Session I. Biotransformation

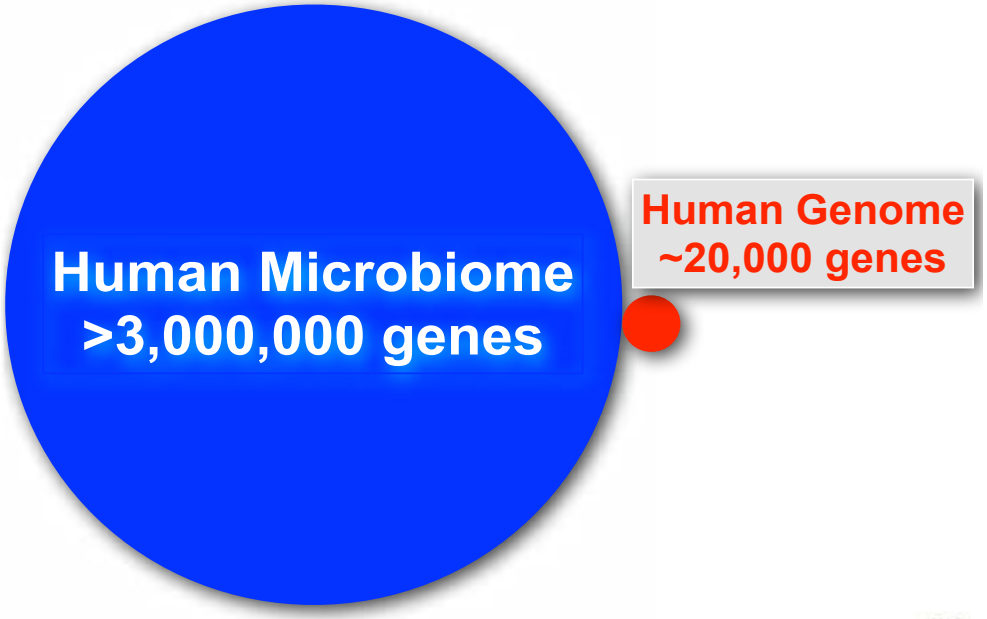
Overview of Biotransformation of Xenobiotics by Microbiota

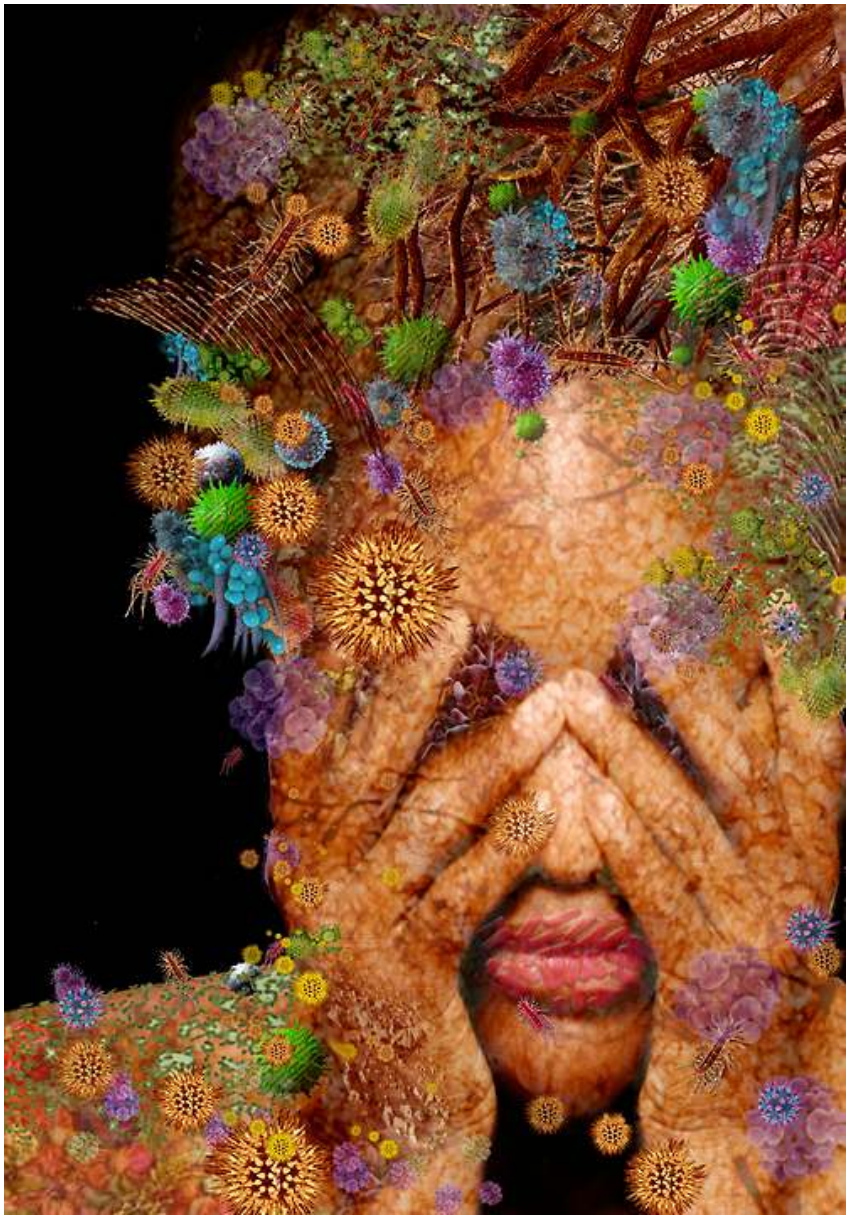
Julia Yue Cui, PhD, DABT

Sheldon Murphy Endowed Assistant Professor
Department of Environmental and Occupational Health Sciences
University of Washington
Email: juliacui@uw.edu

Human Microbiome: Our Second Genome

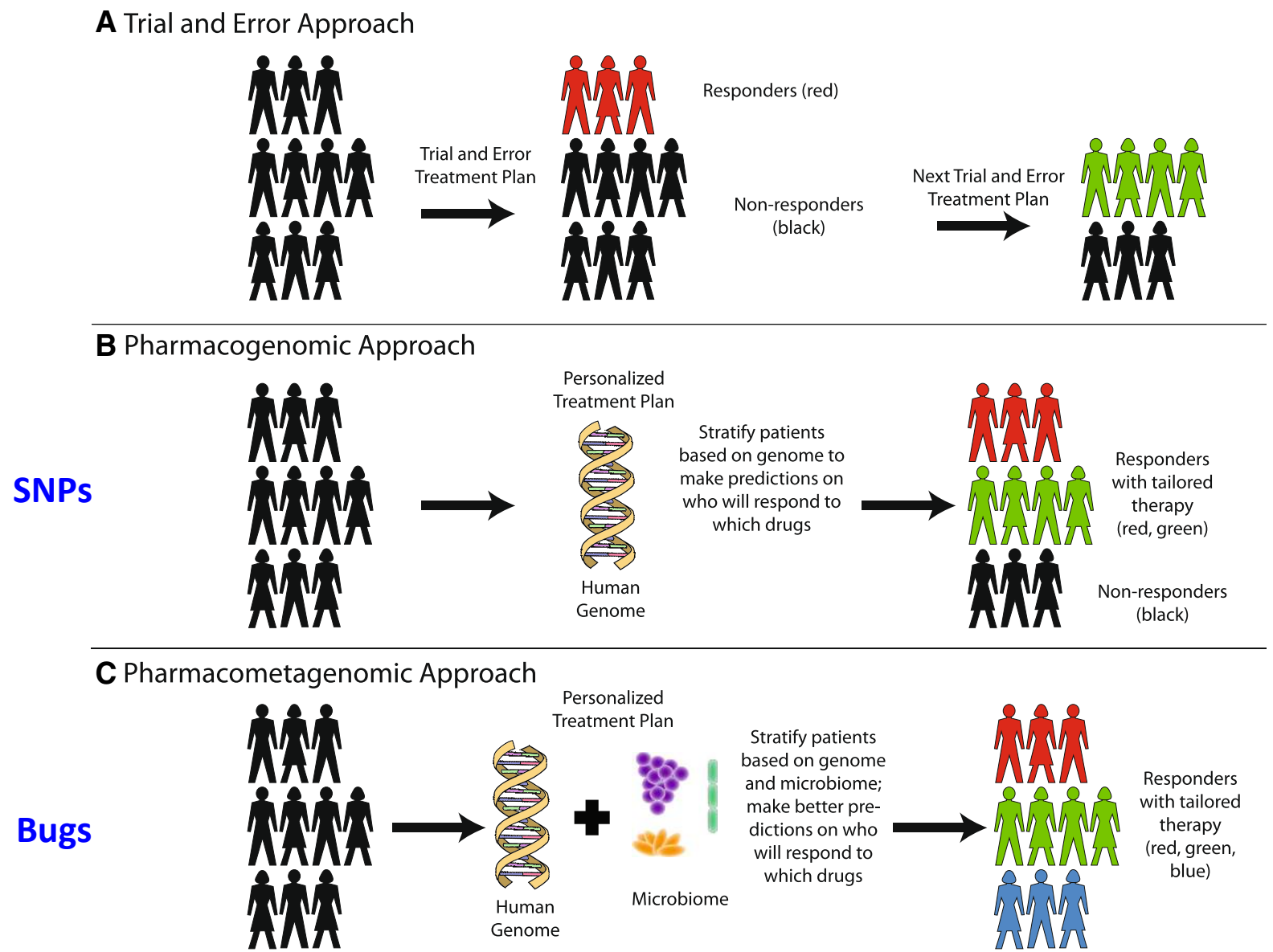
- Human microbiome is a highly dynamic and metabolically active "second genome", estimated to have over 3 million unique genes, that varies in composition due to host genetics, diet, xenobiotic exposure, and location in the body
- The majority of the human microbiome is located in the GI tract





Picture courtesy from Dr. Allan Rettie

Gut Microbiome as the "Second Genome" for Precision Medicine: the Pharmaco-metagenomic Approach



Nayak and Turnbaugh, 2016 *BMC Medicine*

ANNUAL REVIEWS 2018

Annual Review of Pharmacology and Toxicology

Impacts of the Human Gut Microbiome on Therapeutics

Yoshiki Vázquez-Baeza,[1] Chris Callewaert,[2] Justine Debelius,[2] Embriette Hyde,[2] Clarisse Marotz,[3] James T. Morton,[1] Austin Swafford,[4] Alison Vrbanac,[3] Pieter C. Dorrestein,[5] and Rob Knight[1,2,4]

Table 1 Summary of selected studies in which the microbiome was determined to be implicated in the outcome of a treatment. In these cases, the presence or absence of a group of bacteria may hinder or promote the efficacy of a procedure

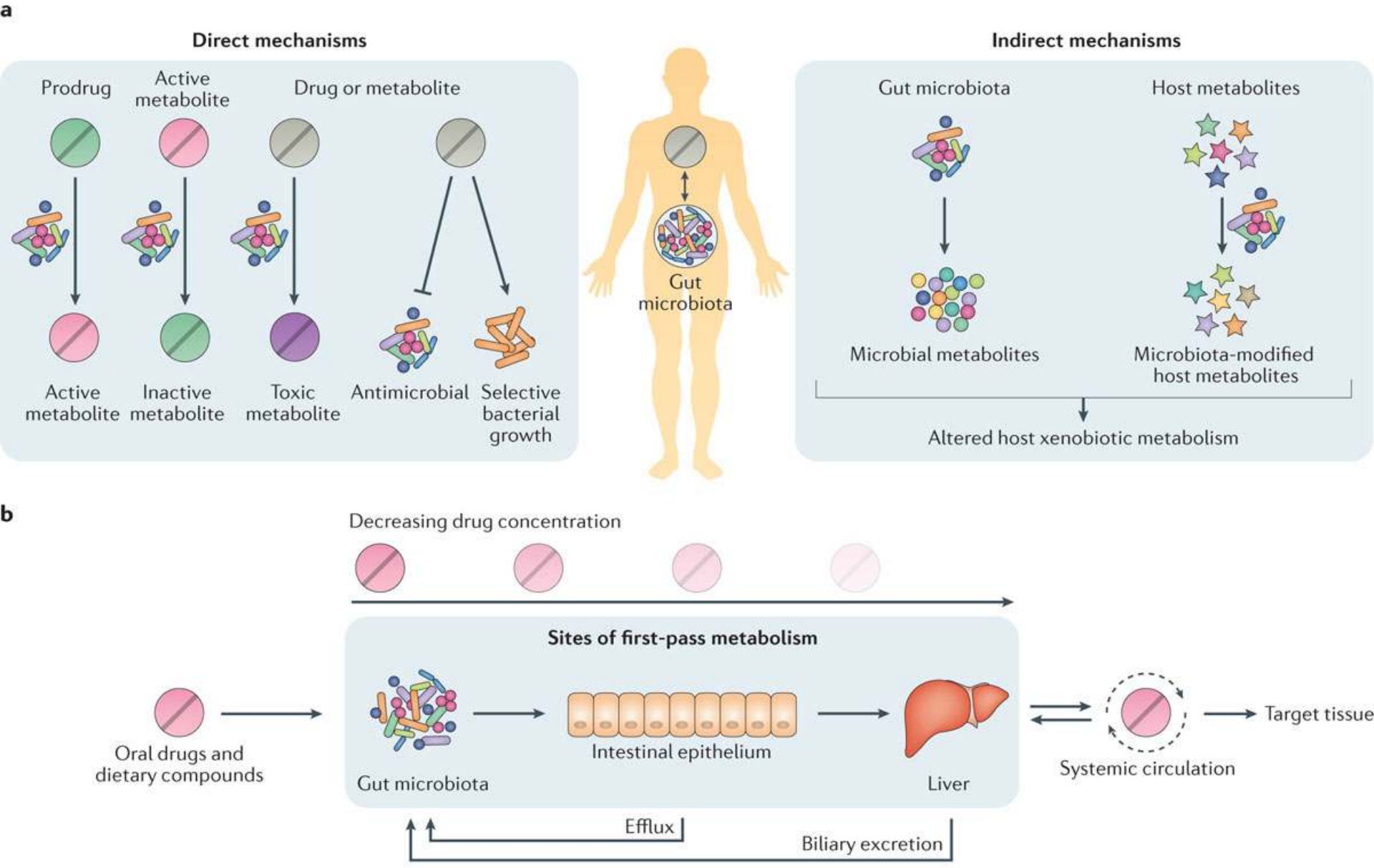
Treatment	Interaction	References
Nonsteroidal anti-inflammatory drugs (NSAIDs)	Increased gram-negative bacteria are associated with increased damage to the small intestine. These lesions can be ameliorated if pretreated with antibiotics.	50, 51
Antibiotics	Treatment effect depends on the host's starting microbiome composition and antibiotic class. Subjects with similar profiles have correlated abundance alterations of *Enterobacter cloacae*.	58, 60
Cardiac glycosides	The bioavailability of digoxin is affected by the presence of *Eggerthella lenta*. This can be regulated by suppressing the reductase responsible for degrading this drug.	62, 63
Metformin	The mechanism driving the effect of this drug is a result of metabolic shifts of the gut microbiome.	66
Fecal microbiome transplant	The efficacy of this treatment is known to benefit from pretreatment with antibiotics.	72, 73
Chemotherapeutics	This treatment causes negative drug-drug interactions by bacterial metabolism and protects from chemotherapy-related bloodstream infection.	136, 137

Early Life Disruption of Gut Microbiome and Delayed Onset of Human Diseases

Disruptive factor	Study	Cohort characteristics	Outcomes
C-section	Sevelsted *et al.*[25]	1.9 million Danish term children, ages 0–15 years	Asthma, systemic connective tissue disorders, juvenile arthritis, IBDs, immune deficiencies and leukemia
	Huh *et al.*[26]	1,255 US children, age 3 years	Obesity, higher body-mass index and sum of skinfolds
	Eggesbø *et al.*[27]	2,803 Norwegian children, 0–2 years	Reactions to egg, fish or nuts, and a fourfold increase in egg allergy
Antibiotic treatment	Risnes *et al.*[43]	1,401 US children, ages 0–6 months	Asthma and allergy
	Hoskin-Parr *et al.*[44]	5,780 UK children, ages 0–2 years	Asthma and eczema
	Saari *et al.*[150]	12,062 Finnish children, ages 0–2 years	Overweight and obesity
	Schwartz *et al.*[151]	163,820 US children ages 2–18 years	Weight gain
	Kronman *et al.*[48]	9 million UK children	IBD development
Probiotics	Maldonado *et al.*[89]	215 Spanish children, ages 0–6 months	Reduction in gastrointestinal and upper respiratory tract infections
	Braegger *et al.*[76]	ESPGHAN Committee on Nutrition	Reduction in nonspecific gastrointestinal infections
Diet supplements	Zimmerman *et al.*[93]	Iron, 139 African children, ages 6–14 years	Intestinal inflammation, lower frequency of colic or irritability
Hygiene	Hesselmar *et al.*[96]	184 children, pacifier cleaning, ages 0–3 years	Lower risk of developing asthma, allergy and sensitization
Pets	Virtanen *et al.*[99]	3,143 Finnish children, ages 0–1 year	Reduction in risk of preclinical type I diabetes

Tamburini et al., 2016 *Nature Medicine*

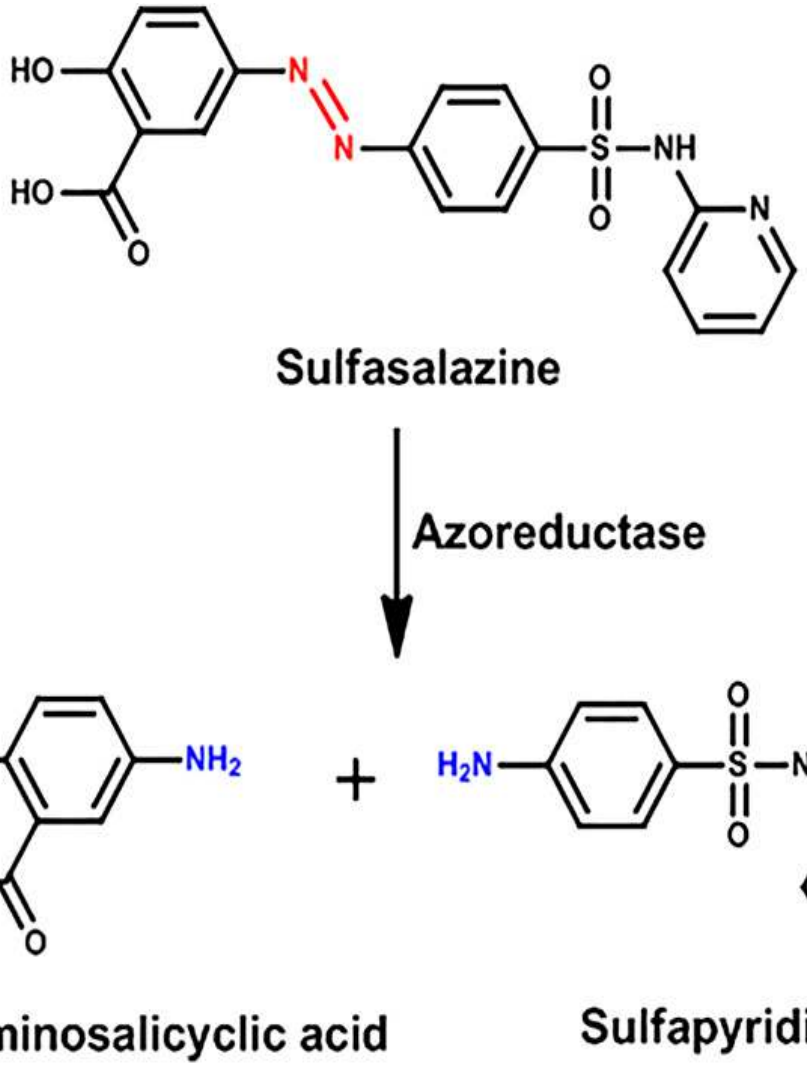
The Gut-Liver Axis in Xenobiotic Biotransformation



Spanogiannopoulos et al., 2016 *Nature Reviews*

Examples for Direct Metabolism by Microbial Enzymes

- A pro-drug that has two therapeutic moieties
- Azo-reduction by gut microbiota
- Generating anti-inflammatory drug and the antibiotic drug
- Used to treat ulcerative colitis



Klaassen and Cui (2015) Drug Metab Dispo 43: 1505-21 PMID: 26261286. Review

Examples for Direct Metabolism by Microbial Enzymes

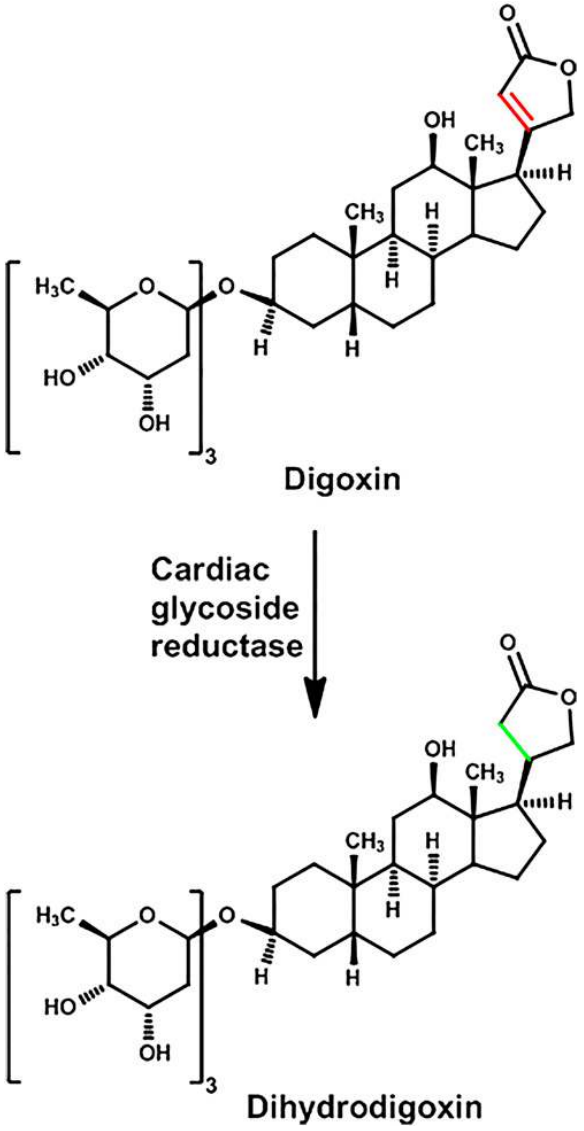
www.sciencemag.org **SCIENCE** VOL 341 19 JULY 2013

Predicting and Manipulating Cardiac Drug Inactivation by the Human Gut Bacterium *Eggerthella lenta*

Henry J. Haiser,[1] David B. Gootenberg,[1] Kelly Chatman,[1] Gopal Sirasani,[2]
Emily P. Balskus,[2] Peter J. Turnbaugh[1]*

Foxglove (digilatis sp.)
Contains digitalis glycosides in leaves and seeds

- **Digoxin is inactivated by the microbial enzyme Cgr operons from *E lenta*.**
- **There is a "drug-bug" interaction if digoxin is taken with oral antibiotics**



Haiser et al., 2013 Science
Klaassen and Cui 2015 Drug Metab Dispo

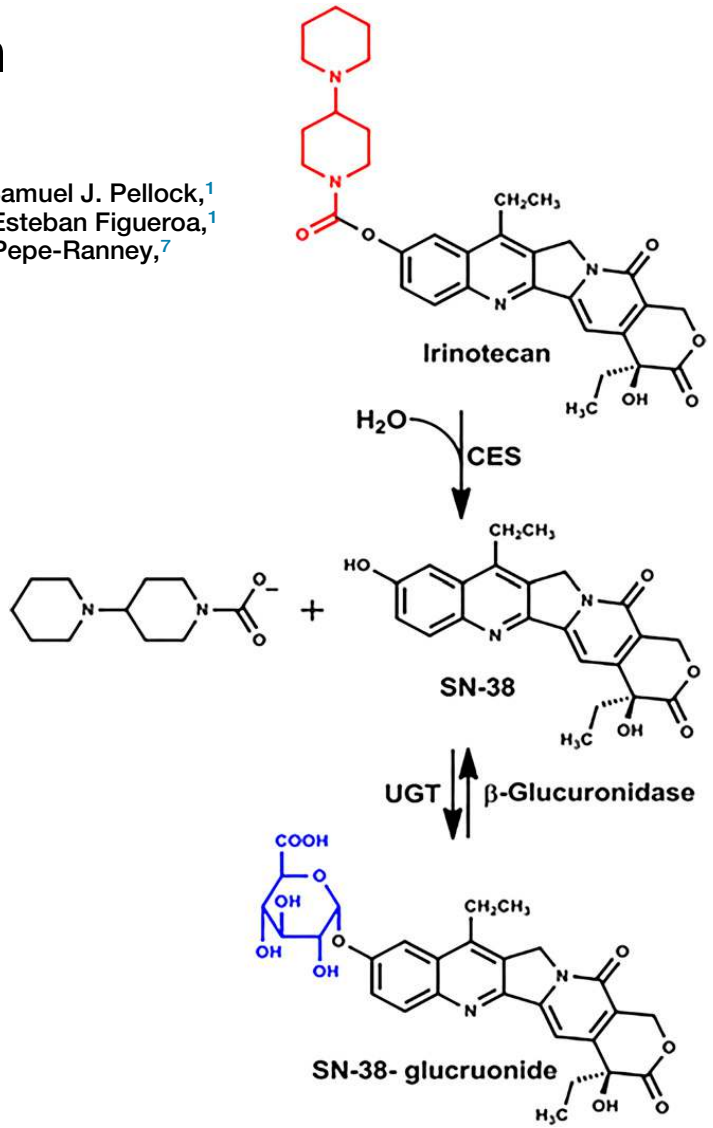
Examples for Direct Metabolism by Microbial Enzymes

Structure and Inhibition of Microbiome β-Glucuronidases Essential to the Alleviation of Cancer Drug Toxicity

Bret D. Wallace,[1,8] Adam B. Roberts,[2,8] Rebecca M. Pollet,[1] James D. Ingle,[1] Kristen A. Biernat,[1] Samuel J. Pellock,[1] Madhu Kumar Venkatesh,[3] Leah Guthrie,[4] Sara K. O'Neal,[5] Sara J. Robinson,[1] Makani Dollinger,[1] Esteban Figueroa,[1] Sarah R. McShane,[1] Rachel D. Cohen,[1] Jian Jin,[6] Stephen V. Frye,[6] William C. Zamboni,[5] Charles Pepe-Ranney,[7] Sridhar Mani,[3] Libusha Kelly,[4] and Matthew R. Redinbo[1,2,*]

Chemistry & Biology *22*, 1238–1249, September 17, 2015

- **Specific chemical inhibitors targeting the microbial beta-glucuronidases can inhibit the deconjugation of SN-38 glucuronide**
- **This leads to reduced toxicity of this anti-cancer drug**
- **Inhibition does not alter serum PK of irinotecan or its metabolites**



Wallace et al., 2015 Chemistry & Biology
Klaassen and Cui 2015 Drug Metab Dispo

Examples for Direct Metabolism by Microbial Enzymes

Environmental chemicals directly metabolized by microbiota:

Table 1. Human exposure to pollutants and their interaction with the GI microbiota

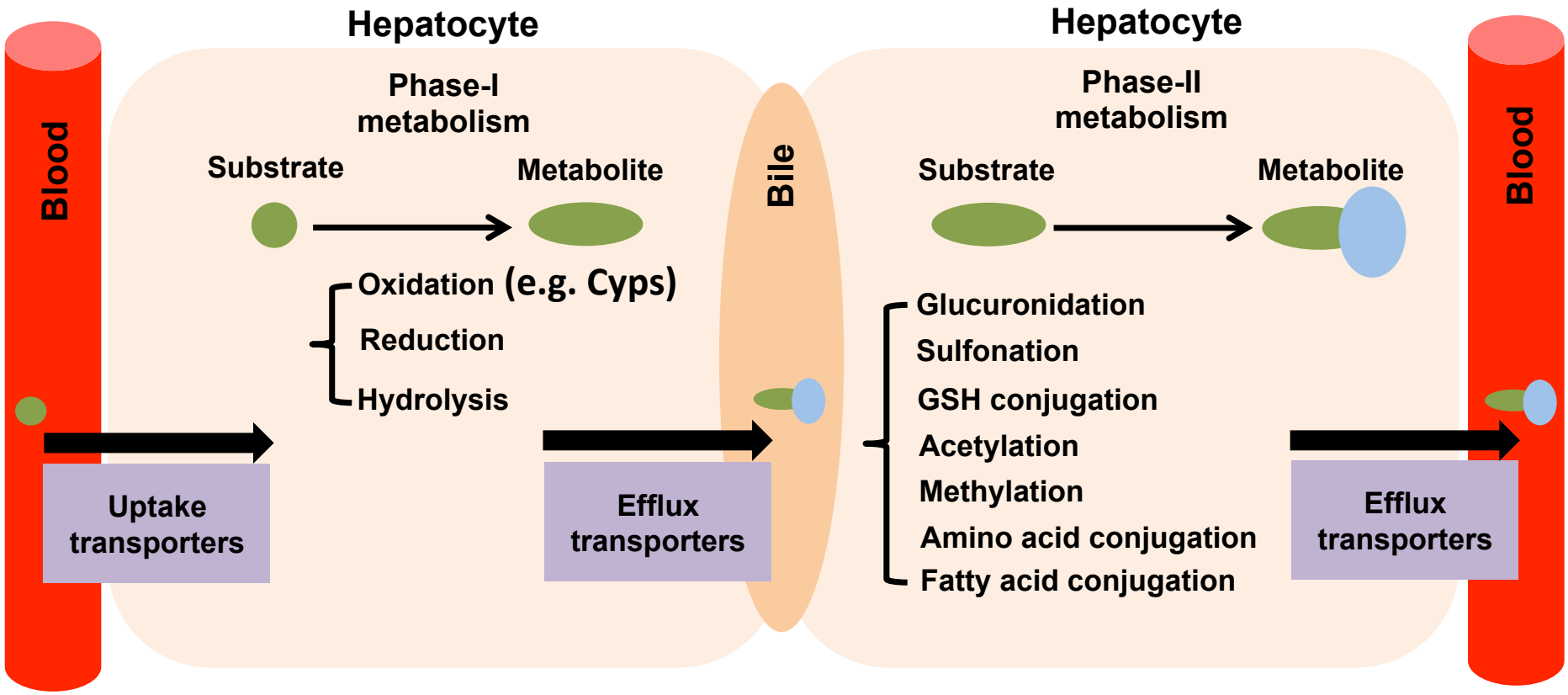
Chemical	*Source*	*Human exposure*	*Metabolism by microbiota*	*Effect on microbiota*	*References*
PAHs	Air and food pollutants resulting from incomplete combustion of fossil fuel, tobacco	Mean total intake of 3.12 mg per day (97% through food, 1.6% air, 0.2% water, 0.4% soil)	*In vitro*: hydroxylation; *in vivo*: deconjugation of liver metabolites, involved in the formation of CH_3S-metabolites		32,104,105
Nitro-PAHs	Air and food pollutants, derivatives of PAHs	Diesel exhaust identified as main source of exposure. 2NF: range from 0 to 92 ng/m^3	Reduction to amine metabolites		36,106
Nitrotoluenes	Intermediates in the manufacture of dyes, chemicals, explosives	Mainly occupational. 2-nitrotoluene: 0.35–0.7 mg/m^3 through air; 420 mg per day through skin	Reduction to amine metabolite and hydolysis of glucuronide conjugates		39,40,107
Pesticides	Pollutants in air and food	Chlorpyrifos: mainly through diet 0.01 to 0.14 μg/kg bw per day; DDT: through diet 0.29 μg/kg bw per day	Dechlorination of organochlorides. Deconjugation of propachlor *in vivo*	Perinatal exposure to chlorpyrifos (1 mg/kg bw per day) induced dysbiosis at adulthood (rat)	45,92,108
PCBs	Industrial chemicals now prohibited but persistent in water sediments and soils	Mainly through diet DL-PCBs: 0.29 pg TEQ WHO_{98}/kg bw per day; NDL-PCBs: 2.71 ng/kg bw per day	Bacterial C–S-lyase plays an important role in formation of methyl sulfone ($MeSO_2$)-metabolites *in vivo*	Mixture of PCBs (150 μM/kg for 2 days) decreased the abundance of many bacteria (mainly Proteobacteria)	55,56,109,110
Metals	Ubiquitous environmental contaminants	Mainly through diet: arsenic 0.78 μg/kg bw per day; lead 0.2 μg/kg bw per day; cadmium 0.16 μg/kg bw per day	Involved in demethylation of mercury, methylation of arsenic and bismuth	Cadmium (20–50 mg/kg bw/d for 45 days); lead (100 or 500 mg/l) or arsenic (10 p.p.m. for 4 weeks) induced dysbiosis (mouse)	93,94,96,109
Azo dyes	Food colourants	Mainly through diet	Azoreduction of the azo bound to produce aromatic amines		74,111
Melamine	Widely used in plastics, illegal food contaminant	TDI: 0.2 mg/kg bw (EU)	Metabolised to cyanuric acid		75
Artificial sweeteners	Food additives	ADI (FDA, US): Aspartame: 50 mg/kg bw; saccharin: 15 mg/kg bw	Cyclamate metabolised to cyclohexamine	Aspartame (5–7 mg/kg/d), sucralose and saccharin (5 mg/kg per day) induce dysbiosis in animals with potential deleterious metabolic effect for the host (mouse and human)	80,98,102,103
Other POPs (e.g., PCDFs)	Pollutants formed during industrial processes	Mainly through diet: PCDD/Fs 0.176 pg TEQ WHO_{98}/kg bw per day		2,3,7,8 TCDF (24 μg/kg) induced dysbiosis and affected the faecal metabolic profiles (mouse)	97,109

Abbreviations: ADI, acceptable daily intake; DL-PCBs, dioxin-like PCBs; EU, European Union; FDA, Food and Drug Administration; NDL-PCBs, Non-dioxin-like PCBs; PCBs, polychlorobiphenyls; PAHs, polycyclic aromatic hydrocarbons; POPs, persistent organic pollutants; TEQ, toxic equivalency; TDI, tolerable daily intake.

Lu et al, 2015 ILAR Journal

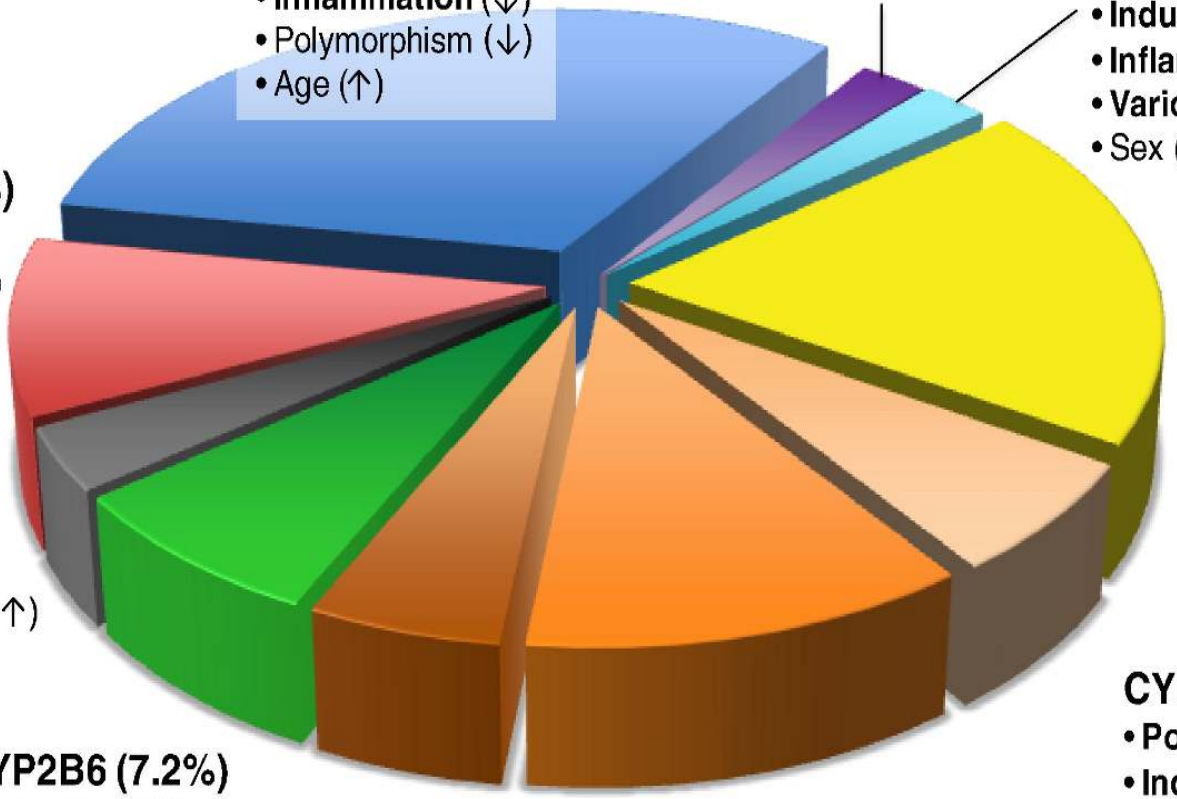
Claus et al., 2016 npj Biofilms and Microbiomes

Introducing the "Xenobiotic-Processing Genome" in Liver



Julia Yue Cui and Cindy Yanfei Li. Comprehensive Toxicology, 3rd Edition. Chapter 9.05

The P450 Gene Superfamily



CYP3A4/5 (30.2%)
- **Induction**
- **Sex (f>m)**
- **Inflammation (↓)**
- Polymorphism (↓)
- Age (↑)

CYP2J2 (3%)
- Polymorphism (↓)

CYP2E1 (3%)
- **Induction (↑)**
- **Inflammation (↑)**
- **Various diseases (↑)**
- Sex (m>f)

CYP1A2 (8.9%)
- **Induction (↑)**
- Inflammation (↓)
- Cholestasis (↓)
- Age (↑)
- (Sex, m>f ?)
- (Polymorphism)

CYP2D6 (20%)
- **Polymorphism (↓↑)**
- Inflammation (↓)

CYP2A6 (3.4%)
- **Polymorphism (↓↑)**
- **Induction (↑)**
- Inflammation (↓)
- (Sex, f>m ?)
- Age (↑)

CYP2B6 (7.2%)
- **Induction (↑)**
- **Polymorphism (↓↑)**
- Inflammation (↓)
- Age (↑)
- (Sex, f>m ?)

CYP2C8 (4.7%)
- **Induction (↑)**
- Polymorphism (↓↑)
- Inflammation (↓)
- Age (↑)

CYP2C9 (12.8%)
- **Induction (↑)**
- **Polymorphism (↓)**
- Inflammation (↓)
- Age (↑)
- (Sex ?)

CYP2C19 (6.8%)
- **Polymorphism (↓↑)**
- **Induction (↑)**
- Inflammation (↓)
- (Sex ?)

Zanger and Schwab (2013) Pharmacology & Therapeutics

Xenobiotica, 2009; 39(4): 323–334

informa
healthcare

RESEARCH ARTICLE

Intestinal flora induces the expression of Cyp3a in the mouse liver

T. Toda[1], N. Saito[1], N. Ikarashi[1], K. Ito[1], M. Yamamoto[2], A. Ishige[3], K. Watanabe[3], and K. Sugiyama[1]

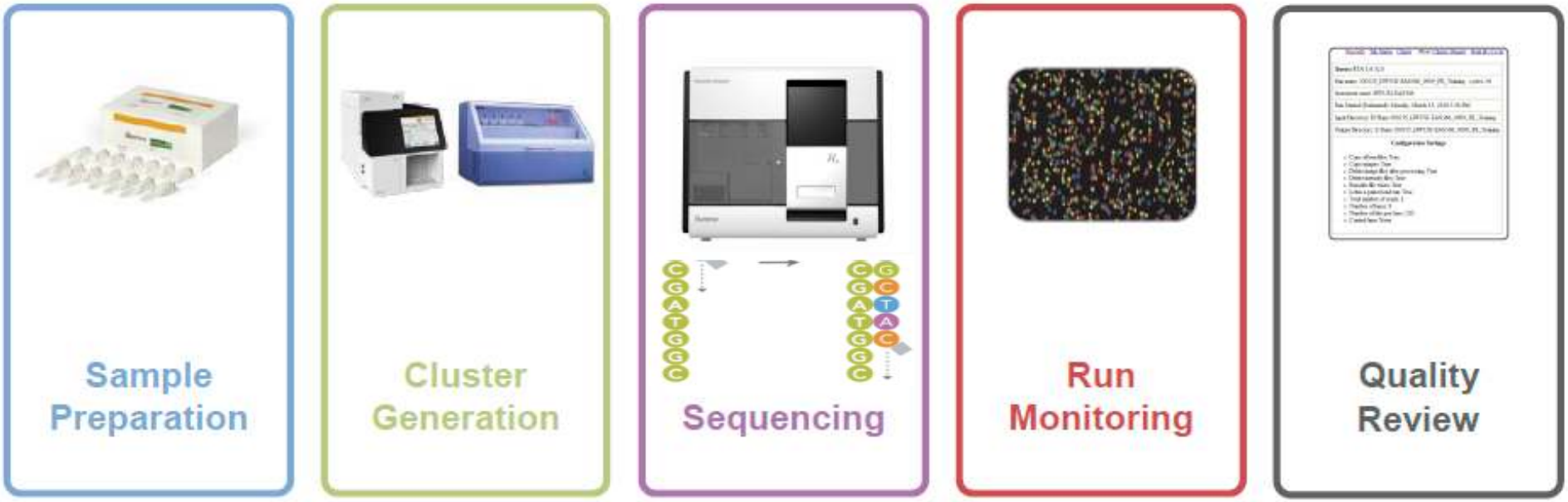
- **Cyp3a: The major Phase-I oxidation enzyme responsible for >50% of prescribed drugs – prototypical target gene of PXR.**

- **Adult conventional mice exposed to ciprofloxacin, or germ free mice have reduced Cyp3a expression (Toda et al., 2009a and 2009b).**

PMID: 19350455

PMID: 19420891

RNA-Seq Pipeline



1.25µg total RNA→ Library construction	PCR amplification	HiSeq 2000 (Sequencing by synthesis)	Illumina Software	QC and error estimation

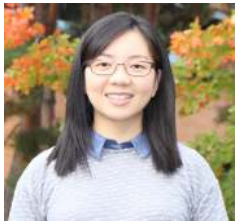
- Library prep: using the TruSeq Stranded mRNA kit using an automated workflow on a Sciclone NGSx Workstation (rRNA depleted using poly-A enrichment)

- Sequencing: 50bp paired-end n=5 multiplexing
- Mapping: HISAT; transcript abundance estimation: Cufflinks (Cuffdiff)

Indirect mechanism: Gut-Liver Axis

Pathway Analysis of the RNA-Seq Dataset in Livers of adult Male CV (Conventional) and GF (Germ-free) Mice

Stranded RNA-Seq: 50bp paired end, > 30 million uniquely mapped reads (HISAT)
Cuffdiff FDR-BH < 0.05



Cindy Yanfei Li (PhD graduate 2017)

GSAA Gene set association analysis

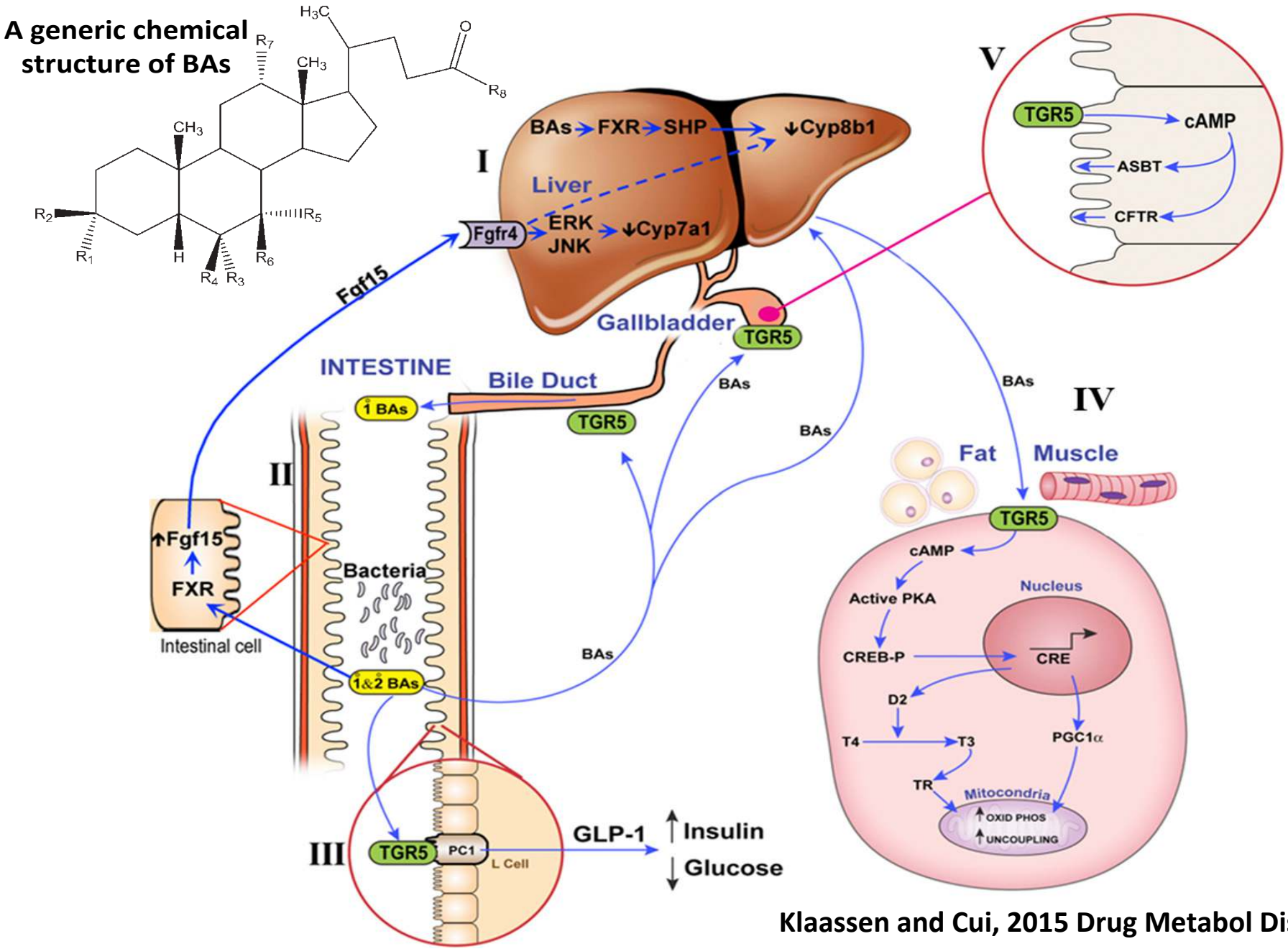
GSAASeqGP (Gene Set Association Analysis for RNA-Seq)

2	HALLMARK_UV_RESPONSE_UP	30	-0.3861713	-3.8617134	0	0	0
★ 3	HALLMARK_BILE_ACID_METABOLISM	37	-0.3304259	-3.3042586	0	0	0
★ 4	HALLMARK_CHOLESTEROL_HOMEOSTASIS	24	-0.8483721	-2.5885663	0	0	0
5	HALLMARK_COAGULATION	27	-0.5166191	-2.4967966	0	0	0
6	HALLMARK_ALLOGRAFT_REJECTION	47	-0.5254555	-1.990955	0	0	0
7	HALLMARK_OXIDATIVE_PHOSPHORYLATION	61	-0.5223557	-1.9362773	0	0	0
8	HALLMARK_MYC_TARGETS_V1	40	-0.1835297	-1.8352966	0	0	0
9	HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	30	-0.4106297	-1.6020466	0	0	0
★ 10	HALLMARK_XENOBIOTIC_METABOLISM	56	-0.3398325	-1.5923129	0	0	0
11	HALLMARK_MTORC1_SIGNALING	44	-0.3743722	-1.5211937	0	0	0
12	HALLMARK_GLYCOLYSIS	40	-0.4032864	-1.4319979	0	0.02888889	0.2

- **Lack of gut microbiome markedly alters the pathways involved in both xenobiotic metabolism and intermediary metabolism (e.g. bile acid metabolism, cholesterol homeostasis).**

Modified from Li et al. (2017) DMD PMID: 28864748; Li et al. (2018) DMD PMID: 29769268

The Gut-Liver Axis in Bile Acid (BA) Homeostasis



Klaassen and Cui, 2015 Drug Metabol Dispo

Central Hypothesis

- **Gut microbiome is a critical interface that modulates the cross-talk between intermediary metabolism and xenobiotic biotransformation of the host liver**

Story 1: Gut Microbiome and Host Drug Processing Genes During Liver Development

Newborns and children are at a higher risk of adverse drug reactions

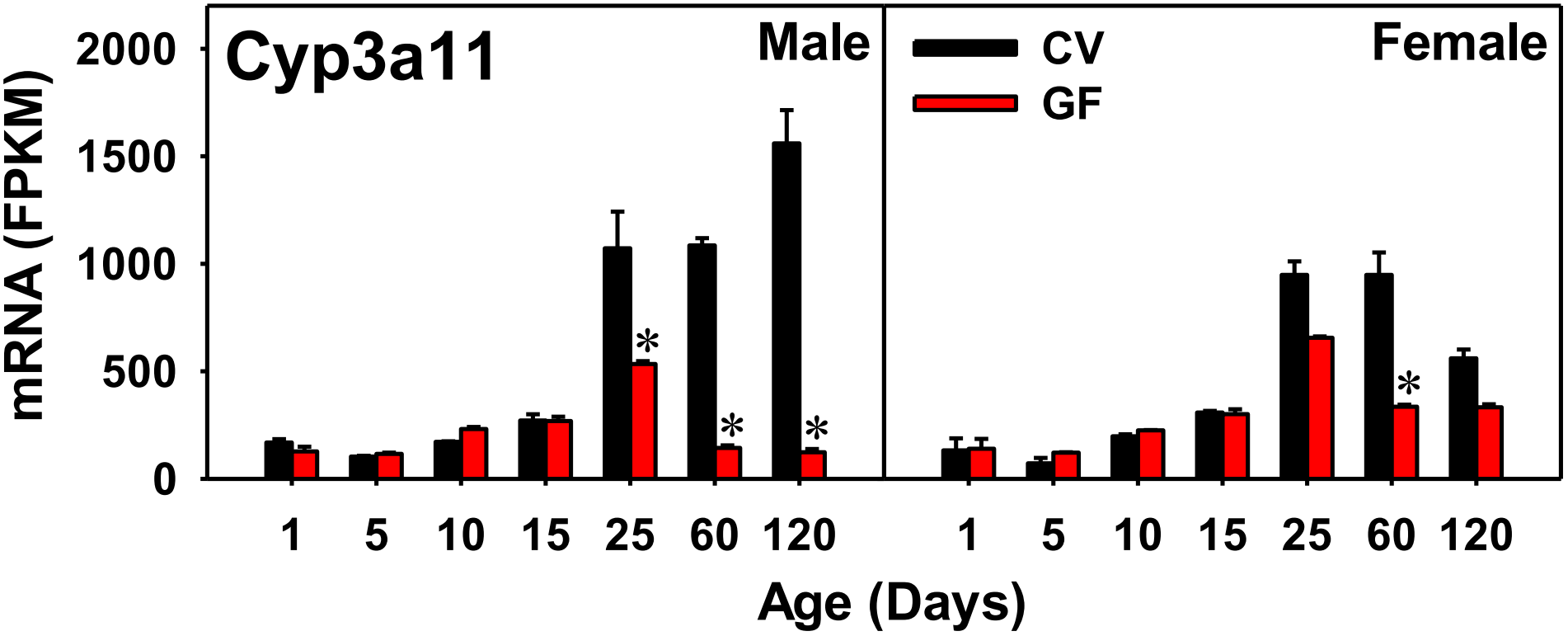
Early life exposure to xenobiotics (drugs, environmental chemicals, dietary factors, etc.)

Developmental reprogramming

Persistent alterations in disease outcomes in adult

- **Neonatal exposure to xenobiotics is associated with increasing risk of human diseases in adults (Jirtle, 2007).**

Lack of Gut Microbiome Reduced Cyp3a11 mRNA Preferably in Adult Male Livers

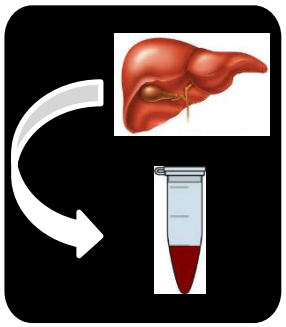


- Method: RNA-Seq (75bp paired-end, > 30 million uniquely mapped reads to mouse mm10 genome [HISAT])
- Asterisks: FDR-BH < 0.05 between CV and GF of the same age and gender (CuffDiff)

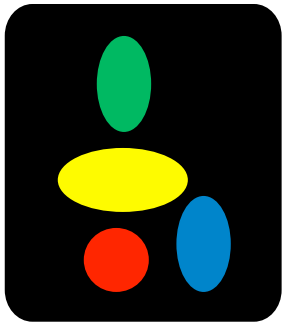
Joseph Dempsey (PhD Student)

Validation of Protein expression in GF and CV Livers Using Quantitative Proteomics

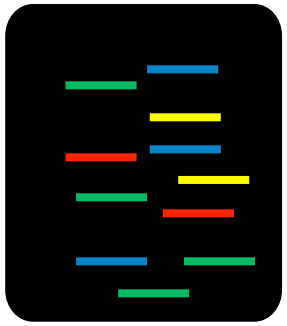
LC-MS/MS quantification of surrogate peptide(s) generated from protein digestion



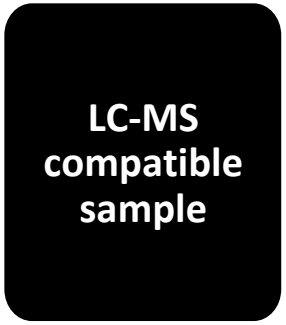
Protein isolation



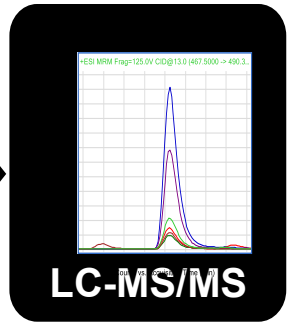
Protein in solution



Protein digestion (trypsin)



Sample cleaning and enrichment

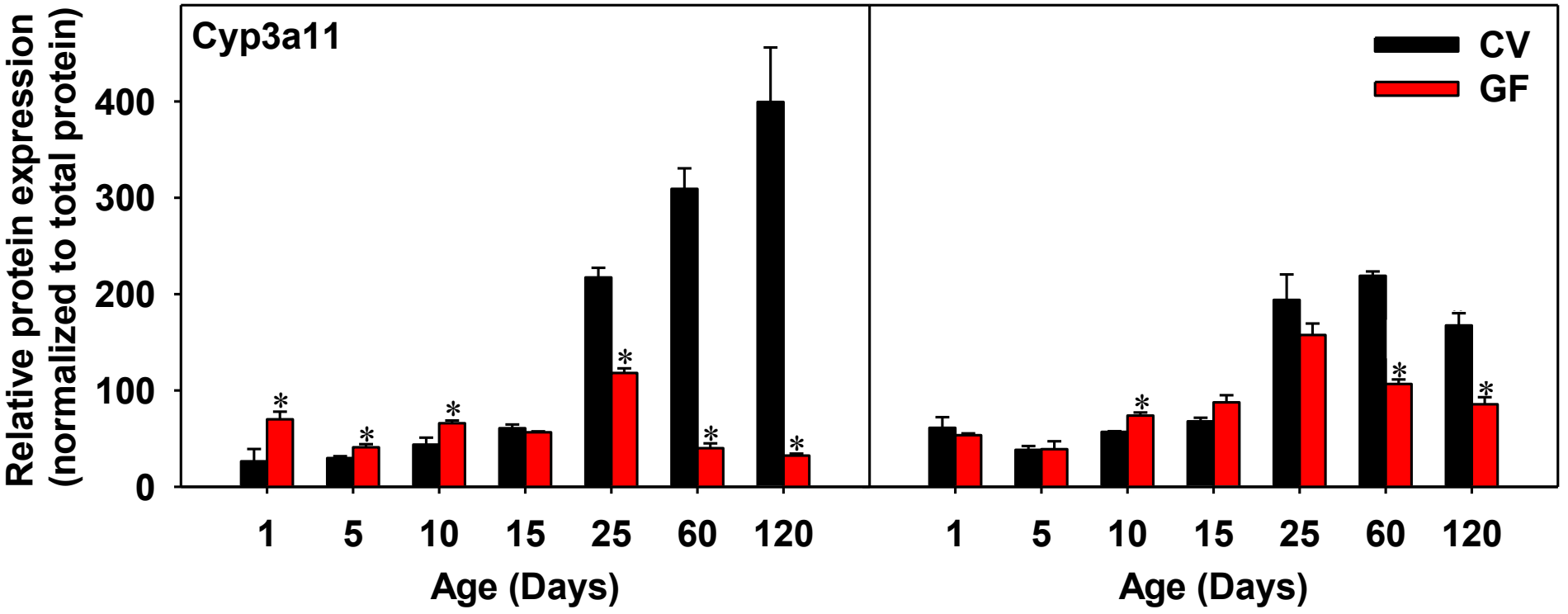


MRM analysis of surrogate peptide

	LC-MS/MS	Western blotting
1. Multiplexed quant	+++	- -
2. Reproducibility	+++	- -
3. Small overall costs	+++	- -
4. Specificity/selectivity	+++	- -

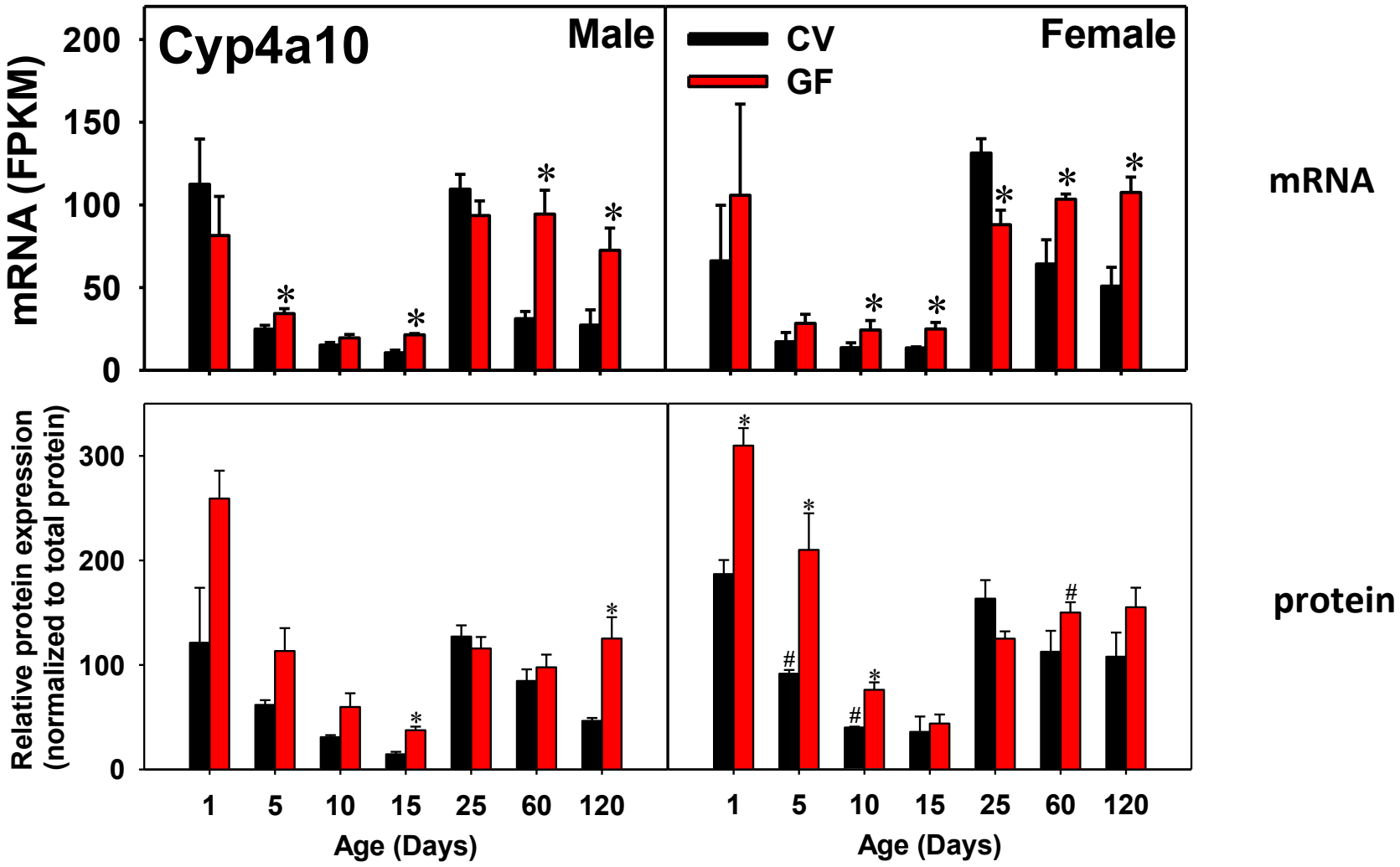
Slide courtesy from Dr. Bhagwat Prasad

Lack of Gut Microbiome Reduced Cyp3a11 Protein Preferably in Adult Male Livers



- Method: LC-MS by quantifying unique peptides specific for Cyp3a11 protein isoform
- Asterisks: FDR-BH < 0.05 between CV and GF of the same age and gender

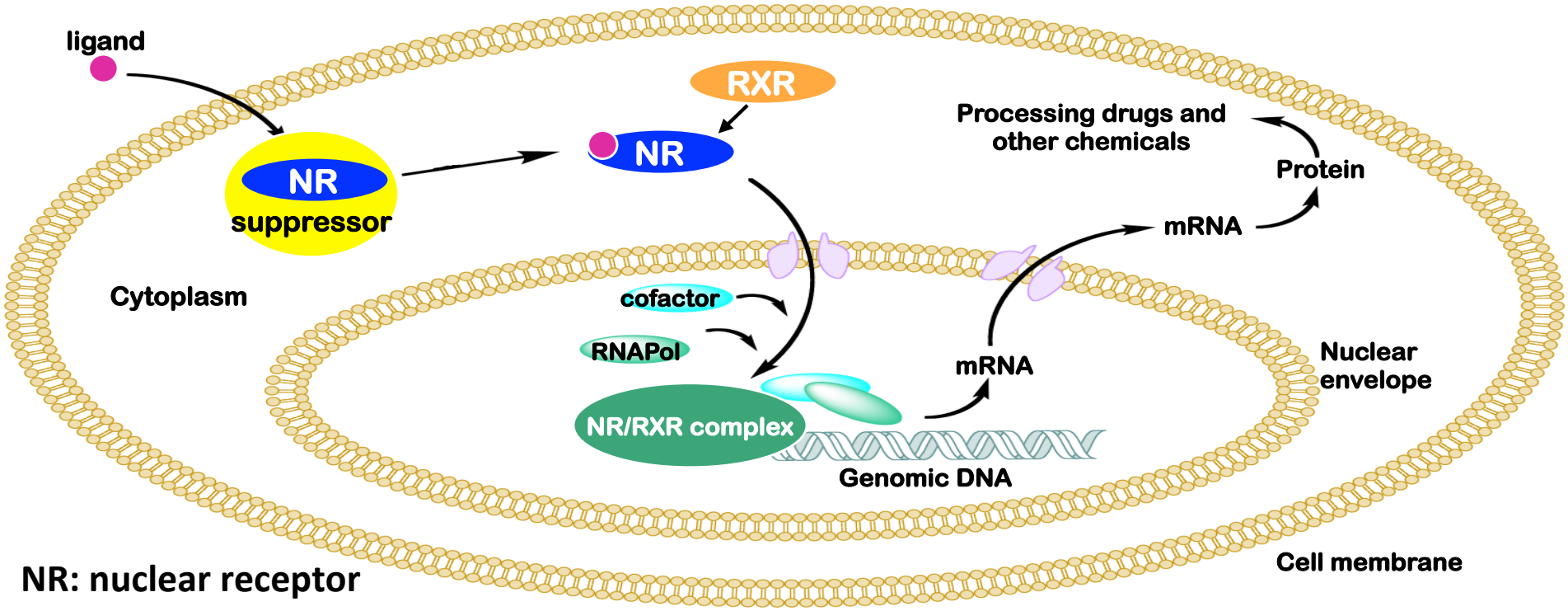
Regulation of Hepatic Cyp4a10 mRNA and Protein by Gut Microbiome during Development



- **Cyp4a10: The fatty acid oxidation enzyme targeted by the lipid-sensing nuclear receptor PPARα**

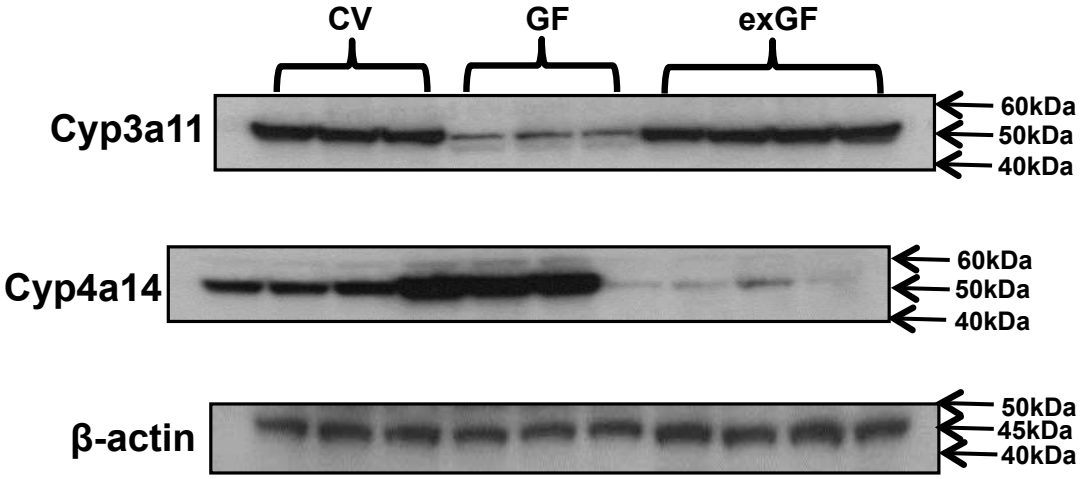
The gut microbiome-derived secondary bile acid lithocholic acid (LCA) was shown to be a PXR activator (Staudiger et al., 2010 PNAS)

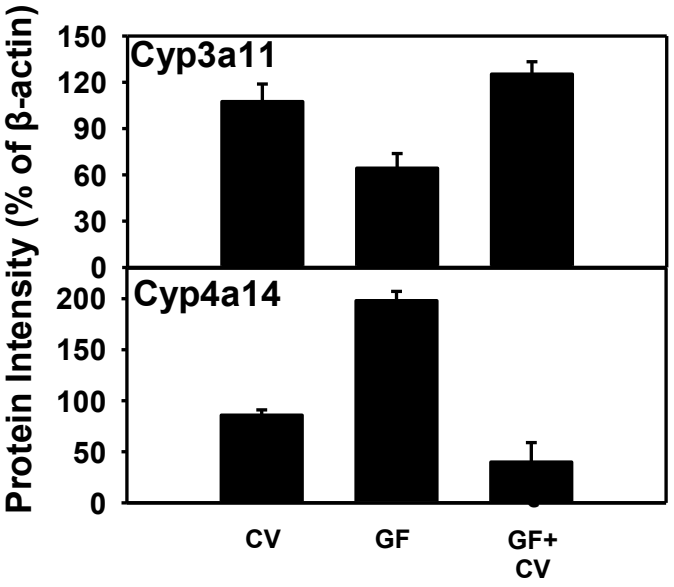
- PXR: key regulator in mediating xenobiotic metabolism and transport in liver
- Restoring LCA can increase the expression of the PXR-target gene Cyp3a



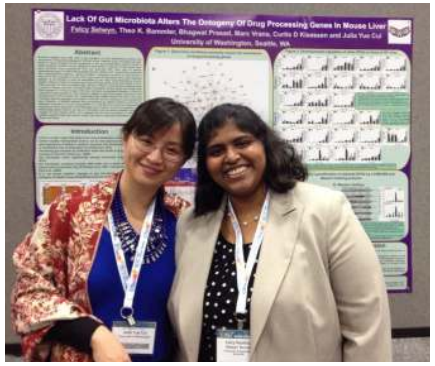
NR: nuclear receptor

Conventionalization Restored the Basal Expression and Enzyme Activity of Cyp3a and Cyp4a in Livers of exGF Mice

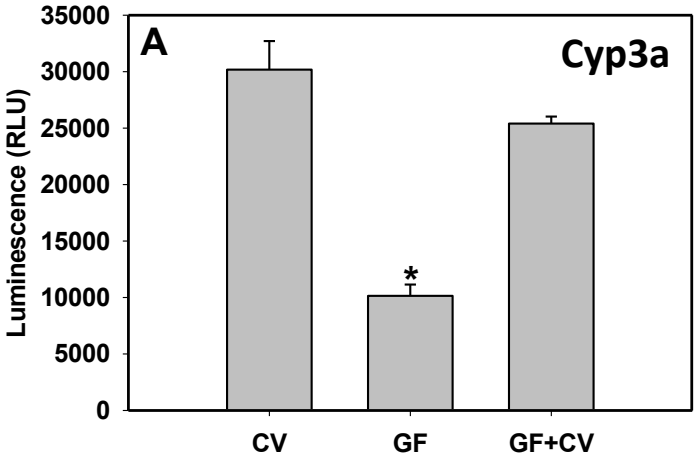


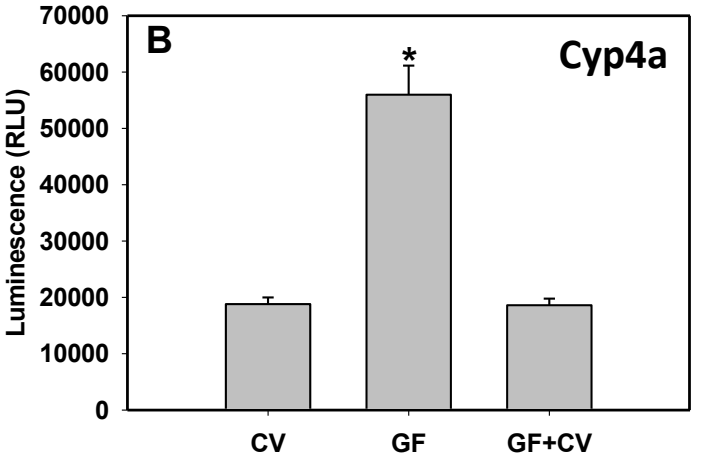


Enzyme Activities:



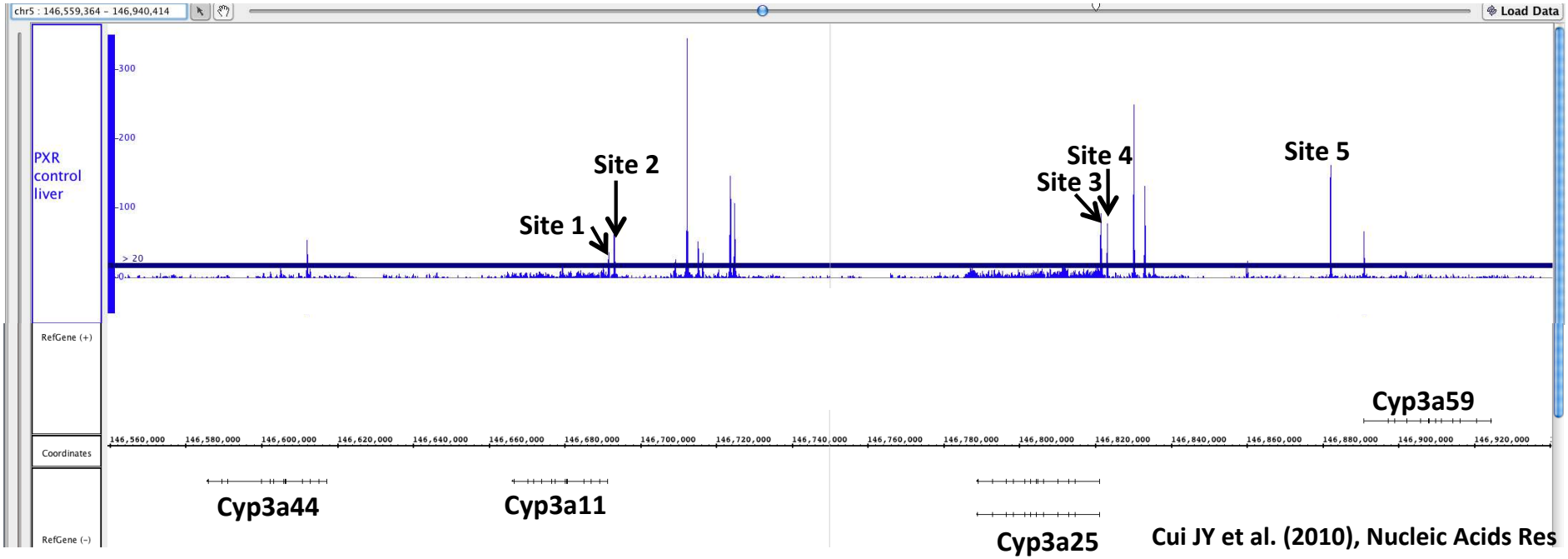
Felcy Selwyn, former research scientist



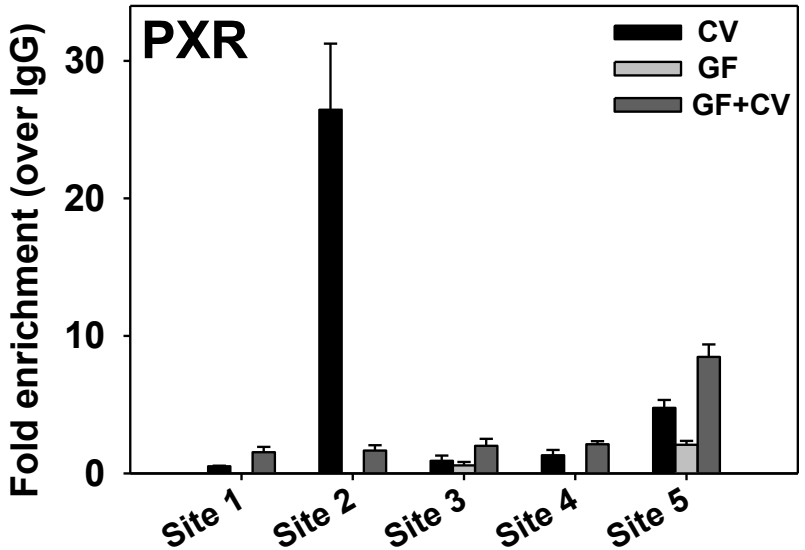


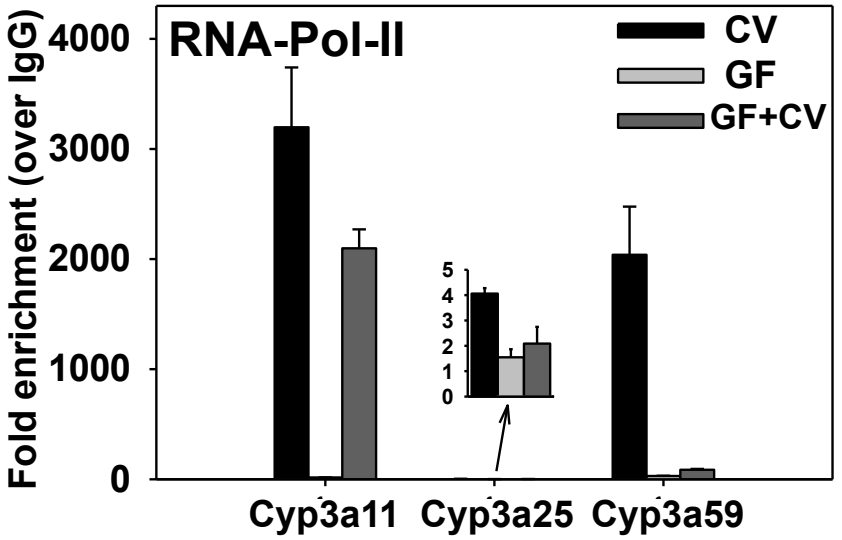
Selwyn, Cheng, Klaassen, and Cui JY*, 2016 *Drug Metab Dispos, 44: 262-74*

PXR-DNA Binding Sites in Conventional Mouse Liver



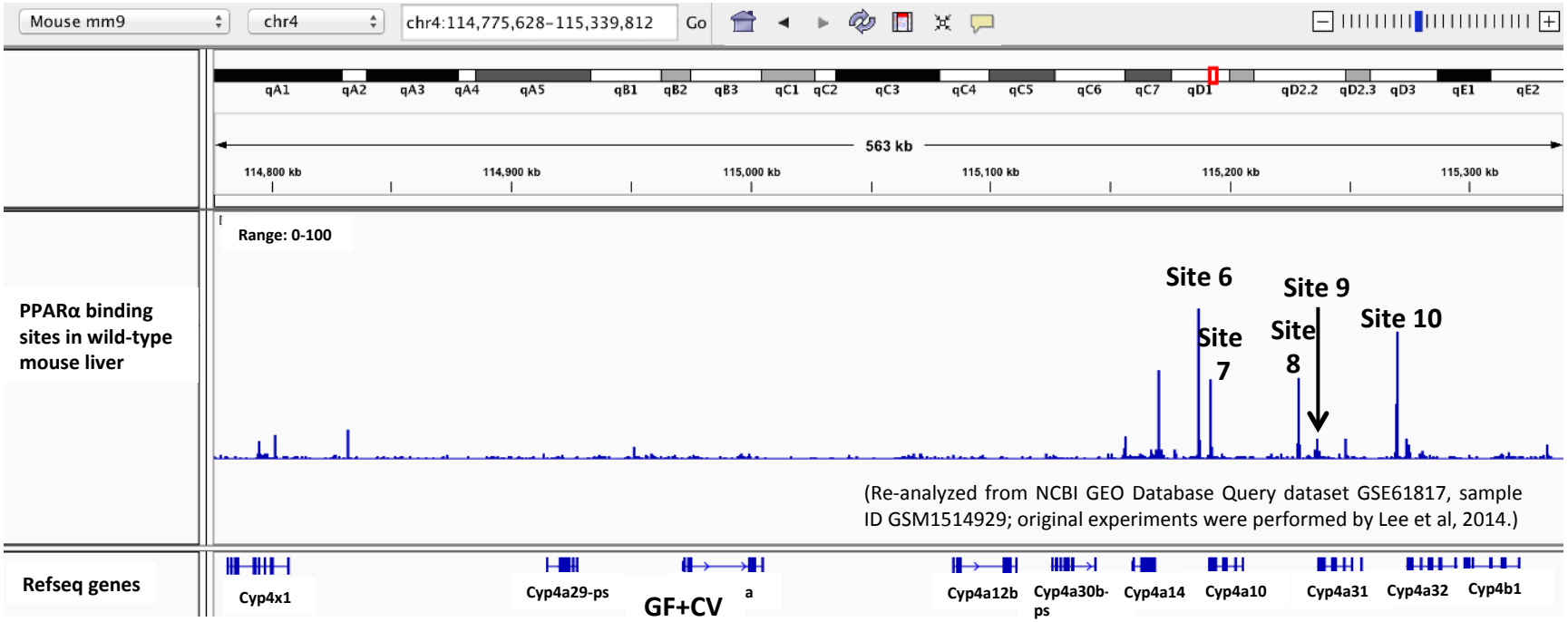
Cui JY et al. (2010), Nucleic Acids Res

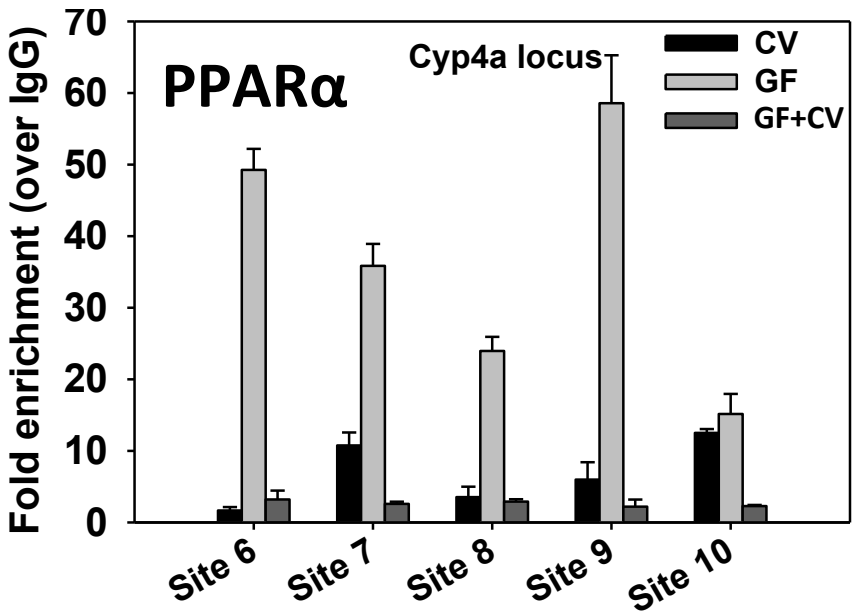


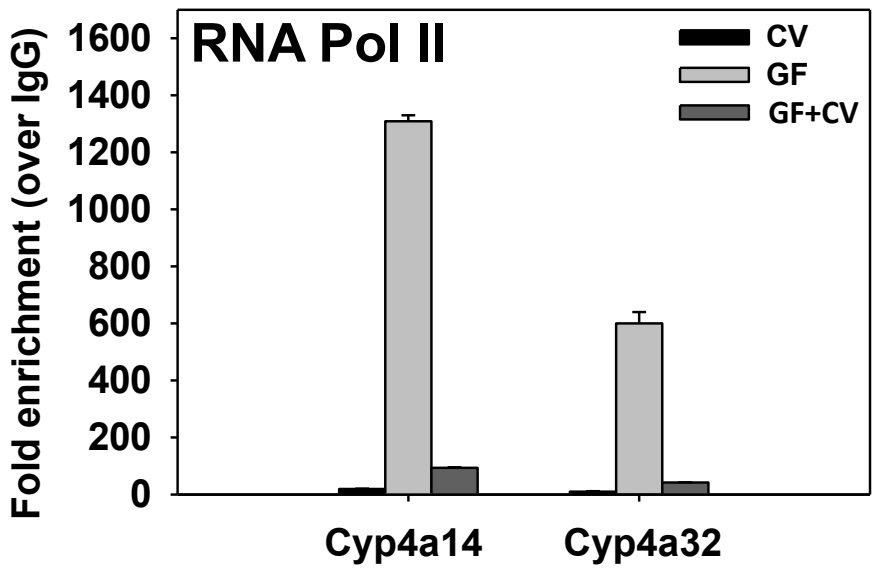


Selwyn, Cheng, Klaassen, and Cui JY*, 2016 *Drug Metab Dispos, 44: 262-74*

PPARα-DNA Binding Sites in Conventional Mouse Liver

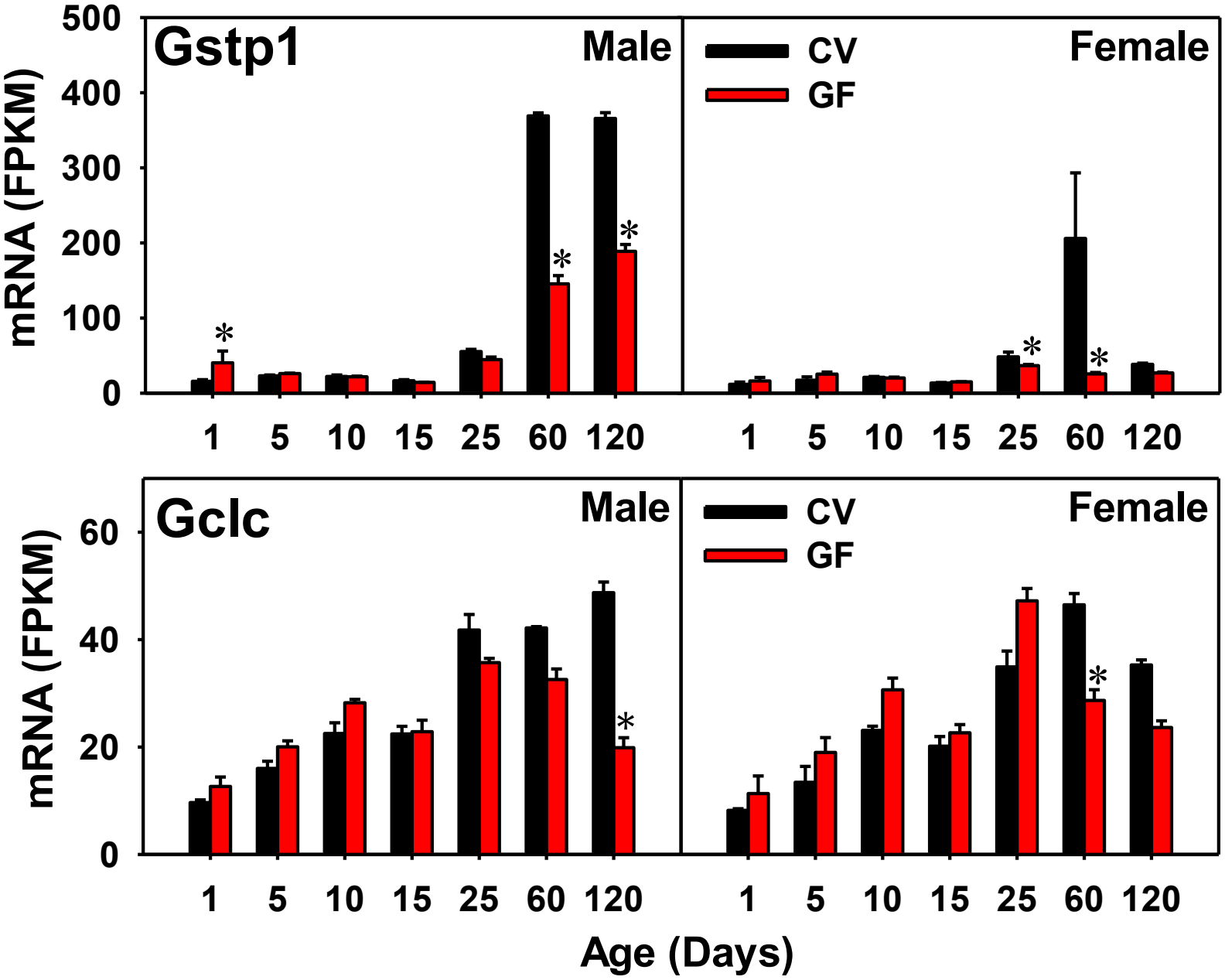




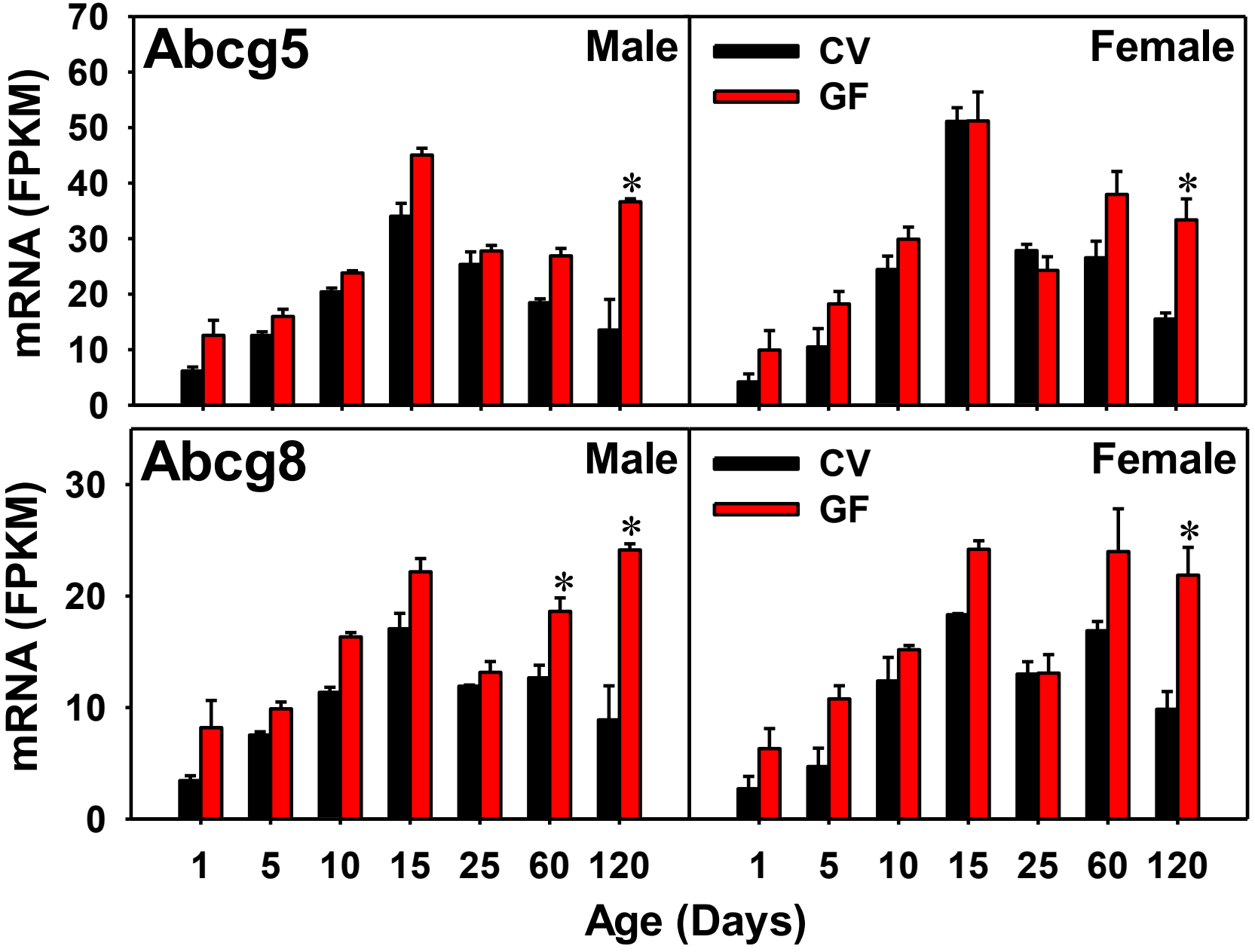


Selwyn, Cheng, Klaassen, and Cui JY*, 2016 *Drug Metab Dispos, 44: 262-74*

Lack of Gut Microbiome Reduced the Expression of Phase-II Gstp1 and the GSH-synthesizing Gclc in Adult Livers



Lack of Gut Microbiome Increased the Expression of the Cholesterol-efflux Transporter Dimers Abcg5/g8 in Adult Liver

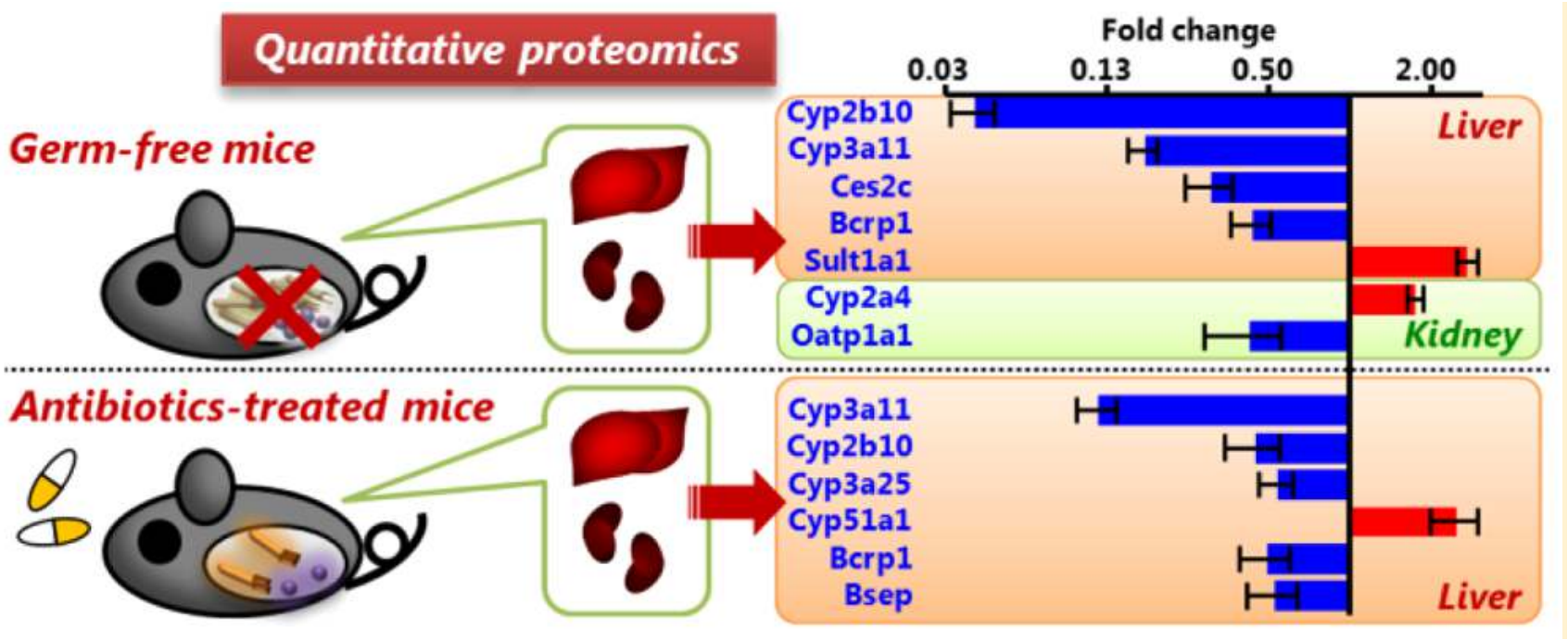


Pathway Analysis of the RNA-Seq Dataset in Intestines of CV and GF Mice

Tissue (DE Gene #)*	GF vs CV (Pathway # Increased/decreased)	Top 6 Associated Pathways (Number of Genes)	NAS
Duodenum (392)	**2/5**	Metabolism of Xenobiotics by Cytochrome P450 (7) ↓	-2.38
		Ribosome (11) ↓	-2.03
		Drug Metabolism-Cytochrome P450 (6) ↓	-2.09
		Metabolic Pathways (34) ↓	-1.82
		Protein Digestion and Absorption (7) ↑	1.94
		Insulin Signaling Pathway (5) ↓	-1.60
Jejunum (2137)	**12/2**	Arginine and Proline Metabolism (14) ↑	2.07
		Alanine, Aspartate and Glutamate Metabolism (9) ↑	2.10
		P53 Signaling Pathway (6) ↑	1.95
		Glutathione Metabolism (10) ↑	1.97
		Amino Sugar and Nucleotide Sugar Metabolism (9) ↑	1.88
		Drug Metabolism-Other Enzymes (7) ↑	1.85
Ileum (329)	**2/5**	Retinol Metabolism (8) ↓	-2.08
		Metabolism of Xenobiotics by Cytochrome P450 (10) ↓	-1.90
		Drug Metabolism-Cytochrome P450 (9) ↓	-1.77
		Glycolysis/Gluconeogenesis (5) ↓	-1.78
		Adipocytokine Signaling Pathway (6) ↓	-1.80
		Steroid Biosynthesis (5) ↑	1.57
Large Intestine (781)	**8/6**	Protein Digestion and Absorption (22) ↑	2.08
		Protein Processing in Endoplasmic Reticulum (7) ↓	-2.04
		Mineral Absorption (7) ↑	2.10
		Starch and Sucrose Metabolism (7) ↑	1.97
		Pancreatic Secretion (16) ↑	1.90
		Neuroactive Ligand-Receptor Interaction (6) ↑	1.79

(Fu et al., Selwyn, and Cui 2017)

Increased protein expression of Cyp2a4 but reduced Oatp1a1 in kidney of GF mice



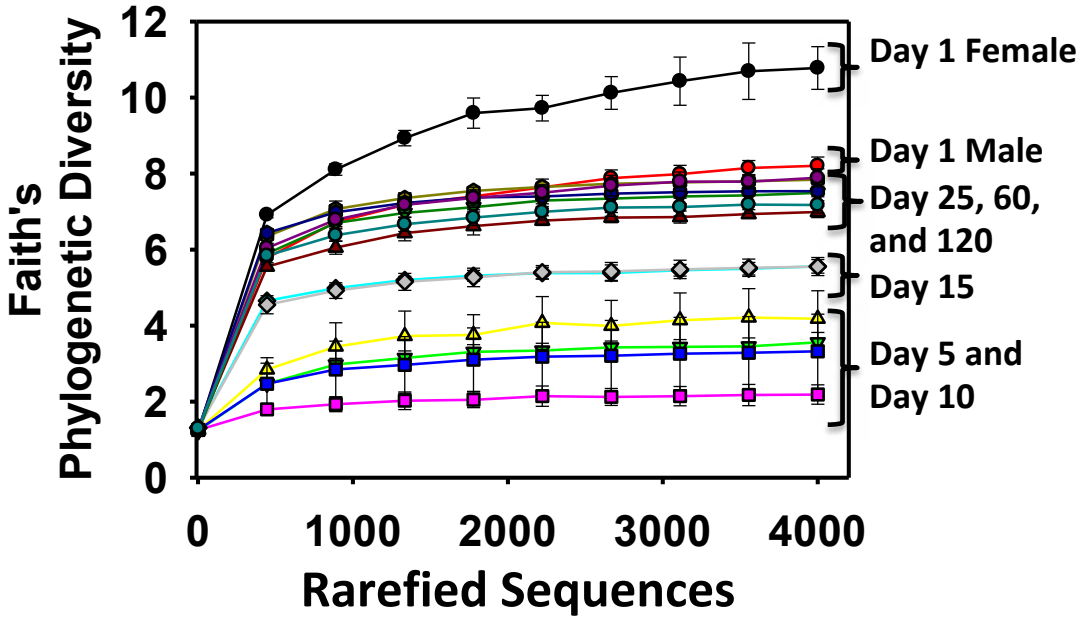
Kuno et al, 2016

Summary

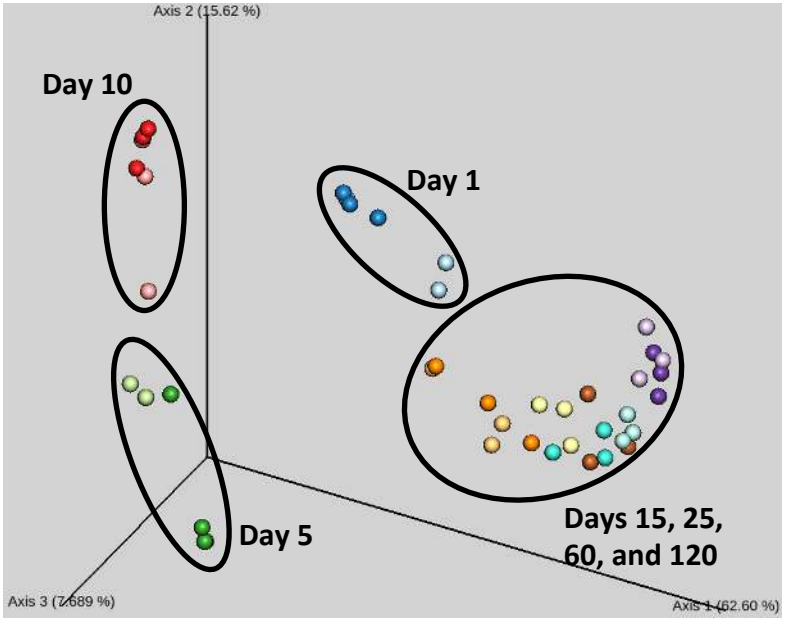
- Lack of gut microbiota regulates the expression of many host drug-processing genes
- Age and gender further modify the interactions between gut microbiome and liver
- Conventionalization normalizes the hepatic Cyp3a and Cyp4a expression activity, corresponding to normalized PXR and PPARα signaling in liver

QIIME2 Gut Microbiota Diversity Increases with Age

α-Diversity



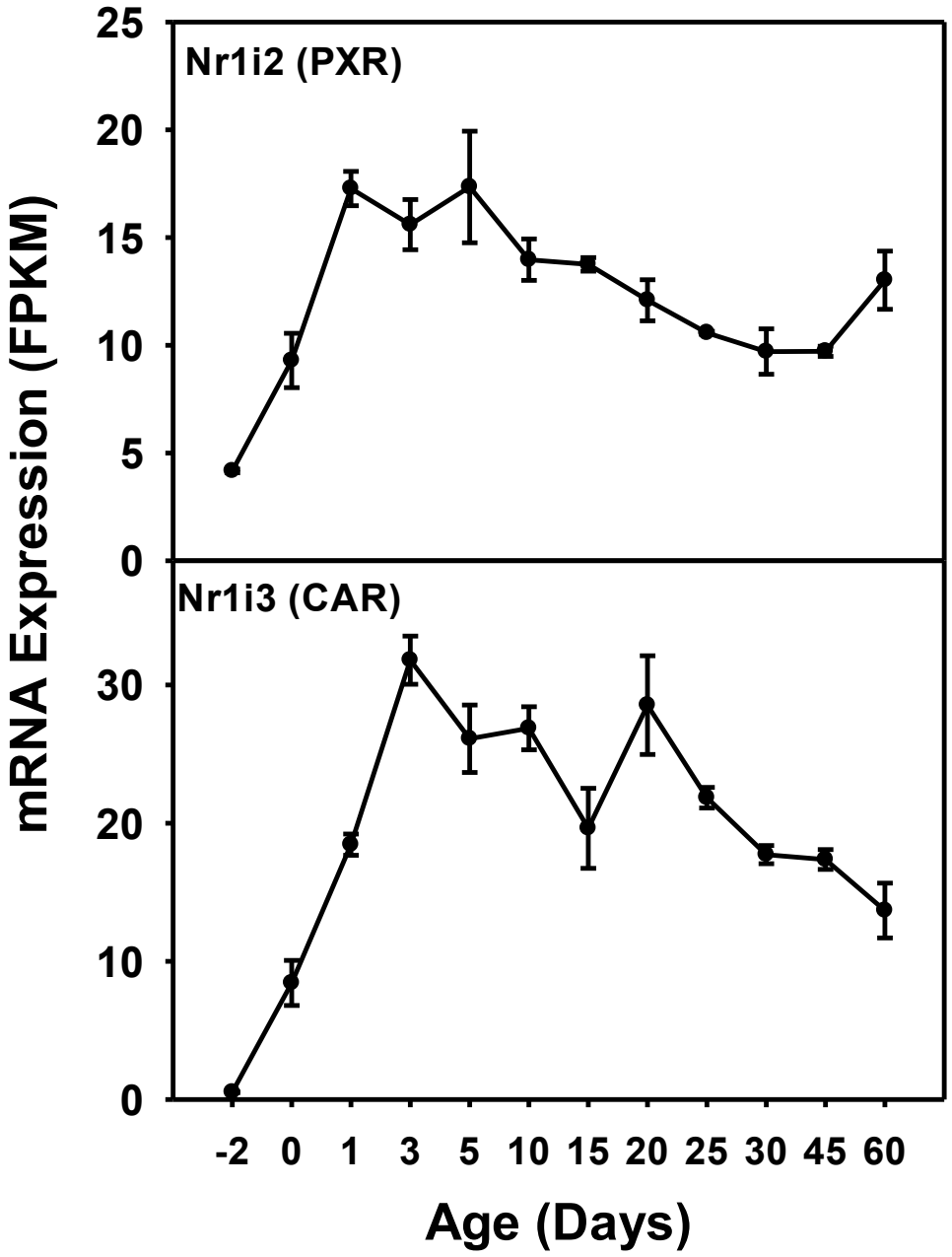
β-Diversity



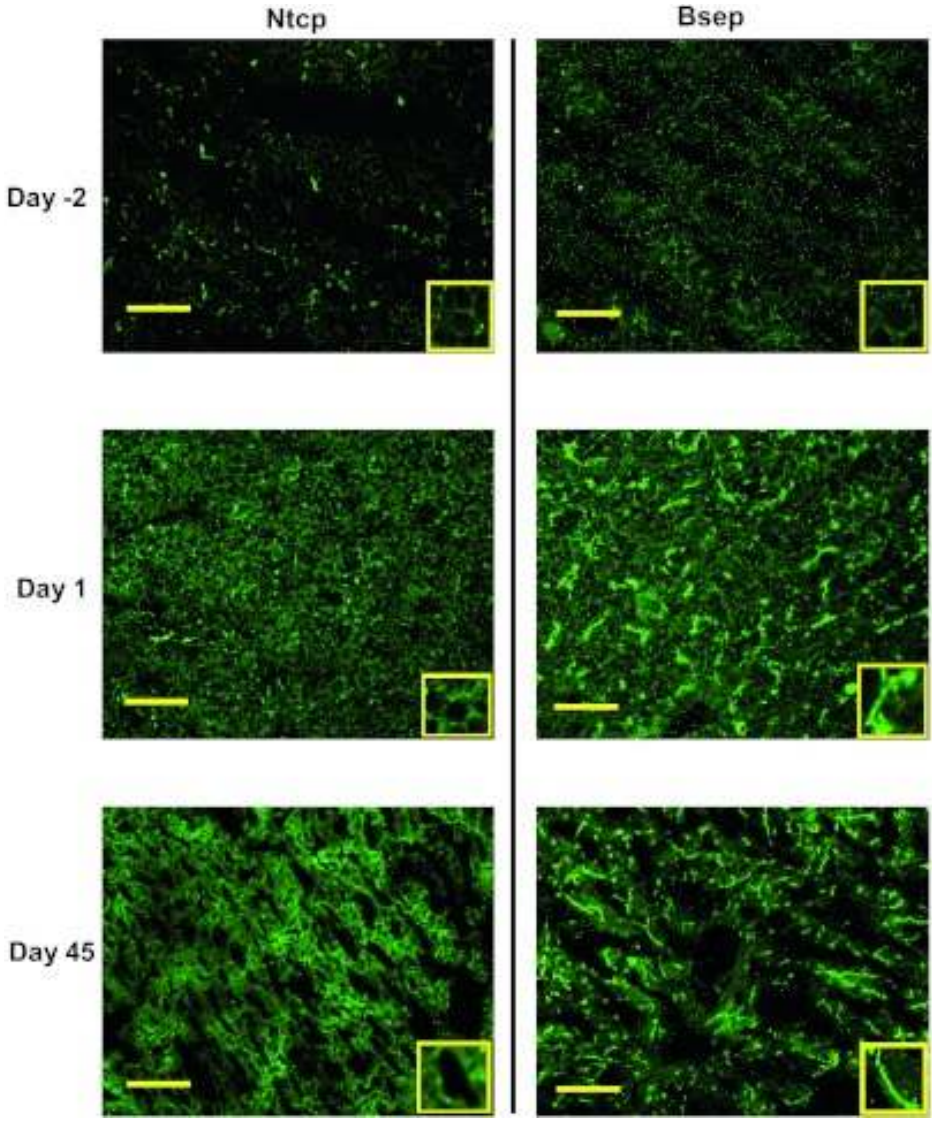
- **Alpha diversity is highest at Day 1, lowest at early developmental ages, and then increases through adulthood.**
- **For species heterogeneity (β-diversity), there are 4 distinct clusters, separating pre-weaning ages and adults.**

Joseph Dempsey (PhD Student)

Ontogeny of PXR and CAR in Mouse Liver

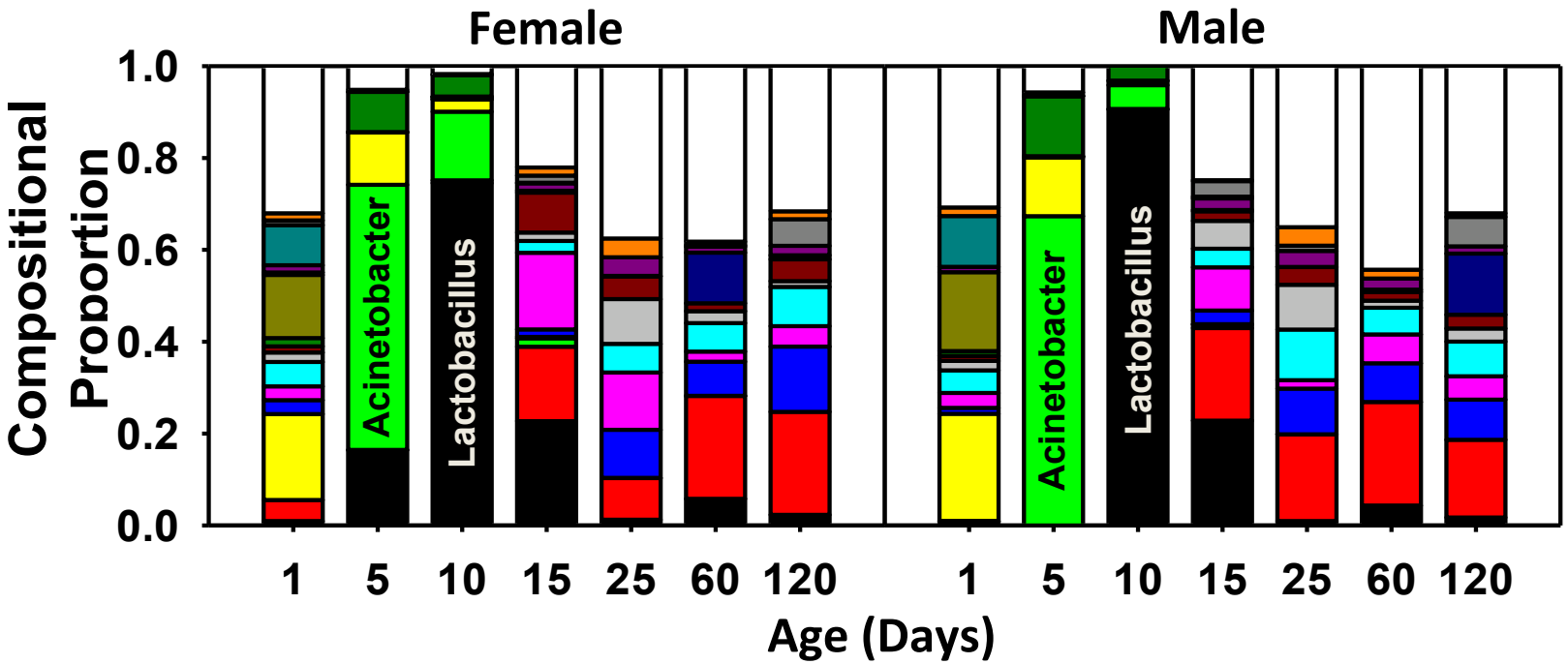


"Neonatal pattern" of major hepatic bile acid transporters in neonatal liver



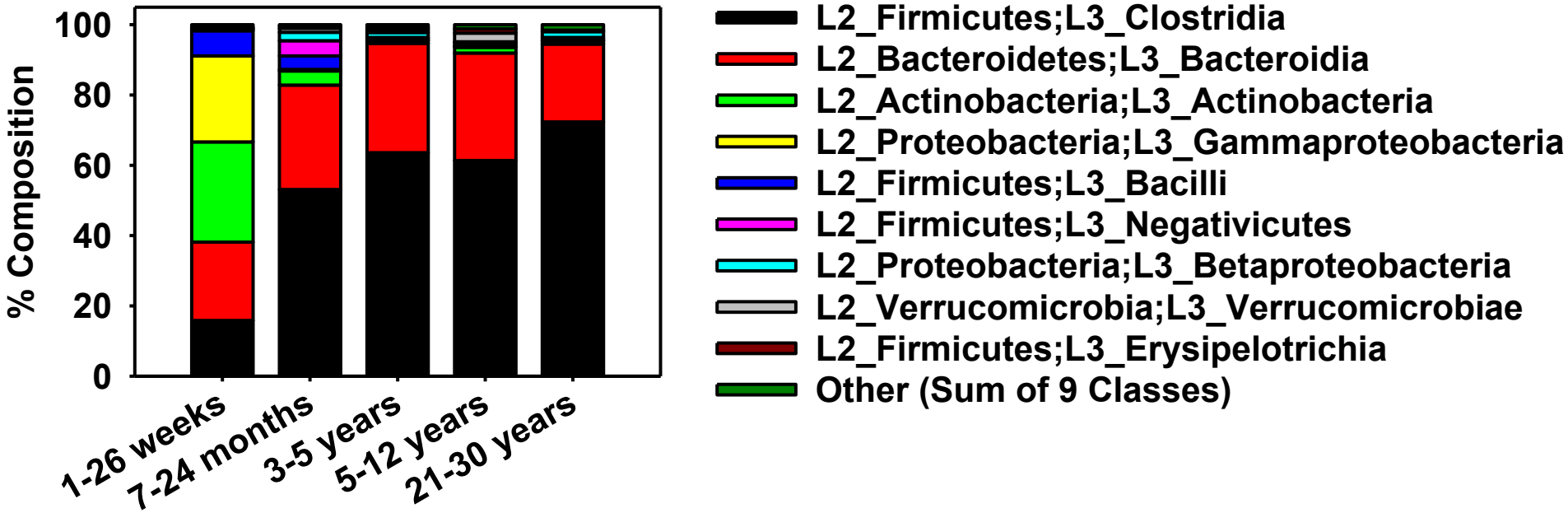
Cui JY et al., (2012) American Journal of Physiology

QIIME2 Ontogeny of Mouse Gut Microbiota



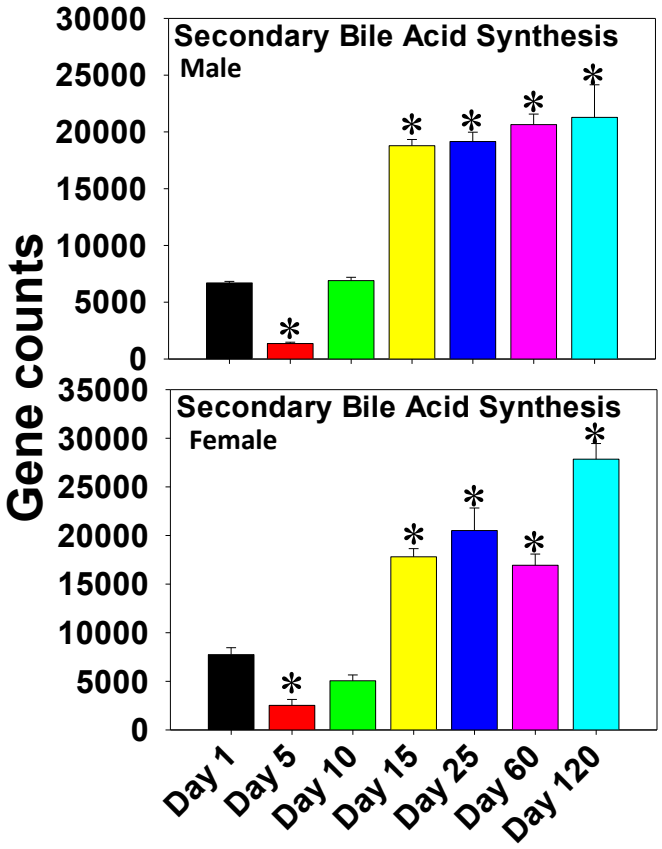
- p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus
- p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidales S24-7 group;g_uncultured bacterium;s_uncultured bacterium
- p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae;g_Acinetobacter
- p_Firmicutes;c_Bacilli;o_Bacillales;f_Bacillaceae;g_Bacillus
- p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Lachnospiraceae NK4A136 group;s_uncultured bacterium
- p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidales S24-7 group;Ambiguous_taxa;Ambiguous_taxa
- p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae
- p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Lachnospiraceae NK4A136 group
- p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_uncultured;s_uncultured bacterium
- p_Firmicutes;c_Bacilli;o_Bacillales;f_Staphylococcaceae;g_Staphylococcus
- p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae;g_Lactococcus
- p_Firmicutes;c_Erysipelotrichia;o_Erysipelotrichales;f_Erysipelotrichaceae
- p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillibacter;s_uncultured bacterium
- p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Enterococcaceae;g_Enterococcus
- p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidales S24-7 group
- p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminiclostridium;s_uncultured bacterium
- Other Taxa (188)

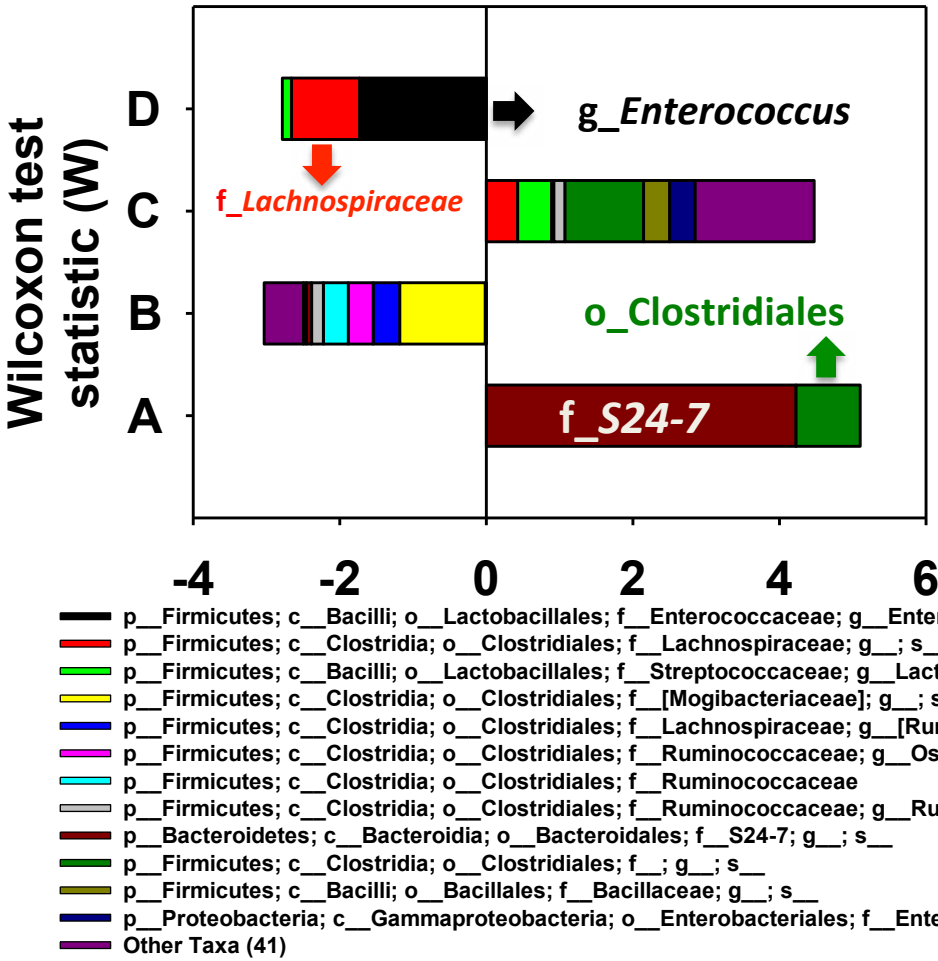
Ontogeny of Human Microbiome



In collaboration with Sheela Sathyanarayana's group at Seattle Children's Hospital

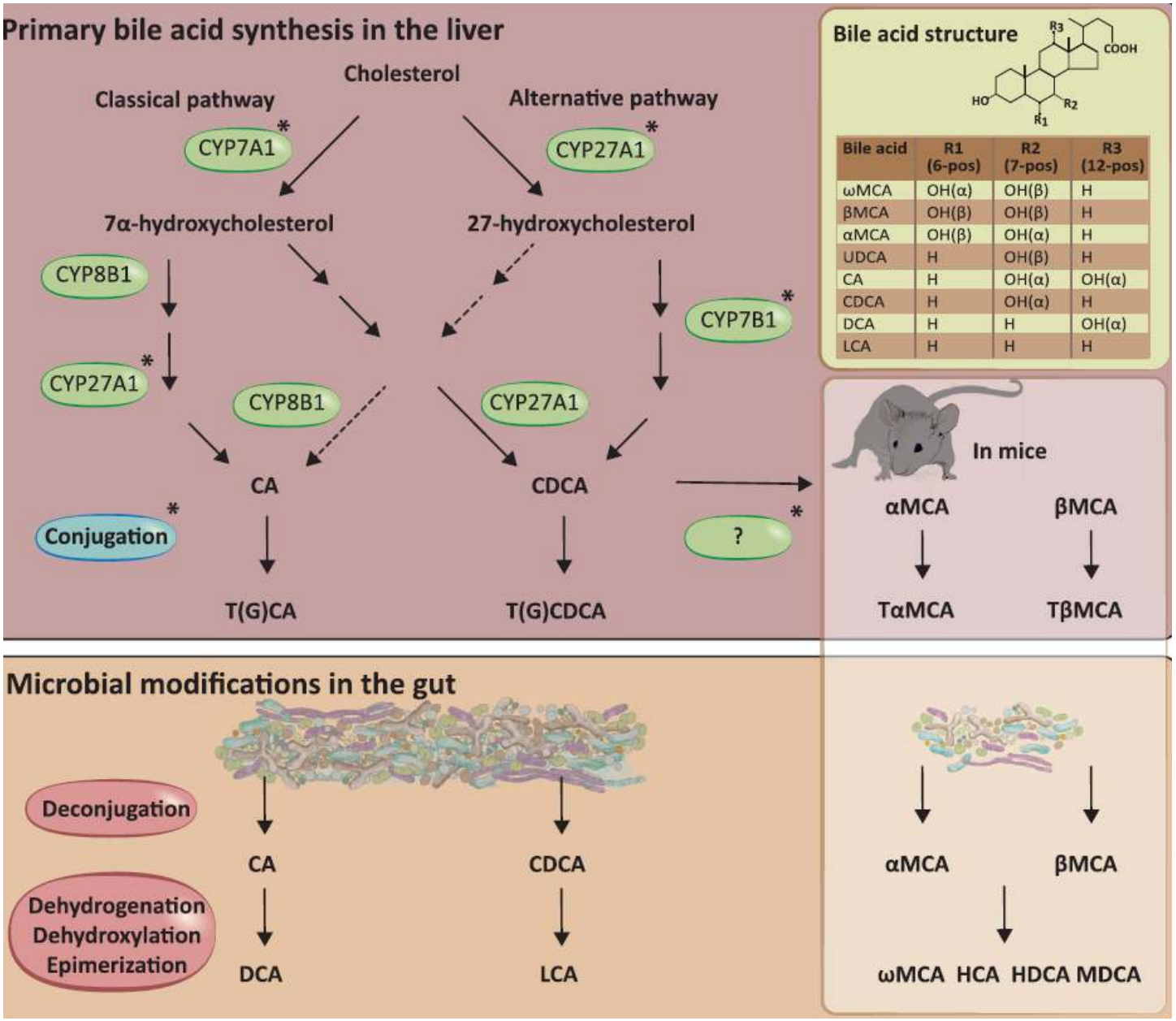
Age-Specific Differences in Microbial Bile Acid Synthesis





- There are 237 differentially regulated metagenomics functions, including secondary BA synthesis (PICRUSt).
- Between Day 1 and 120, there was a significant functional shift in bacteria attributable to secondary BA synthesis (FishTaco).

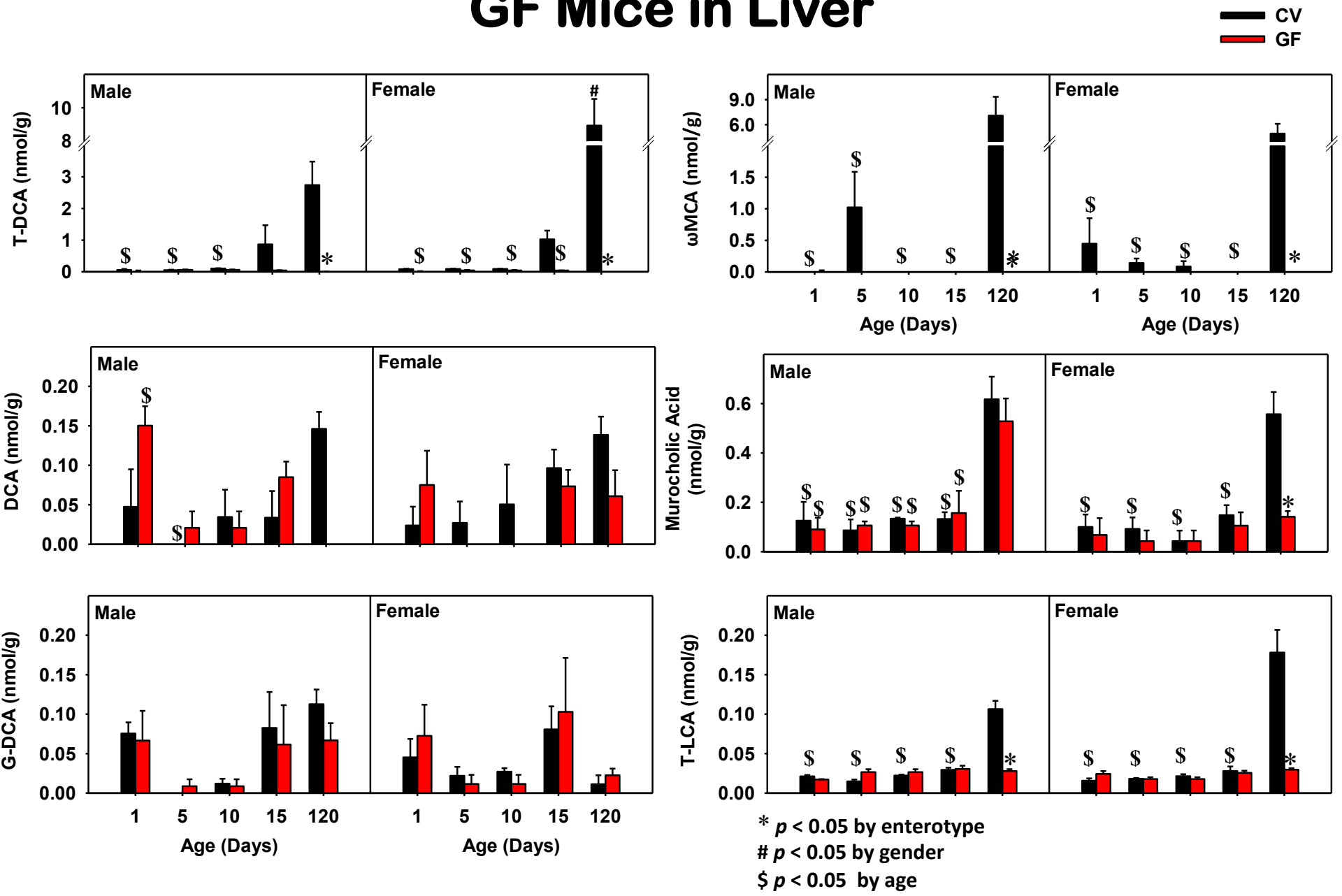
Individual BA profiles



Wahlstrom et al., 2016

- **T-α/βMCAs are FXR antagonists (Sayin et al., 2013)**
- **Gly-MCA is an anti-obesity BA through intestinal FXR inhibition (Jiang et al., 2015)**
- **LCA and DCA are preferable TGR-5 activators than primary BAs (Pols et al., 2013)**

Age-Specificity of Secondary BA between CV and GF Mice in Liver



* $p < 0.05$ by enterotype

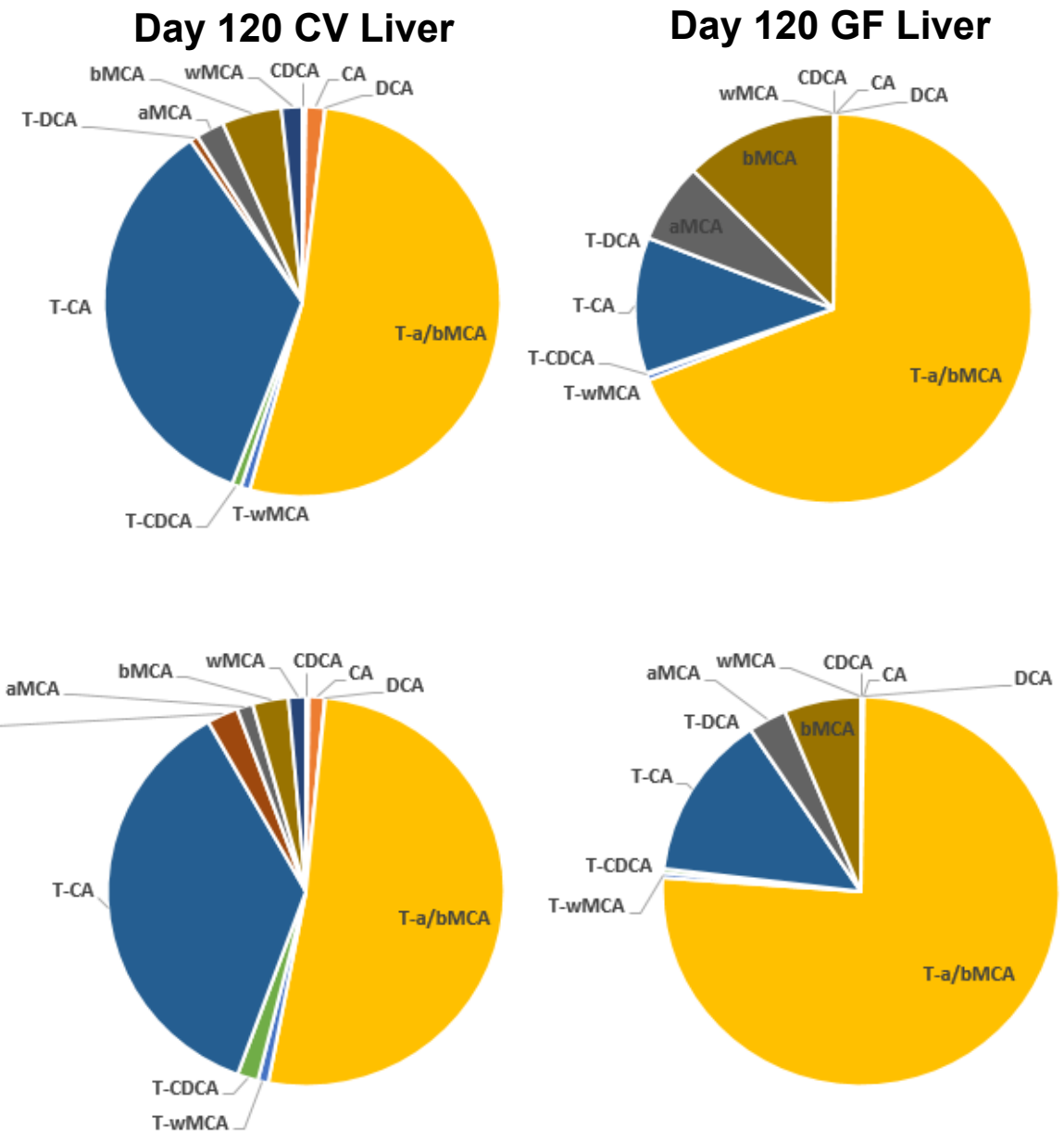
\# $p < 0.05$ by gender

\$ $p < 0.05$ by age

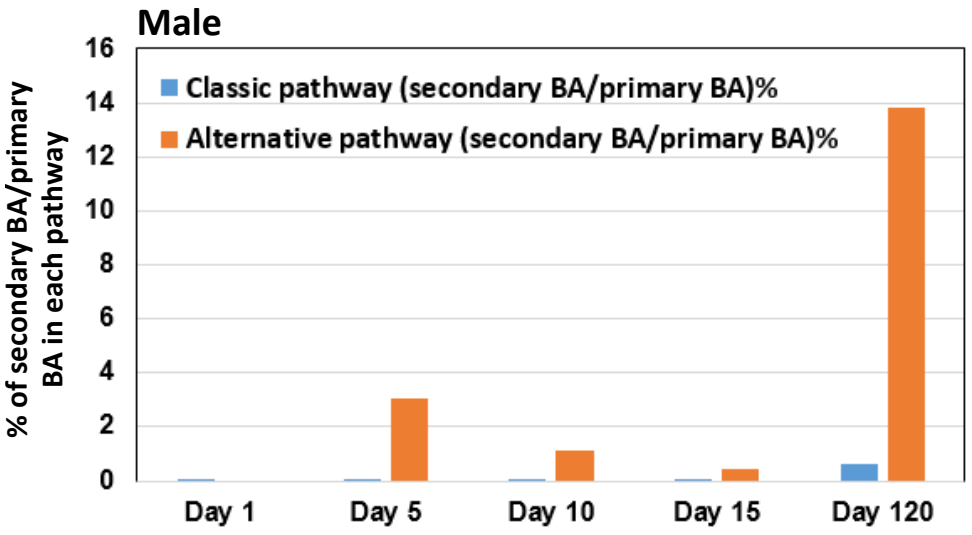
Primary BAs: a marked increase in alternative pathway products in GF mice

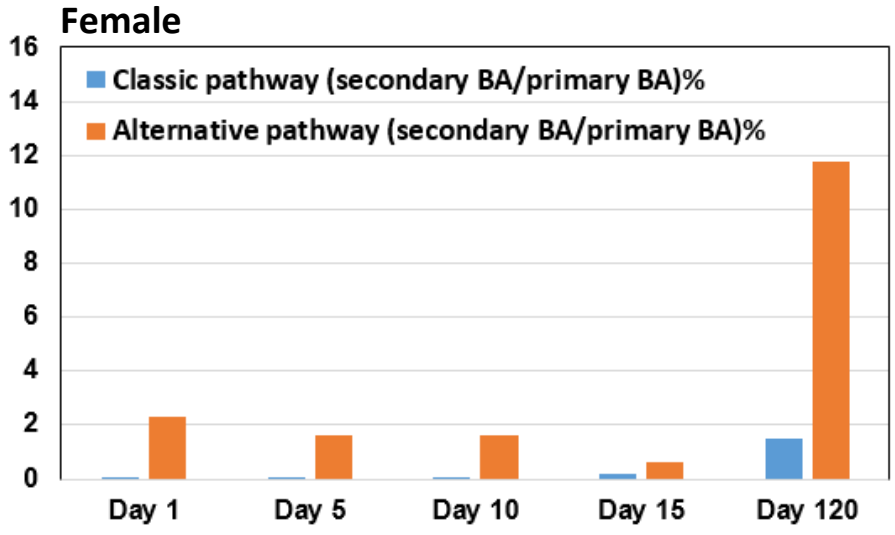
Male

Female



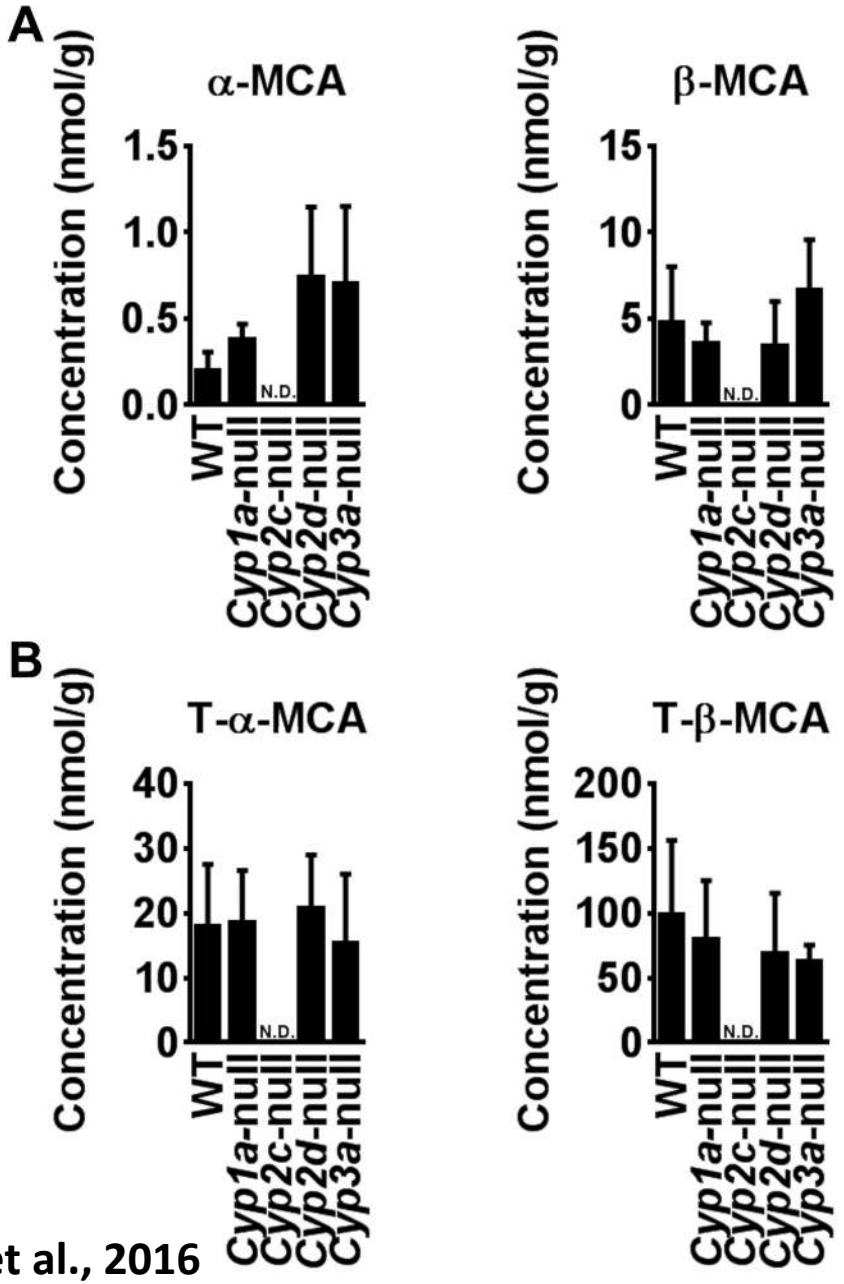
A preferable microbial metabolism of primary BAs from alternative pathway than those from classical pathway in CV mice





- **Classical pathway secondary BA/primary BA % = (DCA + T-DCA) / (CA + T-CA) × 100%**
- **Alternative pathway secondary BA/primary BA % = (LCA + T-LCA + ωMCA + T-ωMCA) / (CDCA + T-CDCA + αMCA + βMCA + T−α/βMCA) × 100%**

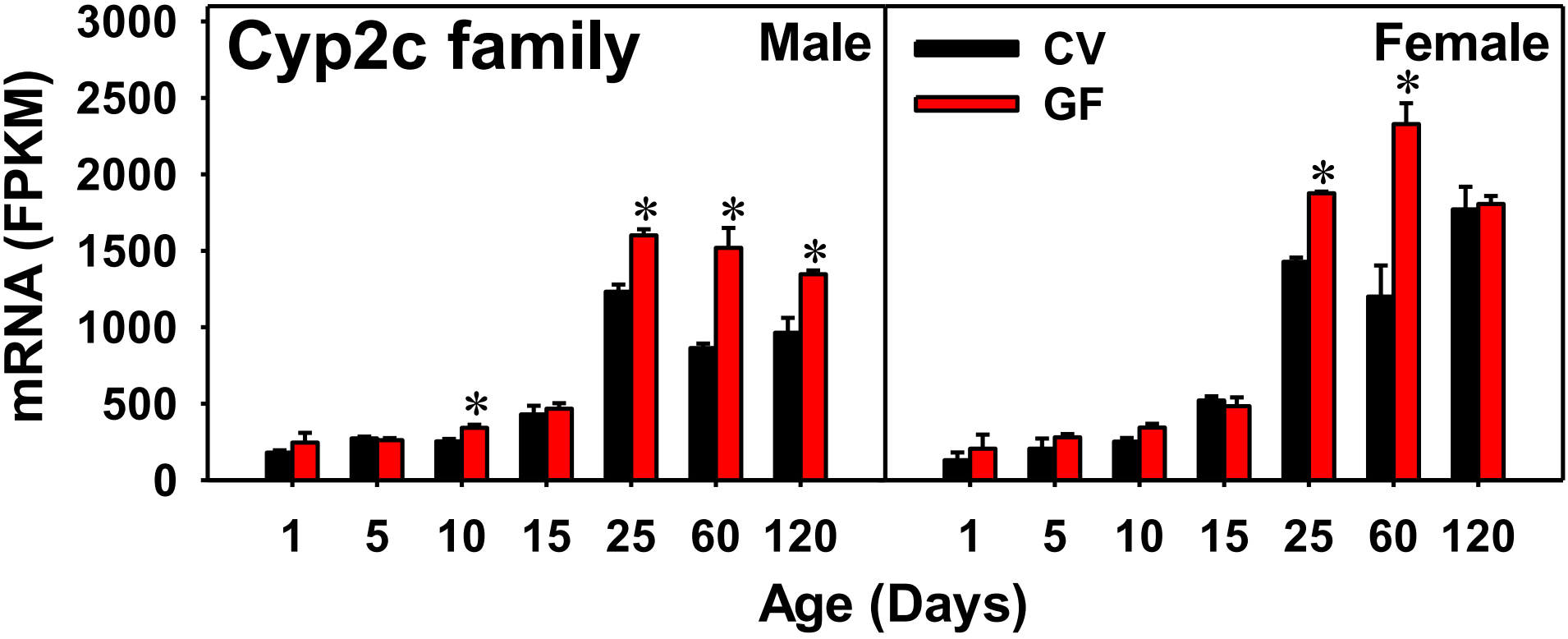
Cyp2c70 is responsible for the species difference in bile acid metabolism between mice and humans (MCA production)



- A breakthrough in resolving the long-standing question regarding which enzyme is responsible for MCA production
- It remains interesting to test whether other Cyp2c members, in addition to Cyp2c70, are involved in MCA production

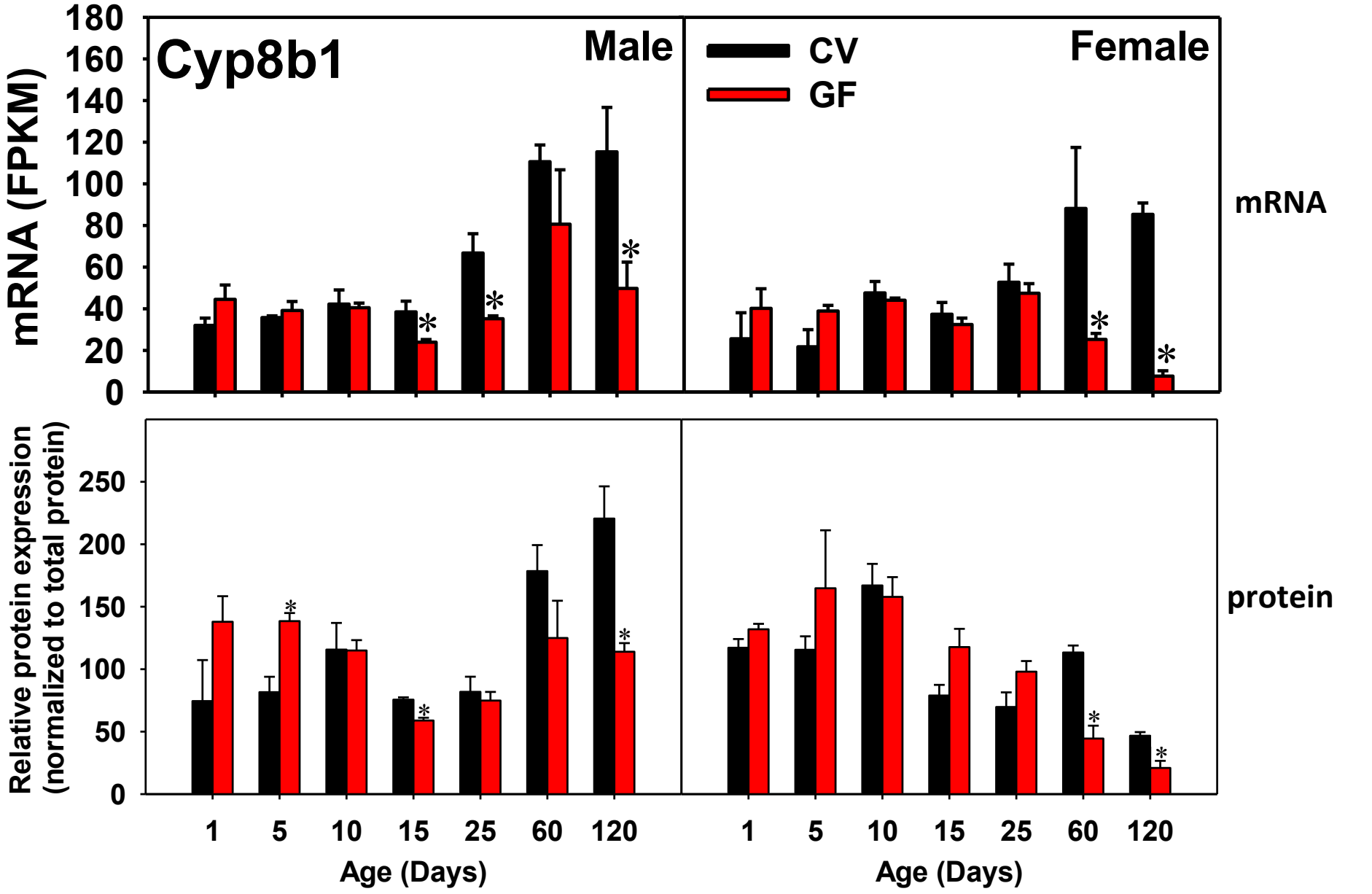
Takahashi et al., 2016

Lack of Gut Microbiome Increased the mRNAs of Multiple Cyp2c Genes in Liver



- Cyp2c family that are differentially regulated: Cyp2c37, 2c54, 2c50, 2c69, 2c67, 2c29, 2c70, 2c44, 2c40, 2c68, 2c55, 2c38, 2c39 (ranked based on FDR-BH)

Cyp8b1: 12α hydroxylase that determines CA/CDCA ratio is down-regulated in livers of GF mice



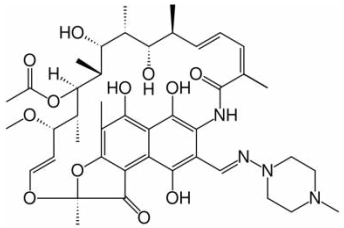
Summary

- **The “Day-1 surge” pattern of gut microbiome richness coincides with the “Day-1 surge” pattern of host hepatic drug receptors (PXR and CAR) and bile acid transporters (Ntcp and Bsep)**
- **Distinct taxa are identified that appear to contribute to age-specific secondary BA synthesis**
- **The absence of secondary BAs (especially LCA) likely contribute to reduced Cyp3a expression in liver in an age-specific manner**
- **There is a clear preference of microbial metabolism from BAs synthesized from alternative pathway over classical pathway**
- **The increased Cyp2c gene expression and reduced Cyp8b1 expression may also contribute to increased T-α/βMCA levels in GF mice**

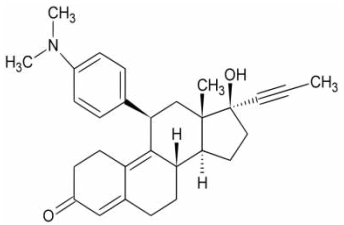
Story 2: Gut Microbiome and Xenobiotic exposure

Introduction: A Wide Spectrum of PXR Activators

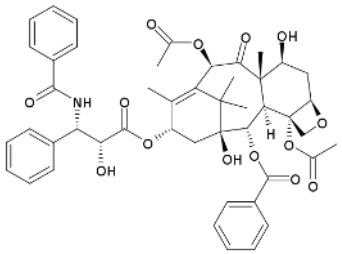
Drugs



Rifampicin

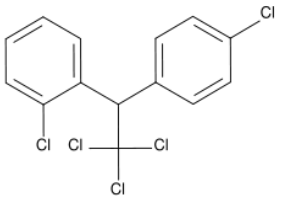


RU-486

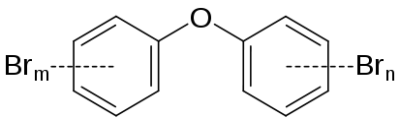


Paclitaxel

Environmental toxicants

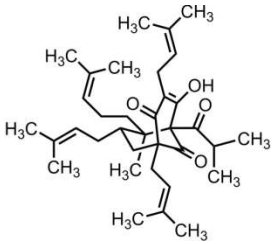


o,p' -DDT

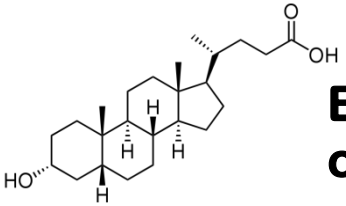


PBDE

Phytochemical



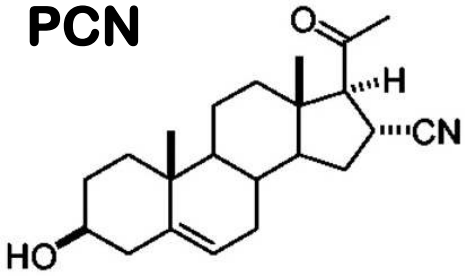
Hyperforin



LCA

Endogenous chemical

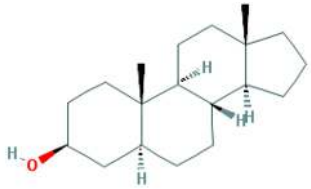
PCN



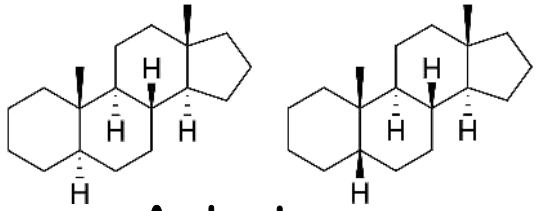
A synthetic compound to recapitulate human PXR activation in mice.

Introduction: A Wide Spectrum of CAR Activators

Endogenous antagonists:

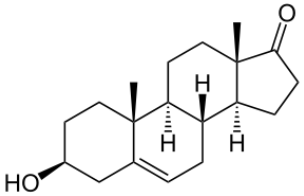


Androstanol



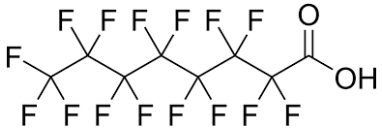
Androstanes

Endogenous agonist:

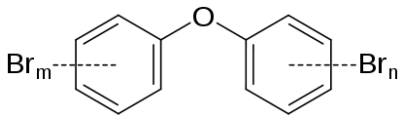


Dehydroepiandrosterone (DHEA)

Environmental toxicants



PFCA



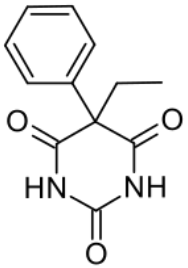
PBDE

Neutraceuticals

Artemisia capillaris (Yin Zhi Huang)

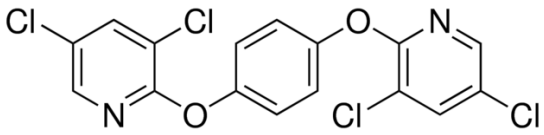
Activation mechanisms:

Indirect activation:



Phenobarbital

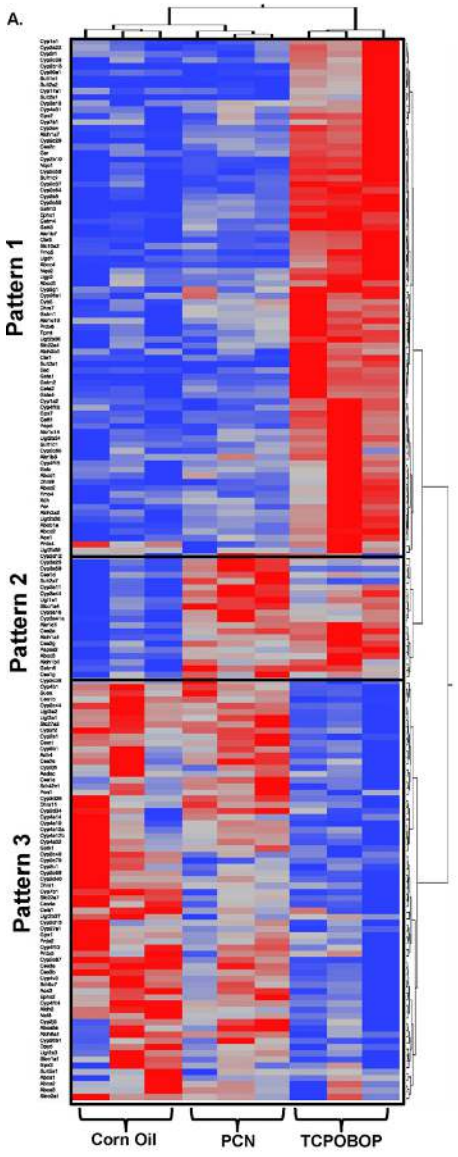
Direct activation:



TCPOBOP

A synthetic compound to recapitulate human CAR activation in mice.

Activation of PXR and CAR increases the expression of many drug-processing genes (DPGs) in host liver



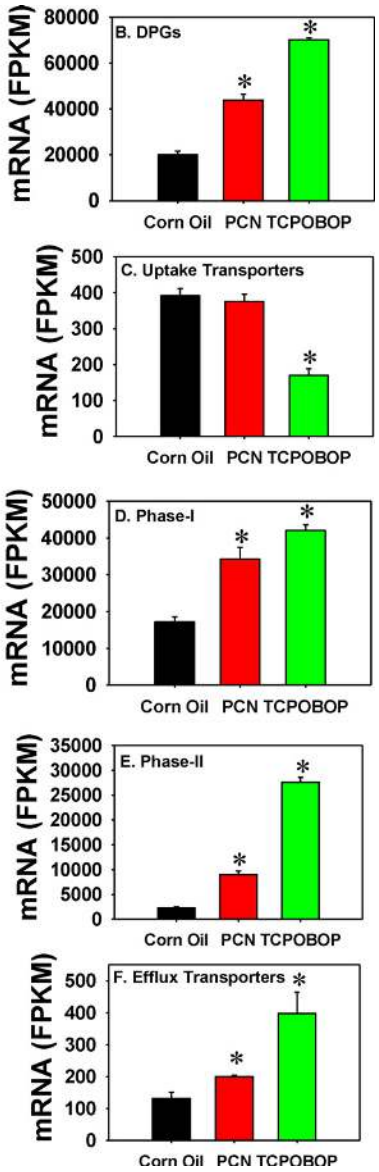
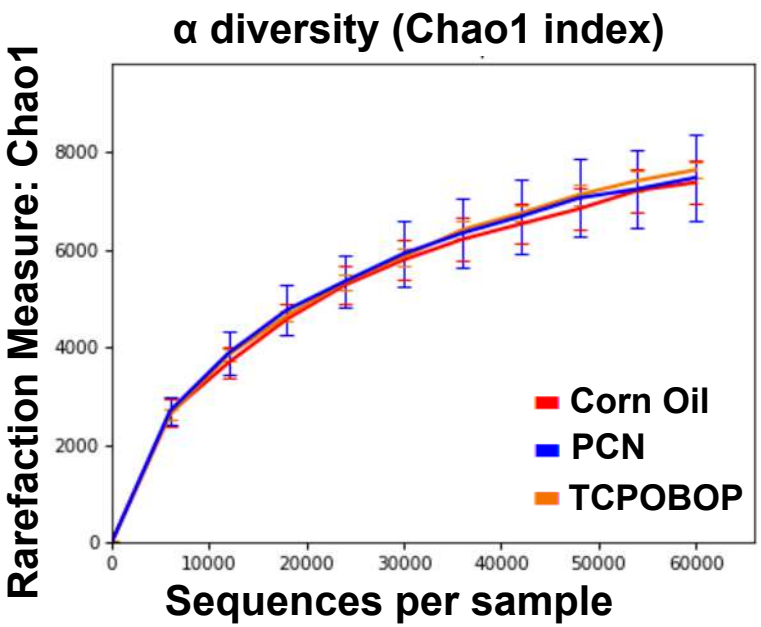


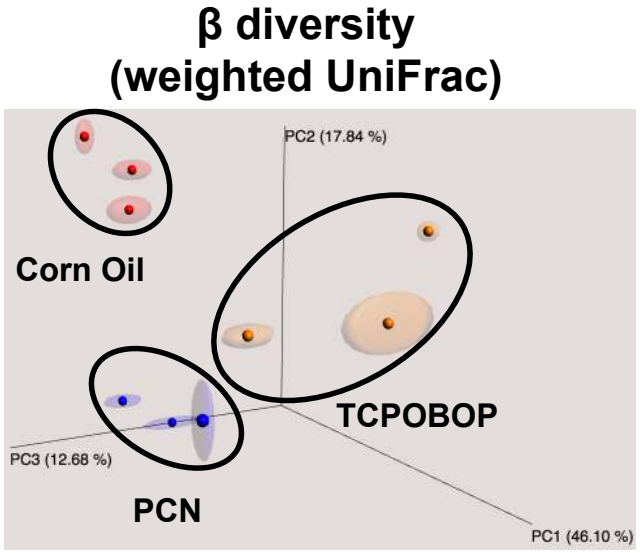
Table 1
Top differentially regulated genes.

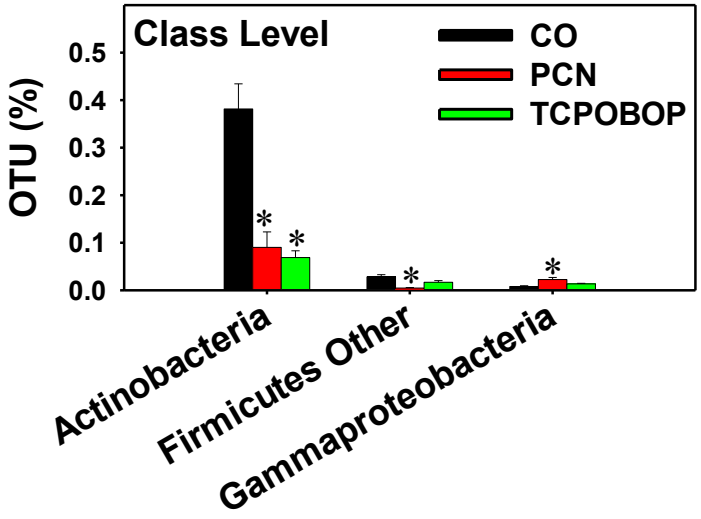
Treatment	Up-regulated		Down-regulated	
	Genes	Fold-increase	Genes	% decrease
PCN	Gstm3	72.32	Mt1	94%
	Cyp2c55	45.56	Alpk1	87%
	Cyp3a16	32.95	Mt2	84%
	Akr1b7	23.39	Moxd1	84%
	Cyp3a59	14.01	Tifa	82%
	Cyp3a44	12.65	Cyp2c69	81%
	Sult2a7	19.28	Tlr2	80%
	Ltf	11.72	9430083A17Rik	79%
	Cyp3a41b	10.03	Gm7334	79%
	Cyp2b10	10.02	Mup9	79%
TCPOBOP	Gsta1	1115	Dmbt1	99.8%
	Gm3776	259.6	Hsd3b5	99.3%
	Gstm3	249.7	Serpina4-ps1	99%
	Cyp2b10	195.3	Obp2a	97%
	Akr1b7	192.3	Mt2	97%
	Cyp2c55	170.1	Slco1a1	97%
	Sult1e1	158.5	Ces4a	96%
	Ccnb1	98.83	Susd4	95%
	Fndc5	95.43	Dct	95%
	Plk1	93.79	Cyp2c40	94%

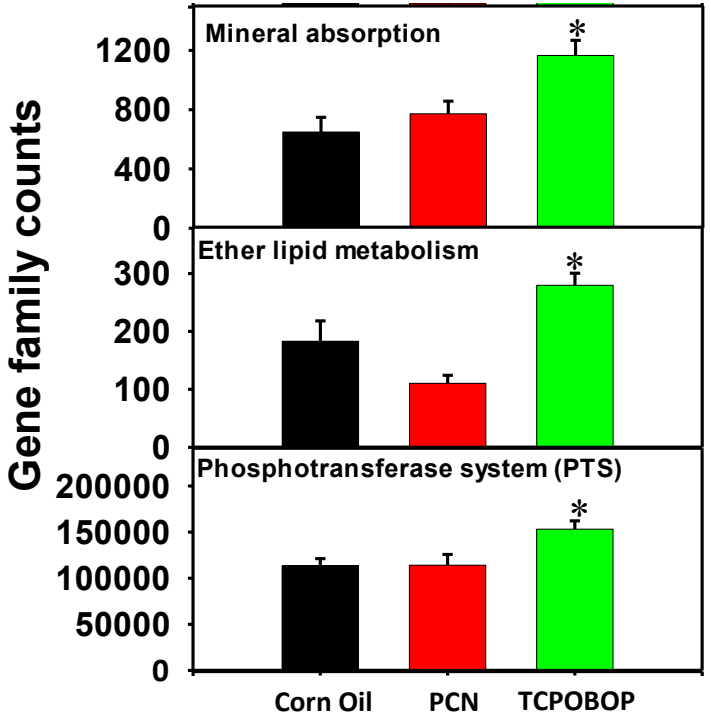
Cui and Klaassen (2016) Biochimica et Biophysica Acta, PMID: 27113289

Activation of PXR and CAR also altered gut microbiome



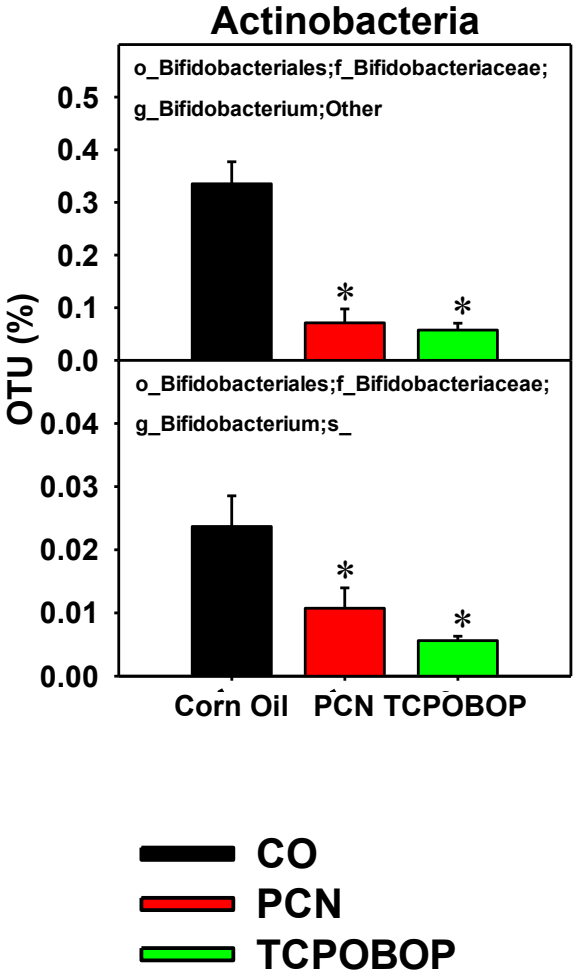


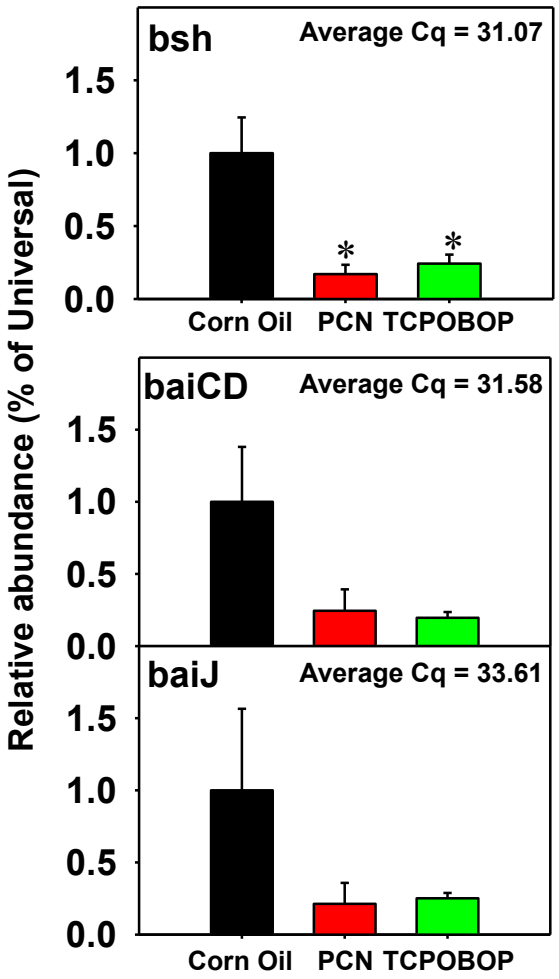


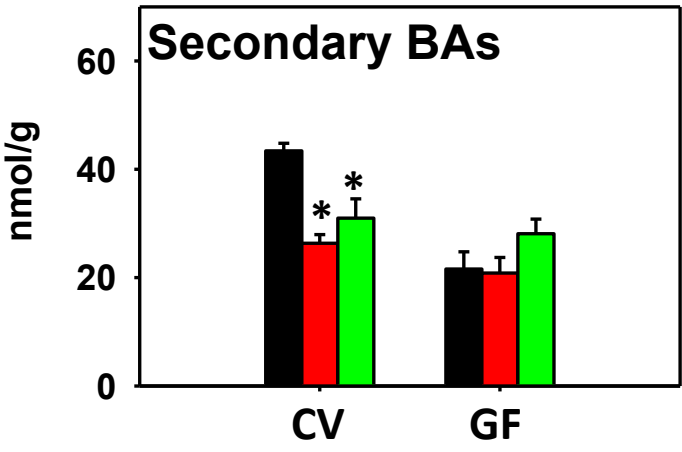


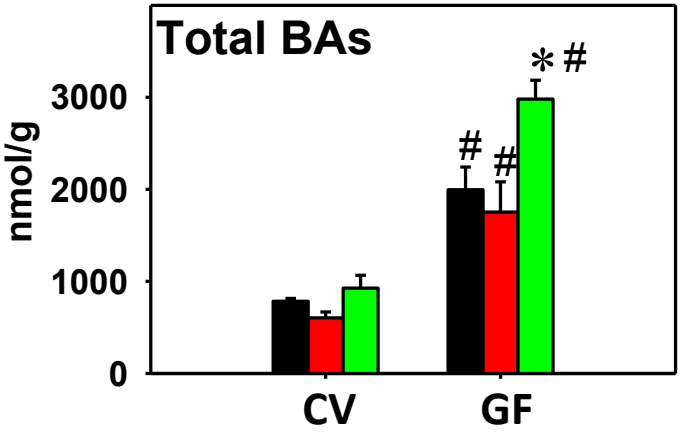
Joseph Dempsey

Activation of PXR and CAR reduces BA-processing bacteria and microbial BA-metabolizing enzymes



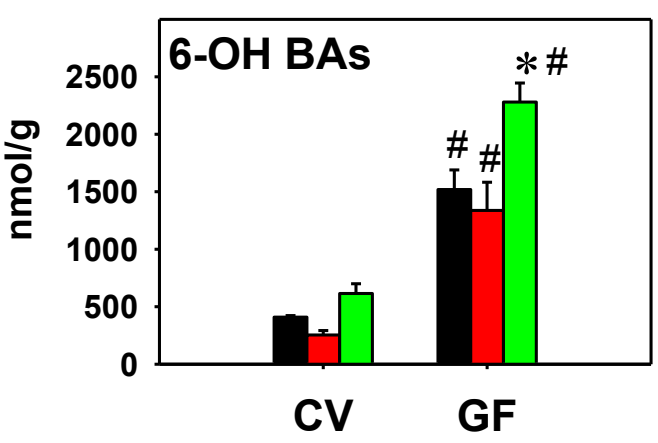


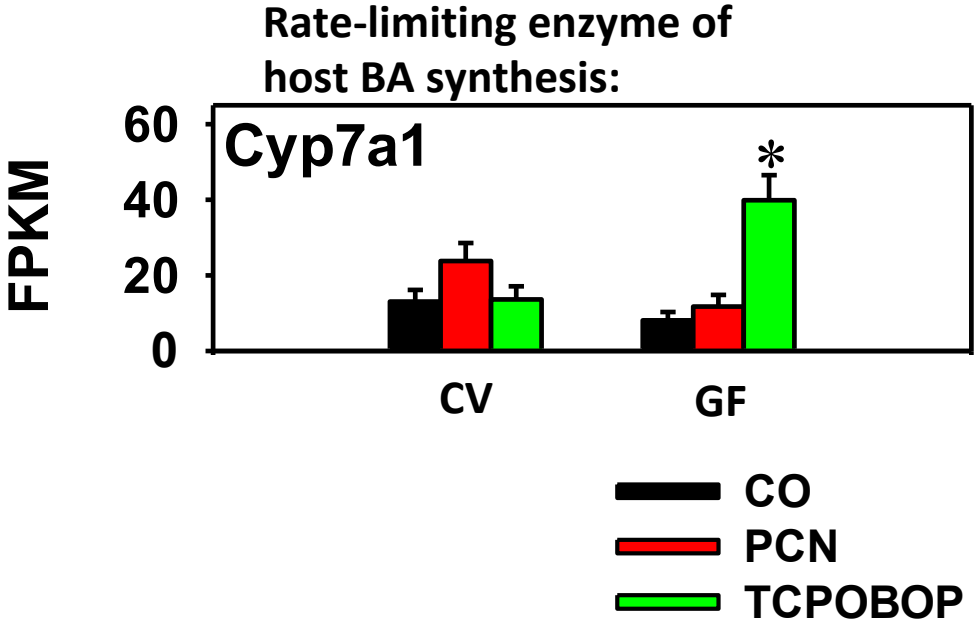




CO
PCN
TCPOBOP

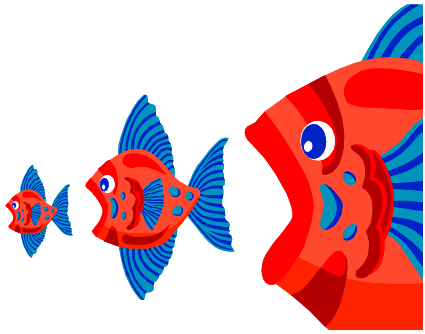
Activation of PXR and CAR reduces BA-processing bacteria and microbial BA-metabolizing enzymes



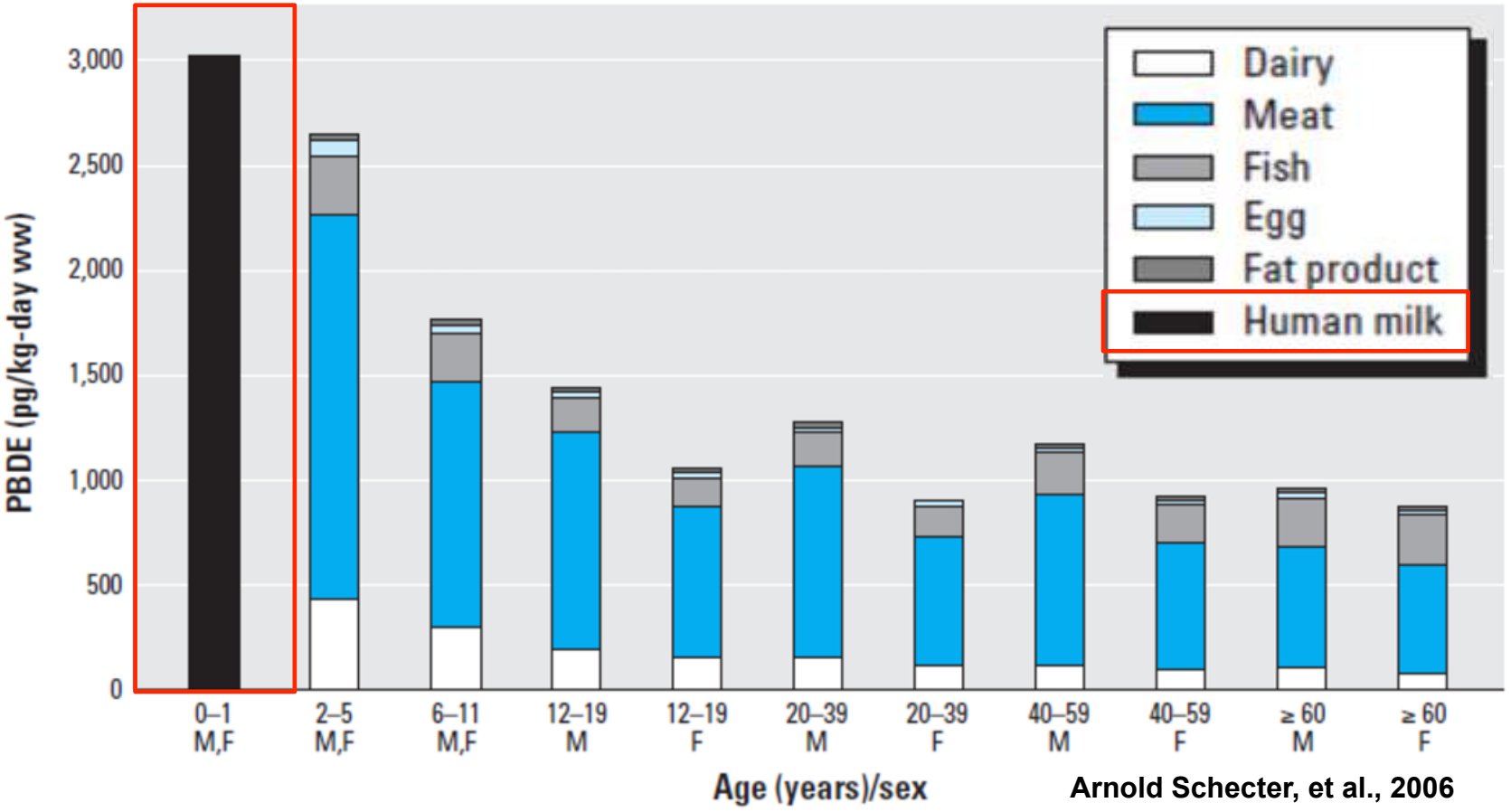


- Lack of gut microbiota potentiated TCPOBOP-mediated increase in 6-OH BAs (including the FXR antagonists T-α/βMCAs, which are the major constituents).
- This may de-repress FXR-mediated repression of hepatic Cyp7a1, leading to an increase in Cyp7a1 mRNA expression in livers of TCPOBOP-exposed GF mice.

PBDEs Exposure in Humans



- Ingestion or inhalation
- Highly persistent and bio-accumulative
- Very high level of PBDEs in adult blood or breast milk

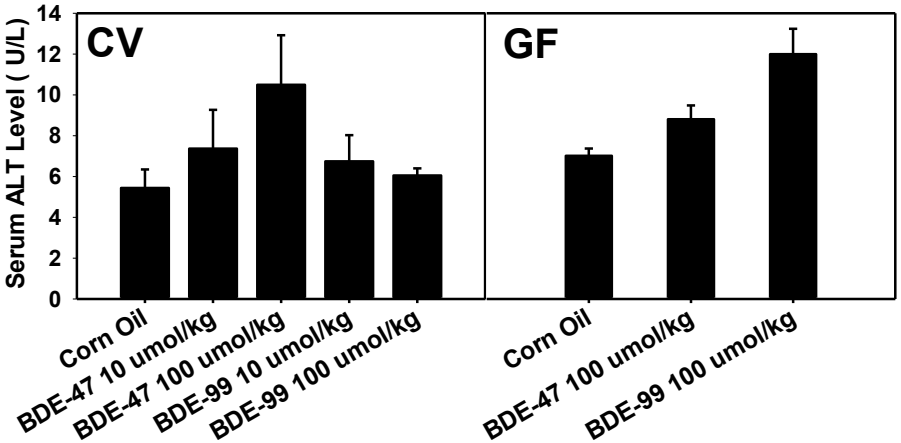


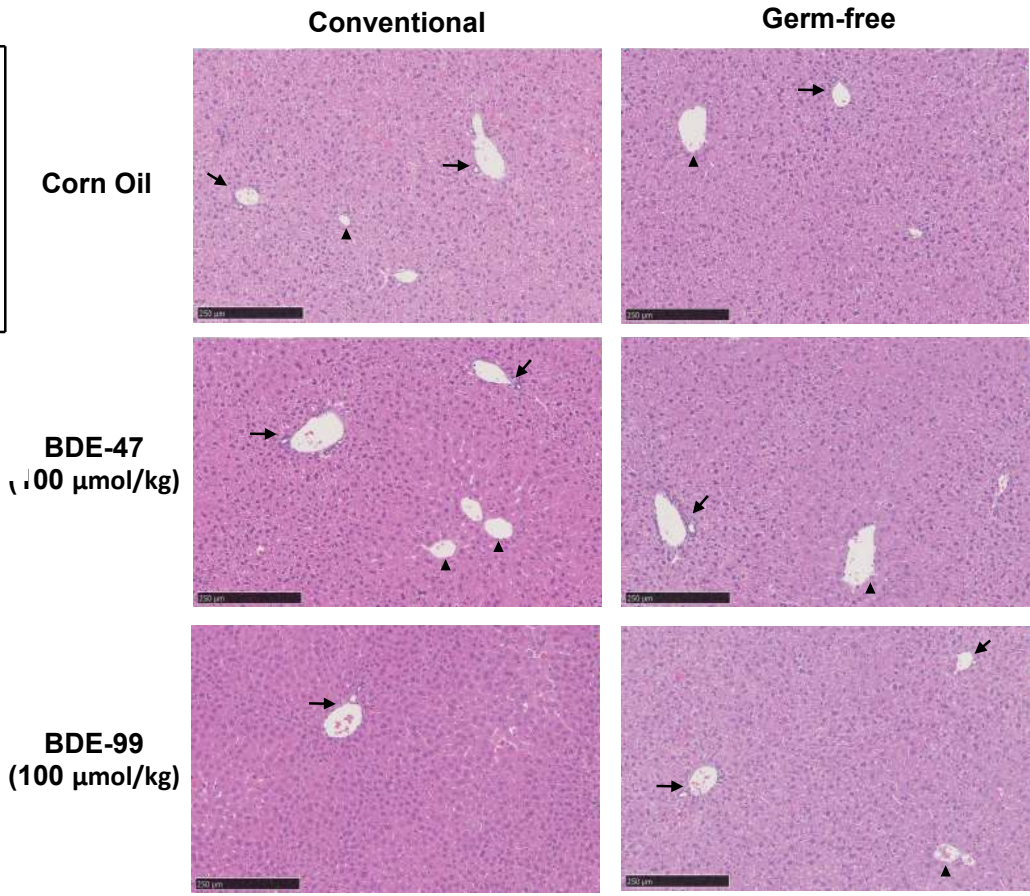
Arnold Schecter, et al., 2006

Major Health Concerns about PBDEs

- **Thyroid hormonal disorders**
  decreased thyroid hormones, which are important for fetal and child brain development (Zhou et al., 2001; Makey et al., 2016; Chevrier et al., 2010)
- **Neurodevelopmental toxicity**
  decreased IQ on children with higher concentration of BDEs 47, 99 or 100 (Herbstman et al., 2010)
- **Reproductive toxicity** (Kim et al. 2010)
- **Liver toxicity**
  - liver hypertrophy in rats (J.K. Dunnick, et al., 2012)
  - hepatic oxidative stress in birds (Fernie et al., 2005)
- **Carcinogenic**
  Long-term exposure in rats caused liver tumor (Dunnick et al., 2016)
- **Diabetes and obesity** (Zhang et al., 2016; Erkin-Cakmak et al., 2015)
- **In liver, certain PBDE congeners activate PXR- and CAR-target genes (Pacyniak et al., 2007; Sueyoshi et al., 2014)**

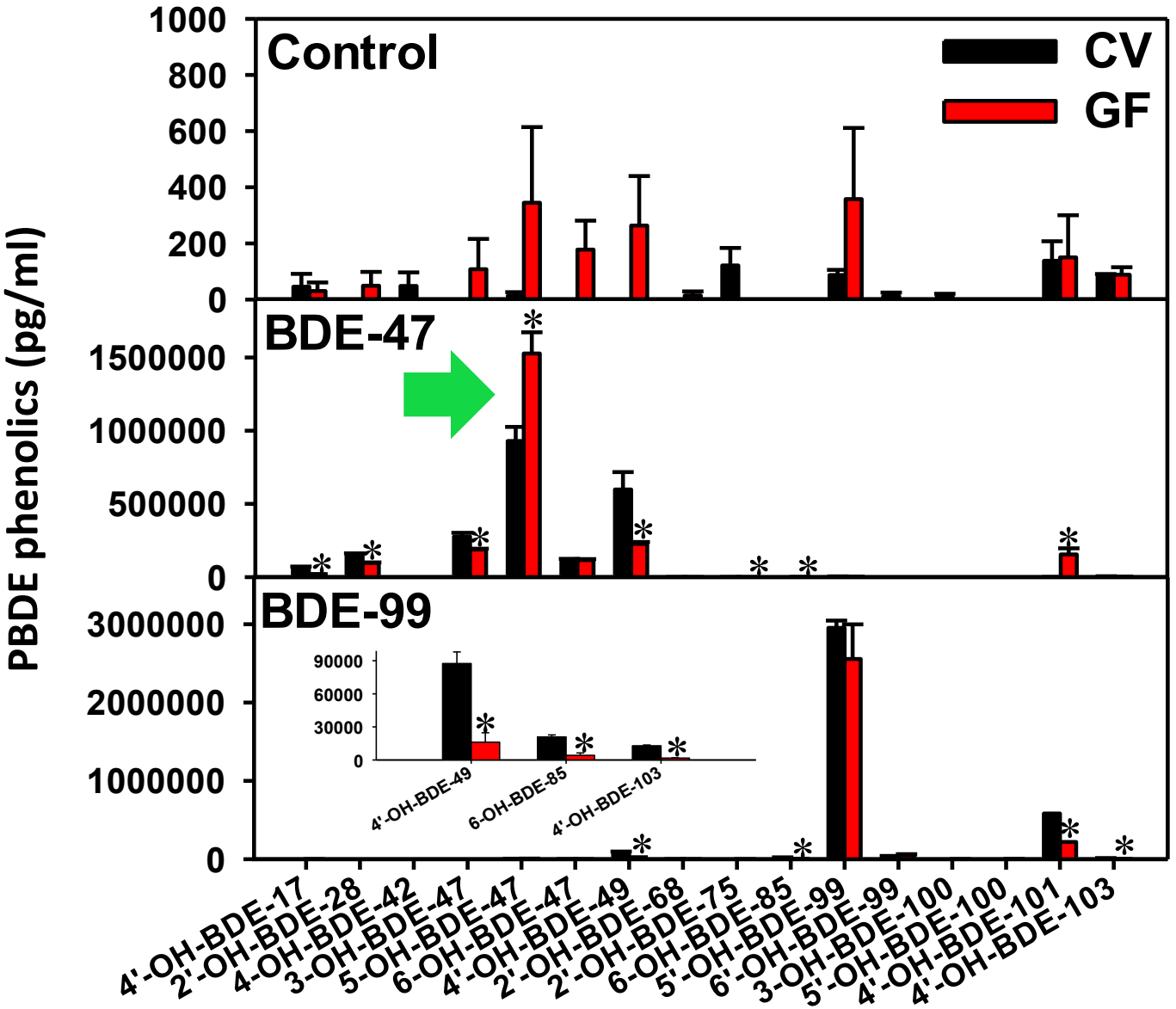
No Apparent Liver Injury Observed at the Given PBDE Dose





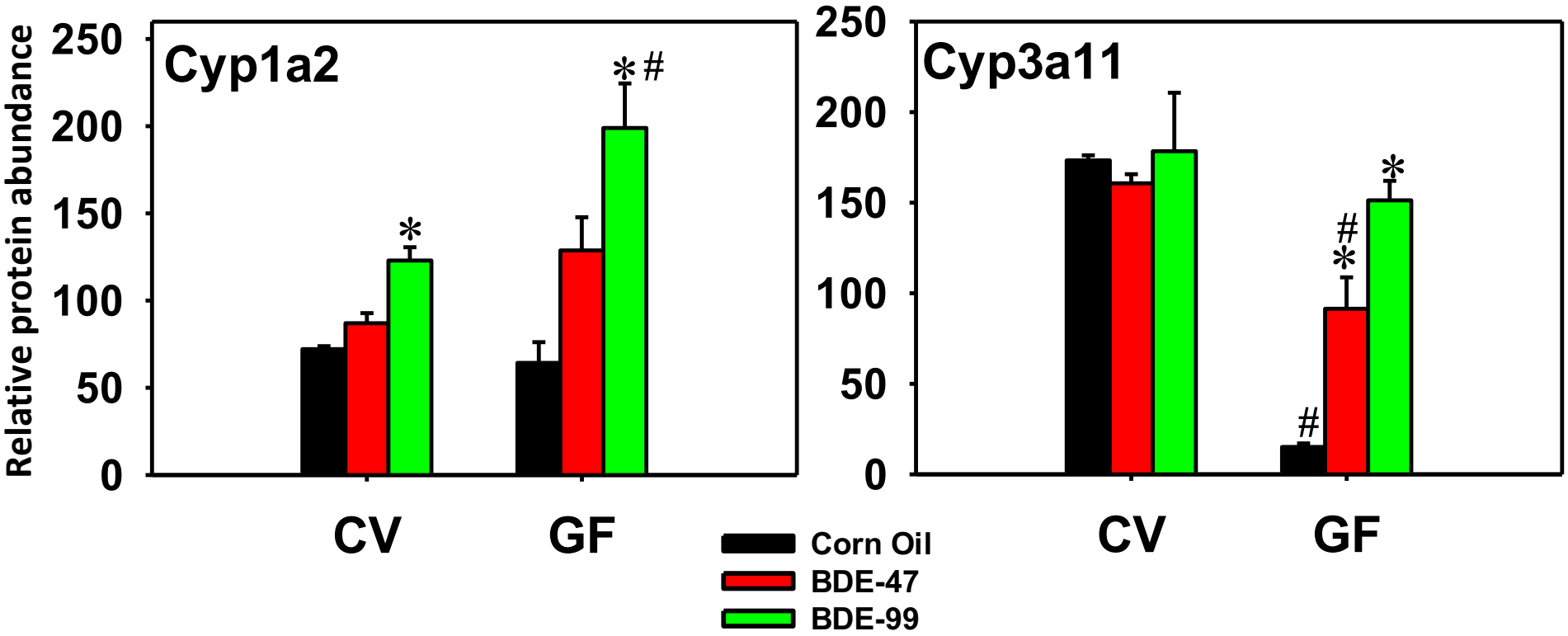
Li et al., 2017 Drug Metab Dispo

Lack of Gut microbiome altered the hepatic oxidation of PBDEs



Li et al., 2017 Drug Metabol Dispo PMID: 28864748

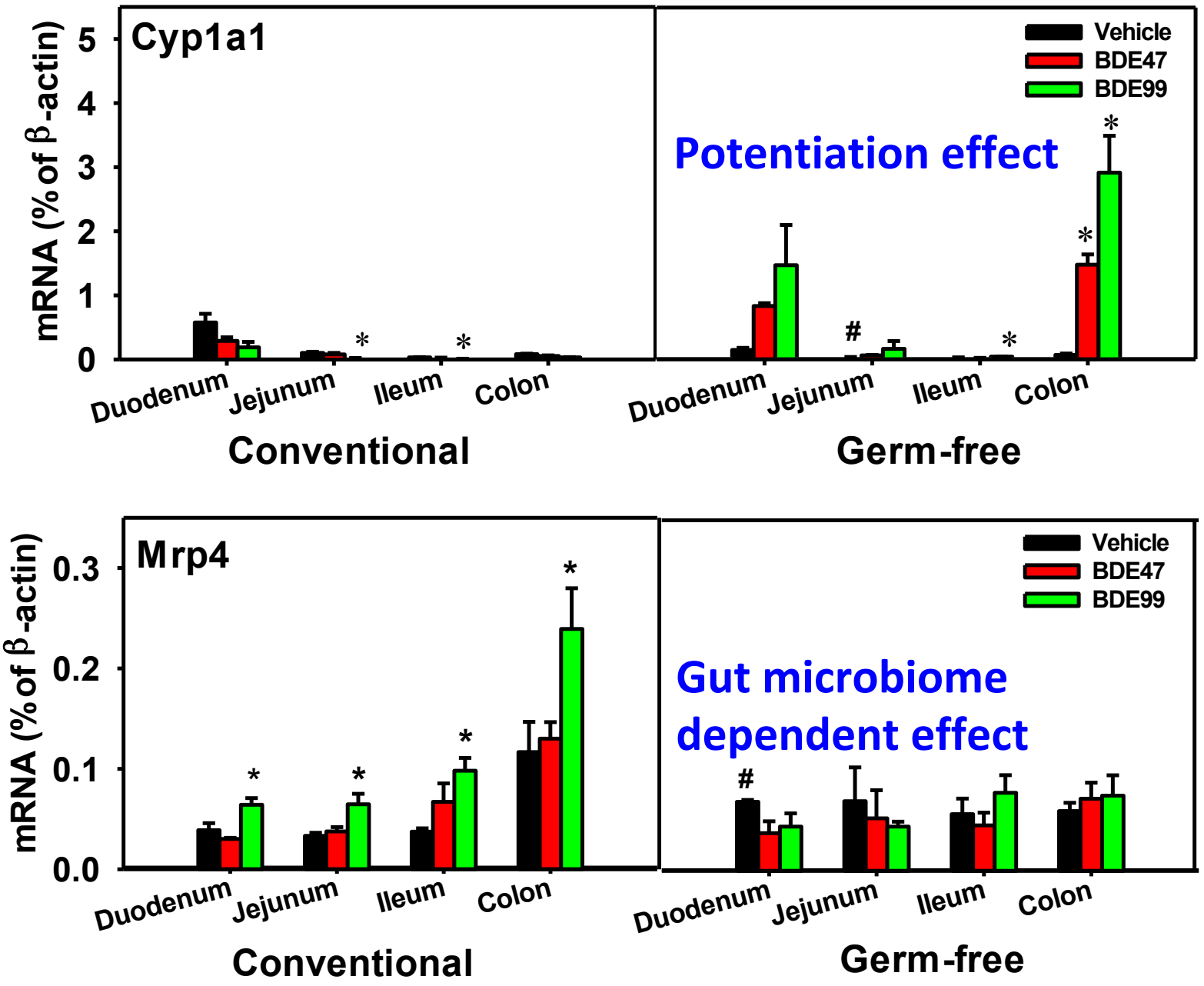
Lack of Gut Microbiome sensitized the Hepatic Response to PBDE-mediated up-regulation of Cyp1a2 and Cyp3a11 proteins



- Gut microbiome may inactivate PBDEs so less AhR and PXR ligands are available to the liver in CV mice.

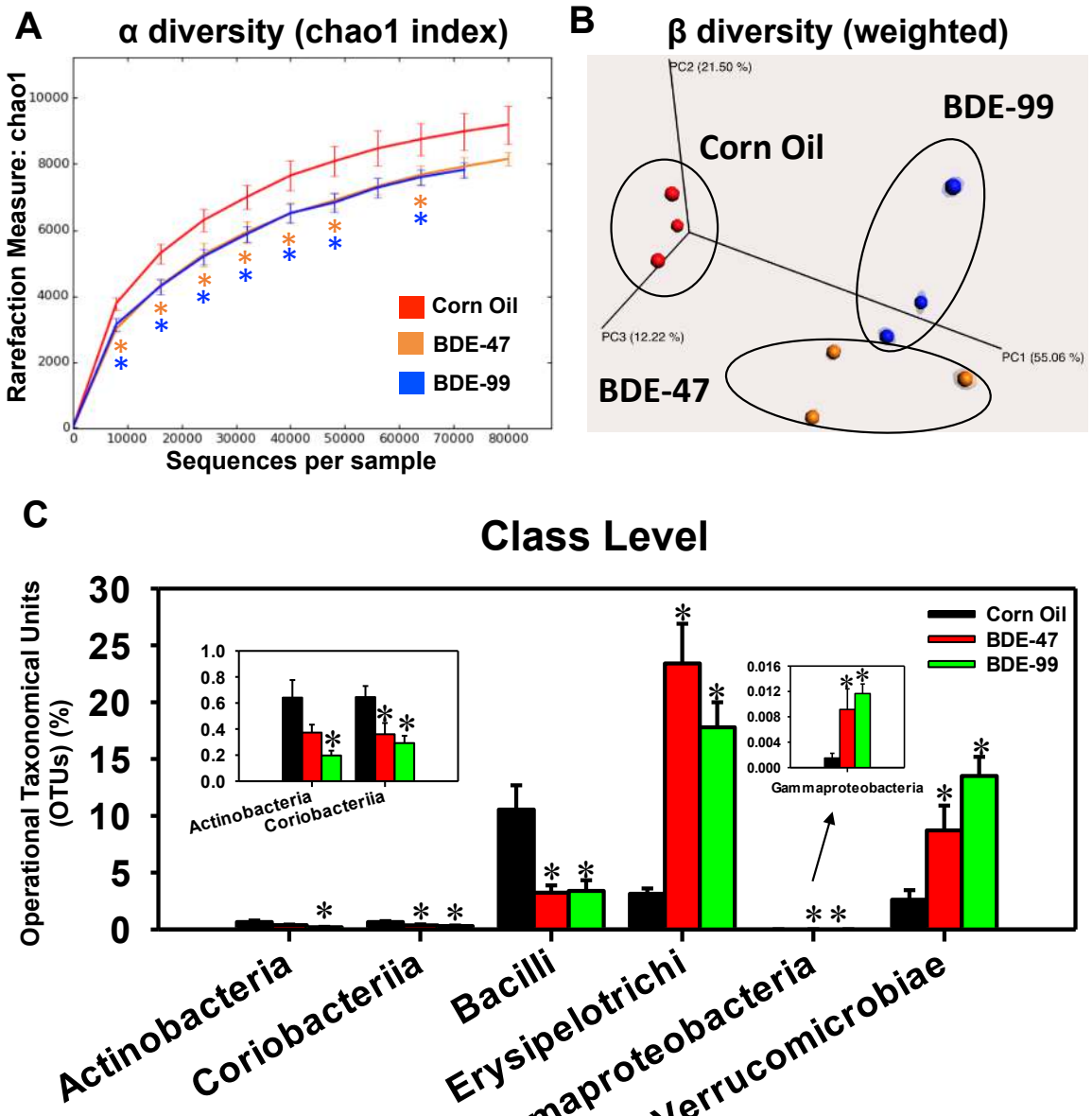
Li et al., 2017 Drug Metabol Dispo PMID: 28864748

Gut microbiome and PBDEs on the Intestinal Gene Expression



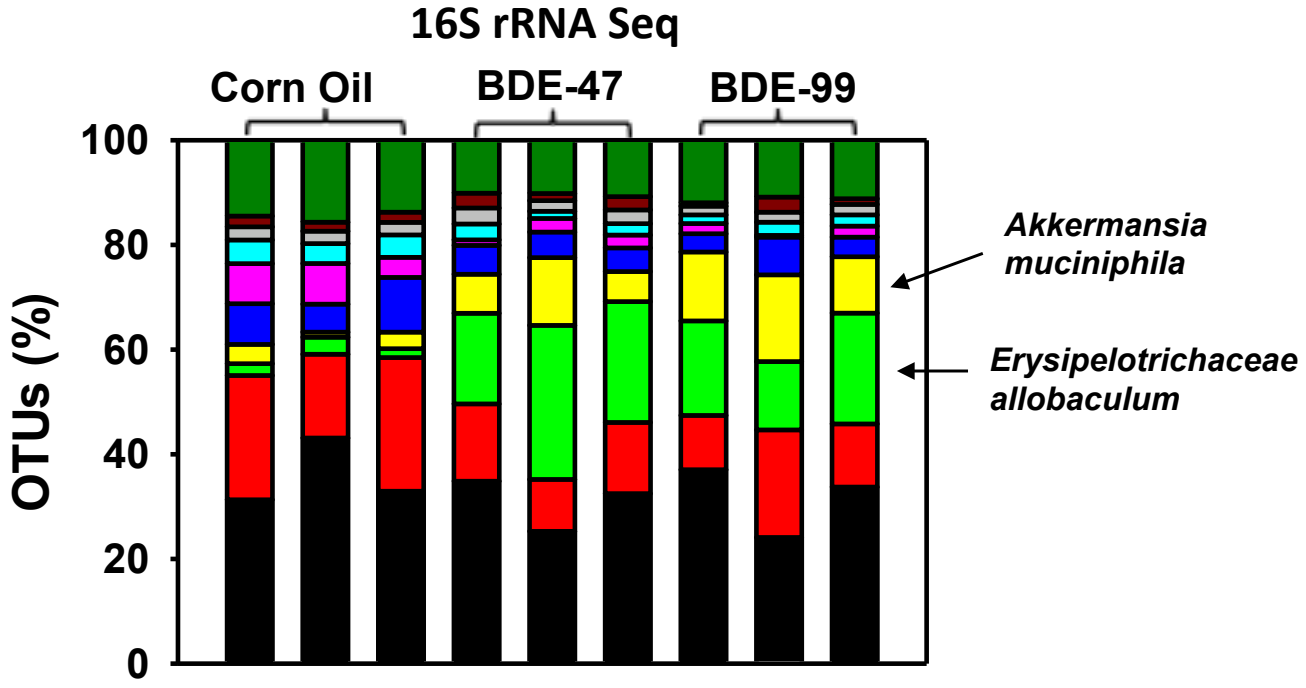
Li et al., 2017 Drug Metab Dispo

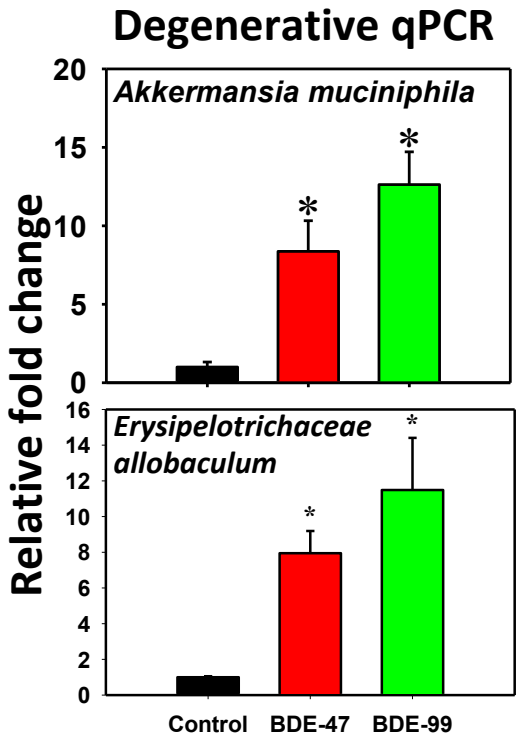
PBDE-mediated Regulation of Gut Microbiome in Large Intestinal Content of CV mice (QIIME)



Li et al. (2018) DMD PMID: 29769268

PBDE-mediated Regulation of Gut Microbiome (Species Level) in Large Intestinal Content of CV mice (QIIME)





p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__S24-7;g__;s__

p__Firmicutes;c__Clostridia;o__Clostridiales;f__;g__;s__

p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Allobaculum;s__

p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae;g__Akkermansia;s__muciniphila

p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__;s__

p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__

p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__;s__

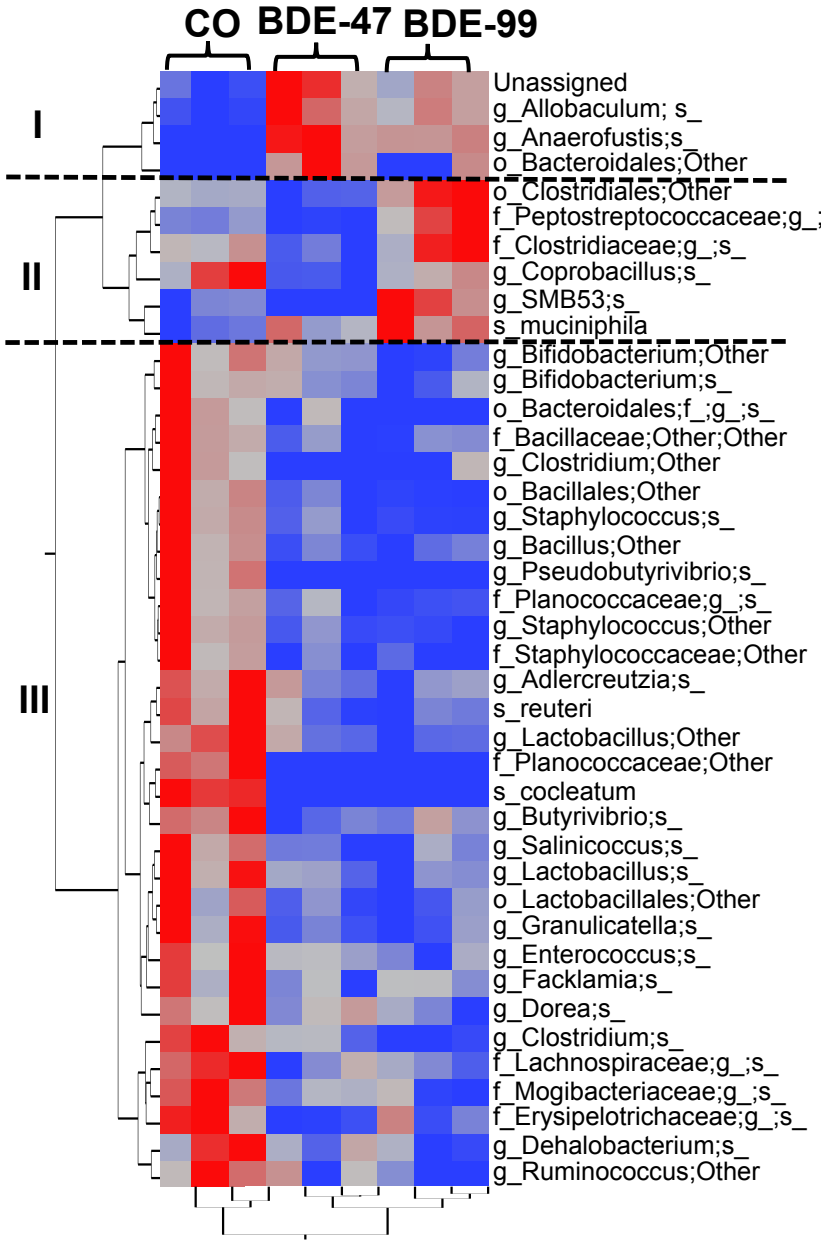
p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__;s__

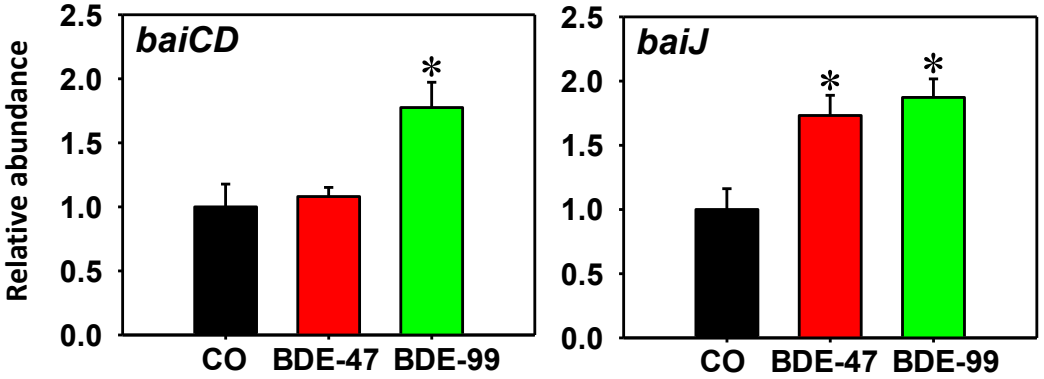
p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus];s__gnavus

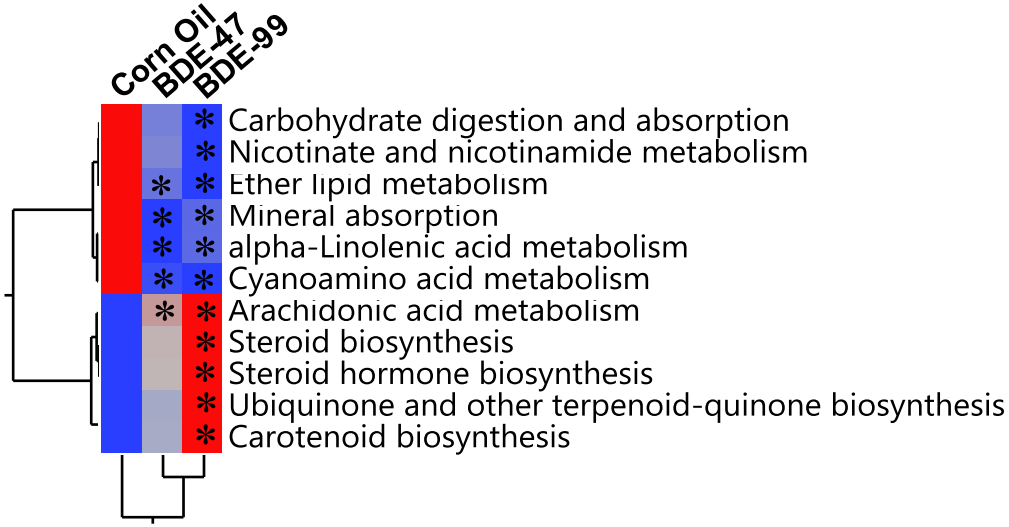
Other taxa

Li et al. (2018) DMD PMID: 29769268

PBDE-mediated Regulation of Gut Microbiome) and BA-metabolizing Microbial Enzymes

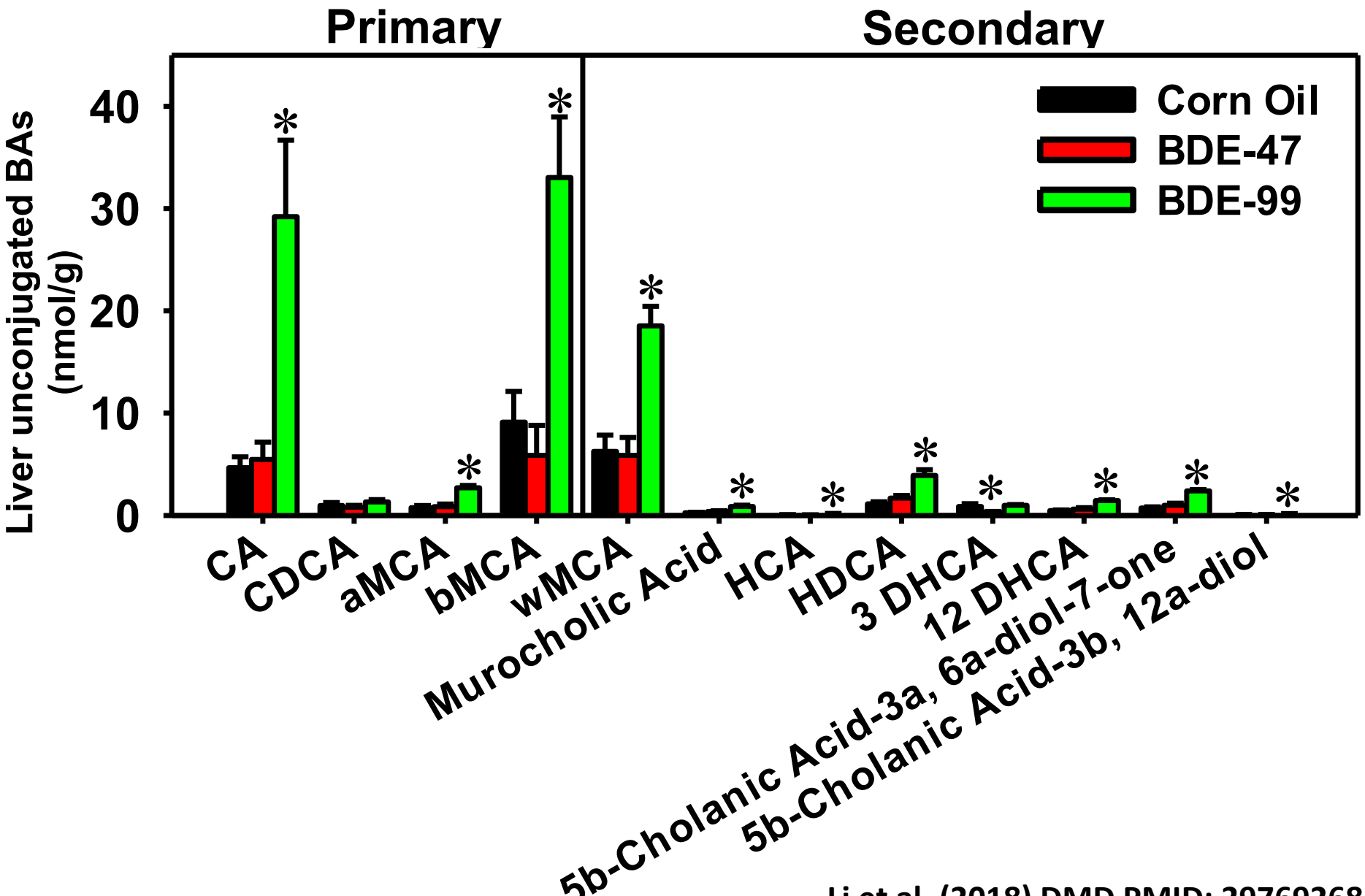






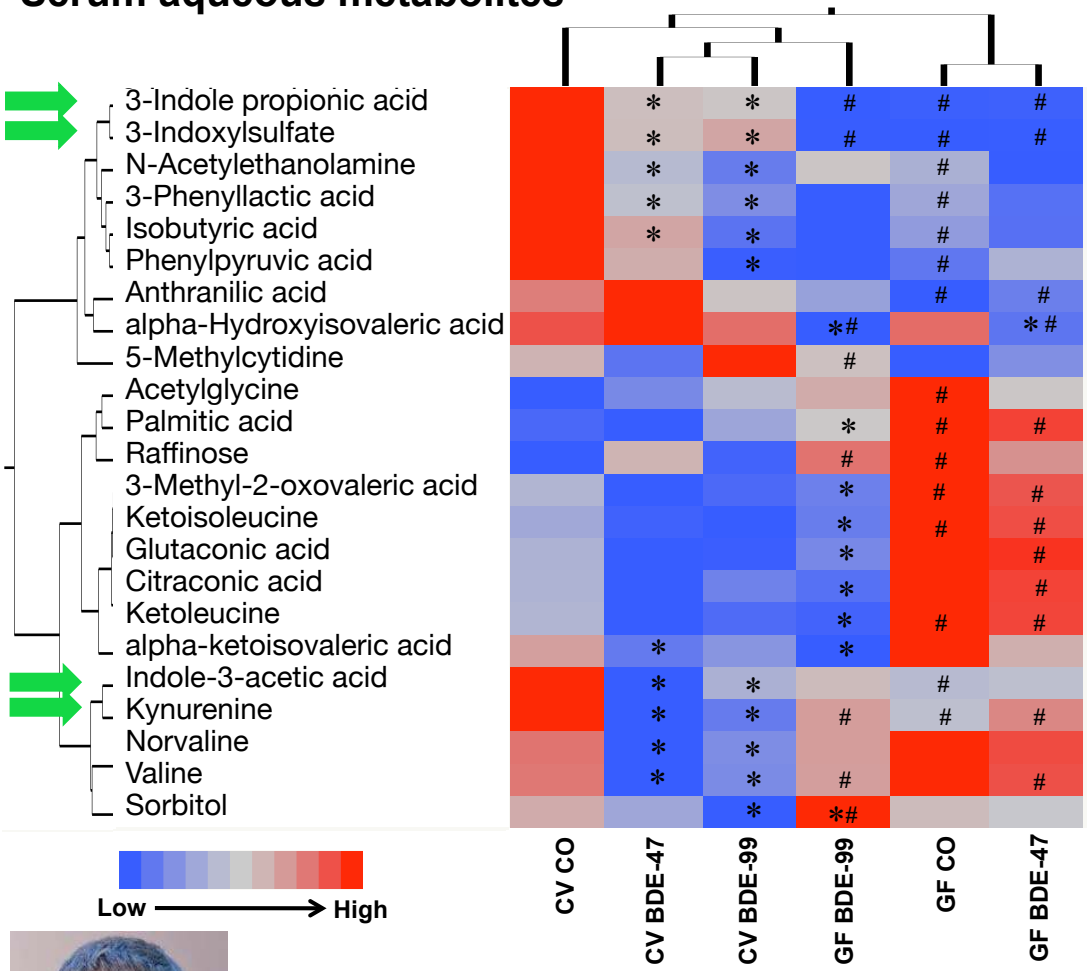
Li et al. (2018) DMD PMID: 29769268

PBDE-mediated Increased Unconjugated BAs in Liver of CV Mice



Li et al. (2018) DMD PMID: 29769268

PBDE-mediated Decrease in Circulating Microbial Metabolites of Tryptophan



- **The tryptphan microbial metabolite indole-3-propionic acid (IPA) is a novel intestinal PXR activator (Venkatesh et al. Immunity 2015)**
- **Serum IPA, and many other tryptophan microbial metabolites, were PBDE exposure in a gut microbiome dependent manner**
- **IPA is anti-inflammatory and protects pancreatic beta cells during diabetes in humans (Tuomainen et al., 2018 Nature)**

David Scoville, Postdoctoral Fellow

Overall Conclusion

- **Gut microbiome is a critical interface that modulates the cross-talk between intermediary metabolism and xenobiotic biotransformation of the host liver**

- **Understanding the interplay between gut microbiome and environmental chemicals within the gut-liver axis is crucial to accurately predict the toxicological outcomes and may aid in the design of novel probiotic therapies in vulnerable populations**

ACKNOWLEDGEMENTS

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- Cindy Yanfei Li, PhD Student (graduated Dec 2017)
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- Khakkhak Khayi, Undergraduate Researcher
- Jennifer Kim, Undergraduate Researcher
- Hannah Bauermeister, Undergraduate Researcher

Former lab members:

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- SooWan Lee, Former Undergraduate Researcher
- Hannah Bolam So, Former Undergraduate Researcher
- Felcy Selwyn, Former Research Scientist
- Yubin Song, Former Undergraduate Researcher
- Daniel Park, Former Undergraduate Researcher
- Shinhee Park, Former Undergraduate Researcher
- Elaine Chen, Former Undergraduate Researcher
- Jessica Mao, Undergraduate Researcher
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