Novel Interactions between Gut Microbiota and Host Hepatic Xenobiotic Biotransformation – Lessons Learned from the Germ-free Mice

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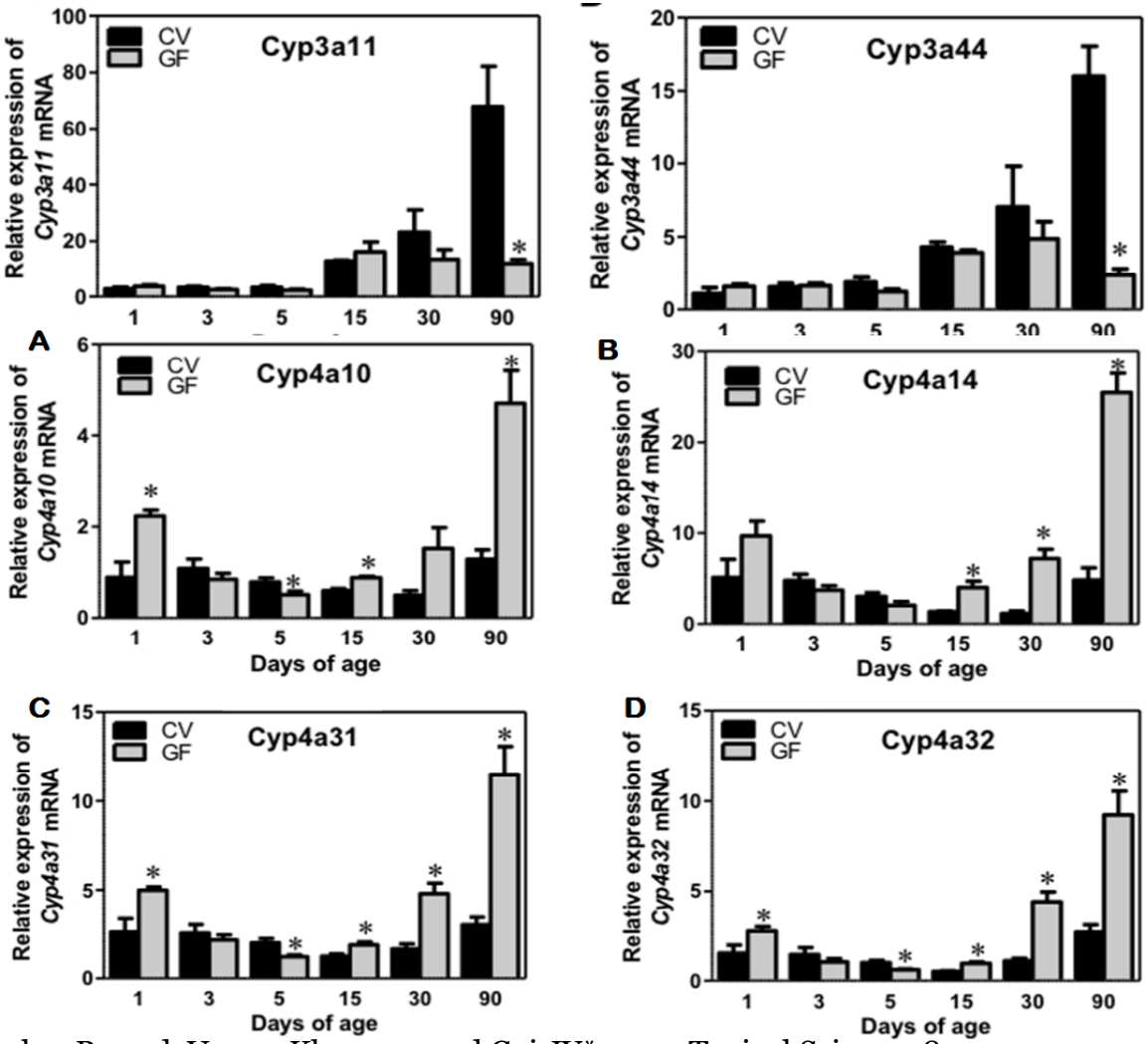
Three examples of the role of gut microbiome in regulating the hepatic drug-processing genes

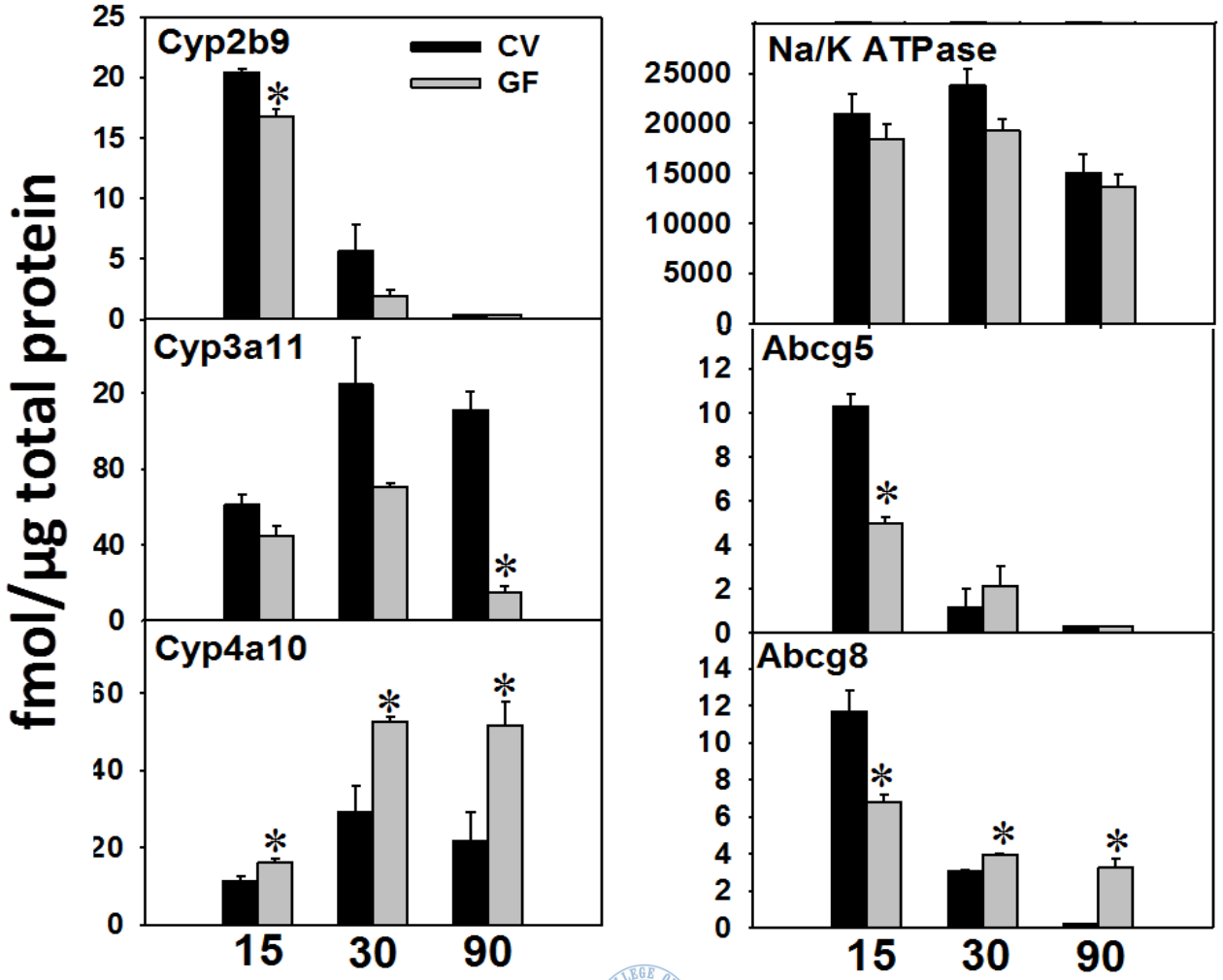
- Gut microbiome and liver development
- Probiotics/conventionalization on hepatic drug-processing gene expression
- Interactions between gut microbiome and environmental chemicals

Liver P450 expression modulated by gut microbiota



Selwyn, Cheng, Bammler, Prasad, Vrana, Klaassen, and Cui JY*, 2015 Toxicol Sci, 147: 84-103

Protein expression was altered in livers of Germ-free Mice as compared to Age-matched Conventional Mice



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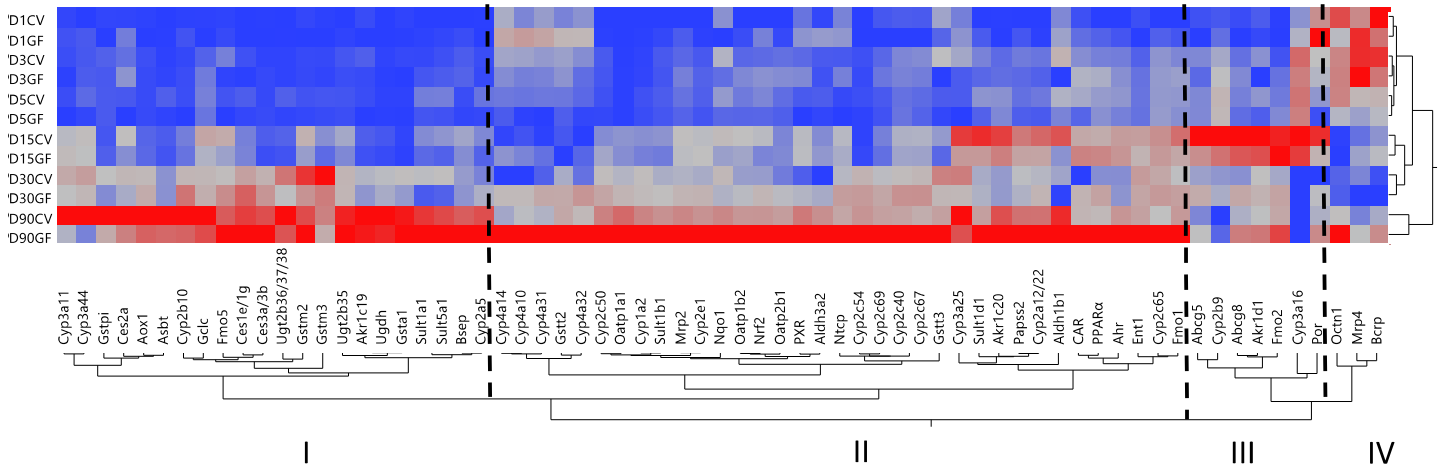
Four Developmental Patterns of Critical Drug Processing Genes in Livers of Conventional and GF mice

Top Networks

A

ID	Associated Network Functions	Score
1	Drug Metabolism, Small Molecule Biochemistry, Carbohydrate Metabolism	52
2	Behavior, Nervous System Development and Function, Small Molecule Biochemistry	42
3	Lipid Metabolism, Small Molecule Biochemistry, Vitamin and Mineral Metabolism	37
4	Cell Death and Survival, Cellular Development, Digestive System Development and Function	37

B



Selwyn, Cheng, Bammler, Prasad, Vrana, Klaassen, and Cui JY*, 2015 Toxicol Sci, 147: 84-103

Age and gut microbiota affect hepatic drug-processing genes

- The absence of gut microbiota produced age-specific effects on the regulation of hepatic drug-processing genes
- The age-specific changes may be due to different types/ratios of intestinal bacteria at various ages during development in CV mice

Regulation of Hepatic Drug-metabolizing Enzymes in Germ-free mice by Conventionalization and Probiotics

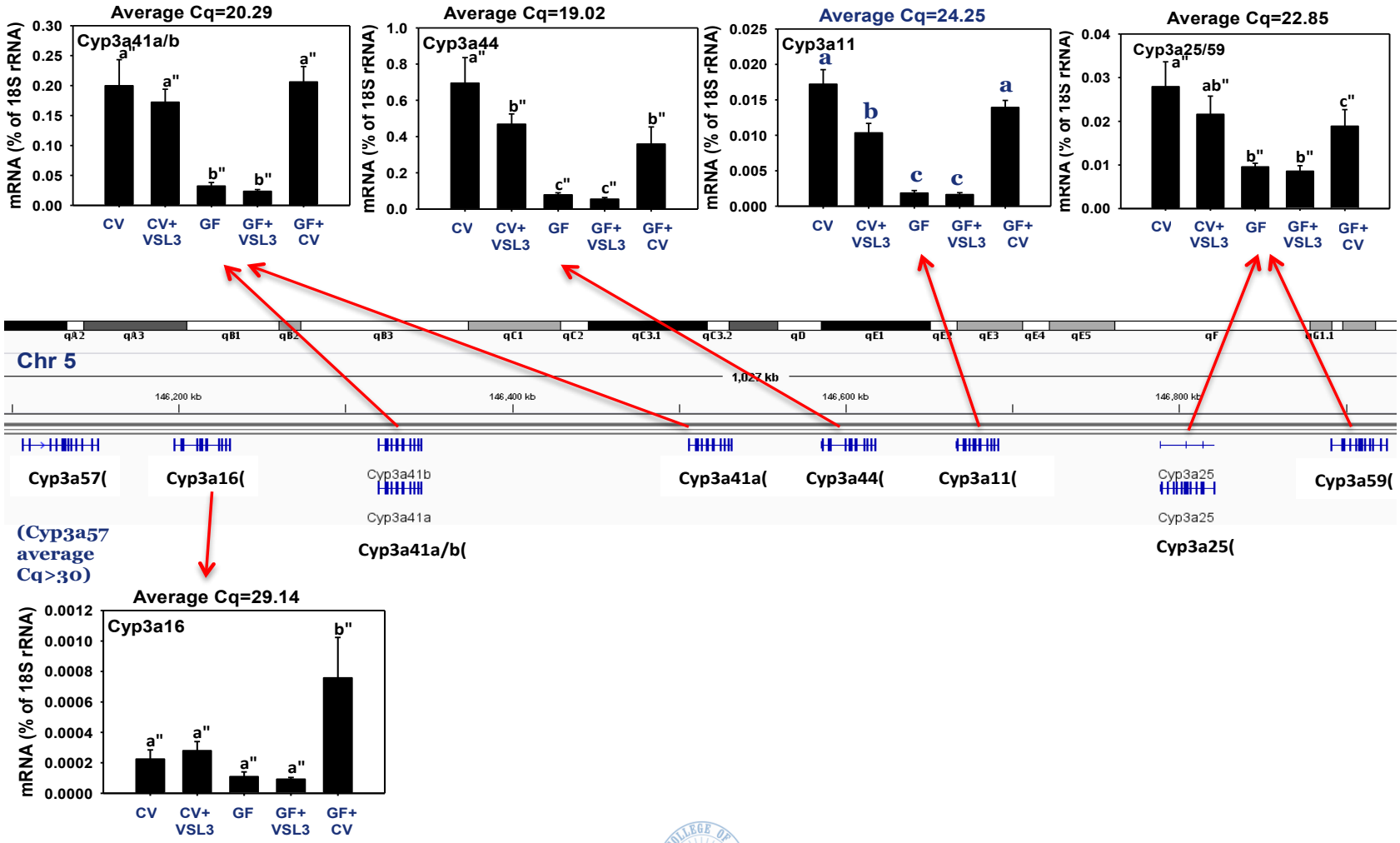
- Probiotics: live microorganisms that confer a health benefit to the host when administered in adequate amount

- VSL3 is a combinatorial probiotic that is used for human intestinal disorders, such as inflammatory bowel disease and ulcerative colitis

Introduction of exogenous bacteria to germ-free mice: Expression of hepatic drug-processing genes

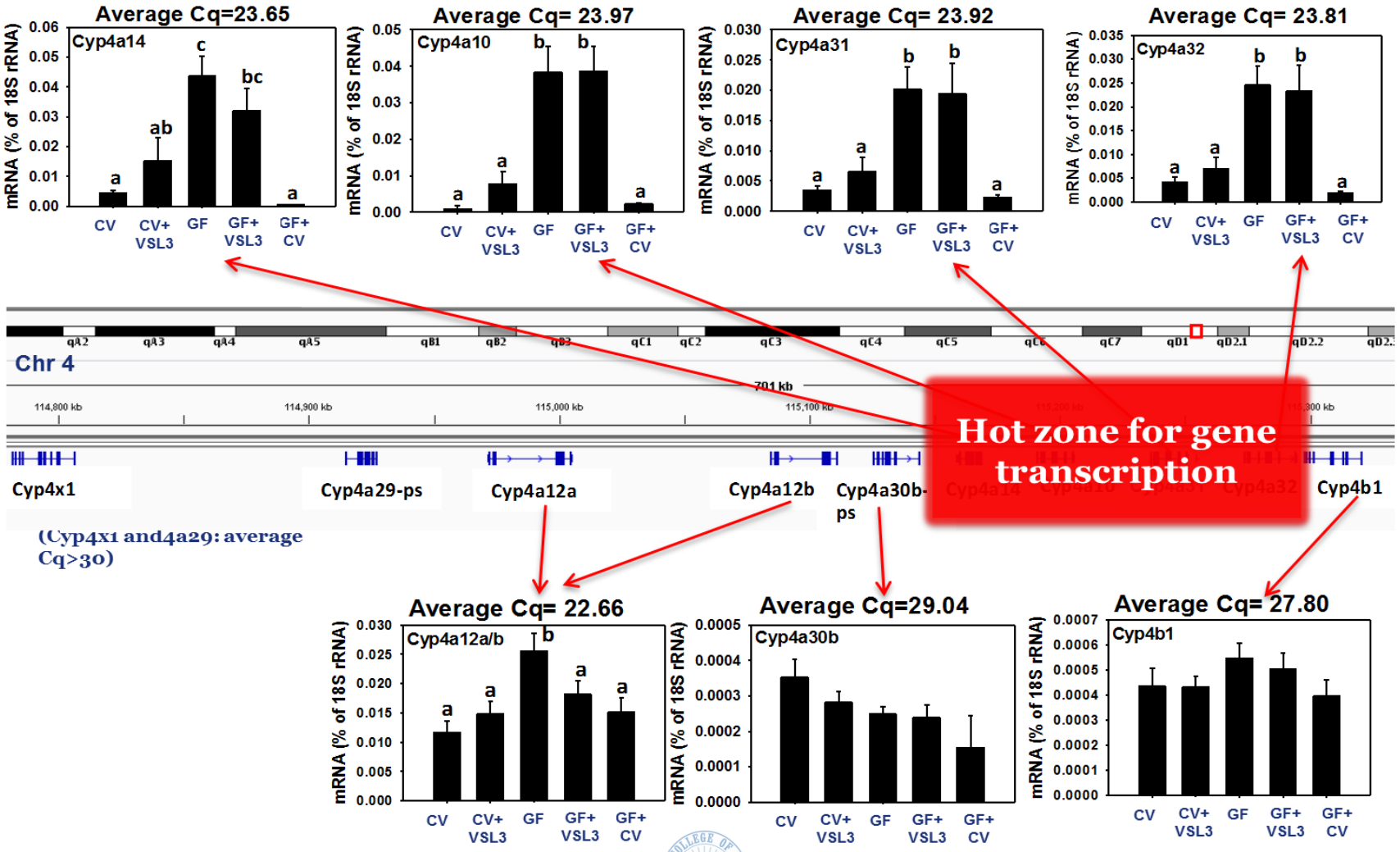
- Age-matched 2-month-old adult conventional (CV) and germ-free mice (males, n=5~8 per group) were administered 4.5×10^6 CFU/ml drinking water for 28-days
- In a separate experiment, 1-month old GF mice were taken out of the isolator and housed with the feces from CV mice for 2-months
- All mice were 3-month-old prior tissue collection.

Regulation of the Cyp3a Gene Cluster by VSL3 and Conventionalization



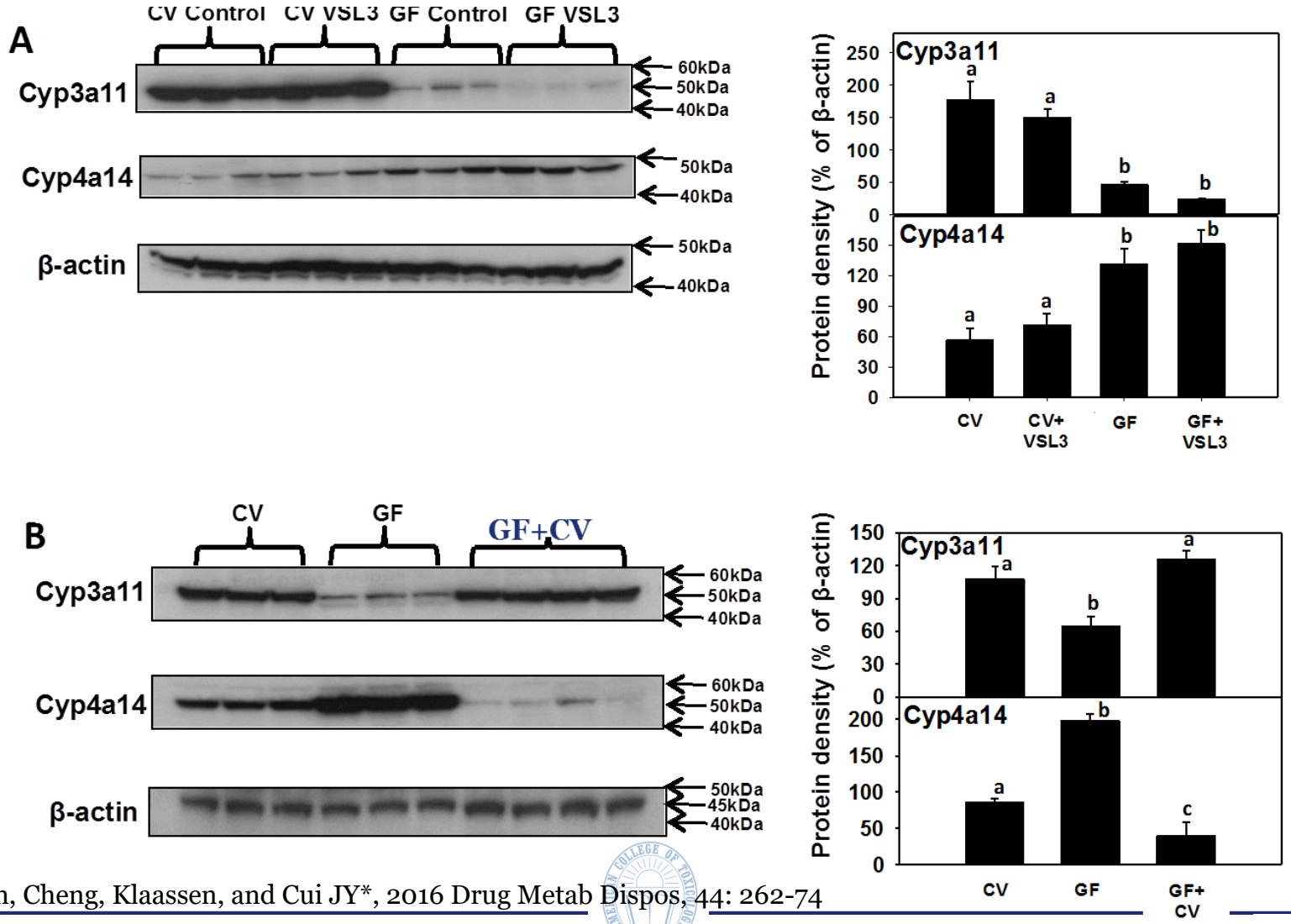
Selwyn, Cheng, Klaassen, and Cui JY*, 2016 Drug Metab Dispos, 44: 262-74

Regulation of the Cyp4a Gene Cluster by VSL3 and Conventionalization



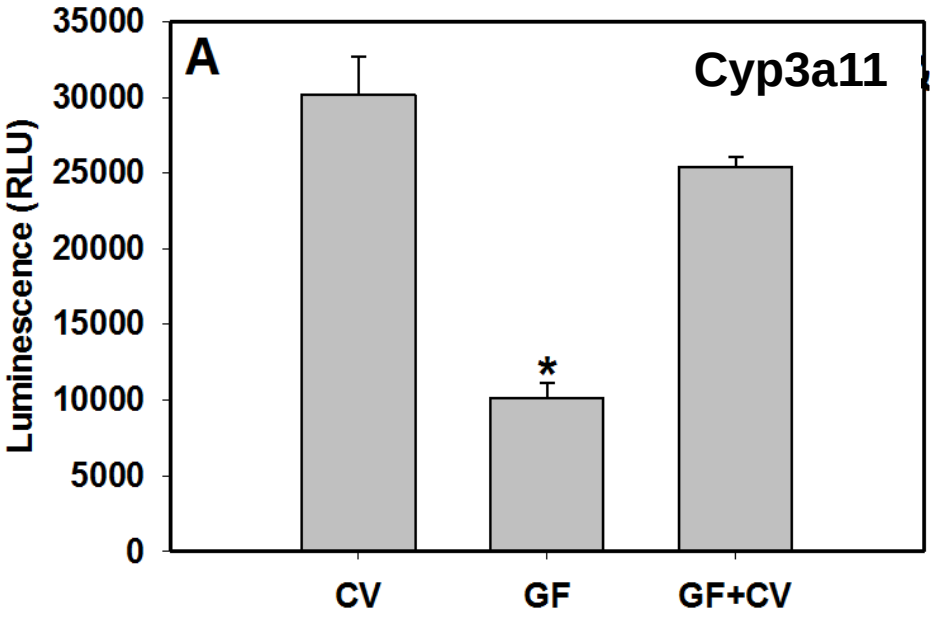
Selwyn, Cheng, Klaassen, and Cui JY*, 2016 Drug Metab Dispos, 44: 262-74

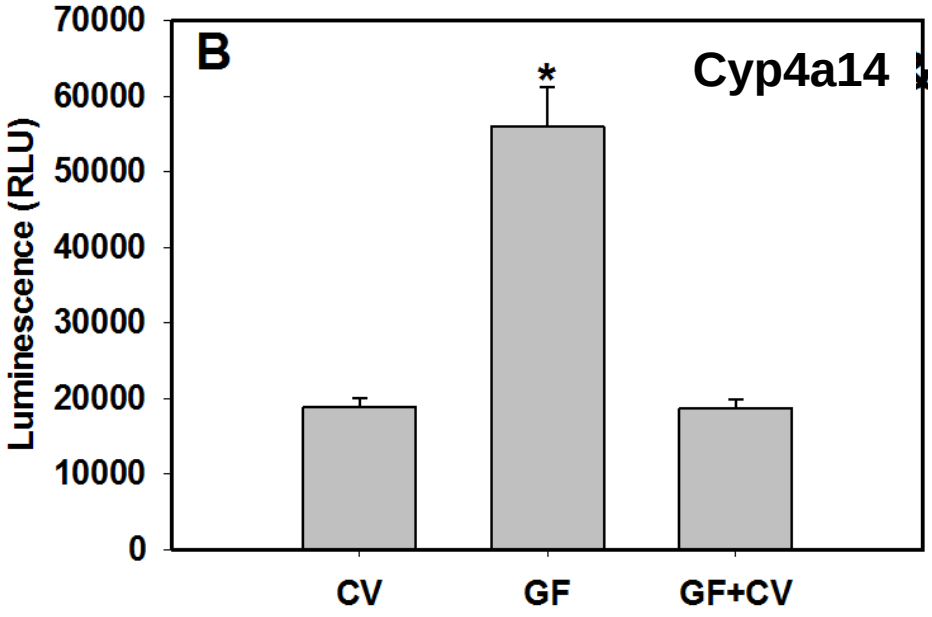
Validation of the Protein Expression of Cyp3a and Cyp4a



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

Validation of Enzyme Activity of Cyp3a and Cyp4a





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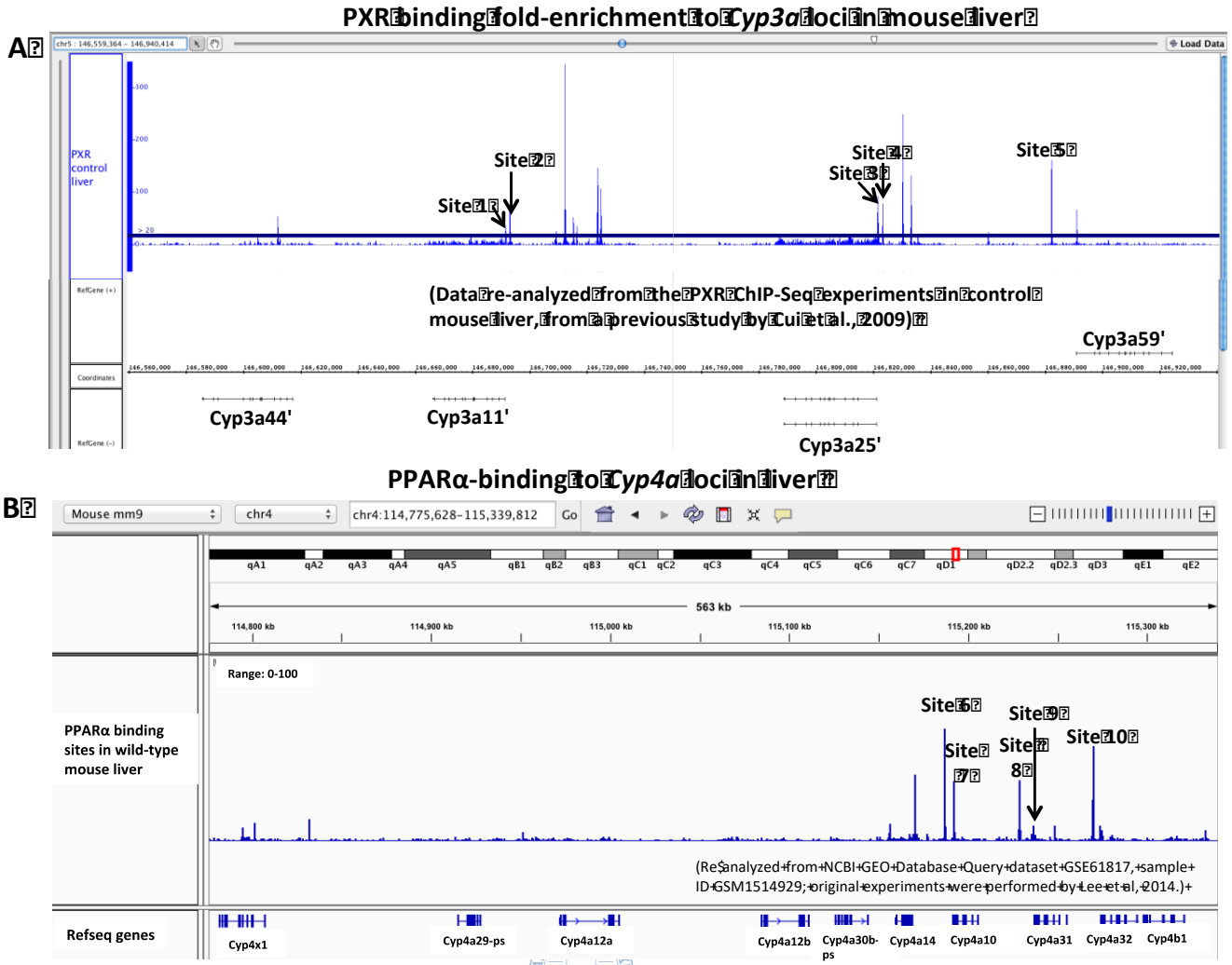
PXR- and PPARα-DNA Binding Sites in Control Mouse Liver



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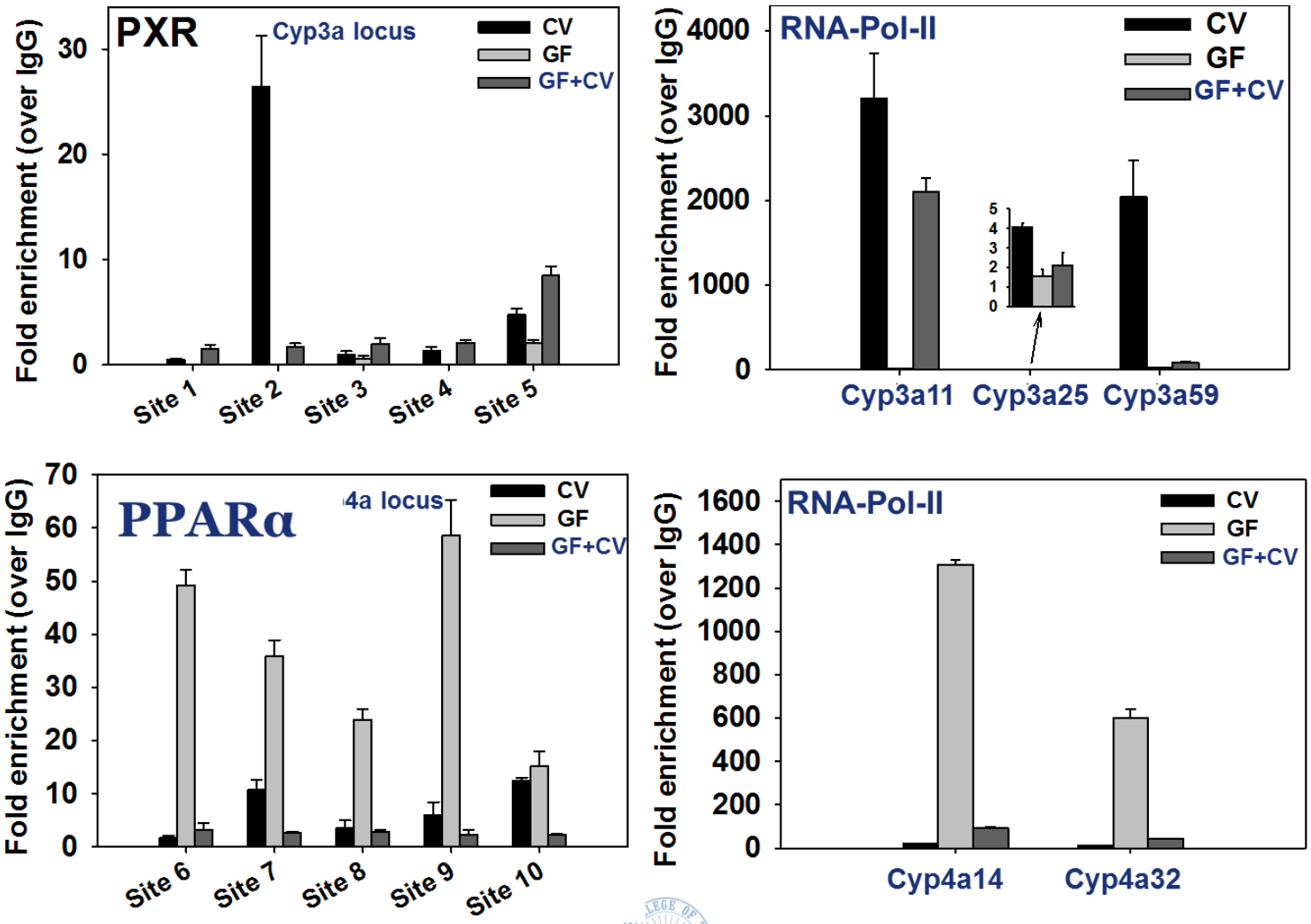
ChIP of PXR and PPARα binding to Cyp3a and Cyp4a loci



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Germ-free and conventionalization have the most prominent effect on the regulation of hepatic drug-processing genes

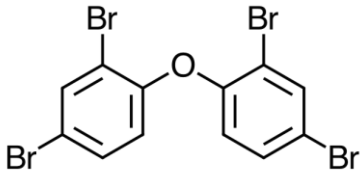
- Germ-free conditions resulted in the most prominent changes in hepatic drug-metabolizing enzyme expression, most notably a consistent down-regulation of many genes in the *Cyp3a* cluster, but a consistent up-regulation of many genes in the *Cyp4a* cluster.

- Conventionalization of GF mice at least partially restores the expression of these genes to CV levels.

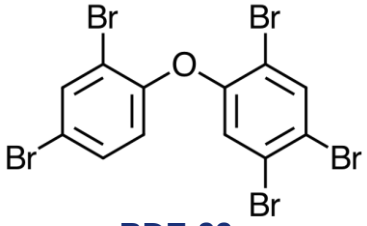
- The GF and conventionalization mediated changes in *Cyp3a* and *4a* genes are associated with altered PXR and PPARα-binding to the targeted DNA sequences within these genes.

Polybrominated Diphenyl Ethers (PBDEs)

- PBDEs are among the most abundant and persistent environmental chemicals in the human population



BDE-47
(2,2',4,4'-Tetrabromodiphenyl ether)



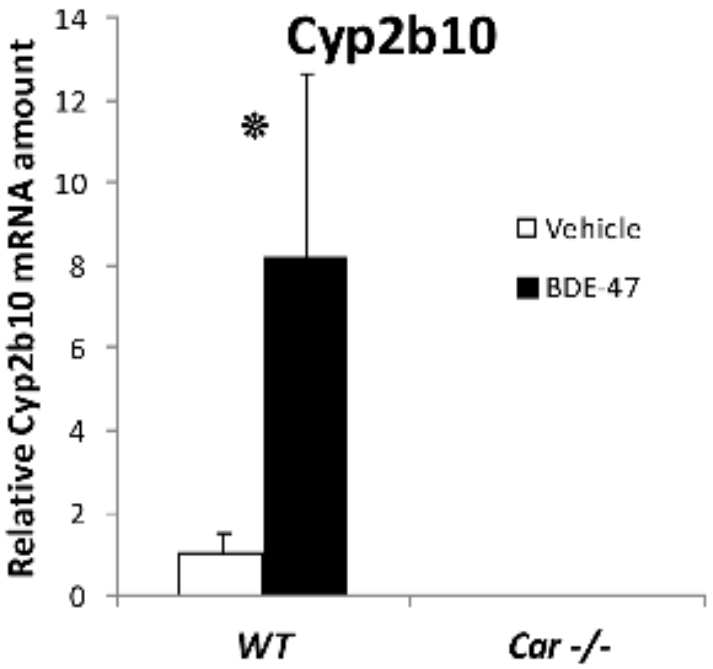
BDE-99
(2,2',4,4',5-Pentabromodiphenyl ether)

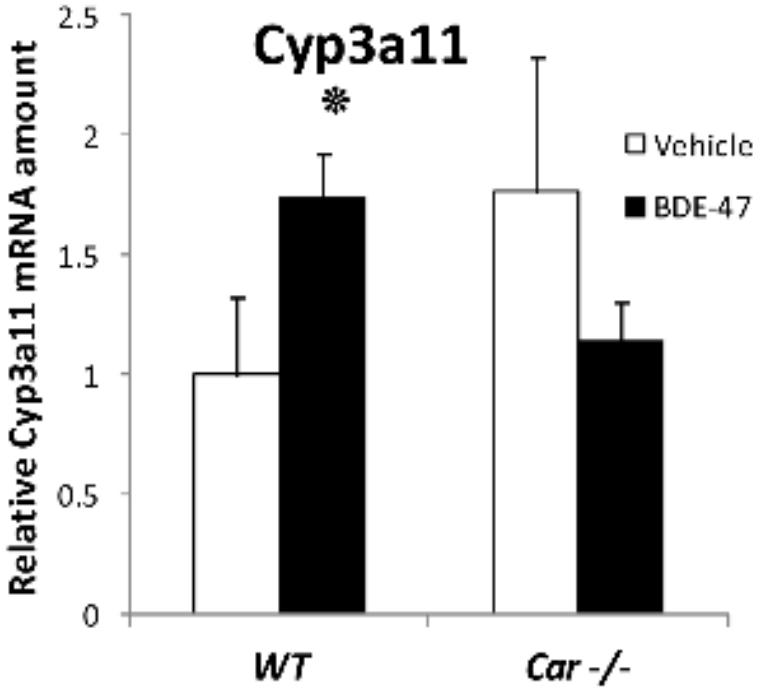
- BDE-47 and BDE-99 are the predominant congeners detected in humans, and they are highly prevalent in seafood and breast milk at worrisome levels humans

Hepatic Biotransformation of PBDEs and Toxicities

- Hepatic uptake: via organic anion transporting polypeptides (OATP) transporters
- Hepatic Phase-I metabolism: by human CYP2B6 to toxic hydroxylated metabolites
- Hepatic Phase-II conjugation (in vitro evidence): glucuronidation, sulfonation or methylation
- Toxicities of PBDEs: thyroid toxicity, neurodevelopmental disorders, hepatic oxidative stress and cancer.

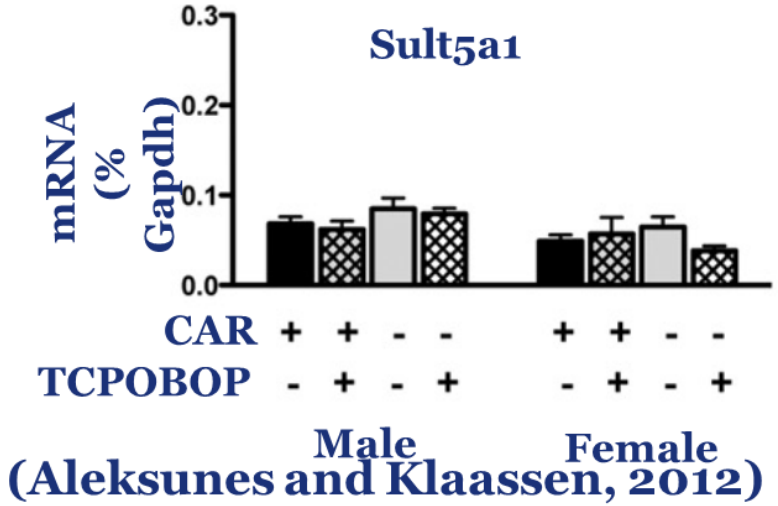
BDE-47 is a CAR activator



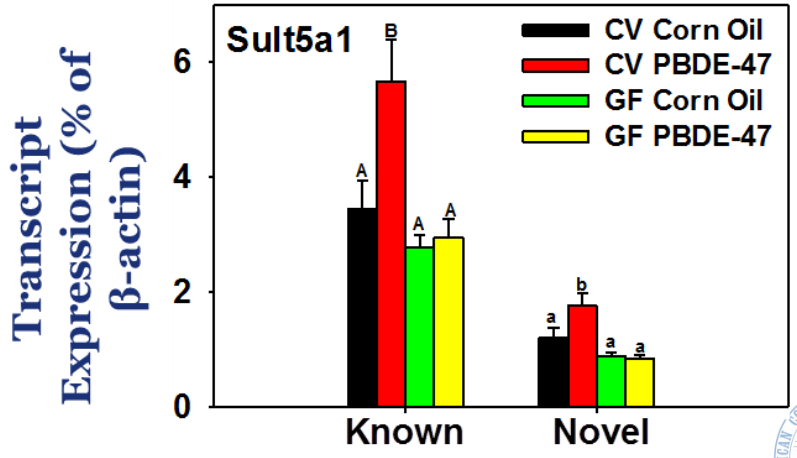


Sueyoshi et al., 2014, Toxicol Sci, 137: 292-302

Sulfotransferase 5a1: important for Phase-II Sulfonation conjugation reaction of xenobiotics



(Aleksunes and Klaassen, 2012)



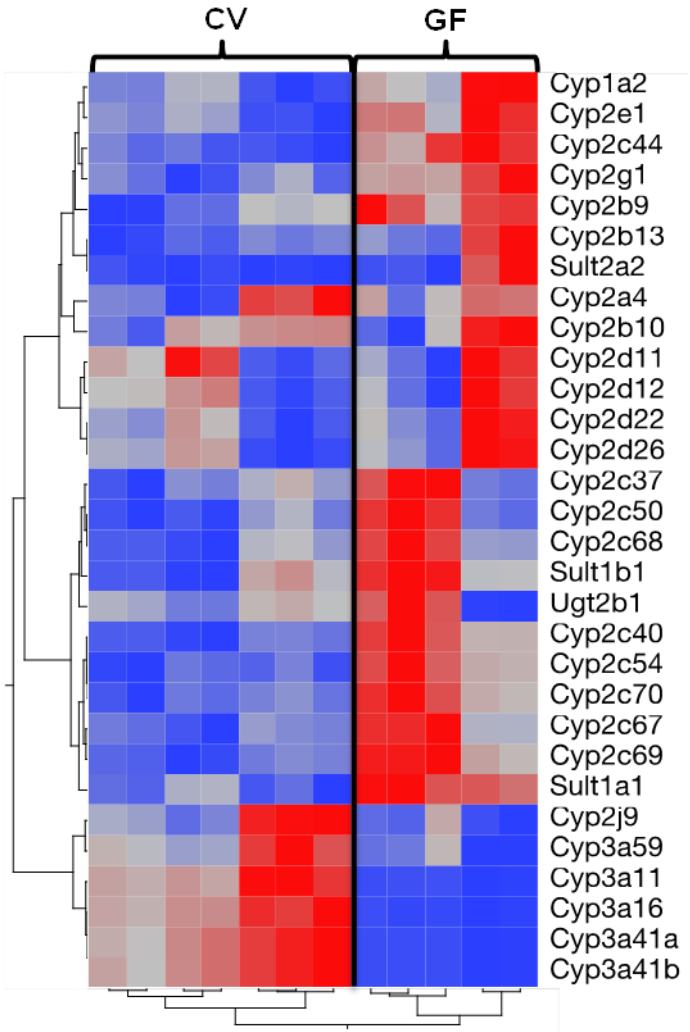
- Sult5a1 is up-regulated by the CAR ligand TCPOBOP in livers of wild-type mice
- The TCPOBOP-mediated up-regulation of Sult5a1 is absent in livers of CAR-null mice
- Sult5a1 is a bona fide CAR-target gene
- PBDE-47 mediated up-regulation of Sult5a1 depends on the presence of gut microbiome.

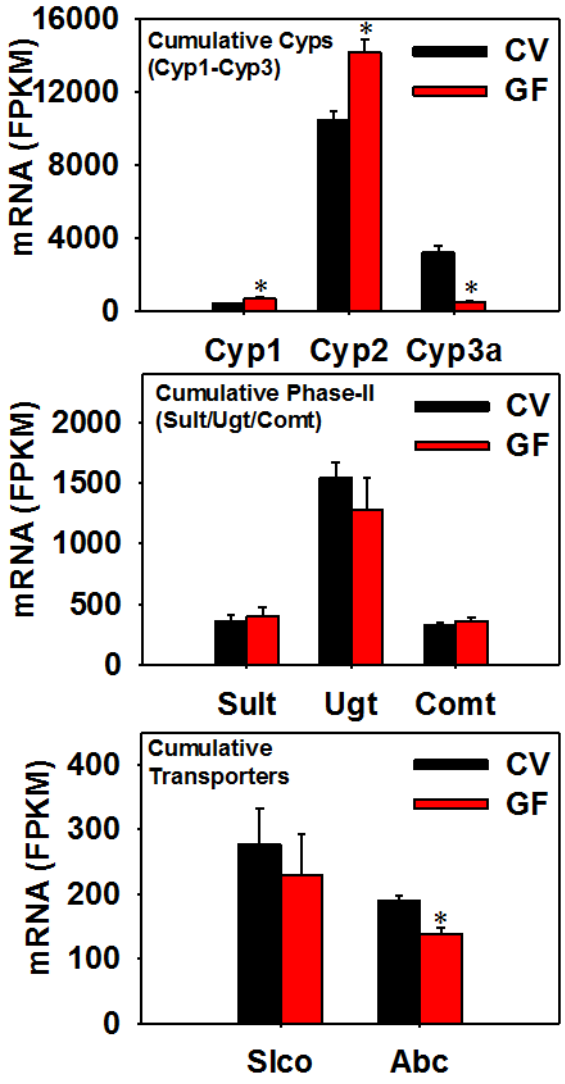
Davis Cowles and Julia Cui

Experimental Design

- CV and GF mice were treated with vehicle (corn oil), BDE-47, or BDE-99 via oral gavage once daily for 4-days
- GC-MS quantification of PBDE metabolites
- RNA-Seq of hepatic transcriptiome

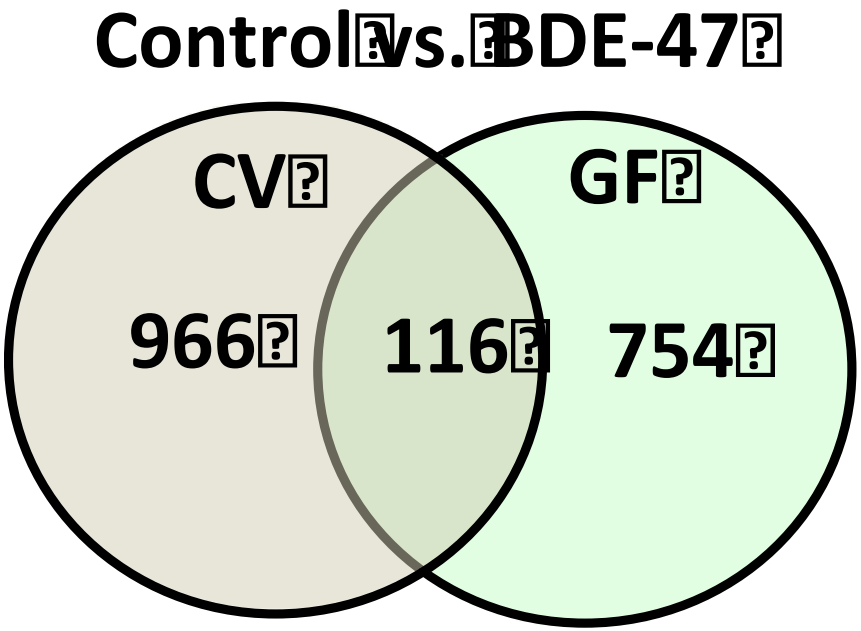
Hepatic Drug-processing Genes Related to PBDE Biotransformation

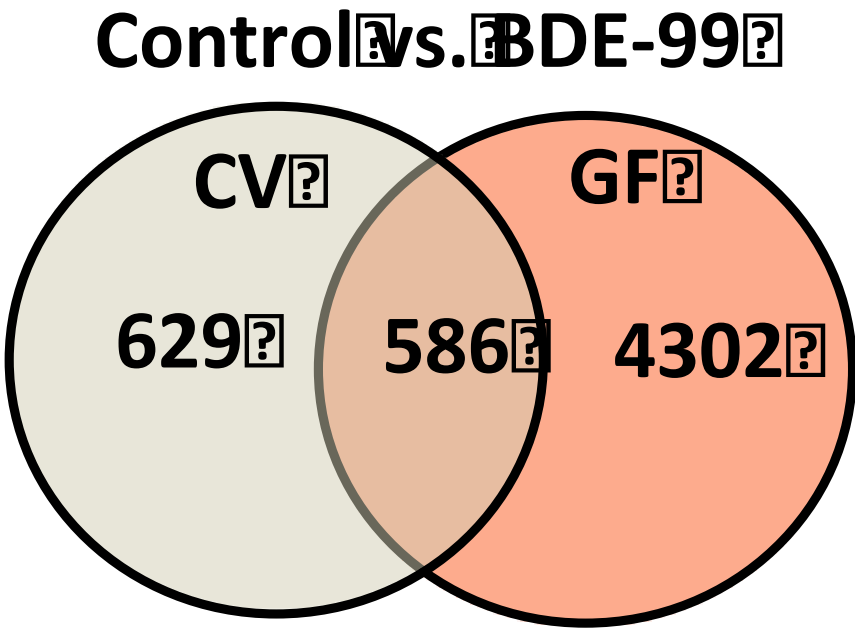




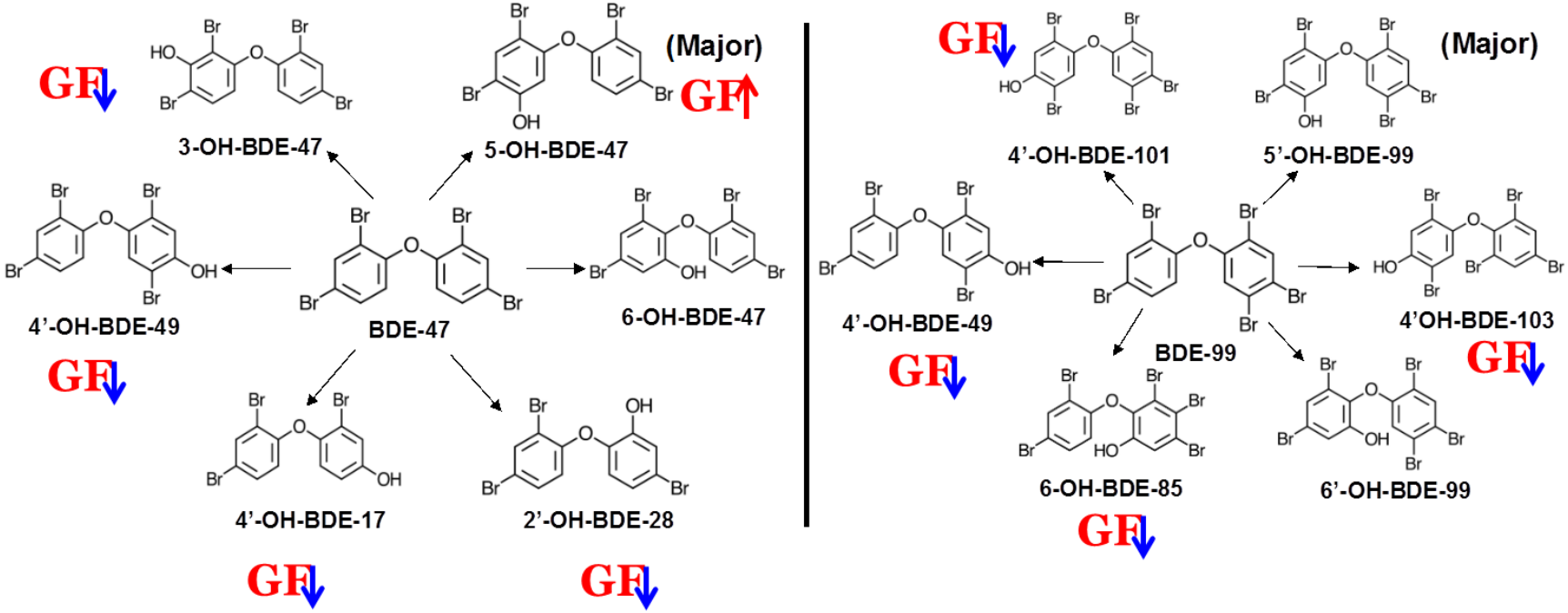
PBDE-mediated regulation of the hepatic transcriptome is profoundly modified by lack of gut microbiota

- Gut microbiota-dependent regulation
- Potentiation effect due to lack of gut microbiota





Summary of PBDE Oxidative Metabolism in Mouse Liver



Overall Conclusion

- Gut microbiota regulates the expression and activity of various drug-processing genes in liver
- PXR and PPARα appear to be the targets which the intestinal bacteria interact with
- The presence of gut microbiota alters the hepatic metabolism of PBDEs, and it is necessary in the PBDE-mediated regulation of many hepatic genes involved in drug metabolism and other critical endogenous pathways
- Future studies will determine which specific bacterial strains and their microbial metabolites interact with the host receptors in liver

Looking into the Future: Microbiome is a Key Player in Exposome

- Microbiome and Toxicology (mice as a research model)
- Microbiome and nutrition
- Microbiome and early life exposure/epigenetics
- Microbiome and “Big Data”
- Microbiome as biomarkers for risk assessment
- Probiotics as preventive therapy to reduce toxic exposure in vulnerable populations

Acknowledgements

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- Khakkhak Khayi, Undergraduate Student
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- Yubin Song, former Undergraduate Student
- Shinhee Park, former Undergraduate Student
- Elaine Chen, former Undergraduate Student
- Davis Houston Cowles, former EHREP Intern

Collaborators:

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- Dr. Irvin Schultz (PNNL)

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Aaron Ericsson, DVM, PhD

Dr. Ericsson received a DVM from the University of Missouri (MU) in 2006 and went on to complete a residency in Laboratory Animal Medicine in 2009, followed by a T32-funded PhD in Area Pathobiology, focusing on the role of the innate immune system in gastrointestinal inflammation and colitis-associated colorectal cancer (CAC).

In 2013, he assumed the role of lead scientist for microbiome research at both the NIH-funded MU Mutant Mouse Resource and Research Center (MMRRC) and Rat Resource and Research Center (RRRC), and received funding to found the MU Metagenomics Center in 2014.

He is primarily funded by a K01 to investigate the influence of certain microbes and polymicrobial communities on CAC, and also collaborates heavily with NIH- and USDA-funded investigators.

Julia Yue Cui, PhD

Julia received her B.S. Degree in Chukechen Honors College, Zhejiang University in Hangzhou, China, and received her Ph.D. Degree with honors in University of Kansas Medical Center. Julia currently is an Assistant Professor in Toxicology in the Department of Environmental and Occupational Health Sciences. She is a recipient of the Sheldon D. Murphy Endowed Chair, and a member of Center of Ecogenetics & Environmental Health. Julia is trained as a toxicologist, specializing in using toxicogenomic and toxicoepigenomic approaches to determine the effects of environmental chemical exposure and reprogramming the gut microbiome on the transcriptional and epigenetic regulation of genes involved in drug metabolism and obesity during development.

Thank you for your participation in the American College of Toxicology Webinar!

We hope to see you at the 37th Annual Meeting of the American College of Toxicology.