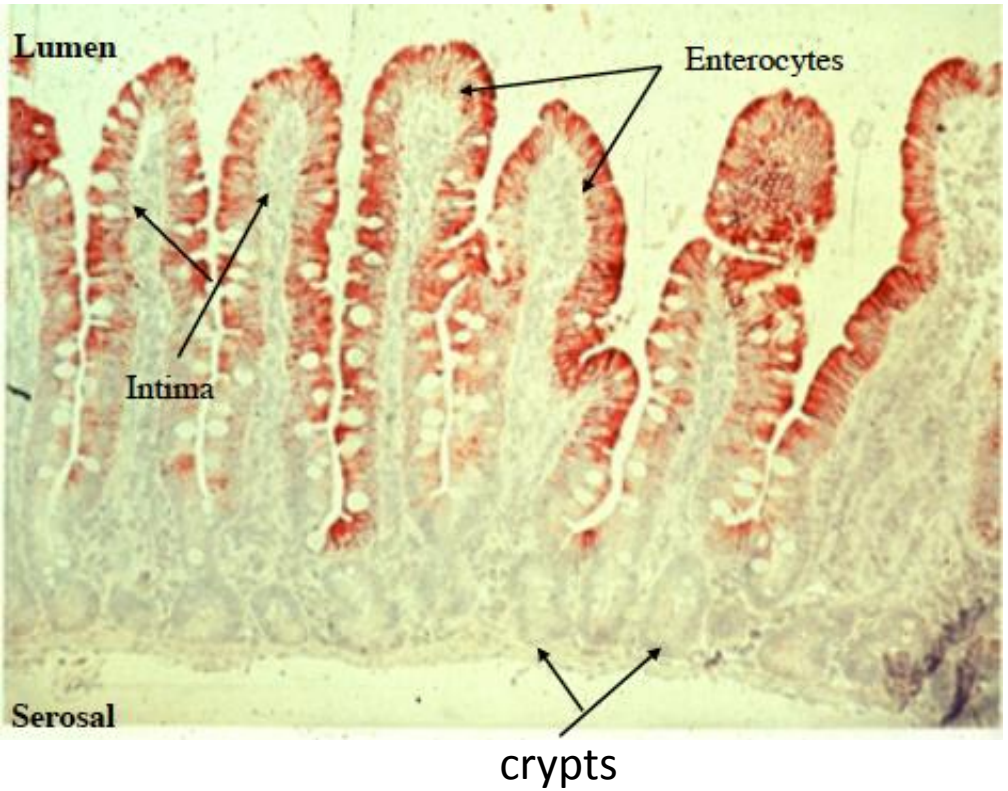
- Graduate School (John Slattery)
  - DDIs and acetaminophen hepatotoxicity
- Post-doctoral Fellowship (John Schenkman)
  - cytochrome P450 biochemistry

Evolving research interest:

- Mechanism(s) of inter-individual differences in CYP3A-dependent drug metabolism and drug response

Hepatic and Intestinal CYP3A





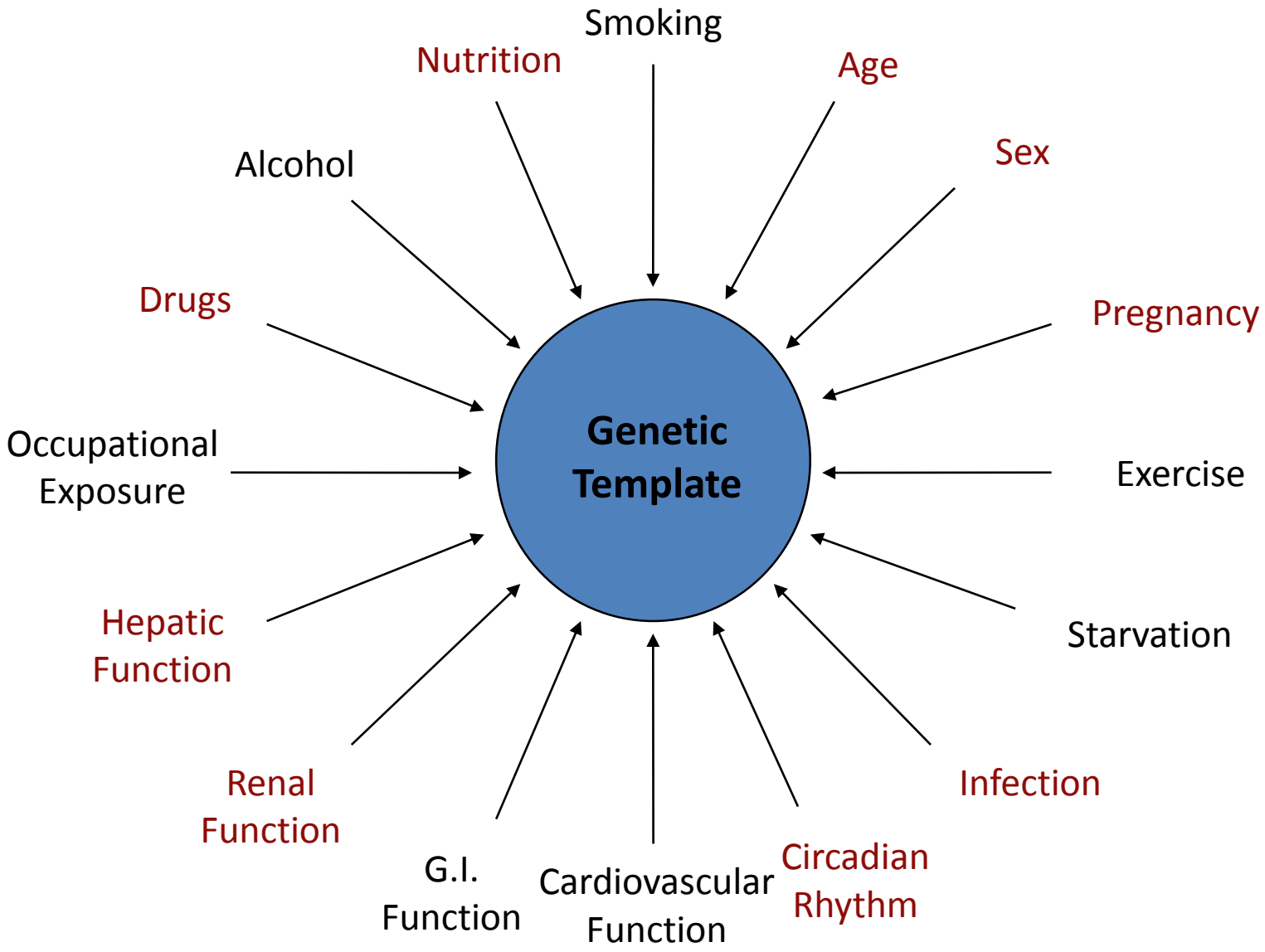
Hepatic CYP3A (brown) is predominantly pericentral; inter-individual differences reflect a variable number of CYP3A (+) pericentral hepatocytes.

(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)

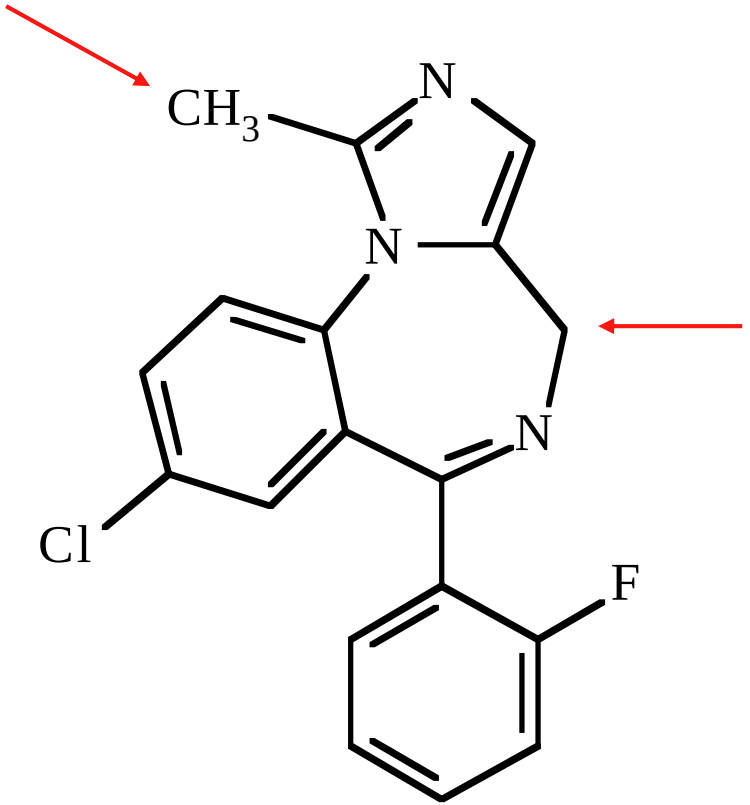
Sources of Interindividual Variability in P450 Expression



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*



Midazolam

Thummel et al. JPET, 1994

In Vitro to *In Vivo* Prediction of MDZ CL_H

$$\frac{V_{\max}(liver)}{K_m} = Cl'_{\text{int}}(liver) \rightarrow \frac{\left(f_u \bullet Cl'_{\text{int}}\right) \bullet Q_H^P}{\left(f_u \bullet Cl'_{\text{int}}\right) + Q_H^P}$$

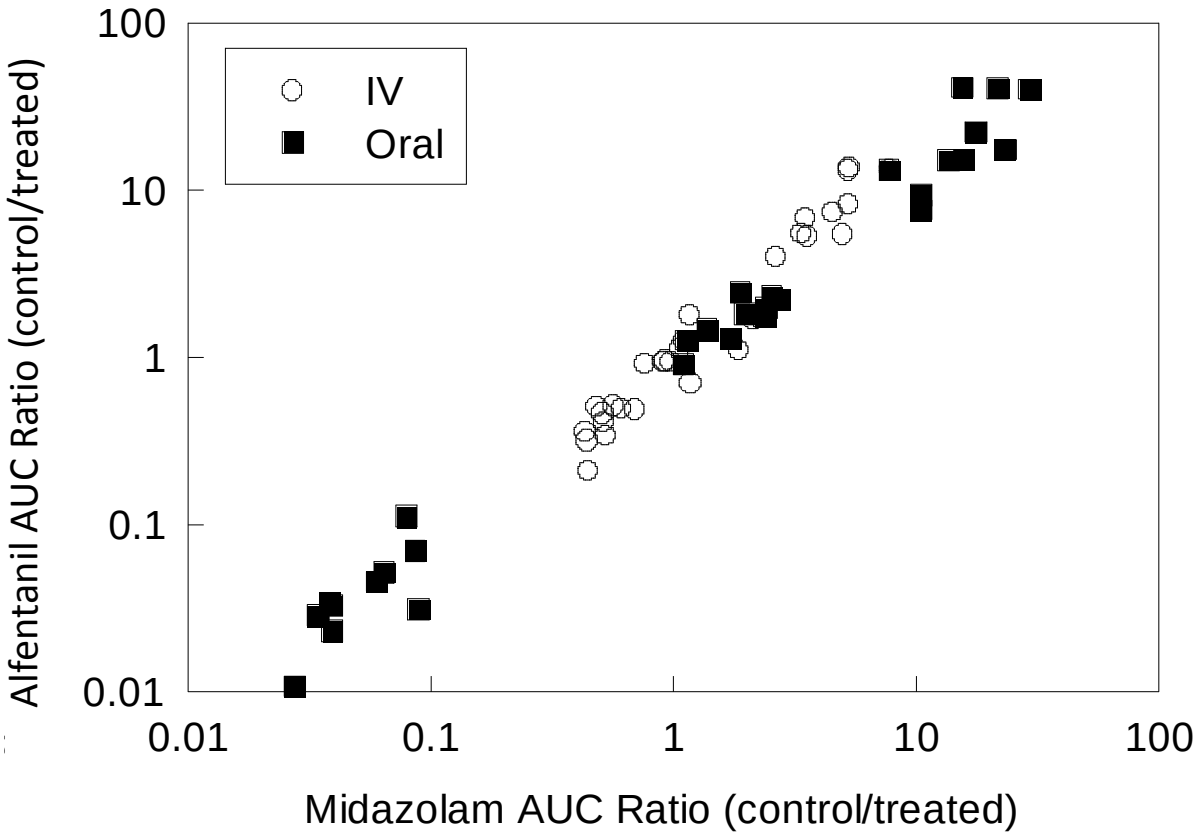
TABLE 3

Predicted hepatic 1′-OH MDZ formation clearance

Subject	Donor Body Weight	Q_H^B [a]	Q_H^P [b]	Predicted $Cl_H^{1'\text{-OH}}$ [c]	Observed Cl_T [d]
	kg			*liter/min*	
R-2	80	1.73	1.21	0.28	0.67
R-6	80	1.73	1.12	0.21	0.15
R-7	64	1.38	0.99	0.44	0.68
R-19	73	1.58	1.07	0.58	0.68
R-22	106	2.29	1.60	0.58	0.79

[a] Calculated as the product: body weight (kg) × 0.0216 liter/kg.
[b] Calculated as the product: (1 − Hct) × (Q_H^B).

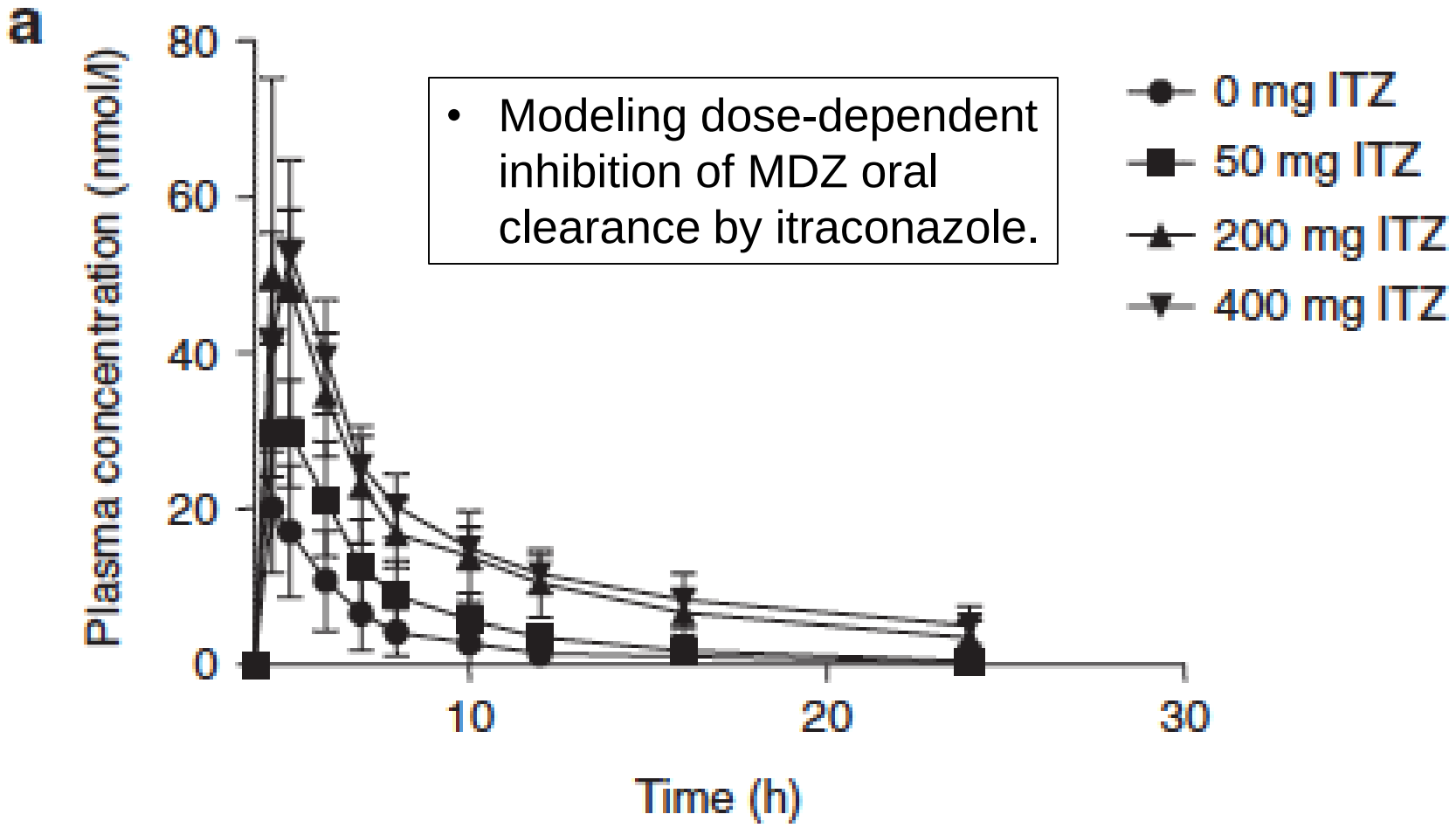
Midazolam Predicts the Disposition of Alfentanil when given with CYP3A Inducers and Inhibitors



- 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.

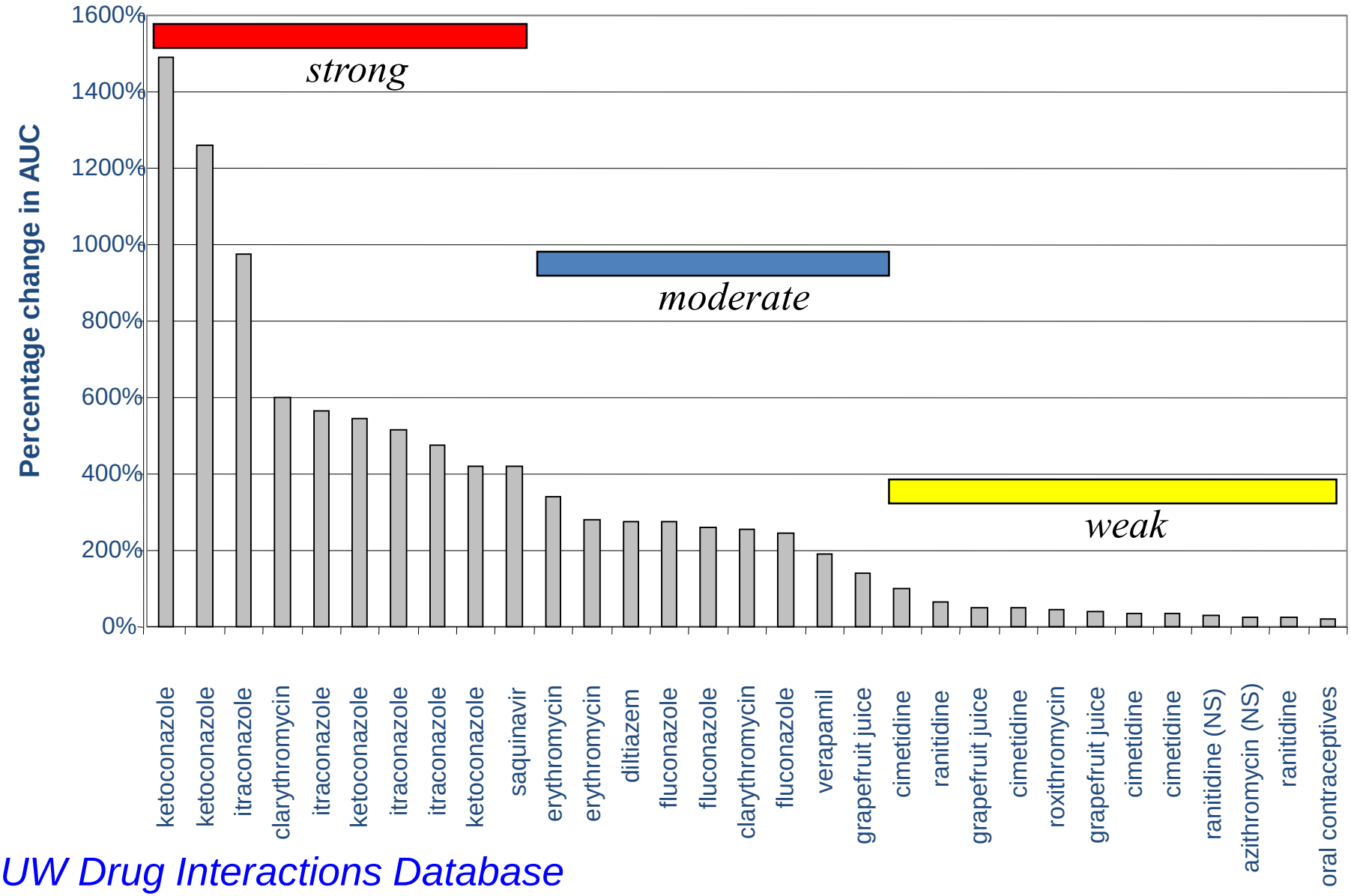
Kharasch et al., CPT, 2004; Proc Assoc Univ Anesthesiologists, 2005

Midazolam is a Sensitive Probe for Characterizing CYP3A Inhibition



Ian Templeton, Nina Isoherranene et al, CPT, 2010

Classifying Inhibitors of CYP3A Based on Inhibition of Oral Midazolam Elimination



UW Drug Interactions Database

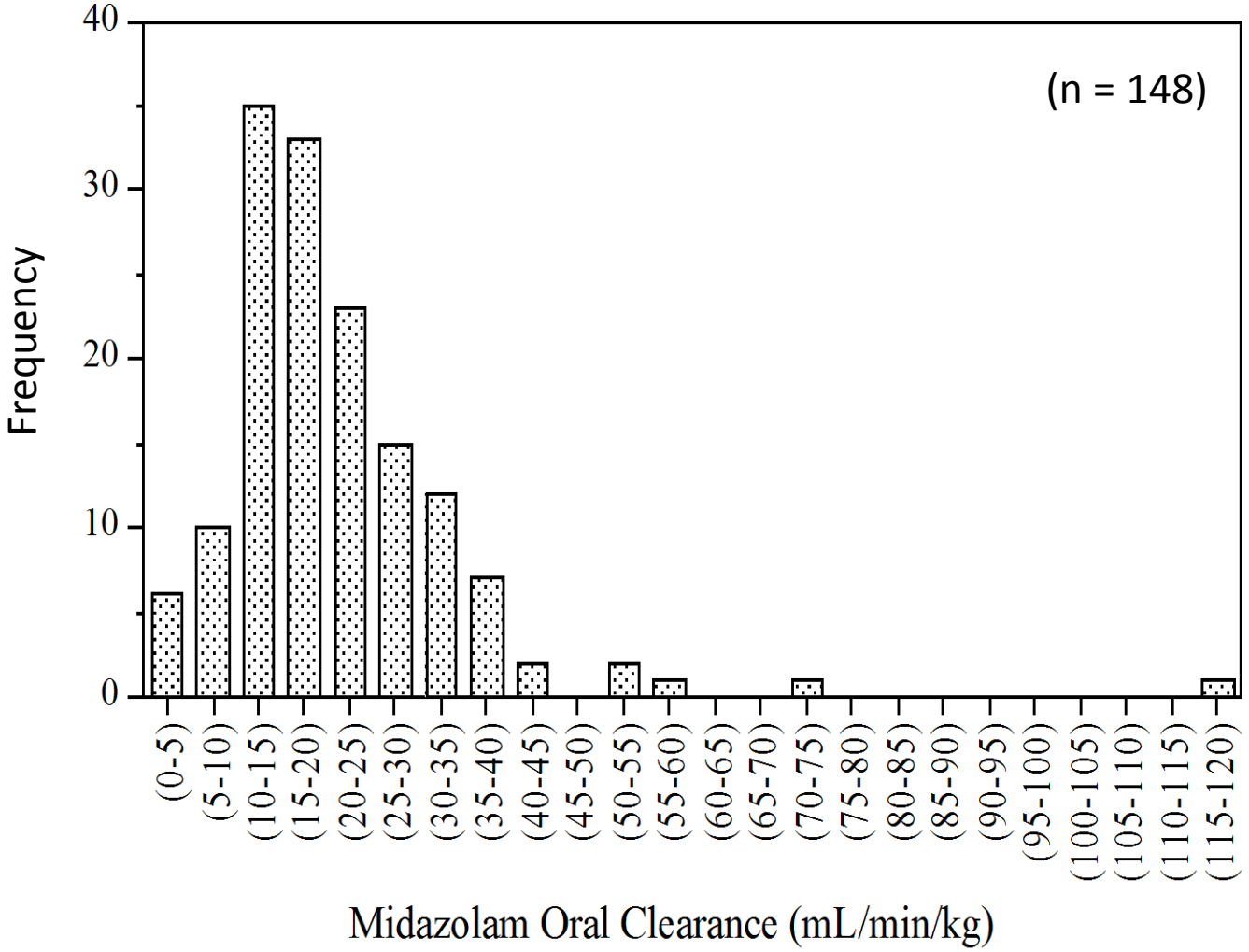
Paul Watkins, MD

- 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

High Plasma Conc/Dose ——————————————— Low Plasma Conc/Dose

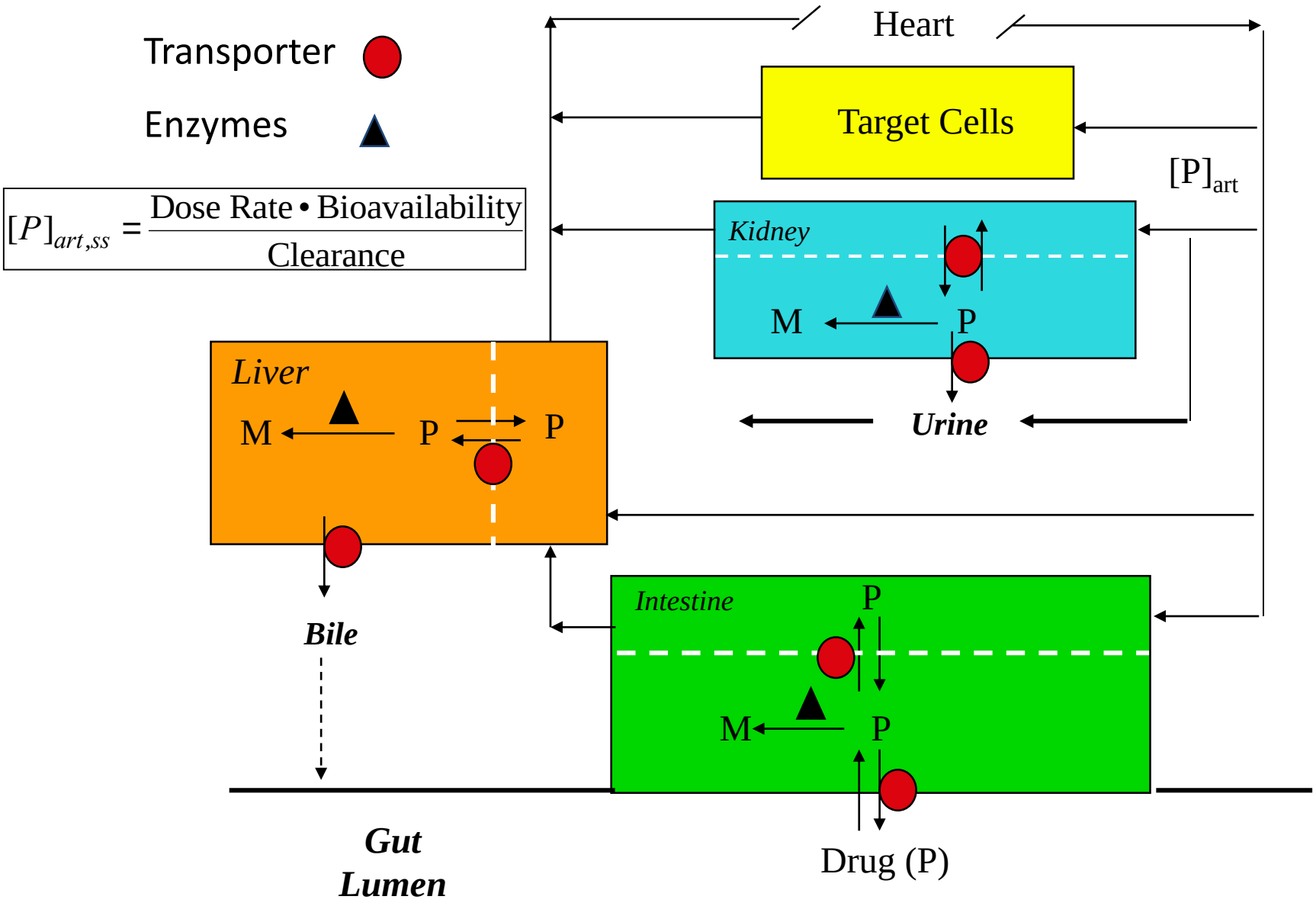


MDZ CL/F depends on:

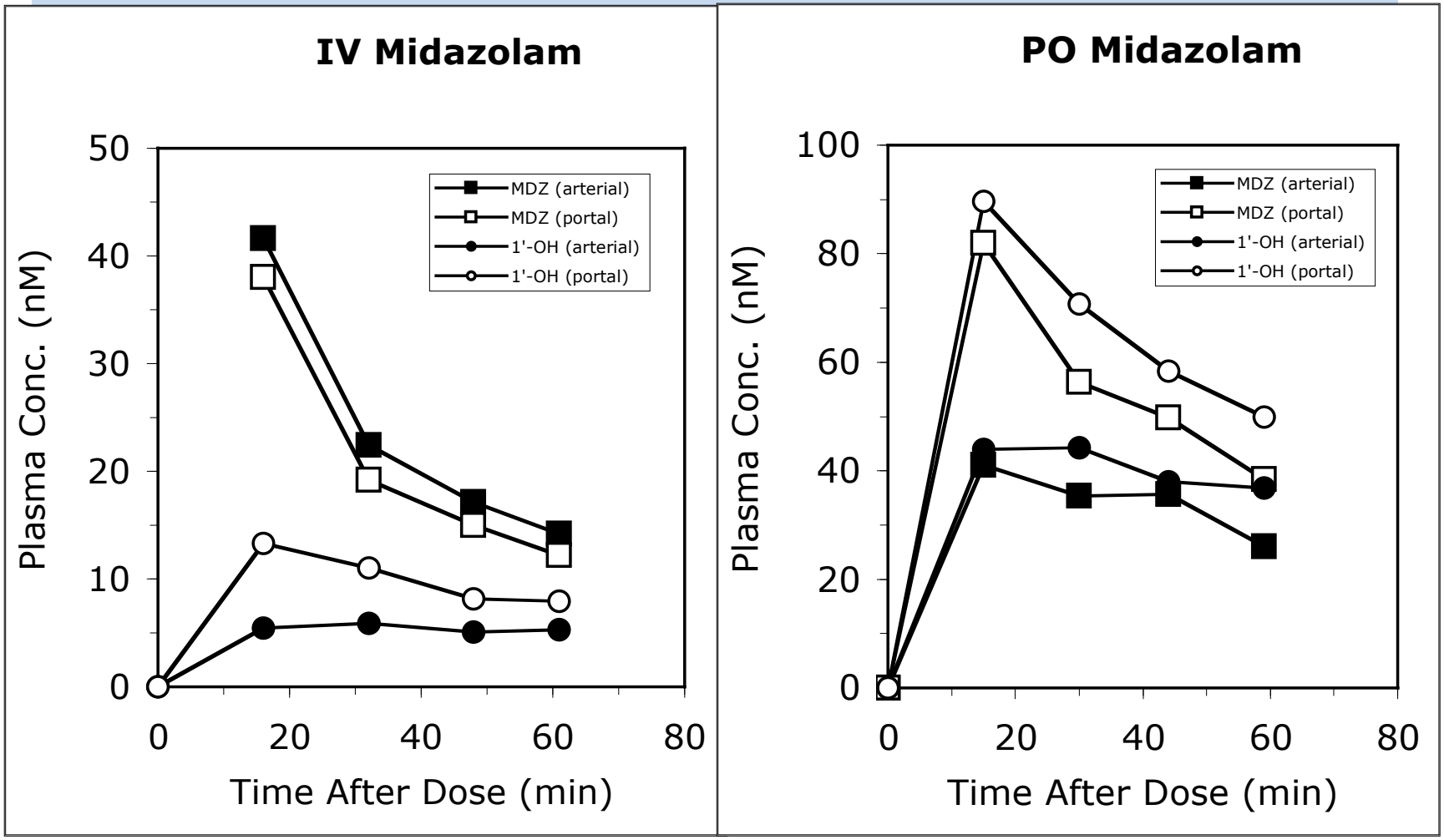
Hepatic CYP3A
Intestinal CYP3A

Yvonne Lin et al., Pharmacogenetics, 2001

Oral Midazolam Disposition



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

Intravenous Dose		*Intraduodenal Dose*	
Subject	E (%)	Subject	E' (%)
3	11.1	1	40.5
4	25.6	2	45.1
6	8.5	5	57.1
7	-2.6	9	58.6
10	-1.8	11	13.6
Mean	8.2	*Mean*	43.0*
S.D.	11.5	*S.D.*	18.1

Mary Paine et al., CPT, 1996

CYP3A Substrates Suspected of Undergoing Extensive First-pass Gut Metabolism

Lovastatin
Simvastatin

Midazolam
Triazolam

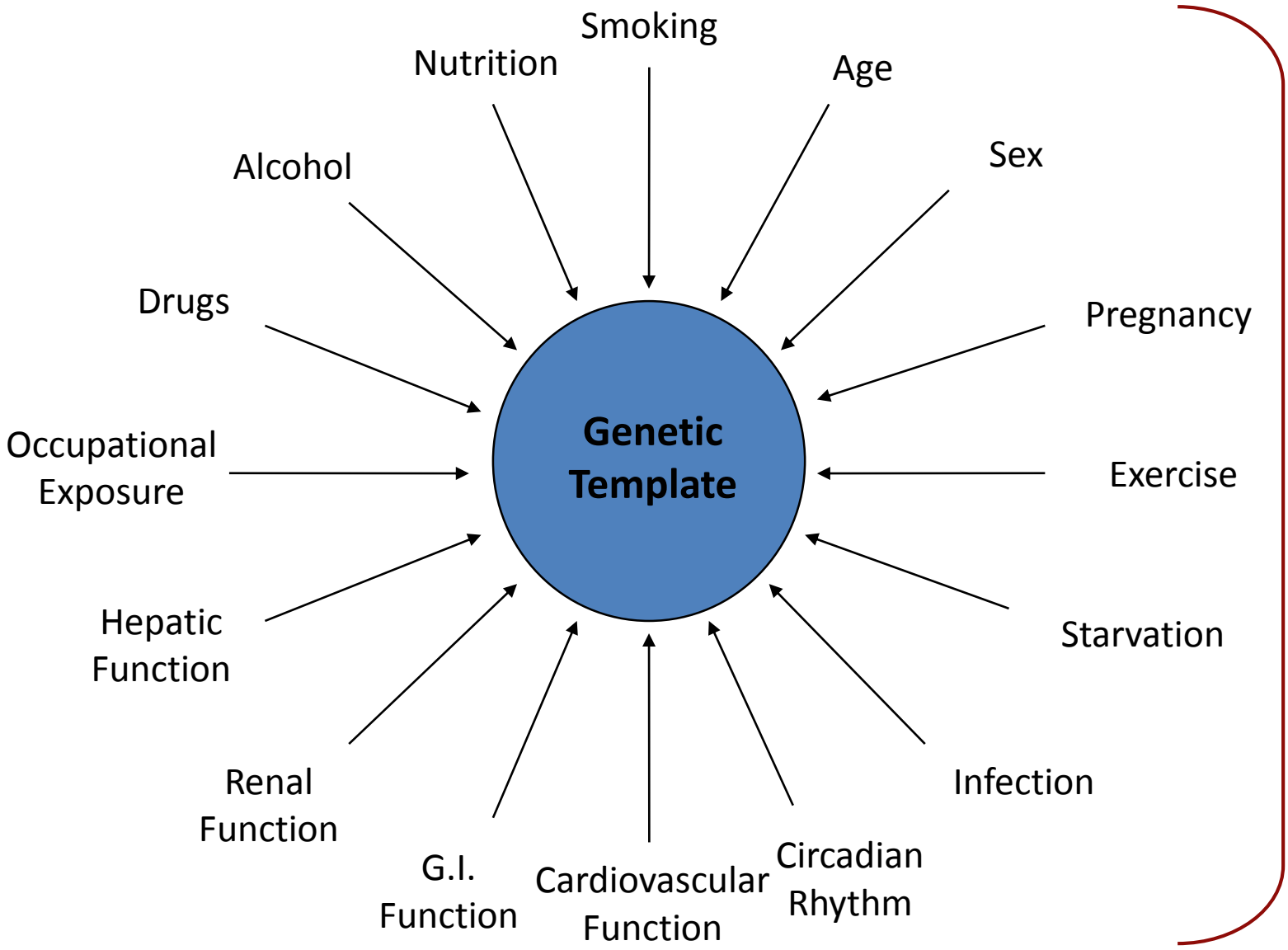
Cyclosporine
Tacrolimus
Sirolimus

Saquinavir
Ritonavir

Felodipine
Verapamil
Nicardipine
Diltiazem

All have an oral bioavailability of < 50%.

Identifying Sources of Variable CYP3A Expression



Variable CYP3A Gene Regulation

Hormones

Cytokines

Paracrine Factors

Transcription Factors

Genetic Variability

~90%?

Adapted from E. Vesell, 1981

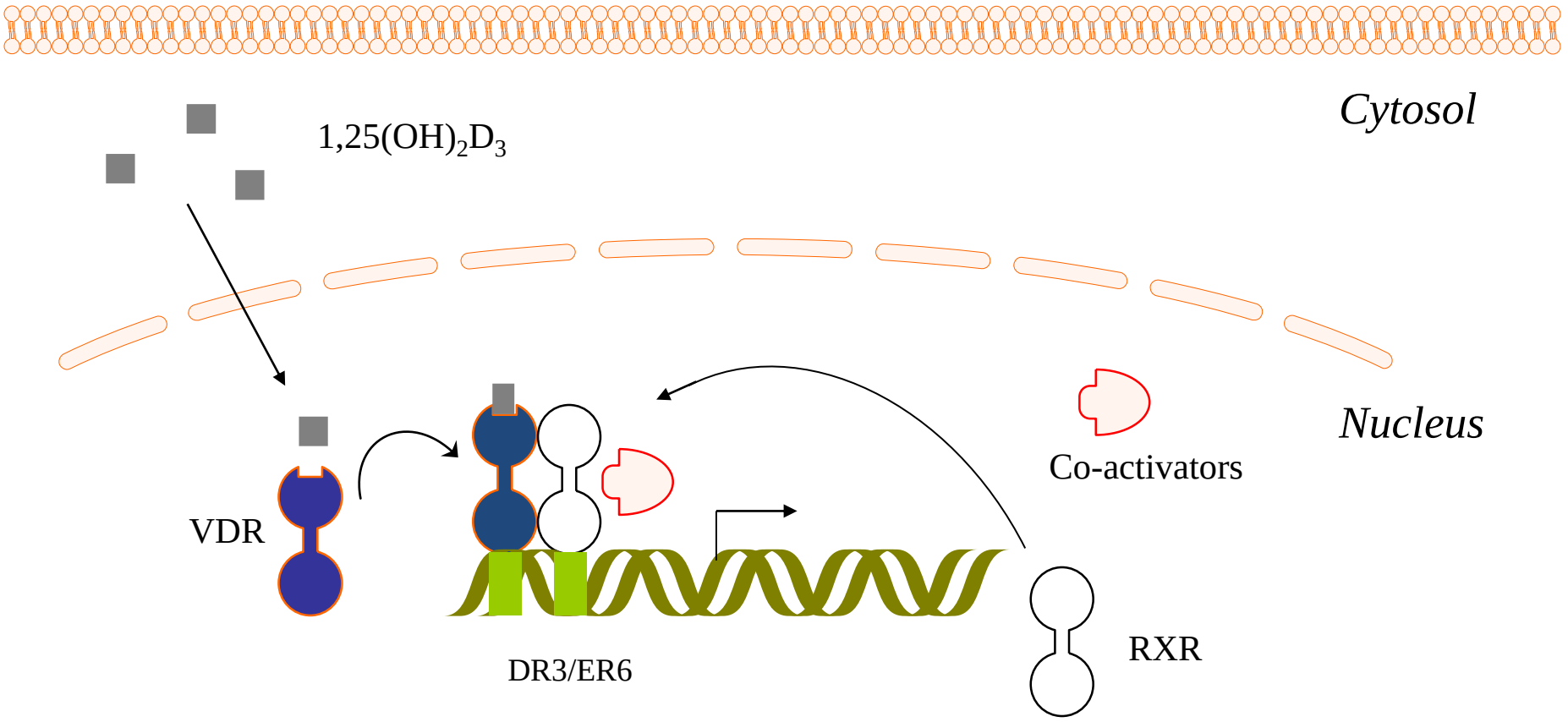
Erin Schuetz, PhD

- St Jude Children's Research Hospital

  Sabbatical - 2000

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

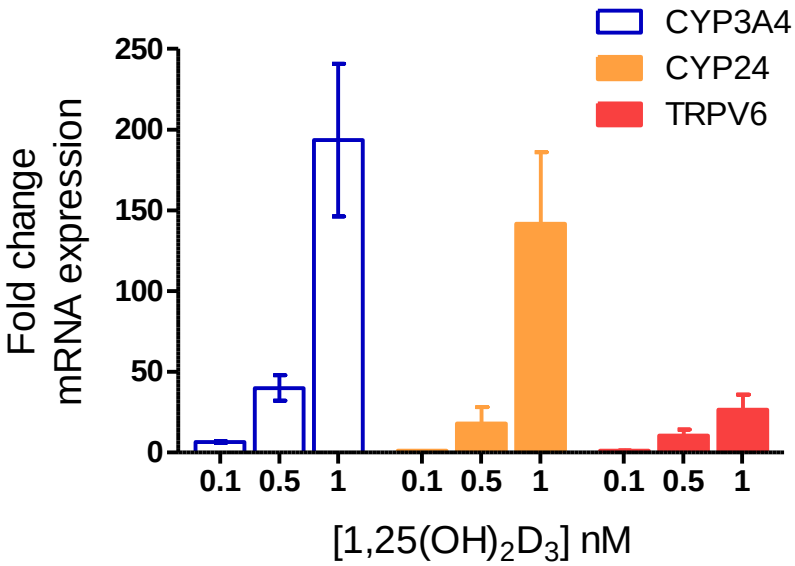
Regulation of Intestinal CYP3A4 by Vitamin D

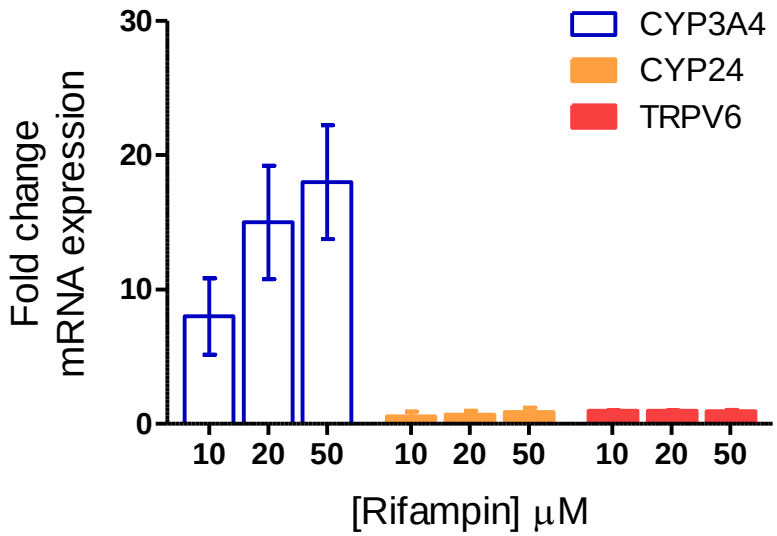


- $1,25(OH)_2D_3$ induces CYP3A4 by a VDR-dependent process *(Schmiedlen-Ren, Jeannine Fisher, Mary Paine et al, Mol Pharmacol, 1997; Thummel, Tauri Senn et al, Mol Pharmacol, 2001).*

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)

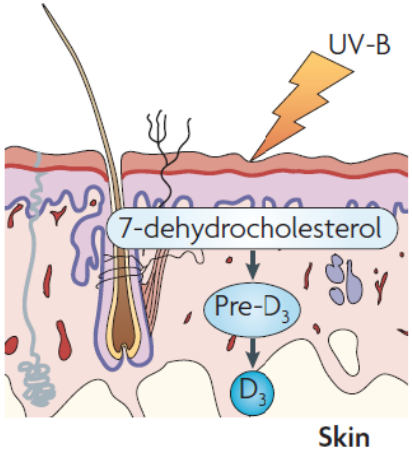




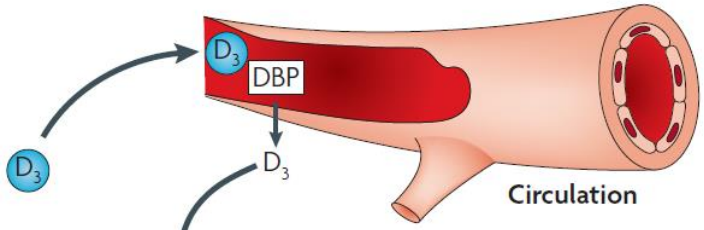
Very low dose $1,25(OH)_2D_3$ induces all 3 VDR gene targets.

Rifampin is a selective CYP3A4 inducer.

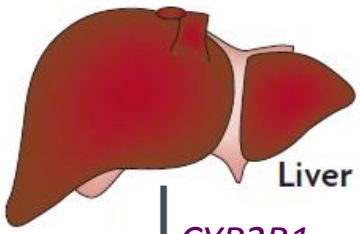
Emily Zheng et al, Biochem Pharmacol, 2012



Vitamin D



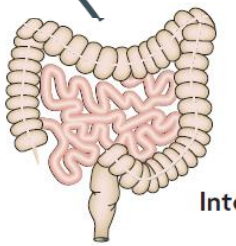
DHCR7

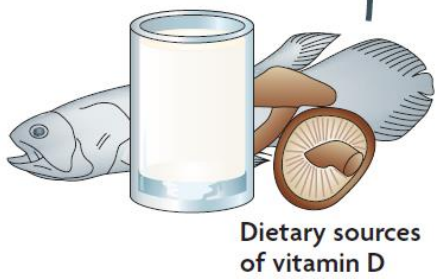


CYP2R1

25(OH)D_3

[DBP]



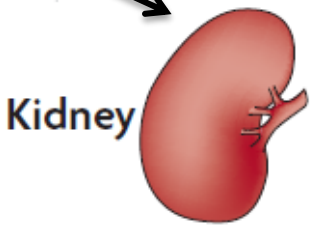


Other Tissues
Skin
Immune cells
Vasculature
Colon

CYP27B1

[DBP] 1,25(OH)$_2$D$_3$

Intracrine



CYP27B1

1,25(OH)$_2$D$_3$ [DBP]

Endocrine

VDR

Adapted from
Deeb et al, 2007

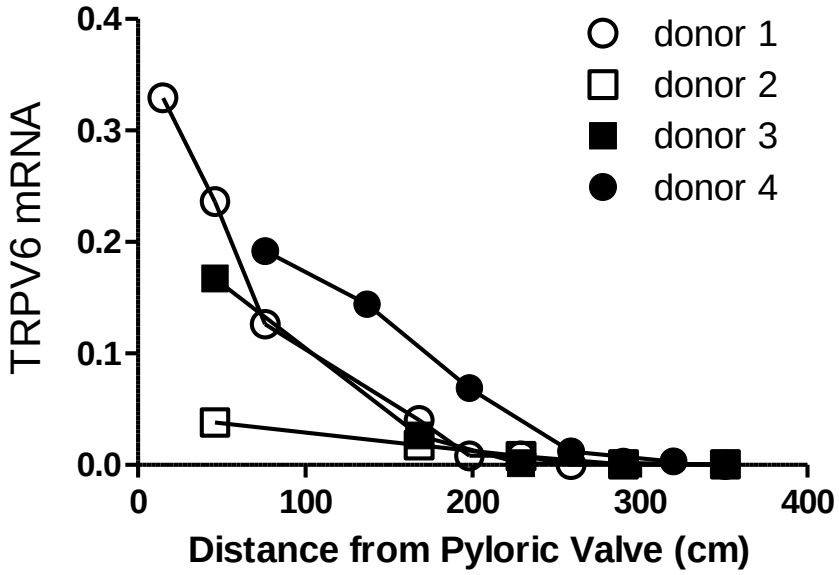
Immune cells
Induces cell differentiation

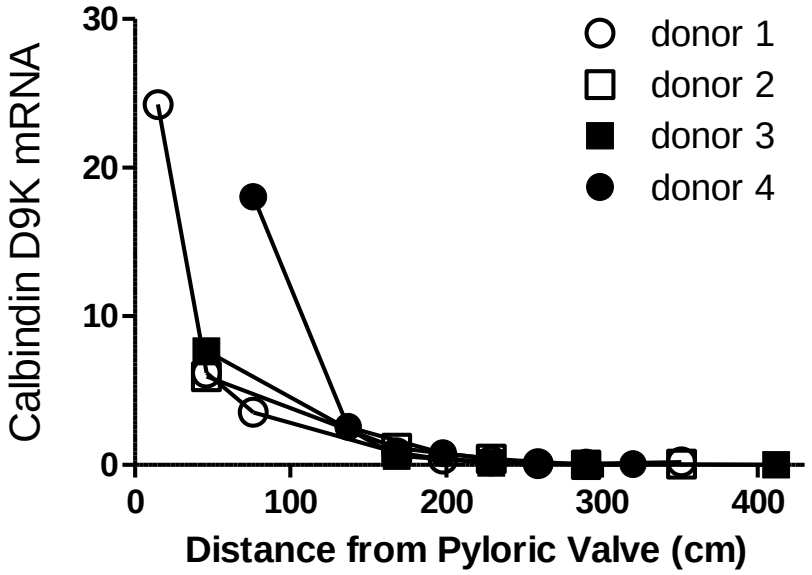
Kidney
Increases reabsorption of Ca^{2+} and Pi

Small Intestine
Increases absorption of Ca^{2+} and Pi, and CYP3A enzyme and activity

Bone
Mineralization and remodeling

Preferential Expression of VDR Target Genes in the Upper Small Intestine

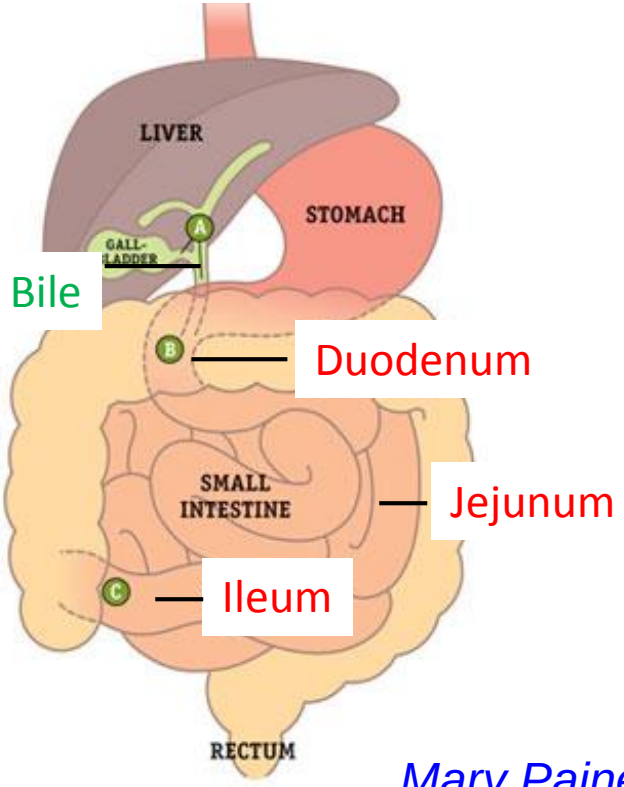


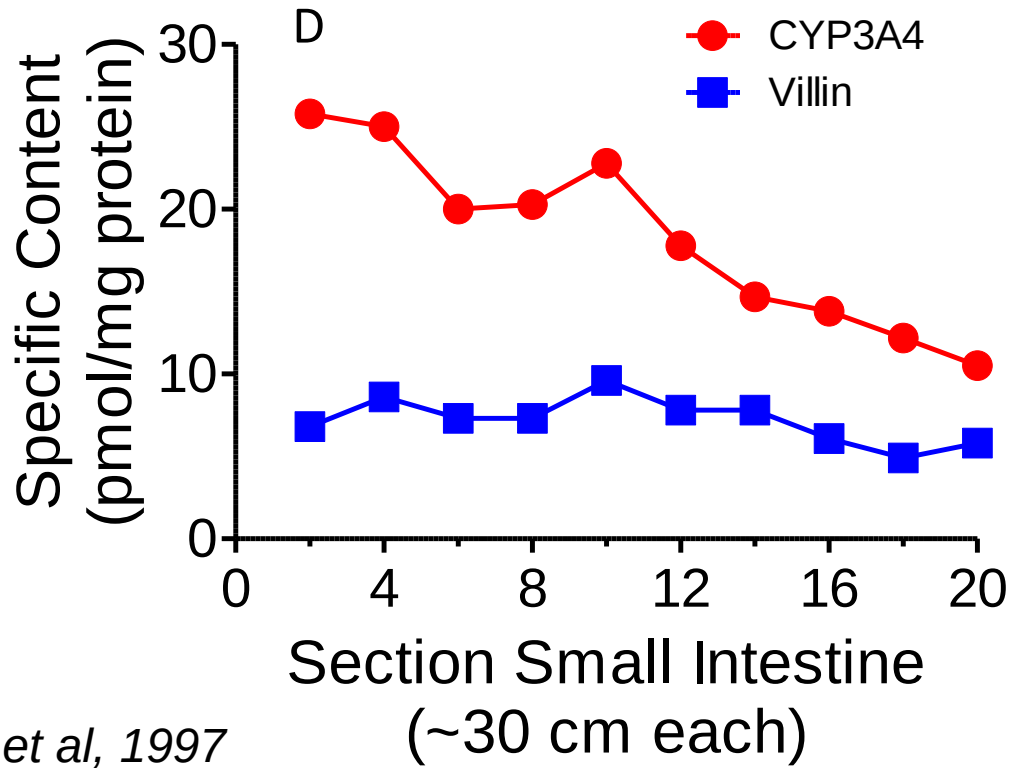


- Heterogeneous expression pattern consistent with primary site of calcium absorption.

Zhican Wang, Yang Xu et al., *J. Steroid Biochem. Mol. Biol.,* 2013

Heterogeneous Distribution of CYP3A4 Protein in Small Intestine

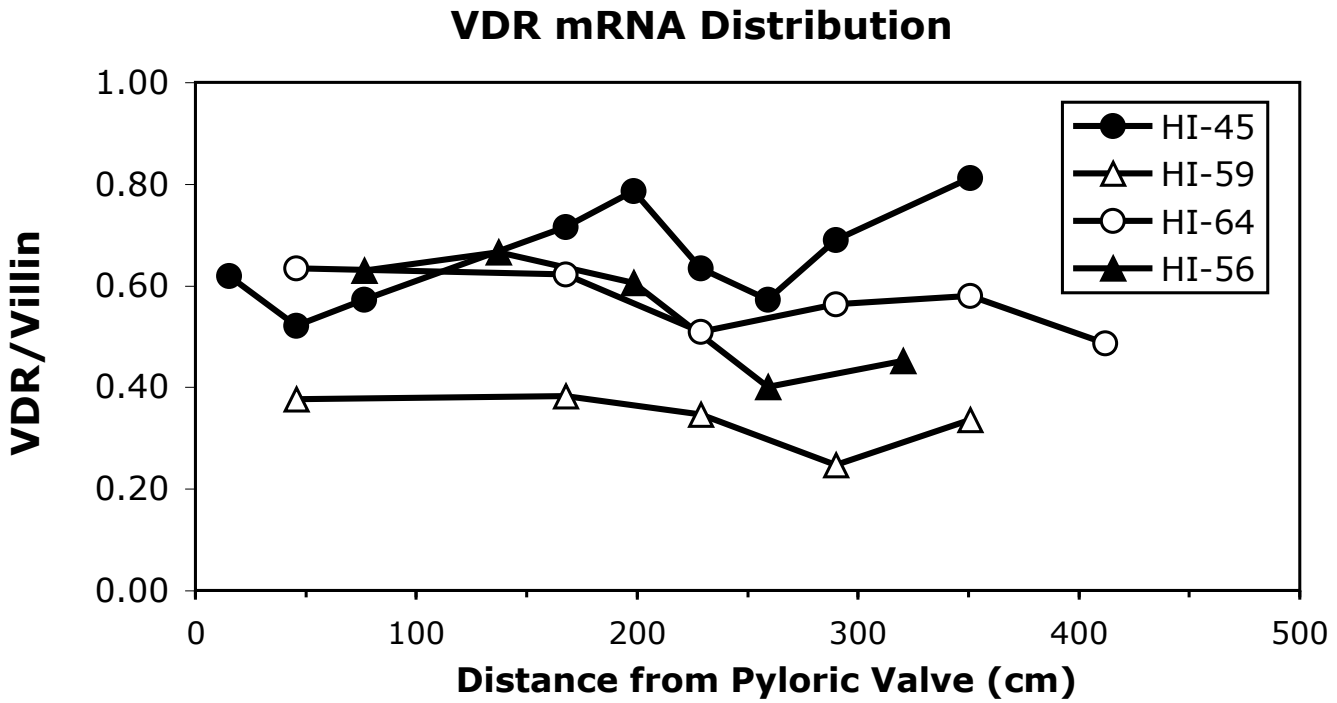




Mary Paine et al, 1997

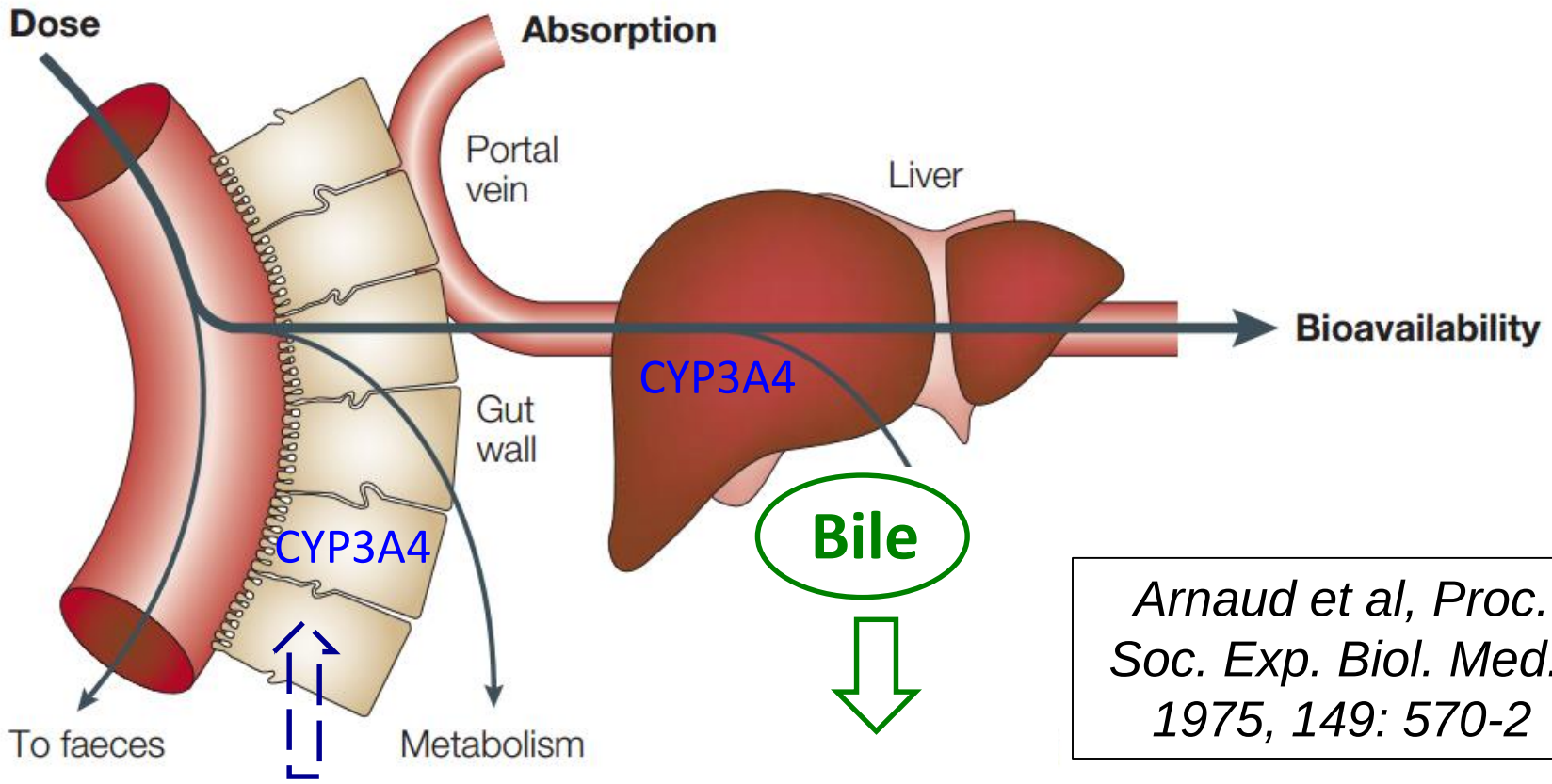
A common VDR signaling pathway for both calcium transport proteins (TRPV6, calbindin D9K) and CYP3A4?

VDR Expression is Relatively Constant Along the Length of the Small Intestine



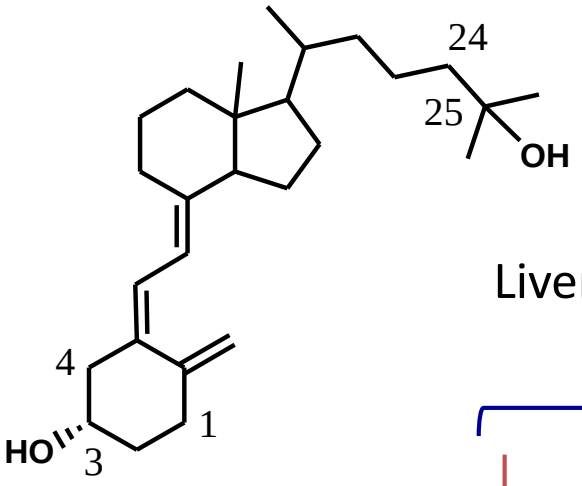
What about delivery of the ligand?

Hypothesis: Biliary Vitamin D Conjugates Regulate Intestinal *CYP3A4* Expression



Arnaud et al, Proc. Soc. Exp. Biol. Med., 1975, 149: 570-2

Major Metabolic Pathways of 25OHD$_3$



Kidney

Liver

CYP27B1 → 1α,25(OH)$_2$D$_3$

CYP24A1 → 24R,25(OH)$_2$D$_3$

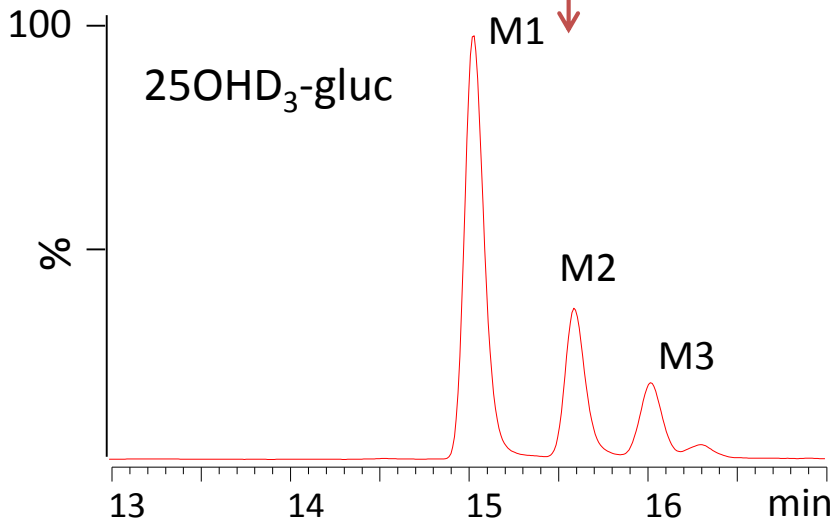
CYP3A4 → 4β,25(OH)$_2$D$_3$

Liver

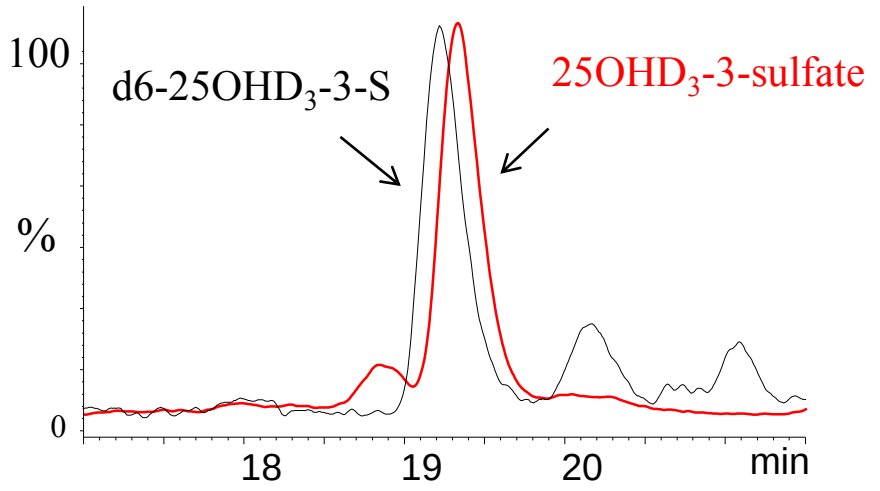
(Zhican Wang et al, Mol Pharmacol, 2012)

UGT1A4/1A3

SULT2A1/1A1

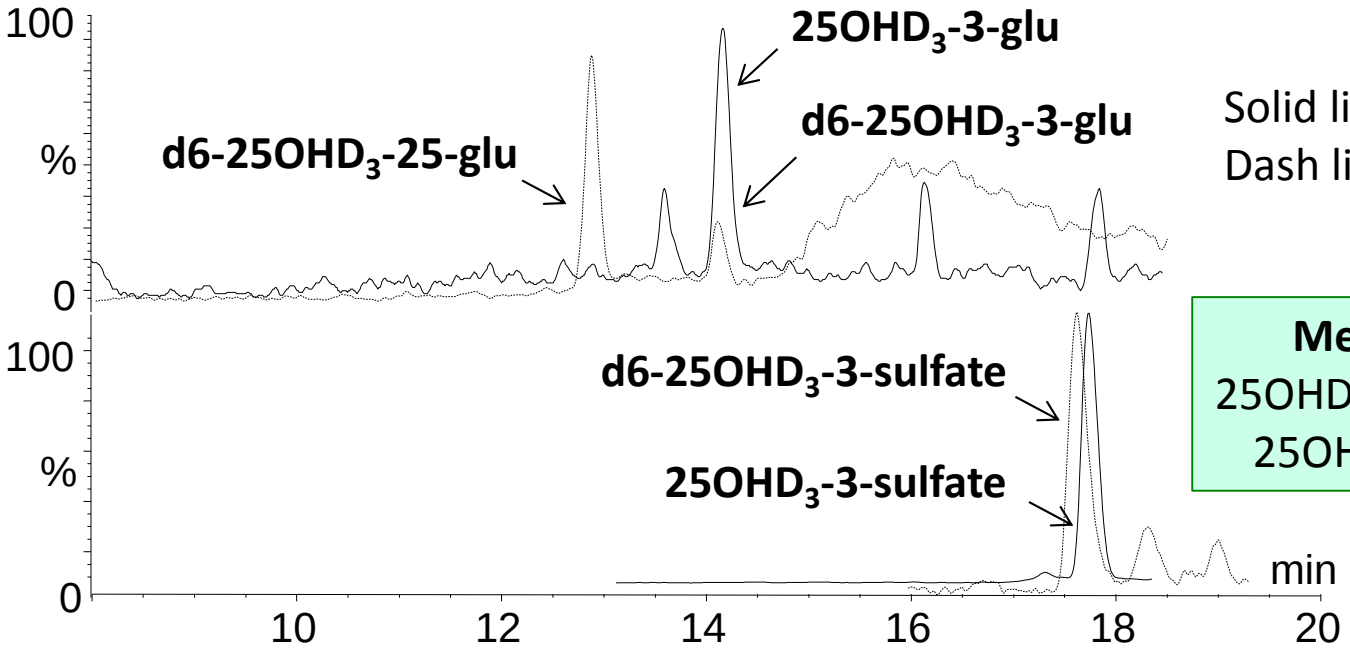


(Zhican Wang et al, Endocrinol, 2014)



(Tim Wong et al, In Preparation)

25OHD$_3$ Conjugates in Human Plasma

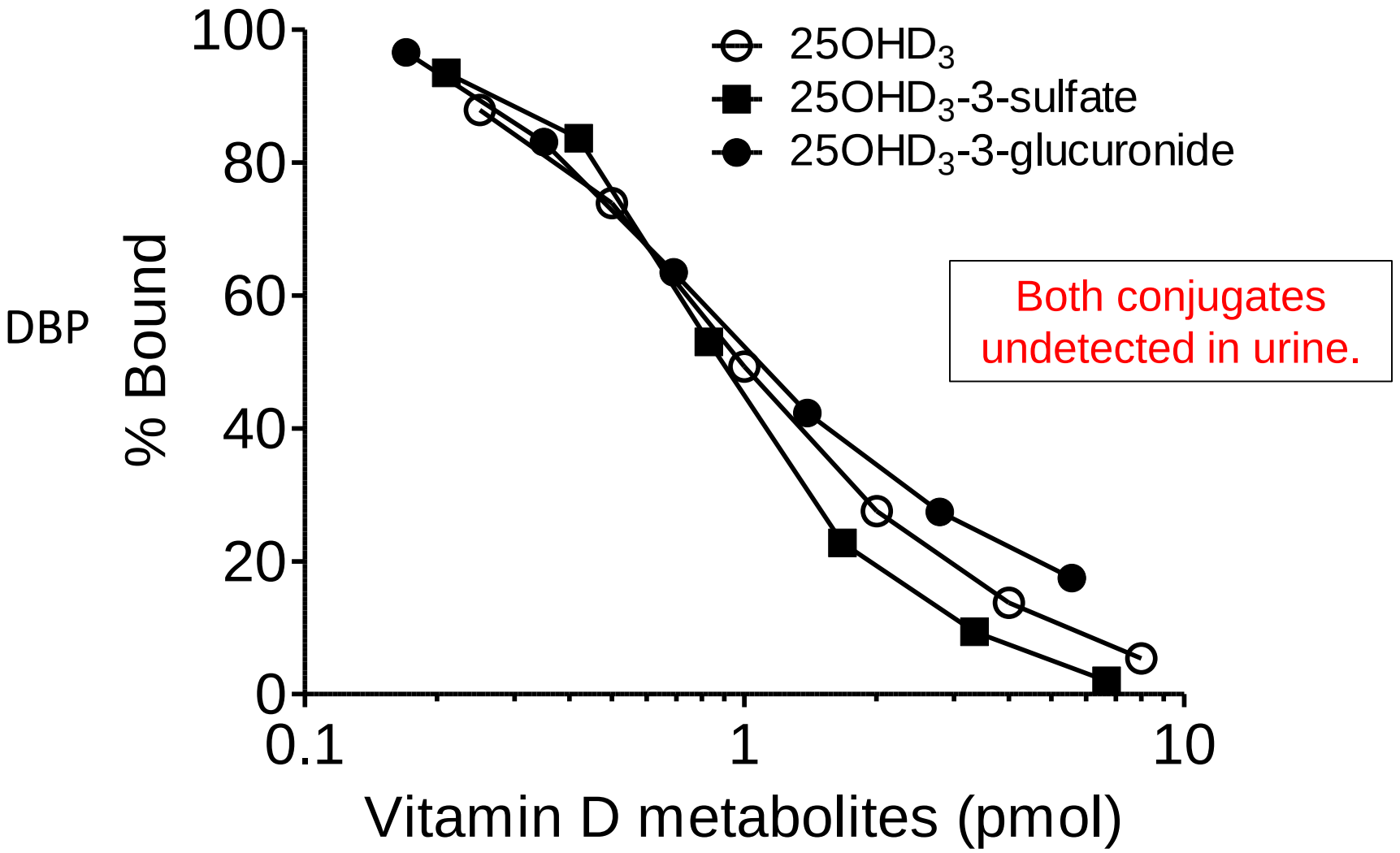


Solid line: *m/z* 575 & 479
Dash line: *m/z* 581 & 485 (I.S.)

Mean concentrations:
25OHD$_3$-3-glucuronide, ~2 nM
25OHD$_3$-3-sulfate, ~40 nM

Conditions	**25OHD$_3$ nM**	**24R,25(OH)$_2$D$_3$ nM (M/P ratio)**	**25OHD$_3$-3-sulfate nM (M/P ratio)**
Healthy Control (n = 21)	52.3 ± 25.2	3.8 ± 2.8 (0.11 ± 0.02)	46.2 ± 21.1 (1.07 ± 0.73)
Liver Disease (n = 20)	40.6 ± 21.8	2.9 ± 1.9 (0.11 ± 0.03)	42.5 ± 30.2 (1.33 ± 0.95)
Kidney Disease (n = 15)	26.5 ± 18.5	1.1 ± 0.4 (0.05 ± 0.02)	28.0 ± 19.0 (1.06 ± 0. 39)

Circulating $25OHD_3$ Conjugates Tightly Bind to Vitamin D Binding Protein (DBP)



25OHD Glucuronides in Human Bile

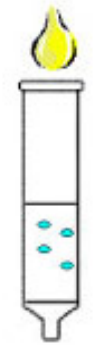
Diluted human bile

↓ **Hexane extraction**

Aqueous solution

↓ **Buffered, sodium acetate (pH 4.0)**

Mixture

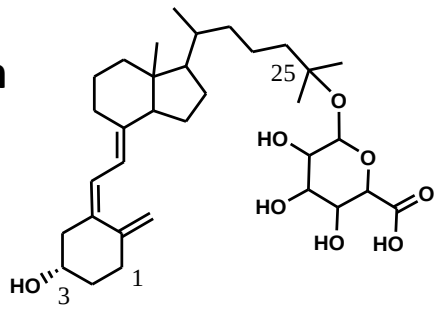


Anion exchange SPE

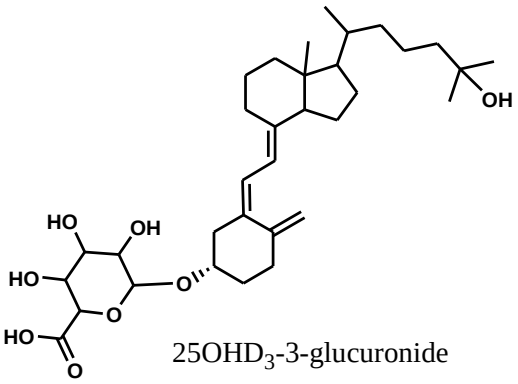
Dried

Derivatization

ESI-LC-MS/MS

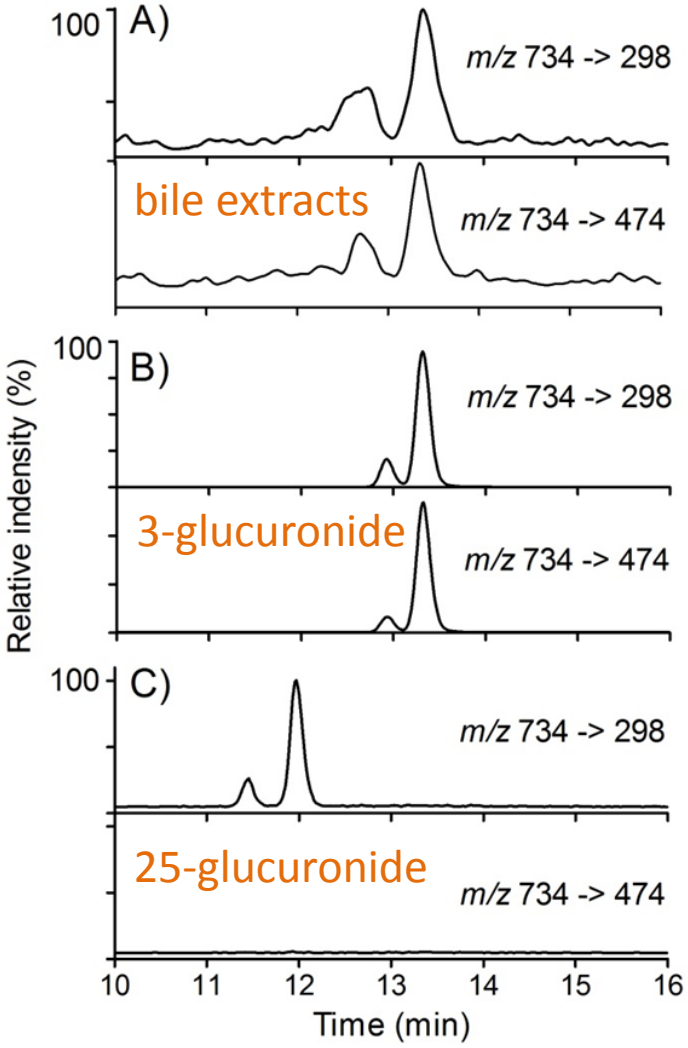


$25OHD_3$-25-glucuronide



$25OHD_3$-3-glucuronide

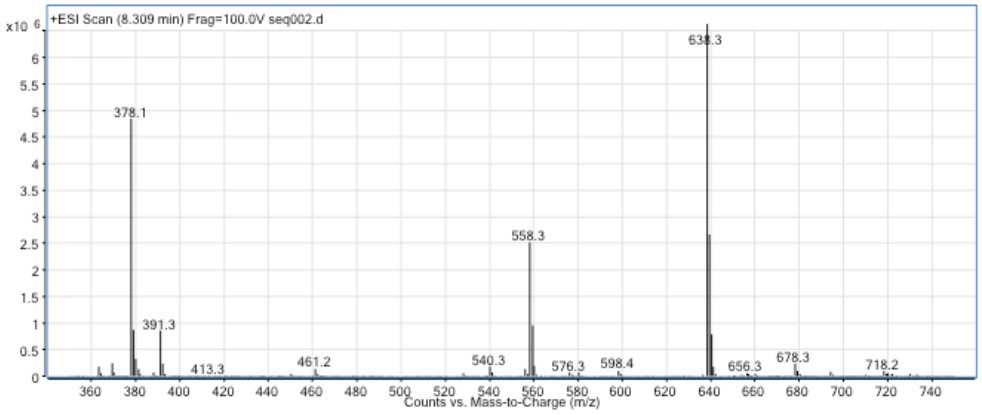
Mass spectra of 25OHD-3-glucuronide in human bile



Zhican Wang et al, *Endocrinology,* 2014

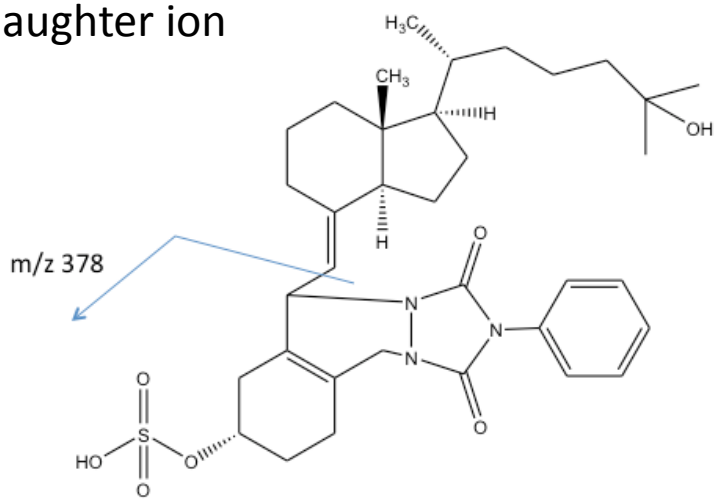
25OHD$_3$-3-O-Sulfate in Human Bile

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

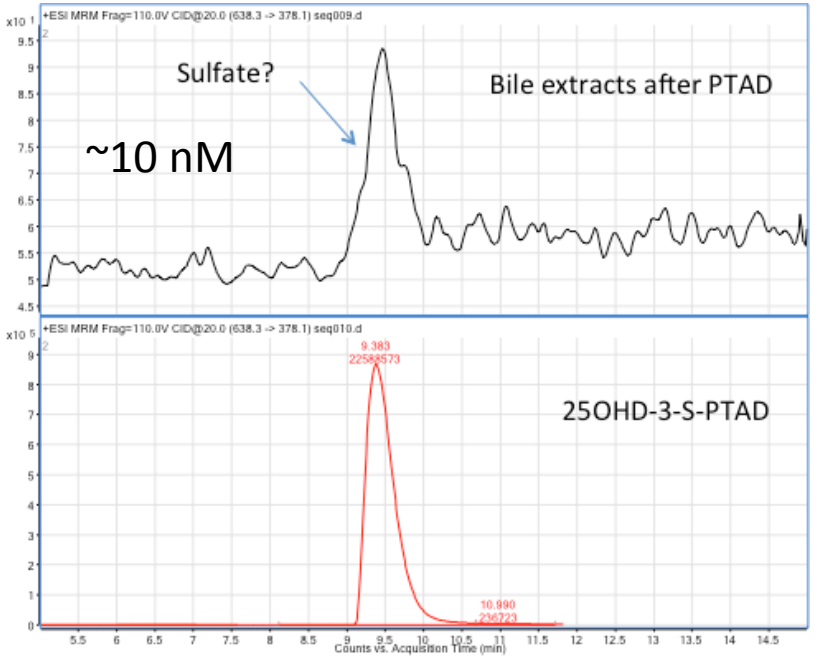


- similar daughter ion scans for bile extract and standard (not shown) suggest the presence of the 3-sulfate metabolite in bile.

diagnostic daughter ion



MRM



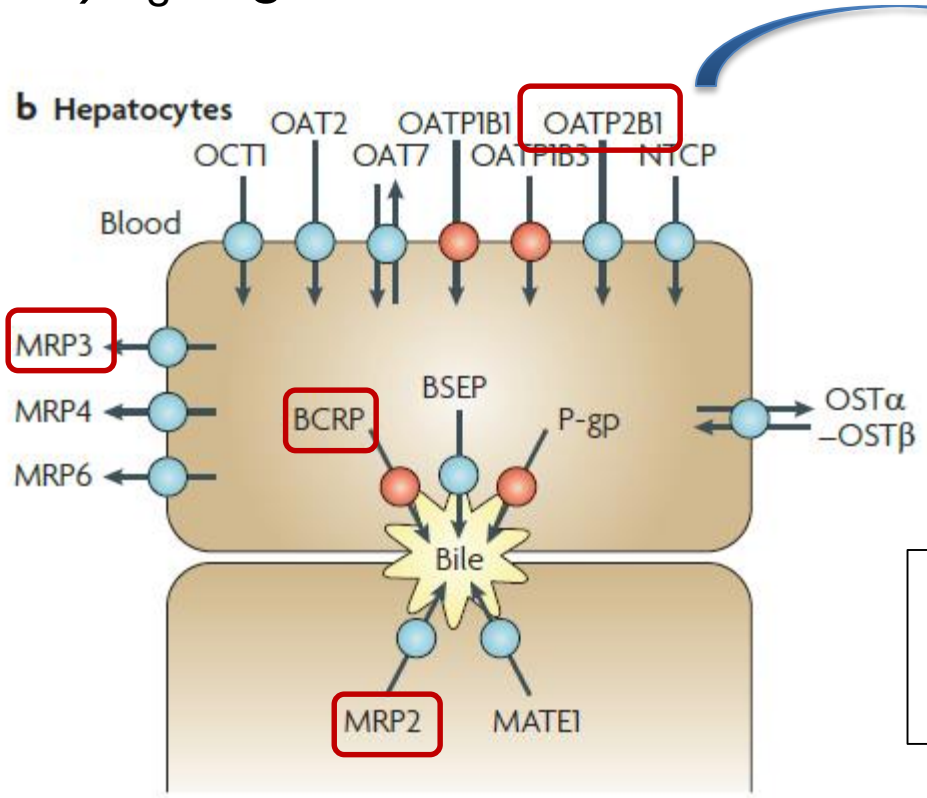
Chunying. Gao et al, Unpublished

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

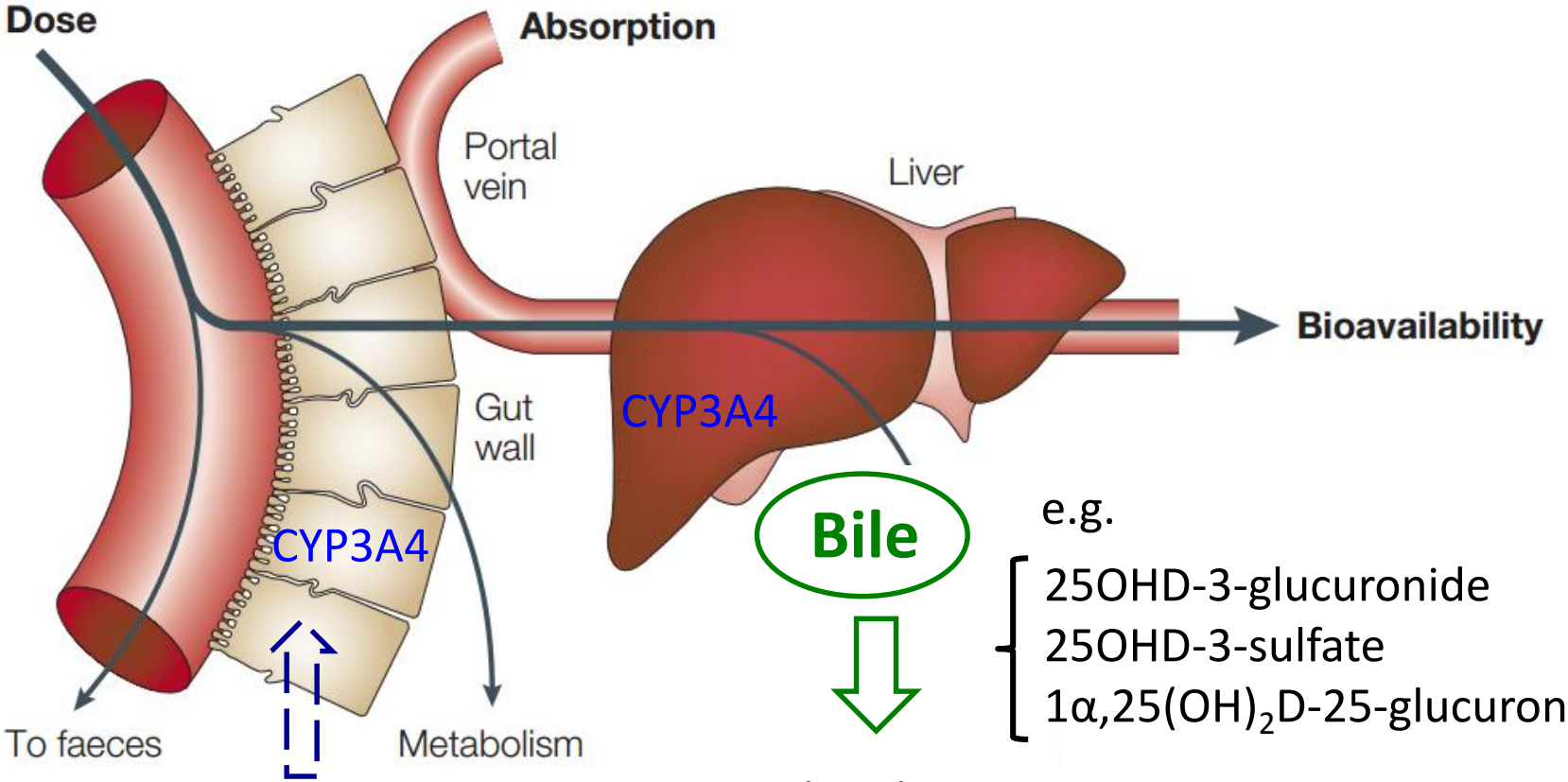
Efflux of 25(OH)D_3-3-glucuronide: MRP2, MRP3



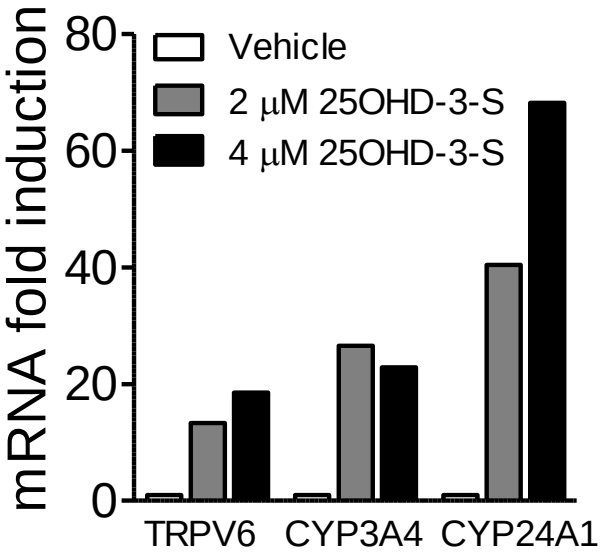
Also expressed on the apical surface of enterocytes.

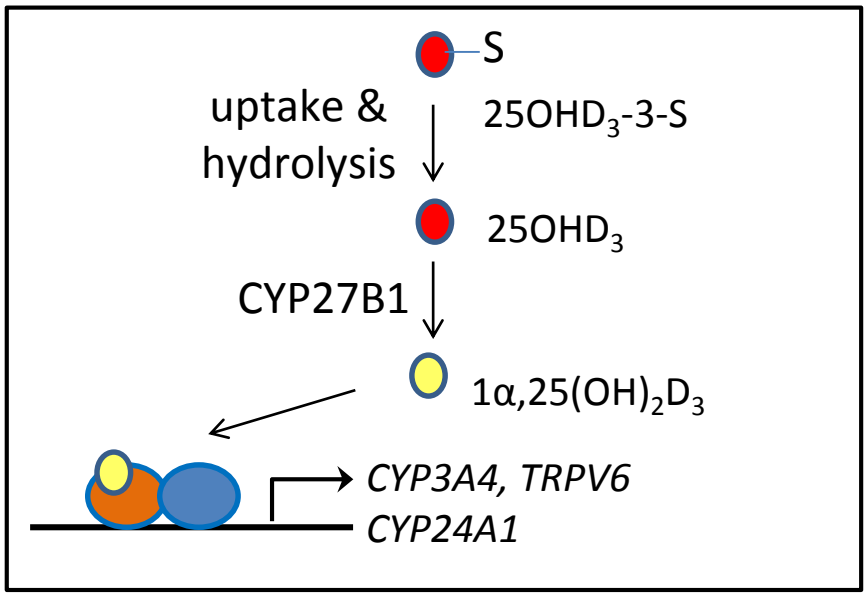
Chunying Gao
Qingcheng Mao
Unpublished Results

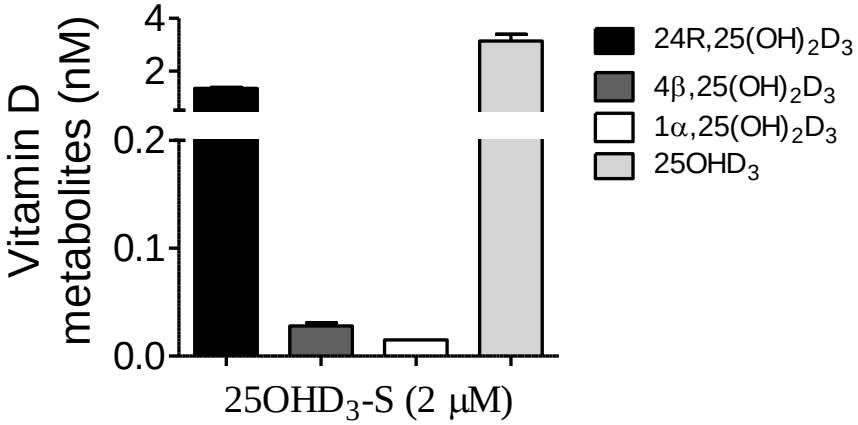
Hypothesis: Biliary Vitamin D Metabolites Regulate Intestinal *CYP3A4* Expression

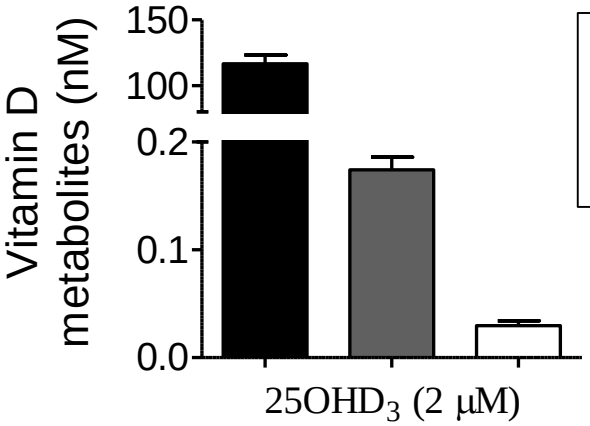


25OHD$_3$-3-sulfate Regulates VDR-responsive Gene Expression in Intestinal LS180 Cells





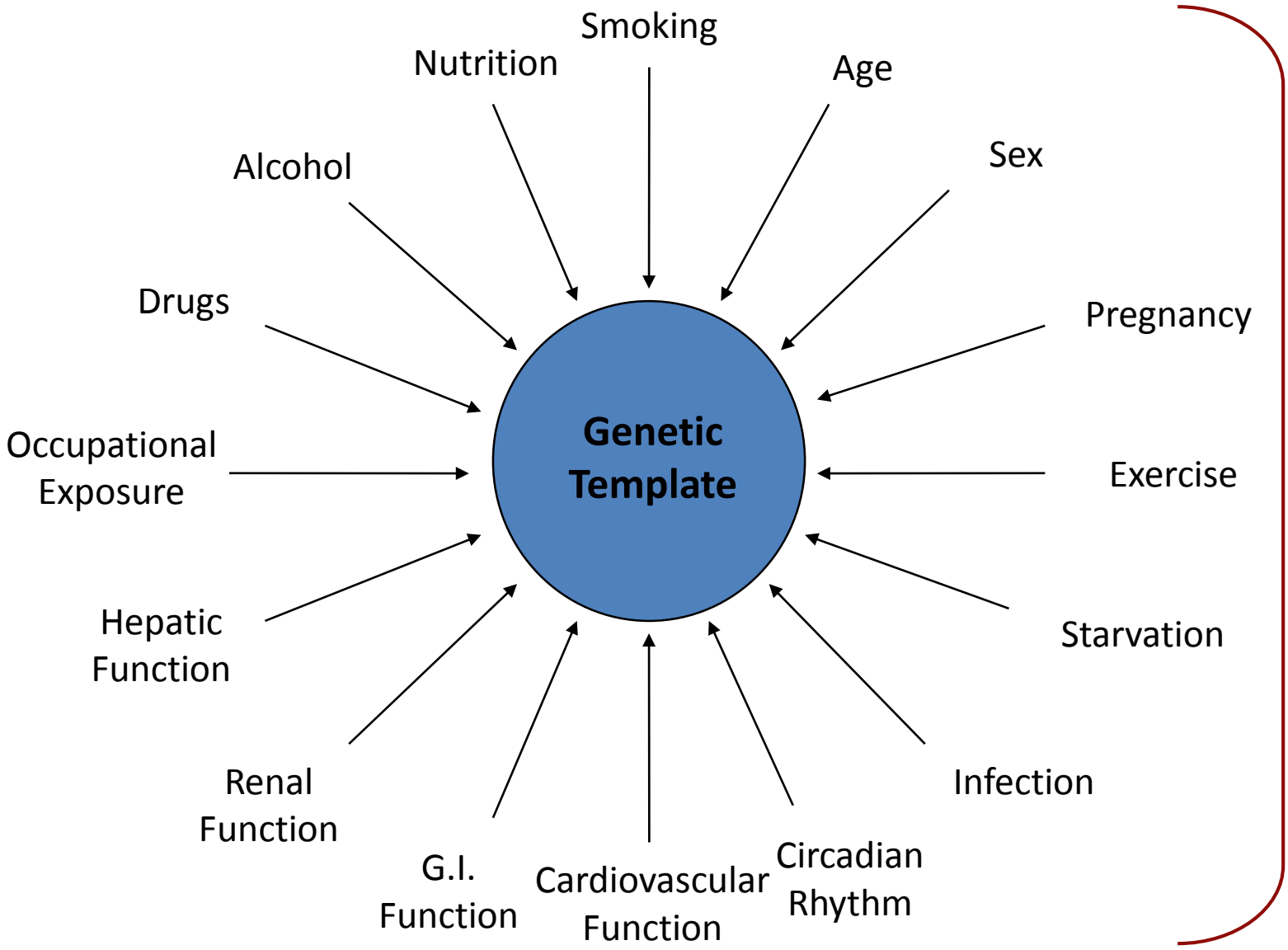




Zhican Wang
Brian Chapron
Unpublished

Cells were treated with 25OHD$_3$ or 25OHD$_3$-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.

Sources of Variable P450 Expression



Variable CYP3A Gene Regulation

Hormones

Cytokines

Paracrine Factors

Transcription Factors

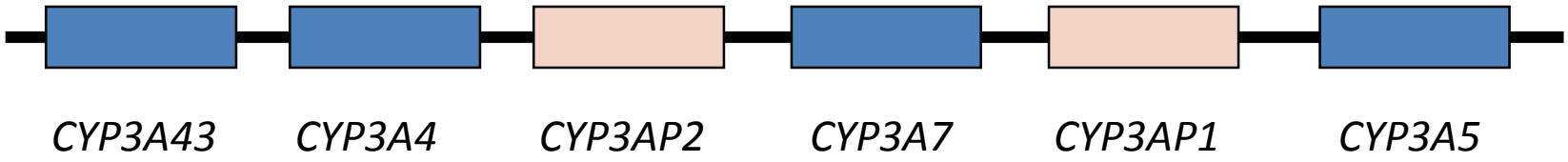
Genetic Variability

~90%?

Adapted from E. Vesell, 1981

Human *CYP3A* Gene Locus on Chromosome 7q

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



- CYP3A4 > CYP3A5 > CYP3A7 are the most important for drug metabolism in the adult
- all three CYP3A enzymes are subject to genetic and environmental sources of variability
  - CYP3A5 exhibits the most obvious polymorphic behavior

Immunodetection of Hepatic CYP3A5

Anti-CYP3A5

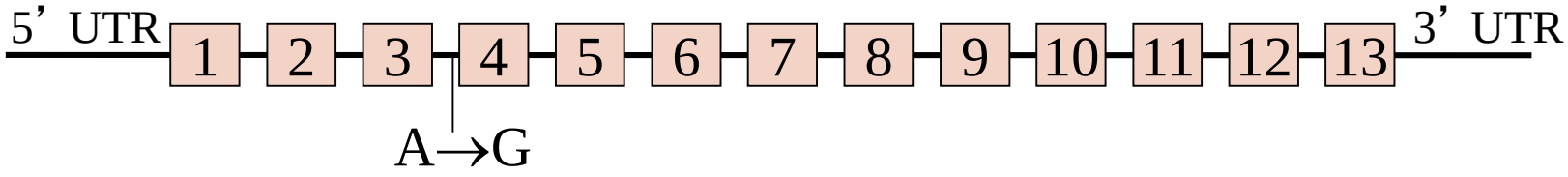
A *B* *C* *D* 1 0.5 0.25 0.125 *E* *F* *G* *H*

3A5 standards (pmol)

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

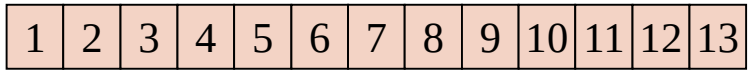
Mary Paine et al., JPET, 1997

*CYP3A5*3* Variant Allele

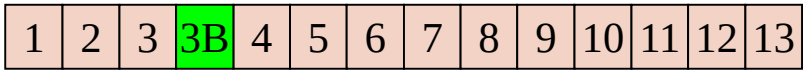


*CYP3A5*1* (A)

*CYP3A5*3* (G)



(wt-CYP3A5 mRNA)



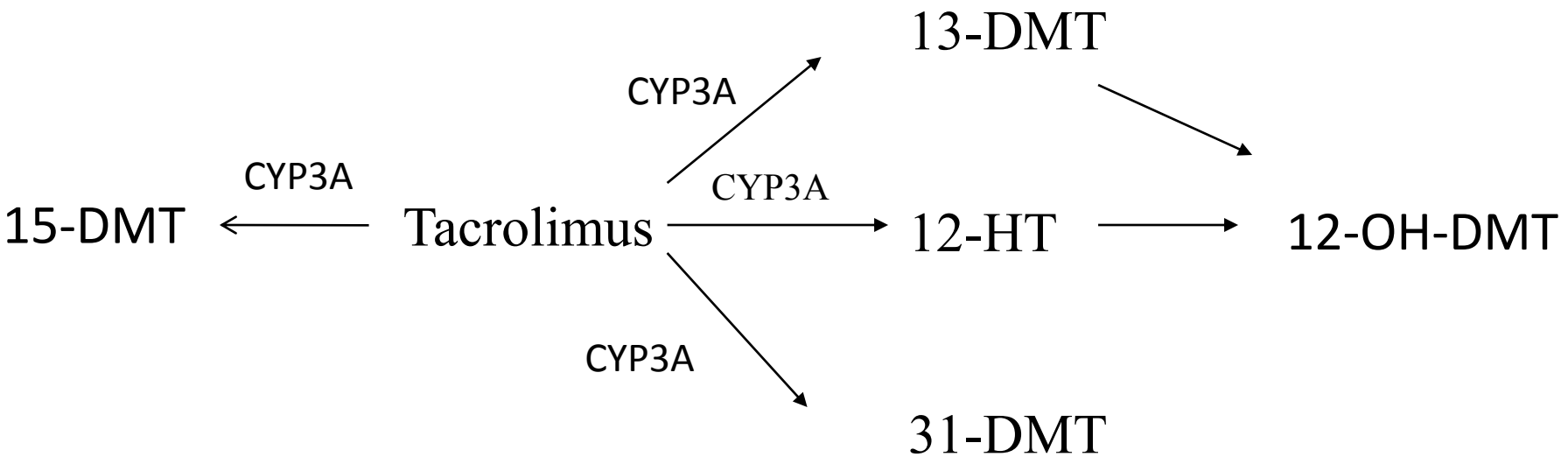
(SV1-CYP3A5 mRNA)

CYP3A5 protein

truncated, inactive protein

Kuehl, Yvonne Lin et al., Nature Genetics,2001; Yvonne Lin et al., Mol Pharmacol, 2002

Metabolic Fate of Tacrolimus



- 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

	13-DMT Formation
CYP3A4	
K_m (μM)	0.21
V_{max} (nmol/min/nmol)	8.0
Cl_{int} (ml/min/nmol)	38
CYP3A5	
K_m (μM)	0.21
V_{max} (nmol/min/nmol)	17.0
Cl_{int} (ml/min/nmol)	82

Unbound K_m and Cl_{int} calculated after correction for nonspecific binding).

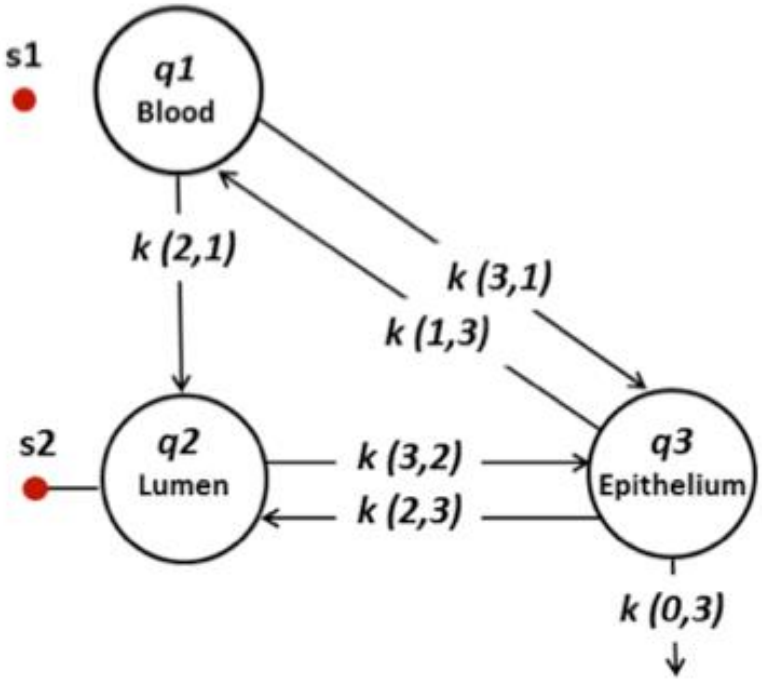
Yang Dai et al., DMD, 2006

Human Liver Microsomes	Tacrolimus Disappearance (nmol/min/mg)
CYP3A4	6.1 (3.6)
CYP3A4 +CYP3A5	15.9 (9.8)

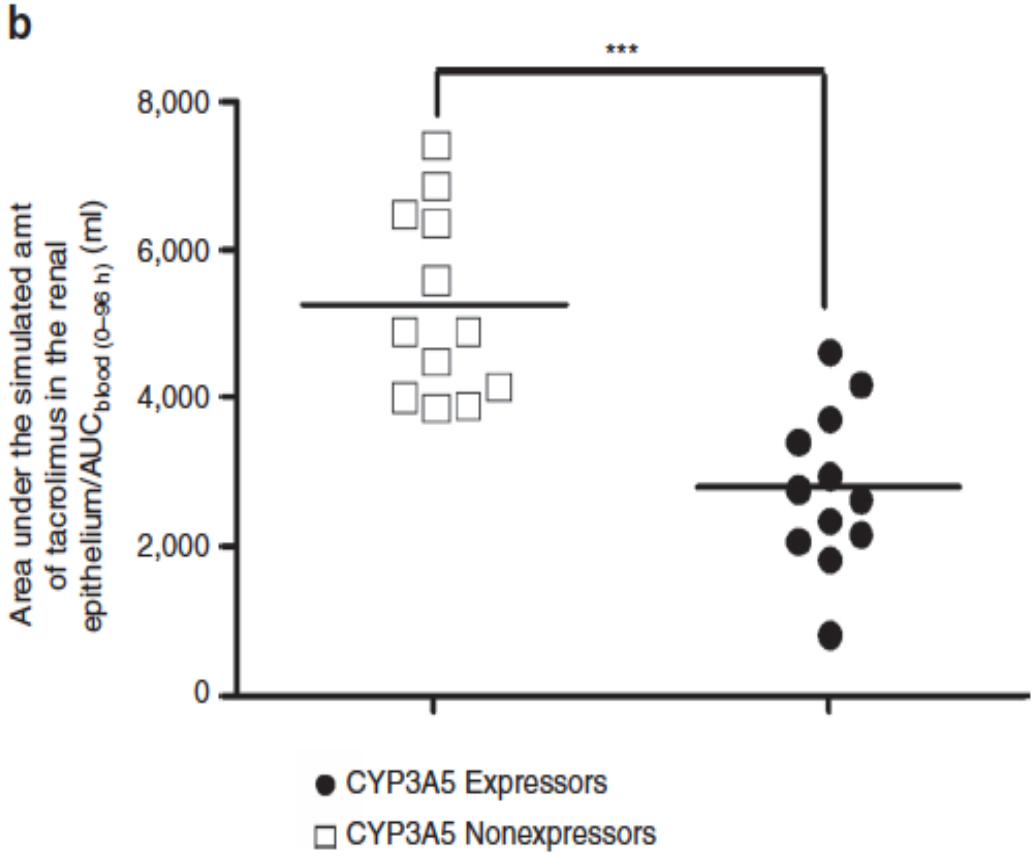
The CYP3A4 content for the 10 matched microsomal preparations represented in each group was equivalent. The nominal initial tacrolimus conc was 0.2 μM; unbound conc determined after measurement of nonspecific binding.

*CYP3A5*1* Affects Intra-Renal Tacrolimus Accumulation

Semi-Physiological Renal Compartmental Model of Tacrolimus Disposition



Ben Zheng et al, CPT, 2012



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

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/
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- **Denise Dillard**
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- **Denise Boudreau**
  Group Health Research Institute

NWA PGRN
Northwest-Alaska Pharmacogenomics Research Network

PGRN

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 D_3**
- Living at 60° 47' N, they experience significant seasonal changes in sunlight exposure and vitamin D_3 synthesis

Study Design

Recruited 1000 Yup'ik study participants

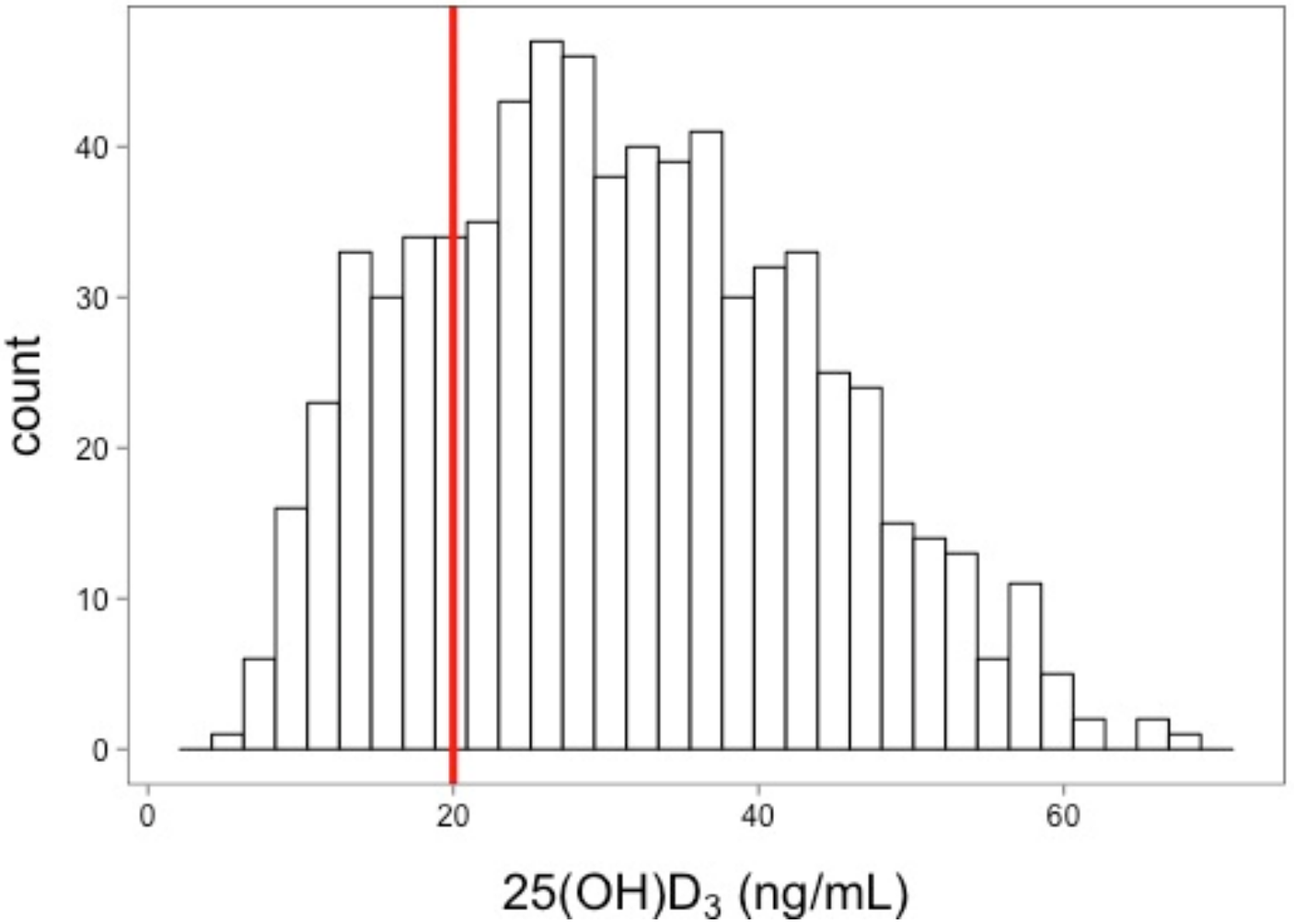
- Collect 5 ml blood: fractionate for DNA, plasma, RBC
- Distribute samples to UA Fairbanks, UW Medicinal Chemistry, UW Laboratory Medicine, UW Genome Sciences

Measure vitamin D status (serum $25OHD_3$)

Determine association with:

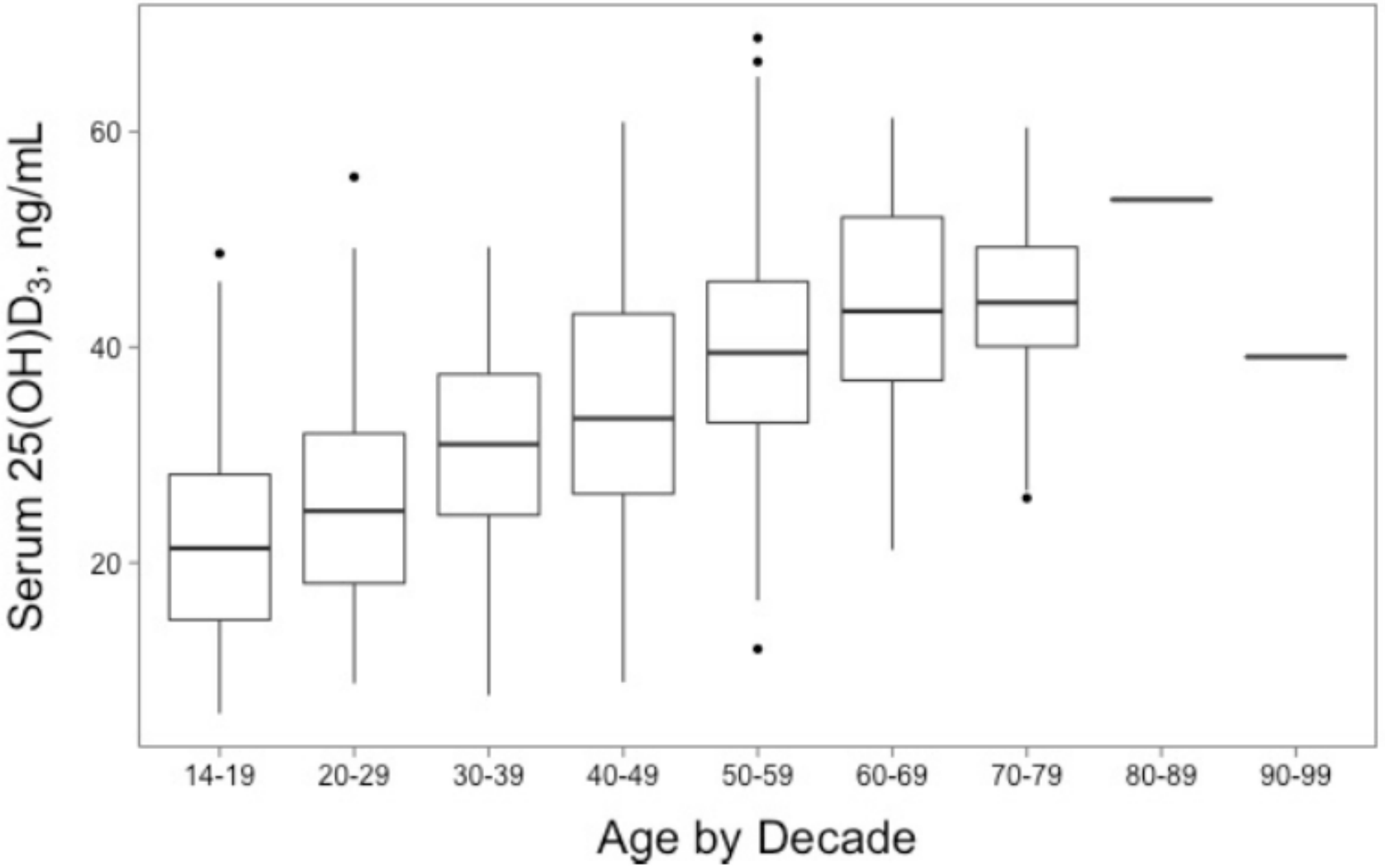
Age, gender, BMI, *CYP2R1, DHCR7* and *DBP* genotypes, dietary ω3 PUFA biomarker ($\delta^{15}N$), season of blood draw

Serum 25(OH)D_3 Concentrations

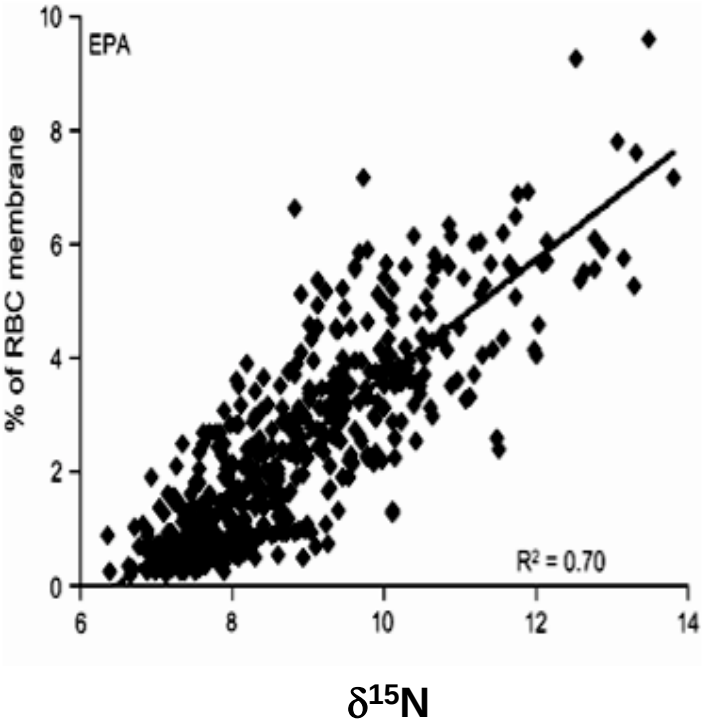


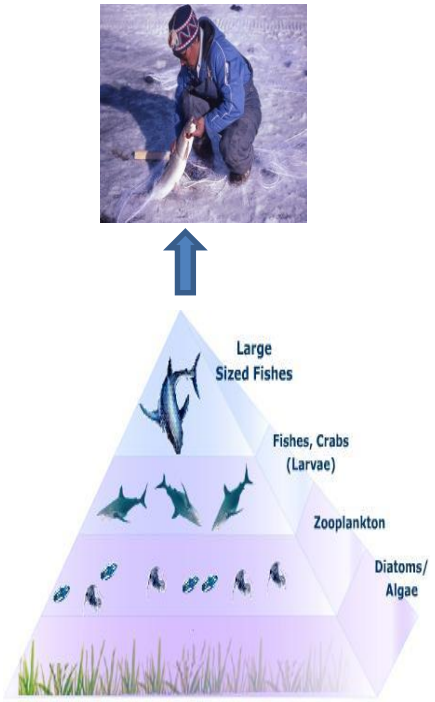
Alie Fohner et al, J Nutrition, 2016

Distribution of Serum 25(OH)D_3 Level by Age



Assessment of Dietary ω3 PUFA Intake – ^{15}N Enrichment ($\delta^{15}N$)





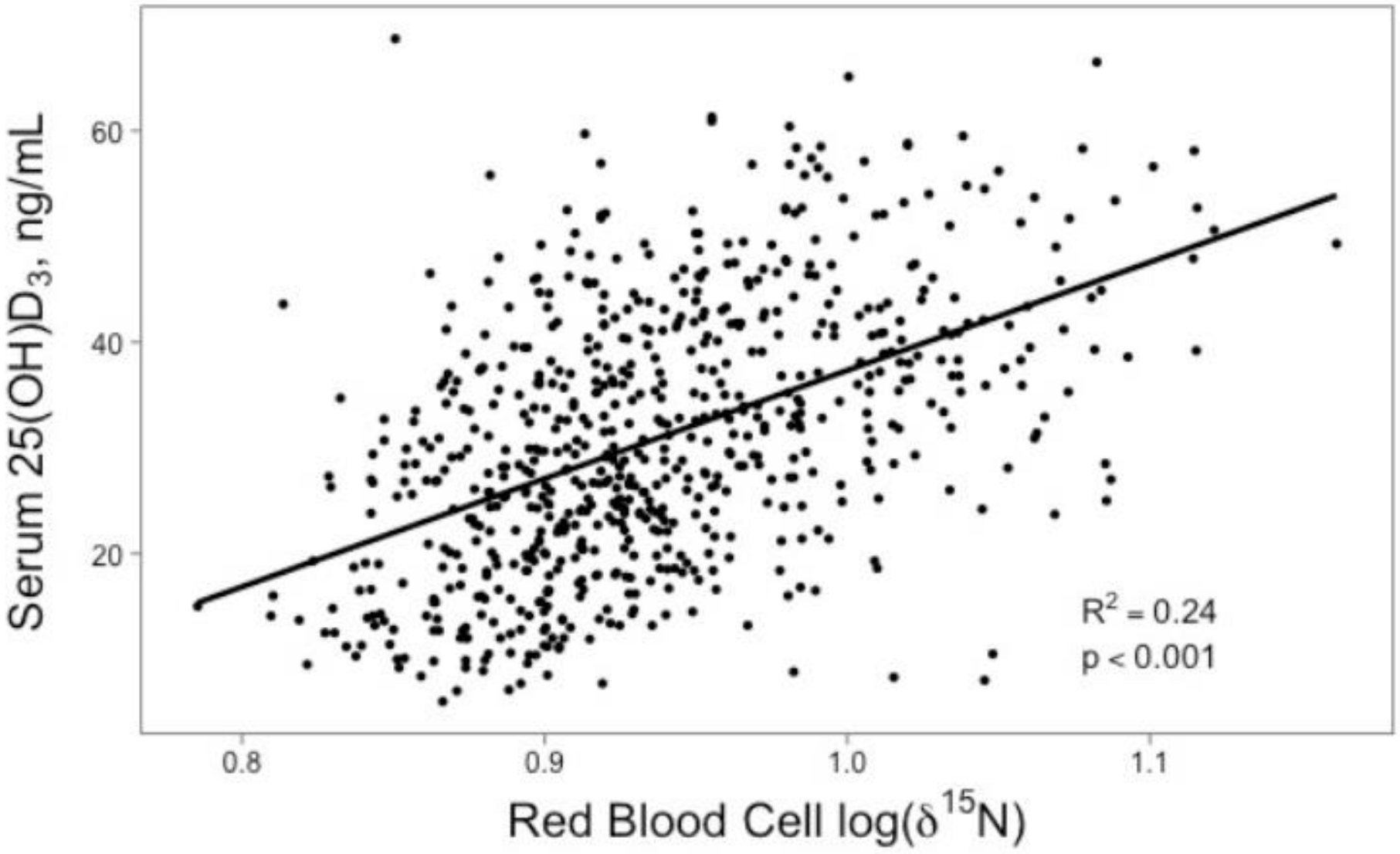
$$\delta^{15}N = \frac{(R_{sample} - R_{standard})}{R_{standard}} \times 1000‰$$

- $R = (^{15}N/^{14}N)$
- Standard = atmospheric N_2, where % abundance of $^{15}N = 0.37\%$

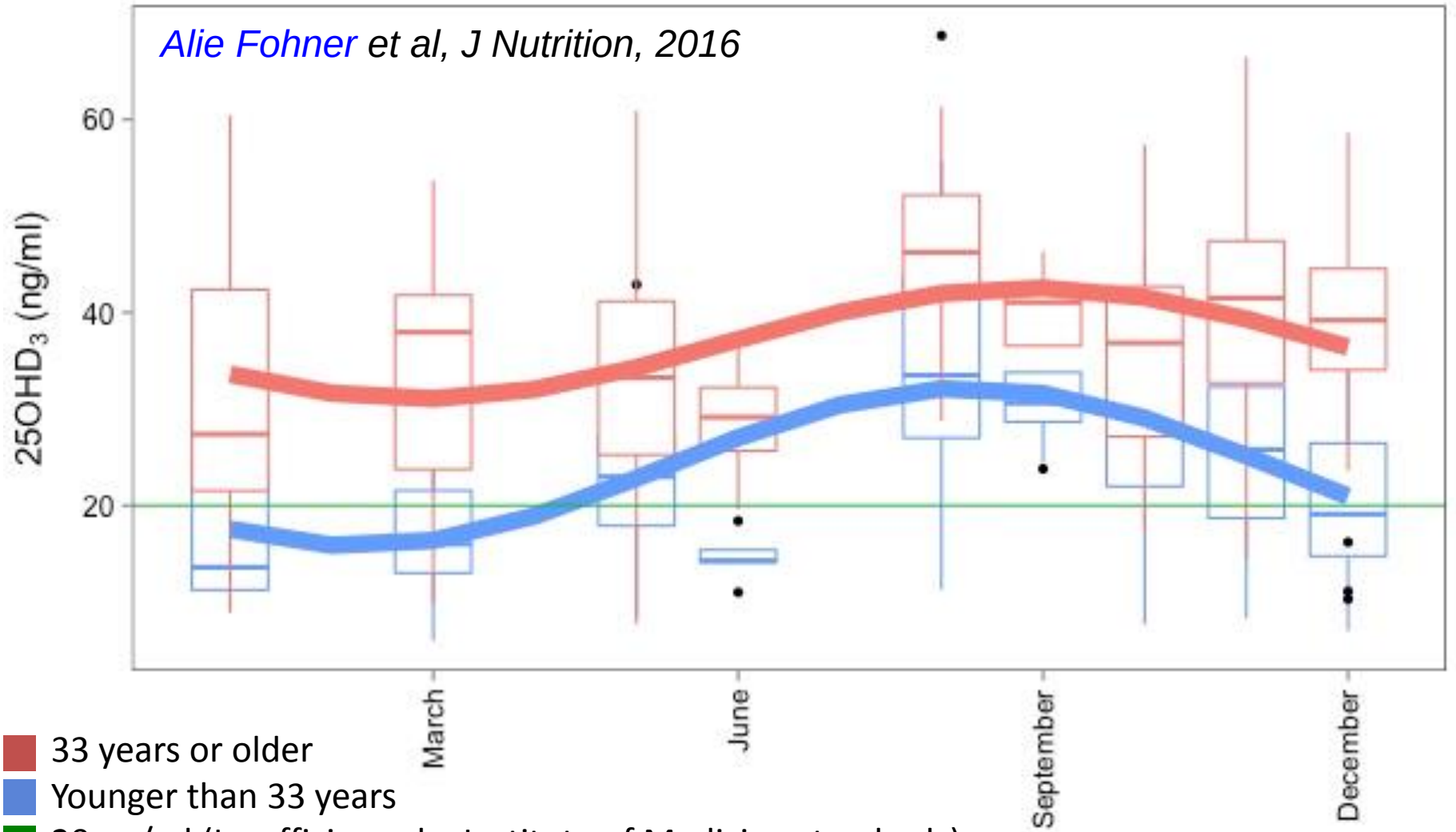
- Continuous-flow isotope mass spectrometry
- Surrogate marker of ω3 PUFA consumption
- Marine environments have more ^{15}N → reflects the length of food chain in predatory fish

O'Brien DM, et al. J Clin Nutr. 2009

Correlation of $25(OH)D_3$ with $\log(\delta^{15}N)$



Sinusoidal Model of Annual Variation



Unrelated Subset Multiple Regression

Covariate	N	Significance in full model	Variability explained (R^2)
Fully adjusted model	**526**	**p < 0.001**	**(0.528)**
Age (younger vs older than 33)		**p < 0.001**	**(0.365)**
Season		**p < 0.001**	**(0.091)**
$Log_{10}(\delta^{15}N$ value)		**p < 0.001**	**(0.205)**
Gender		**p = 0.007**	**(0.00)**
Village location (Coastal vs Inland)		**p < 0.001**	**(0.063)**
BMI		**p = 0.041**	**(0.006)**
***CYP2R1* rs11023374**		**p = 0.016**	**(0.011)**
GC rs4588 (TA haplotype)		**p < 0.001**	**(0.028)**
Age and $log_{10}(\delta^{15}N$ value)			**(0.386)**

- 58% of the variability explained by demographic, diet, season and genetic factors; what explains for the rest?

Conclusions from 25 Years of Studying CYP3A

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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Trainees (1992-2016)