January 2019

Characterization Of Cytosolic Sulfotransferase Expression And Regulation In Human Liver And Intestine

Sarah Talal Dubaisi
Wayne State University, sarahdubaisi@gmail.com

CHARACTERIZATION OF CYTOSOLIC SULFOTRANSFERASE EXPRESSION AND REGULATION IN HUMAN LIVER AND INTESTINE

by

SARAH DUBAISI

DISSERTATION

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

2018

MAJOR: PHARMACOLOGY

Approved By:

_____

Advisor Date

_____

_____

_____

_____

DEDICATION

To Mom and Dad for their love, support, and guidance

To my husband for being by my side and motivating me during this journey

ACKNOWLEDGEMENTS

I would like to thank my mentors, Dr. Melissa Runge-Morris and Dr. Thomas Kocarek, for their tremendous support, guidance, and patience and for helping me develop or improve the skills that I will need to be an independent researcher. I also want to thank them for allowing me to explore new ideas and present my work at national conferences. I am very grateful to my committee members: Dr. Raymond Mattingly, Dr. Lawrence Lash, and Dr. Todd Leff for all their help and support throughout my graduate studies. I am also grateful to Drs. Kathleen Barrett, Hailin Fang, and Elizabeth Rondini for teaching me new lab techniques and for being great friends.

I want to thank Mom and Dad for motivating me to pursue my dreams and for believing in me. I am also grateful for my husband for encouraging me to do my best, helping me find solutions for all of the problems I faced during the past five years, and for being patient. Thank you for my sister and brother for making my life more cheerful and for being supportive.

I would also like to thank the staff and faculty from the Institute of Environmental Health Sciences and Department of Pharmacology and I am also thankful for my friends Ruta and Alex and my collogues at IBio.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AhR, aryl hydrocarbon receptor

ANOVA, analysis of variance

AUC, area under the curve

CAR, constitutive androstane receptor

CDCA, chenodeoxycholate

ChIP, chromatin immunoprecipitation

C_t, cycle threshold

CYP, cytochrome P450

DHEA, dehydroepiandrosterone

DMSO, dimethyl sulfoxide

ER, estrogen receptor

FBS, fetal bovine serum

FMO, flavin monooxygenase

FXR, farnesoid X receptor

GST, glutathione S-transferase

GW3965, 3-[3-[[[2-chloro-3-(trifluoromethyl)phenyl]methyl](2,2-diphenylethyl)amino]propoxy]benzeneacetic acid hydrochloride

GW4064, 3-[2-[2-chloro-4-[[3-(2,6-dichlorophenyl)-5-(1-methylethyl)-4-isoxazolyl]methoxy]phenyl]ethenyl]benzoic acid

GW7647, 2-[[4-[2-[[(cyclohexylamino)carbonyl](4-cyclohexylbutyl)amino]ethyl]phenyl]thio]-2-methylpropanoic acid

HNF4α, hepatocyte nuclear factor 4α

LCA, lithocholic acid

LXR, liver X receptor

MRM, multiple reaction monitoring

PAPS, 3'-phosphoadenosine-5'-phosphosulfate

PPAR, peroxisome proliferator-activated receptor

PPRE, peroxisome proliferator response element

PXR, pregnane X receptor

RACE, rapid amplification of cDNA ends

RIN, RNA integrity number

RNA-seq, RNA sequencing

RQI, RNA quality indicator

RT-qPCR, reverse transcription-quantitative polymerase chain reaction

siRNA, small interfering RNA

SNP, single nucleotide polymorphisms

SULT, cytosolic sulfotransferase

T_3, 3,5,3'-triiodothyronine

T_4, 3,5,3′,5′-tetraiodo-l-thyronine

TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin

TPM, transcripts per million;

TV, transcript variant

UGT, UDP glucuronosyltransferase

VDR, vitamin D receptor

VitD3, 1α,25-dihydroxyvitamin D3

WME, Williams' Medium E

CHAPTER 1: INTRODUCTION

1.1 Drug metabolism in the liver and intestine

The liver and the intestines are among the major organs that determine the bioavailability of xenobiotics and maintain the homeostasis of many endogenous molecules that play important roles in cell signaling and metabolic pathways. A large portion of the hepatic and intestinal metabolic capacity is mediated by xenobiotic-metabolizing enzymes that are capable of detoxifying foreign substances by converting them into hydrophilic metabolites that are excreted through urine or bile, converting drugs into their pharmacologically active forms, bioactivating procarcinogens, and regulating physiological pathways. The process of drug metabolism/transport is generally divided into three phases: (1) phase I reactions that introduce a functional group into their substrates; (2) phase II reactions that conjugate endogenous molecules onto foreign and endogenous compounds; and (3) phase III reactions that facilitate the uptake of molecules into cells and efflux of metabolites through drug transporters.

Phase I reactions are mediated by several families of drug-metabolizing enzymes, including cytochrome P450s (CYPs). CYPs are a major superfamily of phase I drug-metabolizing enzymes and they are abundantly expressed in the endoplasmic reticulum of the liver and intestines. In addition to metabolizing a vast number of drugs and environmental chemicals, CYPs can metabolize a broad range of endogenous molecules, such as steroids (Wang et al., 1997; Lee et al., 2003) and fatty acids (Capdevila et al., 1981; Schwarz et al., 2004).

Phase II reactions are catalyzed by transferases that are classified into six major families of conjugation enzymes: (1) UDP glucuronosyltransferases (UGTs), a superfamily of enzymes that catalyze the transfer of a glucuronic acid molecule from UDP-glucuronic acid to their substrates; (2) N-acetyltransferases (NATs), a multi-gene family of enzymes that catalyze the transfer of an acetyl group from acetyl co-enzyme A to their substrates; (3) glutathione S-transferases (GSTs), a superfamily of enzymes responsible for the transfer of a glutathione molecule from the tripeptide glutathione to its target molecules; (4) thiopurine S-methyltransferases (TPMTs), an enzyme

responsible for the transfer of a methyl group from S-adenosylmethionine (SAM) to its target molecules; (5) catechol O-methyltransferases (COMTs), a class of enzymes that catalyze the transfer of a methyl group from SAM to its substrates; and (6) cytosolic sulfotransferases (SULTs), a superfamily of enzymes that catalyze the transfer of a sulfonate group ($-SO_3$) from 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to their substrates. Conjugation enzymes are widely expressed in human tissues, including liver and intestine, and many of these enzymes exhibit tissue-specific expression patterns (Jancova et al., 2010).

Phase III drug transporters play an essential role in drug absorption, distribution, and excretion. Drug transporters, including P-glycoprotein (Thiebaut et al., 1987), multidrug resistance proteins (Mayer et al., 1995; Konig et al., 1999; Fromm et al., 2000), and organic anion transporting polypeptide 2 (Lu et al., 1996; Abe et al., 1999), are expressed in the liver and intestine and they act as a barrier that regulates drug influx and efflux, thus providing a path for drugs to be eliminated.

The liver is thought to be the major contributor to the first pass metabolism because of its greater weight and CYP microsomal content, compared to the intestine (Lin et al., 1999; Doherty and Charman, 2002). The hepatocytes are the liver's major cell type and they are responsible for 90% of hepatic drug disposition. Expression of drug metabolism is heterogeneously distributed among hepatocytes. The compartmented gene expression results in phenotypic differences among the cells and the metabolic zonation of the liver. Most genes that are expressed in the liver have zone-specific expression patterns, which reflects their function in the liver. CYPs are primarily expressed in the centrilobular zone, whereas phase II enzymes, including UGTs, SULTs, and GSTs, are expressed in the periportal as well as centrilobular region, suggesting that these enzymes play an important role in endogenous metabolism (Gebhardt, 1992; Lindros, 1997).

The intestines which are the site of entry for orally ingested nutrients and xenobiotics, significantly contributes to the first pass metabolism of many of the exogenous molecules that enter the intestine before reaching the liver, thus preventing the uptake of many of these

molecules. A variety of enzymes involved in drug metabolism are expressed in the different segments of the intestine (Ilett et al., 1990; Labroo et al., 1997; Shen et al., 1997; Beyerle et al., 2015). The expression of phase I and II enzymes is mainly localized in the intestinal mucosal epithelial cells that are located in the villous tips of the upper small intestine, the duodenum and jejenum (Ilett et al., 1990). These enzymes are also expressed in the crypts and the lower small intestine (ileum) and colon, but at a lower concentration (de Waziers et al., 1990).

1.2 Characteristics of human SULT genes

SULTs are ubiquitously expressed in mammalian and other eukaryotic organisms (Coughtrie, 2002). They conjugate a sulfonate group to a wide variety of alcohol, phenol, amine, N-oxide, and N-hydroxyl substrates, including cholesterol, hormones, neurotransmitters, drugs, and environmental chemicals (Table 1.1). PAPS, which is the sulfonate donor, is synthesized from an inorganic sulfate and ATP in a two-step process that is catalyzed by the PAPS synthase enzymes, PAPSS1 and PAPSS2 (Klaassen and Boles, 1997).

The SULT superfamily contains 13 human genes that are classified into four families based on their amino acid sequence similarity; SULT1, SULT2, SULT4, and SULT6; and each family is further divided into subfamilies (Fig. 1.1A) (Blanchard et al., 2004). SULT1 and SULT2 are the best characterized families in terms of their substrates, tissue-specific expression, and regulation. The nomenclature and classification of the SULT genes is based on amino acid sequence similarity, whereby SULTs that share 45% and 60% amino acid sequence are classified into the same family and subfamily, respectively. However, clustering analysis of global sequence similarity, local sequence of the substrate-binding site, and catalytic activity profiles demonstrated that enzymes that are closely related in terms of amino acid sequence do not necessarily have similar substrate binding sites or substrate specificity, as shown in Fig. 1.1 (Tibbs et al., 2015). While SULTs within the same family have overlapping substrate specificities, they display markedly different preferences toward exogenous and endogenous compounds. For example, although SULT1A1 and SULT1A3 can sulfonate thyroid hormones, SULT1A1 sulfonation capacity

towards thyroid hormones was greater than that of SULT1A3 (Richard et al., 2001). SULTs can also have distinct substrate profiles (Table 1.1). Numerous single nucleotide polymorphisms (SNPs) were detected in the promoter and coding regions of the SULT genes (Iida et al., 2001; Nowell and Falany, 2006). This is exemplified by the SNPs found in the SULT1A1 gene that were associated with a lower enzymatic activity and increased risk of breast and colon cancer (Bamber et al., 2001; Ning et al., 2005; Shatalova et al., 2006). SNPs were also identified in the SULT2A1 gene and they have been implicated with a decrease in the levels of dehydroepiandrosterone (DHEA) and DHEA-sulfate that could be associated with an increased risk of prostate cancer knowing that the latter is a precursor for the synthesis of androgens and estrogens (Wilborn et al., 2006).

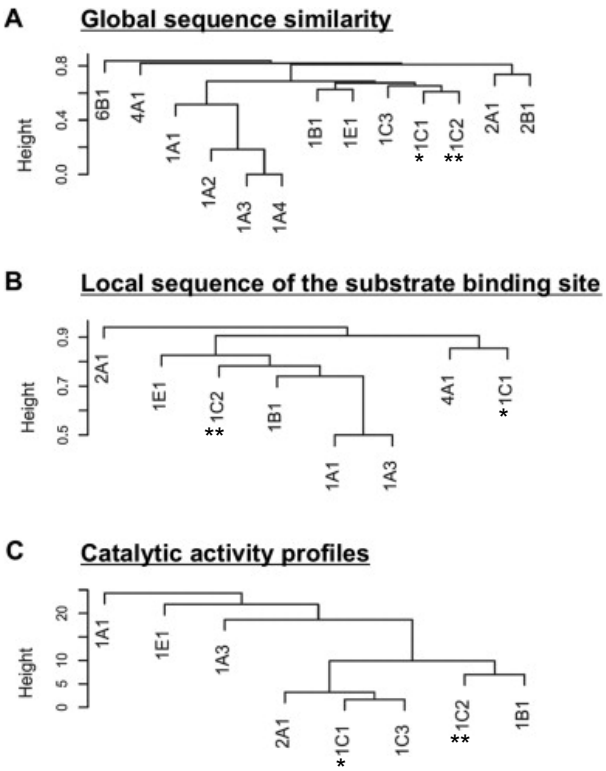


Figure 1.1: Relationship between human SULTs.

Clustering analysis of the human SULTs based on (A) global sequence similarity, (B) local sequence of the substrate binding site, and (C) catalytic activity profiles. * and ** referred to as SULT1C2 and SULT1C4 throughout the dissertation, respectively.
Figure taken with permission shown in Appendix A (Tibbs et al., 2015).

Table 1.1: Endogenous and exogenous substrates of human SULT enzymes

SULT gene	Substrates	
	Endogenous	**Xenobiotics**
SULT1A1	Iodothyronines (Kester et al., 1999), 4-methyl phenol (Allali-Hassani et al., 2007), estradiol, catecholestrogens (Adjei and Weinshilboum, 2002; Hui et al., 2008)	**Drugs, chemicals, and dietary compounds:** p-nitrophenol, m-nitrophenol, p-ethylphenol, p-cresol (Wilborn et al., 1993; Brix et al., 1999), oxymorphone, acetominophen, minoxidil (Tibbs et al., 2015), nalbuphine, nalorphine, naltrexone (Kurogi et al., 2014) **Procarcinogens:** 2,4-dinitrobenzylalcohol, 2-acetylamino-4 hydroxylaminotoluene, N-hydroxy-2- acetylamino-3-methyl-5-phenylpyridine, 2-hydroxylamino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (N-OH-PhIP), 2-nitropropane (Glatt, 2000), 2-hydroxymethylcholanthrene (Banoglu, 2000)
SULT1A2	Estradiol and catecholestrogens (Adjei and Weinshilboum, 2002; Hui et al., 2008)	**Drugs, chemicals, and dietary compounds:** p-nitrophenol (Zhu et al., 1996), naloxone (Kurogi et al., 2012a), minoxidil, β-naphthol (Sundaram et al., 1989) **Procarcinogens:** N-hydroxy-2-acetylaminofluorene (Glatt, 2000)
SULT1A3	Dopamine, norepinephrine (Dajani et al., 1999), tyramine (Brix et al., 1999), Iodothyronines (Kester et al., 1999)	**Drugs, chemicals, and dietary compounds:** Curcumin, demethoxycurcumin (Lu et al., 2015) troglitazone (Honma et al., 2002), morphine, hydromorphone (Kurogi et al., 2014), O-desmethyltramadol (Rasool et al., 2017) **Procarcinogens:** Oxamniquine (Glatt, 2000)

SULT1B1	Iodothyronines (Fujita et al., 1999)	**Drugs, chemicals, and dietary compounds:** Curcumin (Lu et al., 2015), 3-hydroxybenzo[a]pyrene (Wang et al., 2004), 1-naphthol (Wang et al., 1998) **Procarcinogens:** 6-hydroxymethylbenzo[a]-pyrene, 4-hydroxycyclopenta[def]chrysene (Glatt, 2000)
SULT1C2	Iodothyronines (Li et al., 2000), Epinephrine, norepinephrine, 2-hydroxyestradiol, estrone (Allali-Hassani et al., 2007)	**Drugs, chemicals, and dietary compounds:** p-nitrophenol, 4-nitrophenol, 1-naphthol, 2-naphthol, 4-ethylphenol, 2-n-propylphenol, 2-sec-butylphenol, vanillin, resveratrol (Allali-Hassani et al., 2007) **Procarcinogens**: N-hydroxy-2-acetylaminofluorene (Sakakibara et al., 1998)
SULT1C3	Lithocholic acid, α-Zearalenol (Allali-Hassani et al., 2007)	**Drugs, chemicals, and dietary compounds:** 1-naphthol, 4-nitrophenol, 2-ethylphenol, 2-n-propylphenol, 2-sec-butylphenol, a-zearalenol, vanillin (Allali-Hassani et al., 2007), 1,2,3,4 tetrahydro 1-naphthol, tolvaptan (Fang JL, 2016) **Procarcinogens:** 1-hydroxymethylpyrene, (+)-1-(α-hydroxyethyl)pyrene, (-)-1-(α-hydroxyethyl)pyrene, 6-hydroxymethylbenzo[a]pyrene, 6-hydroxymethylanthanthrene, 10-hydroxysafrole (Meinl et al., 2008a)

SULT1C4	Estradiol, estrogen, catechol estrogens (Hui et al., 2008), dopamine (Pai et al., 2002), α-zearalenol, T_3, p-Cresol, tyramine, cholesterol, epinephrine, norepinephrine (Allali-Hassani et al., 2007)	**Drugs, chemicals, and dietary compounds:** Genistein, daidzein, apigenin, chrysin, 6,4′-dihydroxyflavone, BPA (Guidry et al., 2017), 1-naphthol, 2-naphthol, 2-ethylphenol, 4-ethylphenol, 4-aminophenol, 2-n-propylphenol, 2-sec-butylphenol, 4-octylphenol, 4-n-nonylphenol, vanillin, acetominophen, resveratrol (Allali-Hassani et al., 2007), O-desmethyltramadol (Rasool et al., 2017), clioquinol , iodoquinol (Yamamoto et al., 2016), doxorubicin, epirubicin (Luo et al., 2016b), Tapentadol (Bairam et al., 2017), ethanol (Kurogi et al., 2012b), acetaminophen (Yamamoto et al., 2015) **Procarcinogens:** N-hydroxy-2-acetylaminofluorene, 2-hydroxylamino-3-methyl-9H-pyrido[2,3-b]indole, cigarette smoke extract components, 5-hydroxymethylfurfural, 2,5-(bishydroxymethyl)furan, furfuryl alcohol, 5-methylfurfuryl alcohol, (+)-10-Hydroxymethyleugenol, (-)-10-hydroxymethyleugenol, (E)-30-hydroxymethylisoeugenol (Runge-Morris and Kocarek, 2013)

SULT1E1	Estradiol, estrone, catecholestrogens (Adjei and Weinshilboum, 2002), Iodothyronines (Kester et al., 1999), dehydroepiandrosterone, pregnenolone, ethinylestradiol, and 1-naphthol (Falany et al., 1995)	**Drugs, chemicals, and dietary compounds:** troglitazone (Honma et al., 2002) **Procarcinogens:** (−)-1-(α-Hydroxyethyl)pyrene [(−)-1-HEP], (+)-1-(α-hydroxyethyl)pyrene[(+)-1-HEP], 1-acetylpyrene (Glatt, 2000), 7-OH-7,8,9,10-tetrahydrobenzo[a]pyrene, 10-OH-7,8,9,10-tetrahydrobenzo[a]pyrene, 1-hydroxymethylpyrene, S-(-)-1-hydroxyethylpyrene (Banoglu, 2000)
SULT2A1	DHEA, bile acids (Falany et al., 1989), Estrone, Estradiol, and catecholestrogens (Adjei and Weinshilboum, 2002; Hui et al., 2008)	**Drugs, chemicals, and dietary compounds:** Butorphanol , levorphanol (Kurogi et al., 2014), tibolone (Falany et al., 2004), budesonide (Meloche et al., 2002), quinolone drugs (Senggunprai et al., 2009) **Procarcinogens:** Hycanthone (Glatt, 2000), 6-hydroxymethylbenzo[a]-pyrene, (R)-(+)-1-hydroxyethylpyrene (Banoglu, 2000)
SULT2B1	DHEA (Meloche and Falany, 2001), 25-hydroxycholesterol (Bai et al., 2011), estradiol and catecholestrogens (Adjei and Weinshilboum, 2002; Hui et al., 2008)	None reported
SULT4A1	None reported	None reported
SULT6B1	None reported	None reported

Table 1.2: Tissue distribution of human SULT mRNA and protein

hSULT	mRNA	Protein
SULT1A1	Fetal: N/A Adult: Skin, kidney, liver, colon, ovary, brain (Dooley et al., 2000)	Fetal: Liver, lung, kidney, adrenal, small intestine, brain (Richard et al., 2001; Stanley et al., 2005) Adult: Stomach, small intestine, colon, liver, lung, kidney, placenta (Stanley et al., 2005; Teubner et al., 2007; Riches et al., 2009)
SULT1A2	Fetal: N/A Adult: Liver, ovary, lung, kidney, intestine (Dooley et al., 2000)	Fetal: N/A Adult: Liver (Teubner et al., 2007)
SULT1A3	Fetal: N/A Adult: Skin, oral mucosa, liver, lung, ovary, colon, brain, prostate (Dooley et al., 2000; Yalcin et al., 2013)	Fetal: Lung, liver, kidney, small intestine, brain (Richard et al., 2001; Stanley et al., 2005) Adult: Liver, lung, kidney, placenta, stomach, small intestine, colon (Richard et al., 2001; Stanley et al., 2005; Teubner et al., 2007; Riches et al., 2009)
SULT1B1	Fetal: N/A Adult: Liver, kidney, ovary, stomach, small intestine, colon, brain, blood leukocytes, spleen leukocytes (Wang et al., 1998; Dooley et al., 2000)	Fetal: Small intestine (Stanley et al., 2005) Adult: Stomach, small intestine, colon, liver, lung, kidney, blood leukocytes (Wang et al., 1998; Stanley et al., 2005; Teubner et al., 2007; Riches et al., 2009)
SULT1C2	Fetal: Kidney, liver (Her et al., 1997) Adult: Kidney, stomach, thyroid, duodenum, colon, rectum, liver, ovary, brain (Dooley et al., 2000; Bourgine et al., 2012; Hardwick et al., 2013)	Fetal: Kidney, liver, lung, small intestine (Her et al., 1997; Stanley et al., 2005) Adult: Stomach, kidney, thyroid (Her et al., 1997; Teubner et al., 2007)
SULT1C3	Fetal: N/A Adult: Small intestine, colon, rectum (Bourgine et al., 2012; Duniec-Dmuchowski et al., 2014)	Fetal: N/A Adult: N/A
SULT1C4	Fetal: Lung, kidney, and heart (Sakakibara et al., 1998)	Fetal: N/A

	Adult: Kidney, ovary, spinal cord, colon, and liver (Sakakibara et al., 1998; Bourgine et al., 2012; Hardwick et al., 2013)	Adult: N/A
SULT1E1	Fetal: Liver, thyroid, adrenal gland, lung, kidney, heart, intestine (Miki et al., 2002) Adult: Liver, kidney, brain, stomach, adrenal, lung, intestine, estrogen responsive tissues (Falany et al., 1998; Dooley et al., 2000; Miki et al., 2002)	Fetal: Liver, kidney, lung, thyroid, brain (Stanley et al., 2005; Duanmu et al., 2006) Adult: Liver, lung, colon, small intestine, breast, endometrium, prostate, testis (Falany et al., 1998; Miki et al., 2002; Stanley et al., 2005; Teubner et al., 2007; Riches et al., 2009)
SULT2A1	Fetal: Adrenal (Forbes et al., 1995) Adult: Liver, adrenal gland, small intestine (Dooley et al., 2000)	Fetal: Liver and adrenal (Barker et al., 1994; Stanley et al., 2005; Duanmu et al., 2006) Adult: Adrenal gland, small intestine, liver, lung (Tashiro et al., 2000; Teubner et al., 2007; Riches et al., 2009)
SULT2B1	Fetal: Brain (Falany and Rohn-Glowacki, 2013) Adult: Skin, oral mucosa, prostate, colorectal, placenta, lung, trachea, brain (Her et al., 1998; Meloche and Falany, 2001; He et al., 2005; Falany and Rohn-Glowacki, 2013)	Fetal: Brain (Falany and Rohn-Glowacki, 2013) Adult: Prostate, placenta, skin, lung, brain, breast, endometrium, platelets (He et al., 2005; Falany and Rohn-Glowacki, 2013)
SULT4A1	Adult: Brain (Liyou et al., 2003)	N/A
SULT6B1	Adult: Testis (Allali-Hassani et al., 2007)	N/A

N/A: stands for not available

1.2.1 SULT1 family

The SULT1 family includes 9 human SULTs, which are known to sulfonate phenolic compounds. Members of the SULT1 family have been shown to detoxify a myriad of xenobiotics (Table 1.1).

SULT1A

The SULT1A subfamily contains 4 genes, SULT1A1, SULT1A2, SULT1A3, and SULT1A4, which are clustered on chromosome 16 (Blanchard et al., 2004). SULT1A1 was the first member of the subfamily to be identified (Wilborn et al., 1993), and was shown to be one of the most abundant SULT proteins in the liver and the major SULT1A protein in the human body (Riches et al., 2009). There are five SULT1A1 transcript variants (TVs) that are indexed in the NCBI database (to be discussed in chapter 2) that encode two SULT1A1 isoforms: isoform a, which is made up of 295 amino acids, encoded by TVs 1 to 4; and isoform b, which is made up of 217 amino acids, encoded by TV5. SULT1A1 has a hydrophobic substrate-binding pocket that prefers to bind uncharged phenolic compounds, especially simple ones like p-nitrophenol. SULT1A1 is the only SULT1A that is expressed in species other than higher primates, including rodents. This gene has at least fifteen allelic variants that encode four allozymes, which were detected in the human population (Raftogianis et al., 1997; Raftogianis et al., 1999).

SULT1A2 was first cloned from a human liver library and was not detected in any other species (Ozawa et al., 1995). Although SULT1A2 shares more than 93% of its coding sequence with SULT1A1 and SULT1A3, its sulfonation activity towards some of the preferred SULT1A1 and SULT1A3 substrates is variable. SULT1A2 has thirteen allelic variants that encode six allozymes, three of which (allozymes 1 to 3) have different biochemical and physical properties (Raftogianis et al., 1999).

SULT1A3 and SULT1A4, which were generated by a gene duplication event on chromosome 16, are very closely related genes. SULT1A3/4 genes were only found in humans and other higher primates and they encode an identical protein that is made up of 295 amino acids despite some sequence variation at the DNA level. SULT1A3 is primarily expressed in the human intestine and

fetal, but not adult, liver (Stanley et al., 2005; Teubner et al., 2007; Riches et al., 2009). The SULT1A3 substrate-binding site contains acidic residues, and thus prefers positively charged molecules such as dopamine and other monoamines. SULT1A3 has at least eleven allelic variants that encode two allozymes (Thomae et al., 2003).

SULT1B

SULT1B1 was first isolated from a human liver library and was classified into the SULT1B family because of its similarity to rat sult1b1 (74% in the amino acid sequence). SULT1B1, which is located on chromosome 4, is the major SULT in the small intestine (Riches et al., 2009). Its coding sequence encodes a protein that is made up of 296 amino acids.

SULT1C

SULT1C subfamily consist of three members; SULT1C2, SULT1C3, and SULT1C4, as well as a pseudogene, SULT1C2P1; that are located in a cluster on chromosome 2q12 (Freimuth et al., 2004). In earlier studies SULT1C2 and SULT1C4 were referred to as SULT1C1 and SULT1C2, respectively (Blanchard et al., 2004). Although SULT1C mRNA was detected in adult tissues, the expression of these genes is higher in fetal tissues (Her et al., 1997; Sakakibara et al., 1998; Stanley et al., 2005).

SULT1C2 is the first human member of the SULT1C family to be cloned (Her et al., 1997). It has two splice variants that encode two full length proteins, SULT1C2a and SULT1C2b, that are made up of 296 and 307 amino acids, respectively. Although the two SULT1C2 isoforms are 91% identical, the differences, which are in the intermediate amino acid sequences, could have an impact on the proteins' functionality.

There is very little known about the expression, regulation, and substrate specificity of SULT1C3 enzyme, which was identified through computational analysis of the human genome and was predicted to have exon duplications that could theoretically produce four splice variants; SULT1C3a, b, c, and d (Freimuth et al., 2004; Allali-Hassani et al., 2007). Previous studies screening for the splice variant encoding the "SULT1C3d" isoform containing exons 7b/8b failed

to detect this transcript in human tissues (Freimuth et al., 2004; Meinl et al., 2008a). However, SULT1C3a containing exon 7a/8a was detected in human intestinal tissues and LS180 colorectal adenocarcinoma cells (Duniec-Dmuchowski et al., 2014; Rondini et al., 2014).

The full-length SULT1C4 cDNA was originally cloned and characterized from human fetal lung (Sakakibara et al., 1998; Freimuth et al., 2000). SULT1C4 has two transcript variants that are indexed in the GenBank database. The full-length transcript (TV1) encodes SULT1C4 isoform a that is made up of 302 amino acids, and TV2 encodes isoform b that is made up of 227 amino acids. The substrate-binding site of SULT1C4 is similar to that of SULT1A1 and SULT1B1, and thus these three SULTs share many of the same substrates (Dong et al., 2012).

SULT1E1

The SULT1E1 gene is located on chromosome 4 and is only expressed in mammals. Unlike other SULT1 members, SULT1E1 is primarily involved in sulfonation of endogenous compounds, especially estrogens (Kester et al., 1999; Adjei and Weinshilboum, 2002; Hui et al., 2008). The inactivation of estrogen by SULT1E1 reduces the mitogenic effect of estrogen in breast epithelial cells (Falany et al., 1995) and promotes adipocyte differentiation (Wada et al., 2011). SULT1E1 expression is induced in the homozygous cystic fibrosis transmembrane receptor (CFTR) knock-out mouse model as well as a human cholangiocyte–hepatocyte co-culture model, where the knockdown of the CFTR in the cholangiocytes induced SULT1E1 expression in hepatocytes (Falany et al., 2009). The only known SULT1E1 transcript encodes a protein that is made up of 294 amino acids.

1.2.2 SULT2 family

This SULT family includes two human genes, SULT2A1 and SULT2B1, that primarily sulfonate hydroxysteroids and are responsible for regulating cellular functions. Although these two genes are classified into two subfamilies, they are located in the same region of chromosome 19 suggesting that they are a result of a gene duplication.

SULT2A1

SULT2A1 was first identified and characterized in adult liver and it was found to be highly active towards dehydroepiandrosterone (Falany et al., 1989). It is expressed throughout development and is thought to be involved in steroid biosynthesis during fetal stages (Barker et al., 1994; Stanley et al., 2005; Duanmu et al., 2006). There is only one SULT2A1 transcript that encodes a 285 amino acid-long protein.

SULT2B1

Two SULT2B1 transcripts, SULT2B1a and SULT2B1b, that are derived from a single gene were identified and characterized using placental and prostate cDNA (Her et al., 1998). The two transcript variants, which are generated by alternate splicing of the first exon, encode two isoforms that are 350 and 365 amino acids long, respectively (Meloche and Falany, 2001). SULT2B1a and b mRNA is expressed in several human tissues with SULT2B1b mRNA being more abundant than that of SULT2B1a, but only SULT2B1b protein was detected in human tissues (Falany et al., 2006). SULT2B1b is involved in regulating various physiological processes in extrahepatic tissues, where it is predominantly expressed. For example, cholesterol sulfate, which is produced by SULT2B1b, promotes keratinocyte differentiation (Higashi et al., 2004).

1.2.3 Other SULT families

SULT4A1, the only member of the SULT4 family, is a brain-specific SULT and the most highly conserved SULT in vertebrates (Falany et al., 2000). Although SULT4A1 was cloned and characterized in rat, mouse, and human brain almost two decades ago, there are still no known substrates for this enzyme (Falany et al., 2000; Sakakibara et al., 2002). Several studies linked SULT4A1 polymorphisms and deletion to neurological disorders, such as schizophrenia (Brennan and Condra, 2005; Meltzer et al., 2008). SULT4A1 is thought to play a role in neuronal development because its expression is localized in the neurons and knocking out this gene in mice resulted in severe neurological symptoms, including tremor, rigidity, seizures, and death between postnatal days 21 – 25 (Garcia et al., 2018; Hashiguchi et al., 2018). The SULT6B1

gene, which is located on chromosome 2, was first identified by computational analysis, and expression profiling studies detected SULT6B1 mRNA in human testis (Freimuth et al., 2004).

1.3 Consequences of sulfonate conjugation in human liver and intestine

Many SULTs are abundantly expressed in the liver and small intestine, and to a lesser extent in the colon (Teubner et al., 2007; Riches et al., 2009). In these tissues, SULTs can detoxify or bioactivate their target substrates, modulate the activity of endogenous compounds, or regulate the biosynthesis of hormones. There is relatively little information about zonal expression of SULTs in human liver, but in rat liver a SULT that most likely corresponds to SULT1A1 (termed ASTIV) was highest in centrilobular hepatocytes, where most of the enzymes involved in drug biotransformation are expressed (Chen et al., 1995). However, STa (corresponding to a SULT2A protein) expression was highest in periportal hepatocytes (Chen et al., 1995), where a majority of the expressed genes are involved in cellular metabolism (Lindros, 1997), suggesting that this protein is primarily involved in the regulation of metabolic pathways. Although SULTs are considered to be an important line of defense that protects organisms from chemical exposures, these enzymes can contribute to initiation of carcinogenesis and other diseases by bioactivating promutagens (Banoglu, 2000).

1.3.1 Detoxification of exogenous molecules

Out of all thirteen SULTs, SULT1A1 and SULT1B1 are the two most active SULTs in hepatic metabolism of exogenous molecules because of their broad substrate specificities and high expression levels, together accounting for ~70% of SULT protein content (Coughtrie, 2016). Although SULT1A1 and SULT1B1 have overlapping substrates, the sulfonation capacity of the former is greater than the latter. Although SULT1C enzymes have not been studied extensively, the available information about the substrates of the three SULT1C enzymes indicates that SULT1C4 has the highest activity towards xenobiotics, and thus this enzyme plays an important

role in drug and environmental chemical metabolism (Yasuda et al., 2007; Hui et al., 2015; Yamamoto et al., 2015; Luo et al., 2016a; Luo et al., 2016b).

In the small intestine and colon, SULT1B1 and SULT1A3 are the most abundant SULTs (Teubner et al., 2007; Riches et al., 2009). SULT1A1 protein is also present at relatively high levels, but lower than that of SULT1A3 (Teubner et al., 2007). The expression of SULTs in the intestine was highest in the ileum, compared to the other intestinal segments, and was localized in the differentiated enterocytes implicating these enzymes in the elimination of bioactive food-borne ingredients (Teubner et al., 2007).

1.3.2 Regulation of physiological metabolism

SULTs regulate the activity and homeostasis of a wide range of small endogenous substrates, including cholesterol, bile acids, steroids, neurotransmitters, and thyroid hormone. Sulfonated molecules act as signaling molecules or reservoirs that can regulate biological processes in various tissues. For example, DHEA-sulfate that is synthesized in the brain is involved in brain development and functions (Baulieu, 1998; Maninger et al., 2009), whereas the sulfonation of DHEA in the adrenal gland plays an important role in steroid biosynthesis (Rainey et al., 2002). Contrary to SULTs, sulfatases are enzymes that hydrolyze the ester bonds to release the sulfonate group. These enzymes are required to maintain the homeostasis of endogenous molecules, which are substrates of the SULT enzymes. Since the focus of this dissertation is on the role of SULTs in liver and intestine, the following are a few examples of endogenous molecules that are metabolized by SULTs expressed in these tissues:

a. Sulfonation of thyroid hormones

Thyroid hormone is required for normal development and is a major regulator of metabolism in adults (Mullur et al., 2014). Sulfonation is an important pathway through which 3,5,3′,5′-tetraiodo-l-thyronine (T_4) is irreversibly inactivated by stimulation of inner ring deiodination (Visser, 1994). However, sulfonated 3,5,3'-triiodothyronine (T_3) is a reservoir for T_3 hormone that can be hydrolyzed by tissue sulfatases as needed (Visser,

1996). The T_4 metabolite 3,3′-diiodothyronine (3, 3′-T_2), is also extensively metabolized by SULT enzymes (Richard et al., 2001). SULT1A1, SULT1A3, SULT1B1, SULT1C2, and SULT1E1, which are expressed in the liver, have been implicated in the sulfonation of thyroid hormones, including T_3 and T_4 (Wang et al., 1998; Kester et al., 1999; Li et al., 2000).

b. Sulfonation of steroids and bile acids

Estrogens play essential roles in regulating cellular metabolism and growth but can also contribute to carcinogenesis in hormone-sensitive tissues, such as breast and uterus (Zhu and Conney, 1998; Mauvais-Jarvis et al., 2013). The activity of the estrogen hormone forms, including the two most biologically active forms, estrone (E1) and 17β-estradiol (E2), is mediated through the estrogen receptor (ER). The sulfonated estrogens cannot bind to the ER, and thus they are rendered inactive. Although estrogen sulfonation in human tissues can be catalyzed by several SULTs, SULT1E1 is the primary enzyme that can sulfonate estrogens at nanomolar concentrations (Falany et al., 1995). Other steroids, including DHEA, androgens, and cholesterol, are primarily metabolized by SULT2A1 and SULT2B1 enzymes that have high sulfonation capacity towards DHEA and cholesterol, respectively. Since SULT2B1 expression is low in the hepatic and intestinal tissues (Meloche and Falany, 2001), SULT2A1 is the major enzyme that can conjugate steroids and bile acids in these tissues, and thus facilitate their excretion through bile (Teubner et al., 2007; Riches et al., 2009).

1.3.3 Bioactivation of promutagens

The conjugation of a sulfonate provides a good leaving group that generates reactive intermediates that can interact with DNA, RNA, and protein, inducing cancer and other diseases (Banoglu, 2000). Watabe et al. was the first study that reported the bioactivation of a procarcinogen (i.e., 7-hydroxymethyl-12-methylbenz[a]anthracene) by sulfonation (Watabe et al.,

1982). SULT1 and SULT2 enzymes can bioactivate a wide range of promutagens that include drugs (e.g., tamoxifen), environmental contaminants (e.g., hydroxymethyl polycyclic aromatic hydrocarbons and N-hydroxy arylamines), and food-derived procarcinogens (Chou et al., 1995; Banoglu and King, 2002; Yasuda et al., 2007; Srivastava et al., 2010; Beyerle et al., 2015). For example, human SULT1A1 and SULT1A2 can bioactivate N-OH-PhIP, which is a very mutagenic compound that forms while cooking meat (Ozawa et al., 1994). Additionally, SULT1C enzymes are also capable of bioactivating various phenols, drugs, and procarcinogens, such as N-hydroxy-2- acetylaminofluorene (N-OH AAF) and large benzylic alcohols derived from alkylated polycyclic hydrocarbons (Her et al., 1998; Sakakibara et al., 1998; Meinl et al., 2008a).

1.4 The role of SULTs during liver development

The liver is the largest internal organ, and it performs vital metabolic, endocrine, and exocrine functions. The xenobiotic-metabolizing capacity of the human liver varies throughout development. There are xenobiotic-metabolizing enzymes that are expressed during early life and these likely influence the susceptibility of the developing human to the effects of drugs and environmental chemicals (Barker et al., 1994; Miki et al., 2002; Duanmu et al., 2006; Hines, 2007; Sadler et al., 2016) and regulate biological processes, such as steroid biosynthesis and estrogen and thyroid hormone homeostasis, which are essential during development.

1.4.1 Liver development

The process of liver development is evolutionarily conserved, and it begins during embryogenesis, when the endoderm and mesoderm layers emerge from a primitive streak. The endoderm is an uncommitted germ layer that gives rise to a primitive gut tube that is patterned into three domains: foregut, midgut, and hindgut. Hepatogenesis is initiated in the foregut domain, where the inhibition of the Wnt and fibroblast growth factor 4 (FGF4) pathways is required to establish its identity and progenitors. The hepatic mesenchyme, which originates from the mesoderm, secretes various transcription factors and signaling molecules that promote liver

development. The secretion of FGFs and bone morphogenetic proteins (BMPs) occurs from the heart and septum transversum mesenchyme (STM), respectively, and induces hepatic specification in the ventral foregut endoderm. The hepatic endoderm cells, known as hepatoblasts, are bipotent progenitor cells that differentiate into two cell types: (1) the parenchymal cells that are the major cell type (70-80%) in the liver, known as hepatocytes, and (2) the cells that are localized near the portal vein and are involved in the ductal plate remodeling, known as biliary epithelial cells (BECs) or cholangiocytes. The hepatoblasts migrate from the endoderm and invade the STM, thereby triggering liver bud formation. During liver bud growth, the hepatoblasts begin to differentiate into hepatocytes or BECs, and both cell types differentiate further during fetal development to reach maturity during the perinatal period. The other liver cell types, including stromal cells, stellate cells, and Kupffer cells are derived the from mesoderm (Zorn, 2008).

The expression of xenobiotic-metabolizing enzymes in the fetal liver is localized in the hematopoietic stem cells (HSCs) and embryonic hepatocytes. Several CYPs, including CYP1A1 and CYP3A5, are expressed at low levels in the HSCs whereas the expression of multiple GSTs, including GSTM1, M2, M4, and GSTP1, and SULT1A1 was reported to be much higher in the HSCs (Richard et al., 2001; Shao et al., 2007). However, the expression of many xenobiotic-metabolizing enzymes, such as CYP3A7, CYP1A2, and SULT2A1, seems to be restricted to the fetal hepatocytes (Barker et al., 1994; Shao et al., 2007).

1.4.2 SULT expression profiles during liver development

Experimental and epidemiological studies suggest that exposures to environmental stressors during prenatal periods can increase the risk for developing diseases, such as cancer and metabolic syndrome, later in life (Murray et al., 2007; Drake et al., 2010; La Merrill et al., 2013; Merlo et al., 2014; Shen et al., 2014). The impact of xenobiotic exposures on the developing fetus is modulated by the detoxification enzymes that are expressed in mother and fetus. Because many of the xenobiotic-metabolizing enzymes are differentially expressed during liver maturation,

the metabolic capacity of the fetal liver is different from that of the adult liver. Previous studies established that xenobiotic-metabolizing enzymes and transporters are differentially expressed throughout development, and three major patterns of hepatic expression have been described: (1) class I, where expression is highest in prenatal liver, (2) class II, where expression is relatively constant from prenatal to adult life, and (3) class III, where expression is highest in adult liver (Hines, 2013).

The presence of xenobiotic-metabolizing enzymes was detected starting from the first trimester. For example, CYP3A5 and CYP3A7 protein and activity as well as flavin monooxygenase 1 (FMO 1) protein were detected starting from week 8 of gestation (Yang et al., 1994; Lacroix et al., 1997; Koukouritaki et al., 2002; Stevens et al., 2003). However, the expression of many enzymes involved in xenobiotic metabolism is not well-developed in the prenatal period compared to the postnatal period (Hines, 2008).

Unlike other families of xenobiotic-metabolizing enzymes, SULTs are widely expressed in human tissues during development and, therefore, more likely to be responsible for modulating the effects of chemical exposures and regulating cellular processes in human fetuses. Previous studies detected SULT expression in the developing liver and demonstrated that some of these enzymes are preferentially expressed in the fetal stage (Barker et al., 1994; Richard et al., 2001; Stanley et al., 2005; Duanmu et al., 2006). Using a panel of 235 human liver cytosols prepared from donors ranging in age from early gestation to 18 years, our lab previously reported that SULT1E1, 1A1, and 2A1 proteins are expressed with class I, II, and III developmental patterns, respectively (Duanmu et al., 2006). Additional evidence indicated that SULT1A3, SULT1C2, and SULT1C4 are also preferentially expressed in fetal liver (Cappiello et al., 1991; Her et al., 1997; Sakakibara et al., 1998; Stanley et al., 2005). While SULT1B1 appears to be primarily expressed in adult tissues, SULT1B1 mRNA was detected in several fetal tissues, including small intestine and liver, and its protein was identified in fetal small intestine only (Stanley et al., 2005; Riches et al., 2009).

1.4.3 The beneficial and harmful effects of SULTs in the immature liver

Exposure to environmental stressors during fetal life can cause genomic and/or epigenomic alterations that lead to disease later in life. The expression of SULTs during vulnerable life stages, especially early developmental periods, positions these enzymes to be one of the major defense systems that protect the fetus from xenobiotic exposures by promoting their excretion. However, many molecules can interact with SULTs as substrates or inhibitors, altering the metabolic capability of the fetal liver, increasing the mutagenicity of the parent compounds, or perturbing homeostasis by interfering with the metabolism of endogenous molecules. For example, cigarette compounds, such as β-naphthylamine, catechol, and caffeic acid, which are sulfonated by SULT1A1, SULT1A2, SULT1A3, and SULT1C4, reduced the sulfonation capacity of SULT1A1 towards 17β estradiol, thereby disrupting estrogen homeostasis (Yasuda et al., 2007). Sulfonation of cigarette compounds was also shown to increase the mutagenicity of these compounds, which could contribute to the initiation of carcinogenesis (Banoglu, 2000; Wang and James, 2006). An example of a SULT inhibitor is 2,6-dichloro-4-nitrophenol, which can inhibit the activity of SULT1E1, affecting the state of estrogen and thyroid hormone equilibrium (Wang and James, 2006).

SULTs expressed in fetal liver can metabolize endogenous molecules that have critical developmental functions. DHEA sulfate, which is generated by SULT2A1 in the fetal liver and adrenal gland, circulates in the blood and is desulfonated by the sulfatases that are expressed in the placenta to be used as a precursor for estrogen biosynthesis, indicating that sulfonated DHEA plays an essential role in regulating estrogen biosynthesis during the prenatal stages (Barker et al., 1994). Another critical hormone that is regulated by SULTs during fetal development is thyroid hormone, which is sulfonated by hepatic SULT2A1 (Strott, 2002).

1.5 Regulation of SULTs by lipid- and xenobiotic-sensing transcription factors in hepatic and intestinal tissues

Nuclear receptors, which are a superfamily of transcription factors, are known regulators of genes that play essential roles in regulating cellular processes and xenobiotic metabolism (Mangelsdorf and Evans, 1995; Urquhart et al., 2007; Runge-Morris and Kocarek, 2009). These transcription factors can bind as homo- or heterodimers to specific consensus sequences, also known as response elements, and they are classified into four classes based on their mechanism of action (Fig. 1.2). Many nuclear receptors are activated by endogenous (e.g., oxysterols, bile acids, and estrogen) and exogenous compounds (e.g., drugs and environmental toxicants). Constitutive androstane receptor (CAR), farnesoid X receptor (FXR), liver X receptor (LXR), peroxisome proliferator-activated receptor alpha, delta, and gamma (PPARα, δ, and γ), pregnane X receptor (PXR), and vitamin D receptor (VDR) are lipid- and xenobiotic-sensing nuclear receptors that form heterodimers with retinoid X receptor (RXR). They were identified as regulators of many detoxification enzyme systems, including SULTs, in human and rodent tissues (Runge-Morris, 1997; Assem et al., 2004; Jiang et al., 2005; Alnouti and Klaassen, 2008; Sueyoshi et al., 2011; Runge-Morris et al., 2013). These nuclear receptors are expressed in various tissues, including liver and intestine, and they regulate expression of SULTs in a species-, tissue-, and gender- (in case of rodents) specific manner (examples will be provided throughout this section). The mechanisms underlying the regulation of several human SULTs in hepatic and intestinal cells by CAR, FXR, PPARα, PXR, and VDR were detailed by a number of studies using animal and human experimental models.

CAR was initially identified as a regulator of CYP2B expression in mouse and human liver (Honkakoski and Negishi, 1998; Honkakoski et al., 1998; Sueyoshi et al., 1999). Assem et al. demonstrated that CAR can co-regulate MRP4, an ABC transporter, and SULT2A1 in mouse liver and human hepatic HepG2 cells (Assem et al., 2004). CAR can regulate murine SULT2A1

transcription by binding to a FXR, LXR, and PXR response element, which is only found in the 5'-flanking region of the rodent SULT2A1 genes (Saini et al., 2004). More recent studies found that CAR activators induced the expression of several SULTs, including SULT1E1, in rodent, but not in human, liver (Ding et al., 2006; Alnouti and Klaassen, 2008; Radovic et al., 2010; Ghose et al., 2011; Sueyoshi et al., 2011; Aleksunes et al., 2012). In colon adenocarcinoma Caco-2 cells, activated CAR induced SULT2A1 mRNA (Echchgadda et al., 2007). Using chromatin immunoprecipitation (ChIP) and DNase I footprinting analyses, a composite cis-acting response element located at nt -131 to -155 and nt -167 to -190 relative to the SULT2A1 transcription start site that can bind to CAR and a proximal hepatocyte nuclear factor 4α (HNF4α)-binding site between nt -63 to -35 was identified (Echchgadda et al., 2007).

FXR is a lipid-sensing nuclear receptor that is activated by endogenous molecules, such as bile acids, and can regulate genes involved in maintaining cholesterol and bile acid homeostasis (Makishima et al., 1999; Sinal et al., 2000). FXR activation induced the expression of rat SULT2A1 by binding to a response element located in the 5'-flanking region of the gene, between nucleotides -169 and -193 (Song et al., 2001). However, chenodeoxycholic (CDCA)-mediated FXR activation suppressed SULT2A1 expression in mouse liver and HepG2 cells (Miyata et al., 2006). FXR was also recently reported to suppress SULT1E1 expression in HepG2 cells by inhibiting the binding of PPARγ coactivator 1α to HNF4α (Wang et al., 2017).

Like FXR, LXR is activated by lipid molecules, primarily sterols, and can regulate similar physiological functions (Kalaany and Mangelsdorf, 2006). Uppal et al. reported that LXR can induce SULT2A1 expression in mouse liver by binding to the same FXR and CAR response element described above, thereby preventing lithocholic acid (LCA) toxicity. LXR activation induced SULT2A1 expression in primary human hepatocytes and SULT1E1 expression in mouse liver and HepG2 cells (Gong et al., 2007; Uppal et al., 2007; Falany et al., 2009; Li et al., 2009). In colorectal adenocarcinoma LS180 cells, ligand-activated LXR upregulated the expression of SULT1C2 and 1C3 (Rondini et al., 2014).

The three PPARs, PPARα, PPARδ (also called PPARβ), and PPARγ, regulate essential cellular pathways, including energy metabolism, lipid metabolism, and inflammation (Seedorf and Aberle, 2007; Su et al., 2007; Pawlak et al., 2015). While PPARα has been established as a regulator of xenobiotic-metabolizing enzymes, little is known about the role of PPARδ and PPARγ in xenobiotic metabolism (Waxman, 1999; Runge-Morris and Kocarek, 2009; Runge-Morris et al., 2013; Thomas et al., 2013). Rat SULT1E1 protein was decreased in rats fed with PPARα activators (Fan et al., 2004). Injecting mice intraperitoneally with ciprofibrate (PPARα activator) suppressed SULT1E1, SULT2A, and SULT1C mRNA in female mice only, but these effects were not observed in female mice with nullified PPARα expression (Alnouti and Klaassen, 2008; Aleksunes et al., 2012). We previously reported that PPARα upregulates human, but not rat, SULT2A1 transcription through a peroxisome proliferator response element (PPRE) in the distal 5'-flanking region of the SULT2A1 gene (Fang et al., 2005). Our recent analysis indicated that activation of PPARα and PPARγ increased SULT1C3 mRNA in LS180 cells (Rondini et al., 2014).

PXR was initially identified as a xenobiotic-sensing transcription factor that plays an essential role in the regulation of xenobiotic detoxification enzymes, such as CYP3A4 (Lehmann et al., 1998). Later studies demonstrated that ligand-activated PXR can also control endogenous metabolic pathways in the liver (Dussault et al., 2003; Ihunnah et al., 2011). PXR is activated by secondary bile acids and sterols as well as a variety of exogenous compounds, such as rifampicin and hyperforin (Lehmann et al., 1998; Moore et al., 2000). Echchgadda et al. initially determined that PXR activated the transcription of Sult2a1 in mouse livers that were injected intraperitoneally with pregnenolone 16 α-carbonitrile (PCN), a rodent PXR activator (Echchgadda et al., 2004a). While feeding mice with LCA suppressed Sult2a1 expression in a PXR-independent manner, intraperitoneal administration of LCA, which bypasses the gut, induced Sult2a1 through the activation of PXR (Owen et al., 2010). In human hepatocytes, we identified two PXR-responsive elements in the SULT2A1 promoter region that are bound by HNFα in the absence of a PXR

activator (Fang et al., 2007). However, treatment of human primary hepatocytes with rifampicin suppressed SULT2A1 mRNA by interfering with the positive effect of HNF4α on SULT2A1 transcription (Fang et al., 2007). PXR was also established as a regulator of SULT1E1 transcription in primary human hepatocytes and the hepatocellular carcinoma cell line Huh7 (Kodama et al., 2011). This study revealed that HNF4α enhances the expression of SULT1E1 by binding to a distal enhancer sequence located between nt -1000 and -901 relative to the transcription start site of the gene and that ligand-activated PXR targets HNF4α to decrease its binding to the enhancer sequence, thereby repressing SULT1E1 expression (Kodama et al., 2011). In contrast, SULT2A1 expression was upregulated by PXR activation in Caco-2 cells, and this effect was mediated by a response element that can bind PXR and CAR (as mentioned earlier in this section), located in the SULT2A1 promoter region (Echchgadda et al., 2007). Treatment of intestinal LS180 cells with rifampicin induced SULT1C2 mRNA and protein and SULT1C3 mRNA (Rondini et al., 2014).

VDR is involved in the maintenance of calcium and phosphate homeostasis and it also plays a role in regulating key cellular pathways, including differentiation, proliferation, and inflammation (Lin and White, 2004). It is activated by the hormone form of vitamin D_3 (1α,25-dihydroxyvitamin D3), secondary bile acids (e.g., LCA), and dietary compounds such as curcumin (Makishima et al., 2002). The involvement of VDR in regulation of detoxification enzymes was initially established by multiple studies that identified VDR as a regulator of CYP2 and CYP3 expression (Schmiedlin-Ren et al., 2001; Thummel et al., 2001; Drocourt et al., 2002; Thompson et al., 2002). Ligand-activated VDR was also reported to induce the expression of mouse, rat, and human SULT2A1 in HepG2 and Caco-2 cells and mouse hepatocytes (Echchgadda et al., 2004b). VDR activation induced rodent Sult2a1 expression by binding to the same response element that is recognized by CAR, PXR, and FXR (Echchgadda et al., 2004b). In 2006, Song et al. located a composite response element in the 5'-flanking region of human SULT2A1 that consists of

VDR/RXR and CAAT/enhancer binding protein (C/EBP) binding sites that mediate the induction of SULT2A1 by VDR in Caco-2 cells (Song et al., 2006). Rondini et al. recently reported that SULT1C2 transcription is induced by VDR activation in LS180 cells (Rondini et al., 2014). Barrett et al. then demonstrated that the inducible effect of VDR on SULT1C2 mRNA is mediated through a cis-acting VDR response element found ~5 kb upstream of the transcription start site of the SULT1C2 gene that was identified by computational analysis and was predicted to be a PXR-binding site (Barrett et al., 2016).

Aryl hydrocarbon receptor (AhR) is a xenobiotic-sensing transcription factor that belongs to the Per Arnt Sim (PAS) domain family and is responsible for activating a battery of genes that control a broad range of functions, including xenobiotic detoxification as well as cellular proliferation, differentiation, and apoptosis (Marlowe and Puga, 2005). AhR is activated by exogenous compounds, most notably halogenated and aromatic compounds such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo[a]pyrene, and endogenous molecules, such as tryptophan metabolites and low-density lipoprotein (McMillan and Bradfield, 2007b; McMillan and Bradfield, 2007a). In the canonical pathway, AhR forms a complex with the aryl hydrocarbon receptor nuclear translocator (ARNT) and together they bind to consensus sequences located in the promoter region of their target genes, such as CYP1A1 (Reyes et al., 1992). AhR activation was reported to have a suppressive effect on SULT1A1 expression in rat and mouse primary hepatocytes, and suppressed SULT2A expression in rat hepatocytes only (Runge-Morris and Kocarek, 2005). TCDD treatment of HepG2 cells and female mice suppressed SULT1E1 mRNA (Puga et al., 2000).

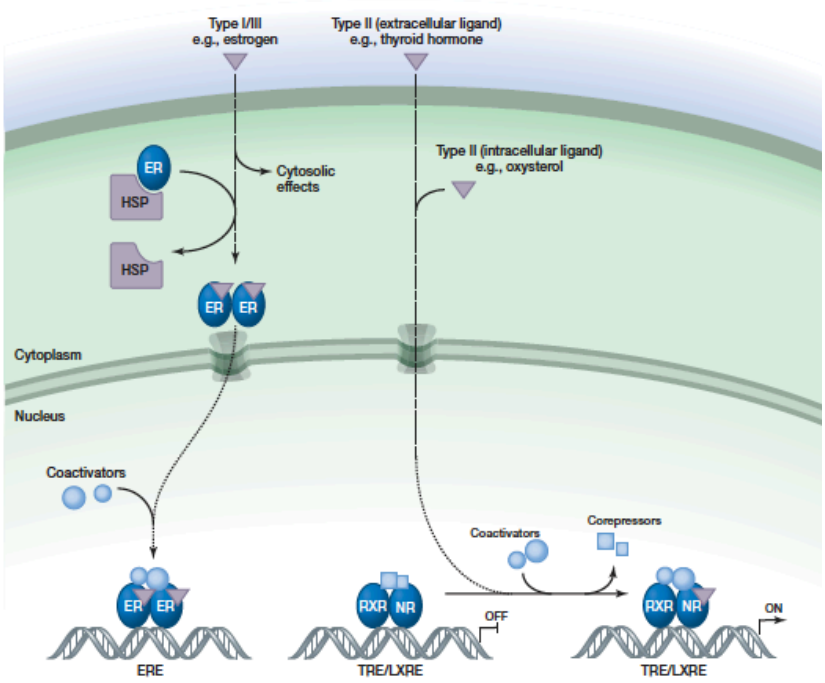


Figure 1.2: Mechanisms of action of nuclear receptors.
There are four types of nuclear receptors that are classified based on their mechanism of action. Type I nuclear receptors (e.g., estrogen receptor) reside in the cytoplasm and/or nucleus and are associated with chaperone proteins, including heat shock protein 90. The binding of a ligand (e.g., estrogen) triggers the translocation of the protein complex to the nucleus, where the chaperone proteins are released and the homodimer is formed. The homodimer will bind to a specific consensus sequence to induce or suppress the transcription of target genes. Type II receptors (e.g., thyroid hormone receptor; TR) are found in the nucleus bound to their DNA consensus sequences as heterodimers with RXR. While some unliganded type II receptors have no impact on the transcription of their target genes, several others (e.g., TR) can suppress transcription of target genes by interacting with corepressors (e.g., NCoR and SMRT). After the binding of a ligand, type II nuclear receptors induce the transcription of the target genes by interacting with coactivators that have intrinsic histone acetyltransferase activity, such as cyclic AMP response element binding protein binding protein (CBP) and p300. Type III and type IV receptors function relatively in the same way as type I receptors. However, type III receptors recognize a different type of response element (direct instead of inverted repeat) and type IV receptors (not shown in the figure) bind as monomers to half-site response element.
Figure taken with permission from (Sever and Glass, 2013).

1.6 Models of human liver development

Differences in the metabolic capacity of the liver between species have been well-documented, and these variations are mostly because of differences in the expression and sequences of xenobiotic-metabolizing enzymes, including SULTs (Honma et al., 2001; Shiratani et al., 2008; Choughule et al., 2015) . For example, mouse and rat SULT1B1 are very homologous, sharing 87.6% of their amino acid sequence, but human SULT1B1 shares only 72.3% and 74% with the mouse and rat proteins, respectively (Saeki et al., 1998; Wang et al., 1998). In terms of expression, rodent SULT1B1 is primarily expressed in the liver, whereas in humans its role appears to be more prominent in the intestine (Dunn and Klaassen, 1998; Saeki et al., 1998; Riches et al., 2009). SULT1Cs also exhibit variations in their sequences across species. While, SULT1C2 shares greater than 90% sequence similarity with its apparent mouse, rat, and rabbit orthologs human SULT1C3 and SULT1C4 share less than 80% amino acid sequence similarity with any of the known SULT1C proteins in the three animal species (Runge-Morris and Kocarek, 2013). Inter-species variation in SULT sequences are reflected in differences in the orthologs' substrate specificities. This is exemplified by SULT1A1, where human SULT1A1 and its presumed mouse, rat, and rabbit orthologs share 79-85% amino acid sequence similarity and a preference for sulfonation of phenolic substrates. However, human SULT1A1 has much higher activity than the animal enzymes toward troglitazone and 2-amino-4'-hydroxy-1-methyl-6-henylimidazo[4,5-b]pyridine (Honma et al., 2001). Therefore, the use of human *in vitro* models and biospecimens is necessary to understand the role of SULTs in the human liver.

In vitro models have been utilized to study chemical metabolism, mechanisms of toxicity, and enzyme kinetics (Iwatsubo et al., 1997; Soldatow et al., 2013). These models were also used to study physiological functions (e.g., proliferation and differentiation of liver cells) and pathophysiological conditions (e.g., non-alcoholic fatty liver disease) of the liver (Hino et al., 1999; Rumin et al., 1999; Yalcin et al., 2013). In this dissertation we used hepatic HepaRG cells and

primary cultures of fetal hepatocytes to examine SULT expression and regulation in immature and differentiated liver cells.

HepaRG is a widely used human cell line that was derived from an Edmonson grade I differentiated hepatocellular carcinoma (Martins-Filho et al., 2017). These cells function essentially as bipotent hepatic progenitor cells that can be differentiated in culture into mature hepatocyte-like and cholangiocyte-like cells, and they express many of the xenobiotic-metabolizing enzymes, transporters, and nuclear receptors that are expressed in normal human hepatocytes (Aninat et al., 2006; Hoekstra et al., 2013; Laurent et al., 2013). Differentiated HepaRG cells exhibit characteristics and gene expression profiles similar to those of adult hepatocytes, whereas proliferating and confluent HepaRG cells behave more like fetal hepatocytes. For example, CYP3A7 and pyruvate kinase muscle isozyme, which are abundantly expressed in fetal hepatocytes, are more abundantly expressed in undifferentiated HepaRG cells while CYP3A4 and CYP2E1, which are preferentially expressed in adult hepatocytes, are more highly expressed in differentiated HepaRG cells (Tsuji et al., 2014; Bucher et al., 2016). These cells have been used to study hepatocellular differentiation, xenobiotic metabolism and toxicity, and development of liver diseases (Sharanek et al., 2015; Nunn et al., 2016; Rodrigues et al., 2016; Sayyed et al., 2016; Xia et al., 2016).

Cultured fetal hepatocytes are another useful model to examine physiological processes and xenobiotic metabolism during prenatal periods because they can maintain hepatocyte-like traits, such as morphology of the cells and gene expression patterns (Carpenter et al., 1996; Lazaro et al., 2003; Chinnici et al., 2015; Tobita et al., 2016). Several studies demonstrated that xenobiotic-metabolizing enzymes that are expressed in the fetal liver, including CYP3A7, CYP3A5, CYP3A4 and CYP2E1, were detected in human and rodent primary fetal hepatocytes (Kremers et al., 1981; Mathis et al., 1986; Carpenter et al., 1996; Chinnici et al., 2015).

1.7 Objective of dissertation, hypothesis, and specific aims

The overall objective of this dissertation is to improve our understanding of the physiological roles of the SULT1 and SULT2 family members in the developing liver as well as adult intestines. Previous findings published by our lab and other groups indicated that several of the SULTs are preferentially expressed in prenatal periods and that SULT expression is modulated by lipid- and xenobiotic-sensing pathways. Based on these reports, we hypothesize that: (1) SULT1 and SULT2 enzymes have markedly different developmental expression profiles and several of these enzymes are preferentially expressed in the early stages of human liver development and the undifferentiated HepaRG cells; (2) SULT1 and SULT2 expression is regulated by lipid- and xenobiotic-sensing transcription factors in confluent and differentiating HepaRG as well as primary cultures of fetal hepatocytes; (3) the expression of the SULT1C4 transcript variants varies throughout human liver development; and (4) SULT1C3 transcription is regulated by PPARγ in human LS180 intestinal cells, and its effect is mediated by a functional PPRE located in the 5'-flanking region of the SULT1C3 gene. These hypotheses will be tested by the following specific aims:

Specific aim 1: Determine the developmental expression patterns of SULT mRNA and protein in the developing liver.

Specific aim 2: Identify the SULT1C4 transcript variants that are expressed in developing liver.

Specific aim 3: Examine the temporal expression profile of SULT mRNA and protein in confluent and differentiated HepaRG cells and evaluate the role of lipid- and xenobiotic-sensing transcription factors in the regulation of SULT mRNA in these two stages.

Specific aim 4: Determine the mechanism underlying the transcriptional regulation of SULT1C3 by PPARγ.

CHAPTER 2: REGULATION OF CYTOSOLIC SULFOTRANSFERASES IN MODELS OF HUMAN HEPATOCYTE DEVELOPMENT

2.1 Introduction

Cytosolic sulfotransferases (SULTs) are expressed during early life and, therefore, metabolize endogenous and xenobiotic chemicals during development. Several human SULTs, including SULT1A1, SULT1A3, SULT1C2, SULT1E1, and SULT2A1, were found to be abundantly expressed in the early stages of development (Cappiello et al., 1991; Barker et al., 1994; Stanley et al., 2005; Duanmu et al., 2006; Ekstrom and Rane, 2015), but little is currently known about the regulation of individual SULTs in the developing human liver. Previous studies demonstrated that the expression of SULT1 and SULT2 enzymes is regulated by lipid- and xenobiotic-sensing transcription factors (discussed in chapter 1.4). In this study we (1) examined the expression of SULT1 and SULT2 enzymes in primary cultures of human fetal hepatocytes and the HepaRG model of liver cell differentiation, (2) investigated the role of AhR, CAR, FXR, LXR, PPARα, PPARγ, PXR, and VDR in the regulation of the SULT1 and SULT2 mRNA in human fetal hepatocytes as well as confluent and differentiating HepaRG cells, (3) studied the role of AhR signaling in regulating the expression of SULT1C2, SULT1C4, SULT1E1, SULT2A1, CYP3A4, and CYP3A7 during hepatocyte differentiation, (4) examined the mechanism underlying the regulation of SULT1C4 by LXR, PXR, and VDR.

2.2 Materials and Methods

Materials: Cell culture media and supplements (except insulin) and Lipofectamine 2000 were purchased from Invitrogen (Carlsbad, CA). Human recombinant insulin (Novolin R) was purchased from Novo Nordisk Pharmaceuticals, Inc. (Princeton, NJ). 3-[2-[2-Chloro-4-[[3-(2,6-dichlorophenyl)-5-(1-methylethyl)-4-isoxazolyl]methoxy]phenyl]ethenyl]benzoic acid (GW4064, purity $\geq$ 97%) and 2-[[4-[2-[[(cyclohexylamino)carbonyl](4 cyclohexylbutyl)amino]ethyl]phenyl] thio]-2-methylpropanoic acid (GW7647, purity $\geq$ 99%) were purchased from Tocris Biosciences (Minneapolis, MN). Chenodeoxycholate (CDCA, purity $\geq$ 97%), 6-(4-chlorophenyl)imidazo[2,1-

b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO, purity ≥ 98%), rosiglitazone (purity ≥ 98%), rifampicin (purity ≥ 97%), 1α,25-dihydroxyvitamin D_3 ($VitD_3$, purity ≥ 99%), 3-[3-[[[2-chloro-3-(trifluoromethyl)phenyl]methyl](2,2-diphenylethyl)amino]propoxy] benzeneacetic acid hydrochloride (GW3965, purity ≥ 98%), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO), and 2,3,7,8- tetrachlorodibenzo-*p*-dioxin (TCDD, purity ~98%) was purchased from Midwest Research Institute (Kansas City, MO). Targets of these drugs is listed in Appendix E. Oligonucleotides were purchased from Integrated DNA Technologies (IDT, Coralville, IA). Other materials were obtained from the sources indicated below.

HepaRG culture and treatments: HepaRG cells were obtained from Biopredic International under a Material Transfer Agreement with INSERM-Transfert (Paris, France). Cells were plated into 6-well plates at a density of 250,000 cells/well in growth medium consisting of Williams' Medium E (WME) supplemented with 10% fetal bovine serum (FBS), 5 μg/ml insulin, 0.1 μM triamcinolone acetonide, 100 U/ml penicillin, and 100 μg/ml streptomycin. Fourteen days after plating, the medium was changed to differentiation medium, consisting of growth medium with 2% DMSO, and the cells were incubated for 14 more days, with medium replenishment every 2-3 days. Differentiated HepaRG cells were then incubated in treatment medium, consisting of growth medium with 2% FBS but without DMSO, 72 hours prior to treatment. Confluent (10 days post-plating) and differentiated (after 72-hour incubation with treatment medium) HepaRG cells were treated with vehicle (0.1% DMSO or 0.1% ethanol) or a transcription factor activator for 48 hours at the concentrations indicated in the figure legends (treatments were renewed after 24 hours). The concentrations used for the various agonists were selected based on previous demonstrations that these concentrations produce optimal regulation of known target genes as well as some of the SULTs (Fang et al., 2007; Rondini et al., 2014; Barrett et al., 2016; Dubaisi et al., 2016).

AhR knockout (KO) HepaRG cells were purchased from Sigma-Aldrich. Around 250,000 AhR KO cells were plated in 6-well plates and maintained in the same media used with the wild-type (WT) HepaRG cells.

Primary human fetal hepatocyte culture and treatments: Experiments with human fetal hepatocytes were done in collaboration with Dr. Alejandro Soto-Gutierrez from the University of Pittsburgh. De-identified tissues were obtained from Magee Women's Hospital (Pittsburgh, PA) and the University of Washington Department of Pediatrics, Division of Genetic Medicine, Laboratory of Developmental Biology (Seattle, WA) after obtaining written informed consent by a protocol approved by the Human Research Review Committee of the University of Pittsburgh (Honest broker approval number HB015 and HB000836). Human fetal hepatocytes were isolated from fetal livers obtained after the termination of pregnancy performed at 12–22 weeks of gestation (Appendix D). Primary human fetal hepatocytes were isolated by digesting the tissue in Eagle's Minimum Essential Medium (Lonza, Walkersville, MD) containing 0.5 mg/ml of collagenase (Type XI, Sigma-Aldrich) on a laboratory shaker for 40 minutes. Viability was assessed by trypan blue exclusion and was routinely >85%. Hepatocytes were plated at a density of 130,000 cells/cm^2 on type I rat tail collagen-coated 12-well plates (Corning, Corning, NY). Cells were cultured overnight with Dulbecco's Modified Eagle Medium (Life Technologies, Carlsbad, CA) containing 100 U/ml penicillin, 100 µg/ml streptomycin, 0.1 µM insulin (Sigma-Aldrich), and 5% bovine serum albumin (Life Technologies). Hepatocytes were then treated with vehicle (0.1% DMSO or 0.1% ethanol) or a transcription factor activator for 48 hours as indicated in the legend to Fig. 2.3 (treatments were renewed after 24 hours).

Human intestinal organoids (HIOs): These organoids were generated from pluripotent stem cells as described previously (McCracken et al., 2011). Briefly, H9 embryonic stem cells (ESCs; Wicell International Stem Cell Bank, Wicell Research Institute) were grown in feeder free conditions on hESC-qualified, matrigel-coated nunclon delta surface 6-well plates and incubated at 5% CO_2 and 37°C. Cells were passaged onto new plates every 4–5 days using dispase (1mg/mL) and treated

with Activin A (R&D Systems) for 3 days to generate endoderm. To promote the patterning of the endoderm into CDX2+ that spontaneously form floating, 3-dimensional aggregates called spheroids, FGF4 (R&D Systems) and Chir99021 (STEMGENT), a Wnt agonist used in place of recombinant Wnt3a, were added to the medium. Spheroids were collected and plated into droplets of Matrigel (BD Biosciences/Corning), a laminin-rich basement membrane complex. Spheroids were cultured in media containing EGF (100ng/mL, R&D Systems), R-Spondin 2, and Noggin (100ng/mL, R&D Systems) for 1 week and then in media containing only EGF and R-Spondin 2 as they grew into HIOs.

RNA isolation and analysis: Total RNA was isolated from HepaRG cells, freshly isolated and cultured human fetal hepatocytes using the Purelink RNA Mini Kit (Life Technologies). RNA was also isolated from the ESCs, definitive endoderms (DE), hindgut (HG; treated with FGF for 4 and 6 days), and HIOs using the same kit. RNA levels were quantified using the TaqMan Gene Expression Assays (listed in Appendix F) from Life Technologies. In each PCR reaction a 2 µl of diluted cDNA (1:2), a TaqMan probe with a FAM or VIC dye label on the 5' end and minor groove binder (MGB), and Universal PCR Master Mix (Applied Biosystems, Foster City, CA). The reaction was performed using the StepOnePlus Real-Time PCR System (Applied Biosystems). Standard thermocycling parameters were 94°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Data were normalized to a reference gene (indicated in the legend of each figure) and to the mean ΔCT of the control (both are indicated in the figure legends) to calculate ΔCT and ΔΔCT, respectively, and then the $2^{-\Delta\Delta CT}$ method was used to quantify the relative changes in gene expression (Livak and Schmittgen, 2001).

Western blot analysis: HepaRG cells were plated into 6-well plates at a density of 250,000 cells per well and harvested after 5, 9, 14, 19, 26, or 30 days for preparation of whole cell lysates, as previously described (Rondini et al., 2014). Protein concentrations were determined using the BCA Protein Assay Kit (ThermoFisher Scientific, Waltham, MA). Proteins (20-30 µg) were resolved on 12.5% sodium dodecyl sulfate-polyacrylamide gels, transferred onto polyvinylidene

difluoride membranes, and incubated for one hour with blocking buffer [2.5% non-fat dry milk in Tris-buffered saline with 0.1% Tween 20 (Sigma-Aldrich)]. The membranes were then incubated overnight at 4°C with mouse monoclonal anti-SULT1C2 (clone OTI5A4; Origene, Rockville, MD) diluted 1:5,000, anti-SULT1E1 (clone E-12; Santa Cruz Biotechnology, Dallas, TX) diluted 1:2,000, or anti-SULT2A1 (clone OTI4D7; Origene) diluted 1:5,000. SULT1C2 and SULT2A1 antibodies detect only the human SULTs whereas SULT1E1 antibody can detect the human, mouse, and rat SULTs. Membranes were then incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (sc-2005; Santa Cruz Biotechnology) diluted 1:20,000 (for membranes probed with anti-SULT1E1 or anti-SULT2A1) or 1:25,000 (for membranes probed with anti-SULT1C2). Enhanced chemiluminescence and a FluorChem E detection system (ProteinSimple, San Jose, CA) were used to visualize the immunoreactive bands. The blots were then incubated in stripping buffer (60 mM Tris-HCl, 70 mM sodium dodecyl sulfate, and 100 mM β-mercaptoethanol) at 37°C to remove the antibodies and re-probed with β-actin antibody (clone AC15; Sigma-Aldrich) diluted 1:40,000 followed by horseradish peroxidase-conjugated goat anti-mouse IgG diluted 1:100,000. Band densities were quantified with ImageJ32 software (Schneider et al., 2012).

RACE analysis: This analysis was performed using the SMARTer RACE 5'/3' kit (Takara Bio USA Inc., Mountain View, CA) and RACE-ready cDNA that was prepared from RNA of Caco-2 and HepaRG cells. A SULT1C4-specific reverse primer was designed for the 5'-RACE analysis (Appendix G). The PCR reactions were run on a 0.8% agarose gel and the bands were recovered and ligated into the pGEM-T Easy plasmid (Promega Corporation, Madison, WI). Individual clones were sequenced at the Wayne State University Applied Genomics Technology Center.

Preparation of SULT1C4 reporter plasmids: Genomic DNA was isolated from MCF10A human breast epithelial cells using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Genomic DNA (100 ng), primer sets predicted to amplify ~2.2 Kb (nucleotides -1890:+350) and ~0.3 Kb

(nucleotides -290:+53) fragments containing the core promoter, and the HotStarTaq DNA polymerase from Qiagen were used to PCR amplify two fragments from the SULT1C4 5'-flanking region. These fragments were ligated into the XhoI and HindIII site of the promoterless pGL4.10[luc2] firefly luciferase reporter plasmid (Promega Corporation, Madison, WI). The reporter plasmid containing the 0.3 Kb fragment was used as the backbone to prepare a series of reporters (A through I) that each contain ~ 2 Kb (A, B, E, F, G, H, I) or ~ 1 Kb (C and D) fragments from the SULT1C4 5'-flanking region starting from nt -1630 up until nt -15,174 (a schematic representation of these fragments is shown in Fig 2.11A). The fragments were amplified by PCR from genomic DNA and inserted into the KpnI and XhoI sites upstream of SULT1C4 core-promoter fragment in the pGL4.10 vector using the In-Fusion HD Cloning Kit (Clonetech, Mountain View, CA). All primer sequences are listed in Appendix G. The sequences of all SULT1C4 clones were confirmed using the services of the Applied Genomics Technology Center at Wayne State University.

HepG2 culture: HepG2 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, and non-essential amino acid mix (all purchased from Life Technologies, Grand Island, NY). Cells were incubated in a humidified atmosphere of 95% air, 5% CO_2 at 37°C.

Transient transfection analysis and treatments: Approximately 100,000 HepaRG cells/ well were plated in 12-well plates and maintained in 1 ml of supplemented WME. At day 9 post-seeding cells were transfected with a complex containing 4µl Lipofectamine 2000, 1.6µg of a firefly luciferase reporter containing one of SULT1C4 fragments (shown in Fig. 2.11A), and 1ng pRL-CMV (Promega) per well diluted in 400µl Opti-MEM (Life Technologies). To transiently transfect HepG2 cells, around 250,000 cells were plated in 12-well plates and maintained in 1 ml of supplemented DMEM. 24-48 hours post-plating, cells were transfected with the same complex used when transfecting HepaRG cells. 1 ng of PXR-pSG5 (provided by Dr. Steven Kliewer, University of Texas Southwestern, Dallas, TX) or VDR-pcDNA3.1 (Barrett et al., 2016) expression

plasmids were cotransfected into HepG2 cells when evaluating the effect of PXR or VDR, respectively, on SULT1C4 transcription. Luciferase reporters containing LXR-responsive element (LXRE) , PXR-responsive regions (referred to as xenobiotic-responsive enhancer module, XREM; provided by Dr. Bryan Goodwin, GlaxoSmithKline, Research Triangle Park, NC), or VDR-responsive element (VDRE) that are located in the promoter region of SREBP1c (Barrett et al., 2013), CYP3A4 (Goodwin et al., 1999), or SULT1C2 gene (Barrett et al., 2016), respectively, were used as positive controls. 24 Hours after transfection of HepaRG and HepG2 cells, fresh supplemented medium was added containing either DMSO (0.1% final concentration), GW3965 (10 μM), rifampicin (10 μM), or $VitD_3$ (0.1 μM). Treatment medium was changed after 24 hours. Cells were lysed and collected after 48 hours of treatment, and firefly and Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) and a Glomax Luminometer (Promega). For each sample, the firefly luciferase value was normalized to the corresponding Renilla luciferase value.

Statistical analysis: All experiments, except where indicated in the figure legends, were repeated at least three times. Gene expression data are presented as means ± SEM (with three or more independent experiments) or range (with two independent experiments) relative to control. Statistical analyses were performed using Prism (version 6; GraphPad, La Jolla, CA). Data were analyzed using two-tailed unpaired t-tests or one- or two-way analysis of variance followed by Tukey's *post hoc* test, with $p<0.05$ considered significantly different.

2.3 Results

2.3.1 Expression and regulation of SULTs in primary cultures of human fetal hepatocytes. To study the regulation of SULTs in a model of human fetal liver, hepatocytes were isolated from five fetal livers, placed into primary culture, and then treated for 48 hours with a vehicle (0.1% DMSO or 0.1% ethanol) or panel of nuclear receptor activators. SULT mRNA levels were then measured, together with CYP3A7 and CYP3A4 for comparison, since these genes are well-known to be

predominantly expressed in fetal or adult liver, respectively. Because several SULT1A1 transcript variants (TVs) have been described (5 confirmed mRNAs, NCBI SULT1A1 UniGene Hs.567342), we used two different TaqMan Gene Expression Assays to measure TV1 separately, since this variant is described as being most abundant, and TV5 separately, since the transcription start site of this variant is distinct from that of TVs 1-4 (i.e., more than 10 Kb upstream). The mRNA levels measured in the DMSO-treated hepatocytes were considered as estimates of basal expression. As expected, CYP3A7 was highly expressed [as estimated by cycle threshold (C_t) values] in the cultured fetal hepatocytes (C_t= 23.6) while CYP3A4 expression was minimal (C_t= 32.7) (Fig. 2.1). Of the SULTs, SULT1C4 was most highly expressed (C_t= 25.9), followed by SULT1E1 (C_t= 26.4), while SULT1A1 TV1 (C_t= 28.2), SULT2A1 (C_t= 28.4), and SULT1C2 (C_t= 28.6) mRNA levels were somewhat lower but still readily detectable. SULT1A1 TV5 (C_t= 35.8), SULT1B1 (C_t= 30.7), SULT1C3 (C_t= 36.6), and SULT2B1 (C_t= 32.6) mRNA levels were low or barely detectable (Fig. 2.1). To evaluate the impact of placing freshly isolated human fetal hepatocytes into primary culture on SULT expression, the ratios of the mRNA levels in cultured relative to freshly isolated hepatocytes were calculated. Culturing the fetal hepatocytes reduced the expression of SULT1C2 (by 82%), SULT1E1 (92%), and SULT2A1 (94%) but increased the expression of SULT1A1-TV5 (3-fold), SULT1B1 (14-fold), and SULT2B1 (6-fold) (Fig. 2.2). CYP3A7 and CYP3A4 expression was also decreased by primary culture, by approximately 91% and 38%, respectively (Fig. 2.2).

Treatment of the human fetal hepatocyte cultures with a panel of nuclear receptor activators produced several effects that were reproducibly seen across the five preparations. Treatment with the LXR agonist GW3965 (10 μM) significantly increased the amount of SULT1A1 TV5 by an average of 7.2-fold (relative to DMSO-treated control) and treatment with the VDR agonist $VitD_3$ (0.1 μM) significantly increased SULT1C2 and SULT2B1 mRNA content by an average of 2.2-fold and 2.0-fold, respectively (relative to ethanol-treated control). $VitD_3$ treatment also increased CYP3A4 and CYP3A7 mRNA levels (by 2.7- and 2.9-fold, respectively). While not significant, treatment with the PPARα agonist GW7647 (10 μM) or PPARγ agonist rosiglitazone

(1 μM) increased the amount of SULT2A1 mRNA by 1.9-fold, while treatment with the FXR agonist GW4064 (10 μM) decreased SULT2A1, CYP3A4, and CYP3A7 mRNA levels (by 78 to 85%). None of the treatments produced clear changes in the levels of SULT1A1 TV1, SULT1B1, SULT1C4, or SULT1E1 mRNA (Fig 2.3).

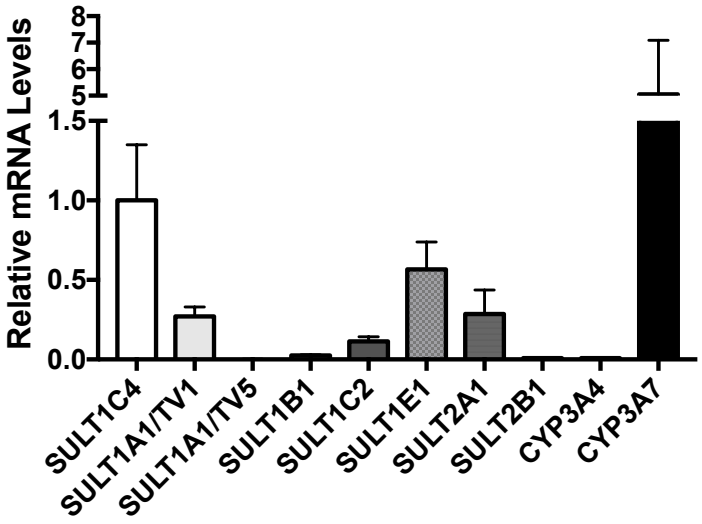


Figure 2.1: SULT, CYP3A4, and CYP3A7 expression in primary cultured human fetal hepatocytes.
Freshly isolated hepatocytes from five fetal livers were incubated in medium containing 0.1% DMSO for 48 hr, after which the cells were harvested and SULT, CYP3A4, CYP3A7, and GAPDH (used as normalization gene) mRNA levels were measured using TaqMan Gene Expression Assays. Each bar represents the mean relative mRNA level ± SEM for the five independent experiments compared to SULT1C4, which had the highest expression of the SULTs. Relative CYP3A4 and 3A7 mRNA levels are shown for comparison.
Figure taken with permission from (Dubaisi et al., 2018).

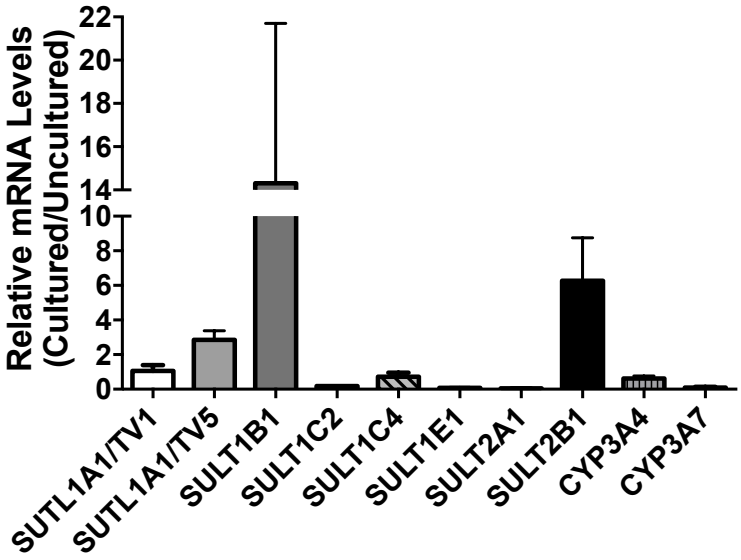


Figure 2.2: Effects of placing freshly isolated human fetal hepatocytes into primary culture on SULT, CYP3A4, and CYP3A7 expression.
mRNA levels from three preparations of freshly isolated fetal hepatocytes and the corresponding DMSO-treated primary cultured fetal hepatocytes were measured using TaqMan Gene Expression Assays. mRNA levels were normalized to 18S RNA and are expressed as ratios of the mRNA levels in cultured to uncultured hepatocytes.
Figure taken with permission from (Dubaisi et al., 2018).

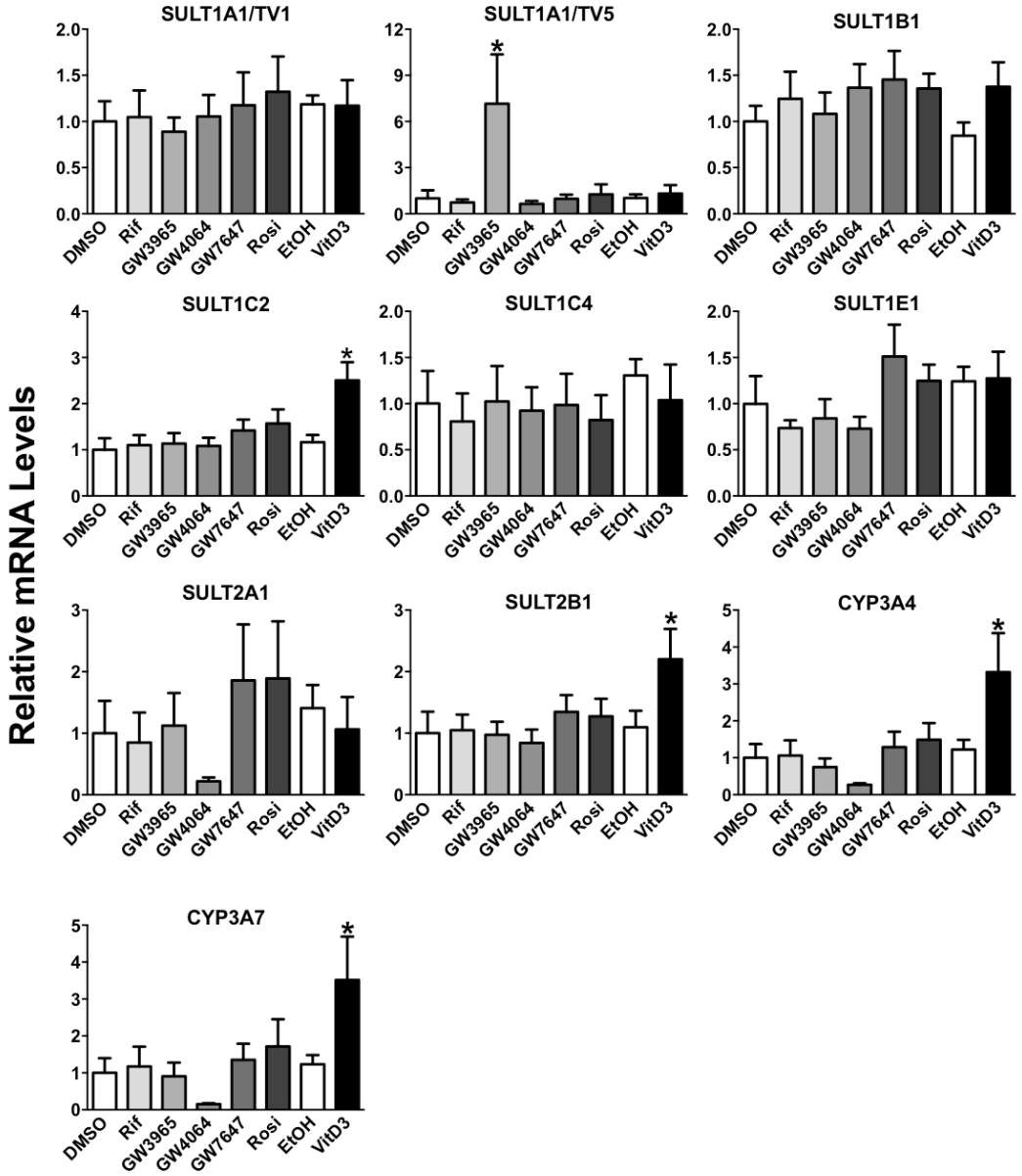


Figure 2.3: Effects of nuclear receptor agonists on SULT, CYP3A4, and CYP3A7 expression in primary cultured human fetal hepatocytes.
Freshly isolated hepatocytes from five fetal livers were incubated in medium containing 0.1% DMSO, 0.1% ethanol (EtOH), 10 μM rifampicin (Rif), 10 μM GW3965, 10 μM GW4064, 10 μM GW7647, 1 μM rosiglitazone (Rosi), or 0.1 μM $VitD_3$ for 48 hr, after which the cells were harvested and SULT, CYP3A4, CYP3A7, and GAPDH (used as normalization gene) mRNA levels were measured. Each bar represents the mean relative mRNA level ± SEM compared to control (0.1% ethanol for $VitD_3$; 0.1% DMSO for other agonists) for the five independent experiments. *Significantly different from control, $p< 0.05$.
Figure taken with permission from (Dubaisi et al., 2018).

2.3.2 Temporal expression of SULTs in HepaRG cells. The approximately one-month protocol for converting proliferating cultures of HepaRG cells into a mixed population of hepatocyte-like and cholangiocyte-like cells consists of growing the cells to confluency, maintaining them at confluency for several more days, and then incubating them in DMSO-containing medium (Fig. 2.4A). To characterize the temporal expression of the SULTs in HepaRG cells as they progressed through the differentiation process, cells were plated and then harvested every 2-3 days for mRNA measurements (Fig. 2.4B). Again, CYP3A4 and CYP3A7 mRNA levels were measured for comparison. CYP3A4 expression was low in the proliferating cultures, increased somewhat during the confluent phase, and then further increased during the differentiation phase (Fig. 2.4B). By comparison, CYP3A7 expression was highest during the confluent phase and then decreased during the differentiation phase (Fig. 2.4B). The SULT2A1 expression profile was comparable to that for CYP3A4, with highest expression occurring in the differentiated cells (Fig. 2.4B). Most of the other SULTs were expressed with patterns resembling those for CYP3A7 (i.e., SULT1B1, SULT1C2, SULT1C3, SULT1C4, and SULT1E1), where expression was highest in the confluent cultures and then reduced in the differentiated cultures (Fig. 2.4B). The expression of SULT1A1 transcripts and SULT2B1 did not vary

markedly throughout the differentiation process (Fig. 2.4B). SULT1A1 TV1 mRNA was abundant, while SULT1A1 TV5 and SULT2B1 mRNAs were low.

The protein levels for three of the SULTs (SULT1C2, SULT1E1, and SULT2A1) showing the two major expression patterns that were observed at the mRNA level were also measured at several time points. Consistent with the patterns observed at the mRNA level, SULT1C2 and SULT1E1 protein contents were highest in the confluent cells and then decreased after differentiation was induced by DMSO, while SULT2A1 protein reached its highest level in the differentiated HepaRG cells (Fig. 2.5).

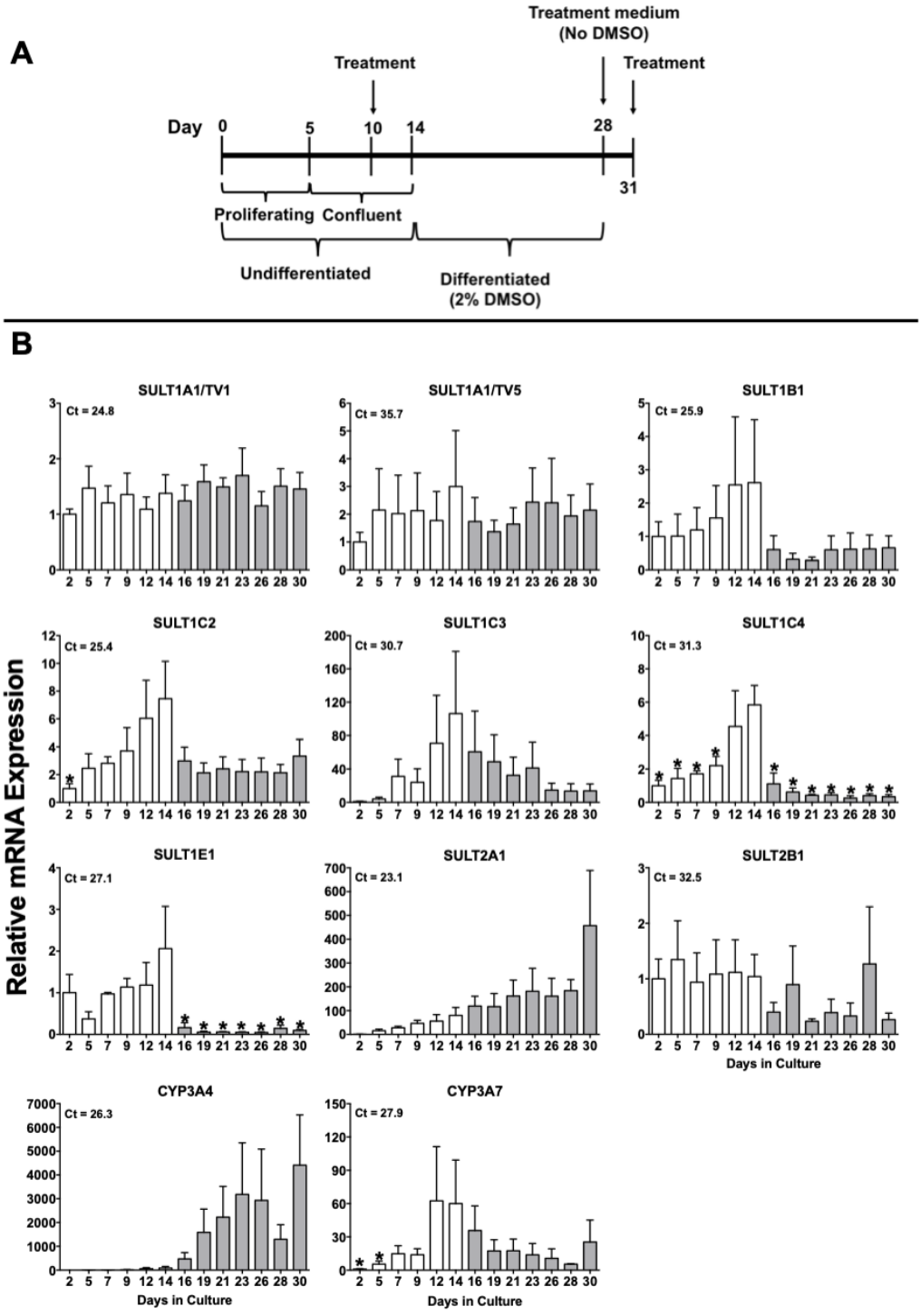


Figure 2.4: Temporal expression of SULTs, CYP3A4, and CYP3A7 in HepaRG cells. (A) HepaRG differentiation protocol showing proliferating, confluent, and differentiated phases and times when treatments were begun. (B) HepaRG cells were plated (day 0) and harvested on the indicated days for measurement of mRNA levels. mRNA levels were normalized to the

levels measured on day two (i.e., first harvest day). The data show the expression patterns of eight cytosolic SULTs as well as CYP3A4 and CYP3A7 from the proliferative to the confluent phase (open bars) and then through the differentiation phase (gray bars). The cycle threshold (Ct) values for the various genes measured on day 14 (i.e., time of highest expression for several of the genes) are shown on the graphs as estimations of their relative expression levels. Data were normalized to GAPDH and are shown as means ± SEM from three independent experiments. *Significantly different from day 14 mRNA level, p<0.05.
Figure taken with permission from (Dubaisi et al., 2018).

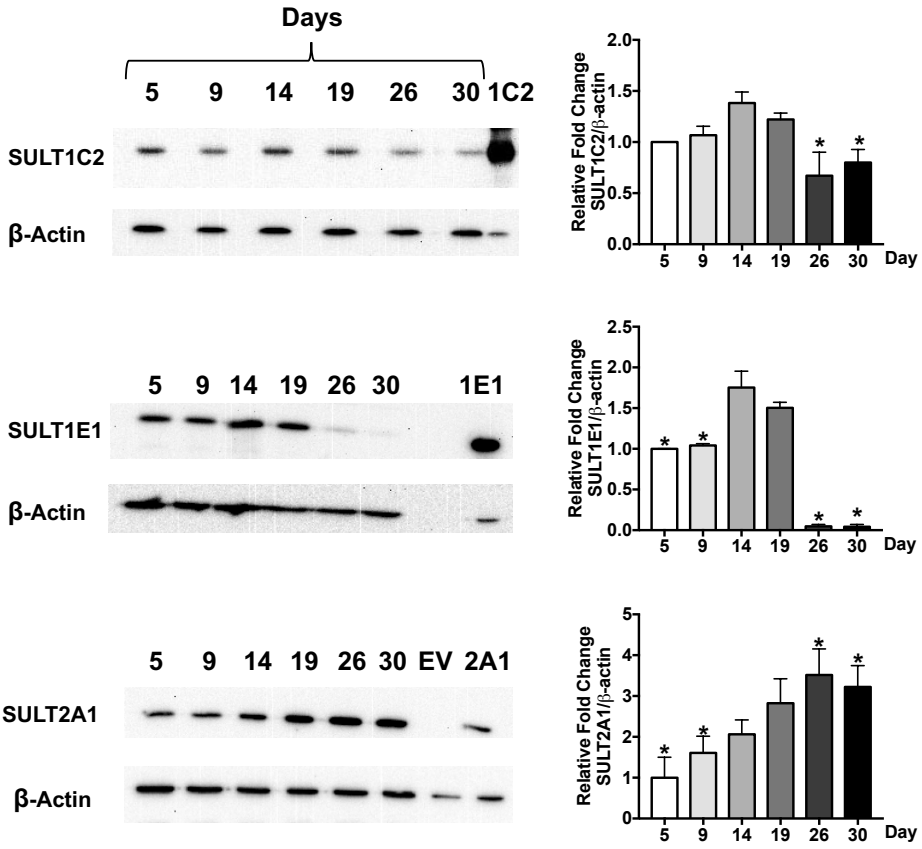


Figure 2.5: SULT1C2, SULT1E1, and SULT2A1 immunoreactive protein levels in HepaRG cells harvested at different time points.
HepaRG cells were plated (day 0) and harvested on the indicated days for measurement of SULT1C2, SULT1E1, and SULT2A1 protein levels by Western blot analysis. β-actin was used as the loading control. The images shown are from one representative experiment. For each protein, the last lane contains a standard consisting of whole cell lysate prepared from SULT cDNA-transfected HEK293 cells (EV, empty vector-transfected HEK293 cells). Band densities were quantified using image J, and data are shown normalized to the protein levels measured at day 5. Each bar represents the mean ± SEM from three independent experiments. *Significantly different from day 14 (P< 0.05).
Figure taken with permission from (Dubaisi et al., 2018).

2.3.3 Temporal expression of SULTs in a model of intestinal development. We examined the temporal expression of SULT mRNA at different time points during the process of differentiating ESCs into HIOs, which mimic fetal intestinal cells (Finkbeiner et al., 2015). Most SULTs, including SULT1B1, SULT1C2, SULT1C4, SULT1E1. SULT2A1, and SULT2B1 mRNA was readily detectable in the HIOs (Appendix H). In addition, SULT1C2, SULT1C4, and SULT2B1 expression was readily detectable in the ESCs and increased during the differentiation process (Appendix H).

2.3.4 Effects of lipid- and xenobiotic-sensing receptor activators on SULT expression in HepaRG cells. To identify nuclear signaling pathways that regulate SULT expression in the HepaRG model of human liver cell differentiation, cells were treated for 48 hours with activators of the AhR and several lipid- and xenobiotic-sensing nuclear receptors that have been reported to regulate SULT expression in other human cell systems (Song et al., 2001; Higashi et al., 2004; Fang et al., 2005; Jiang et al., 2005; Fang et al., 2007; Fu et al., 2011; Rondini et al., 2014; Barrett et al., 2016; Dubaisi et al., 2016). Both confluent and differentiated HepaRG cells were treated to consider the possibility that cells in the two stages could differ in their responses due to differences in their content of transcriptional machinery. However, measurement of marker transcripts for the various nuclear signaling pathways indicated that all treatments activated their targeted transcription factors by comparable amounts in confluent and differentiated cells (Figs. 2.6 and 2.7).

As shown in Fig. 2.8, treatment of confluent cells with the AhR agonist TCDD (0.01 μM) significantly decreased the mRNA levels of all SULTs that were measured, except for SULT1C3, and decreases of at least 50% were seen for SULT1A1 TV5 (69% decrease), SULT1B1 (51%), SULT1C4 (77%), SULT1E1 (86%), and SULT2A1 (86%). Treatment with the CAR agonist CITCO (1 μM) also significantly decreased the mRNA levels of several SULTs (SULT1B1, SULT1C3, SULT1C4, SULT1E1, and SULT2B1), although these decreases were generally modest and none exceeded 38%. Treatment with the PXR agonist rifampicin (10 μM) significantly increased SULT1C4 mRNA content (by 2.8-fold) and decreased the mRNA levels of SULT1B1 (63%

decrease), SULT1C3 (68%), SULT1E1 (63%), and SULT2A1 (62%). GW3965 treatment (LXR agonist, 10 μM) significantly increased the amount of SULT1A1 TV5 (by 1.7-fold) and SULT1C4 mRNA (1.9-fold) and decreased the mRNA levels of SULT1C3, SULT1E1, SULT2A1, and SULT2B1, with the reduction of SULT1C3 mRNA being especially pronounced (>90% decrease). GW4064 treatment (FXR agonist, 1μM) significantly decreased the expression of several SULTs (SULT1B1, SULT1C2, SULT1C3, SULT1C4, SULT1E1, SULT2A1), with the largest decreases seen for SULT1C3 (64%) and SULT2A1 (68%). Treatment with CDCA (50 μM), another FXR agonist, produced effects that were comparable to those of GW4064, with the largest effects being reduction of SULT1C3 (60% decrease) and SULT2A1 (55%) mRNA levels. GW7647 treatment (PPARα agonist, 10 μM) significantly increased SULT1B1 mRNA content, although the increase was only 1.2-fold, and decreased SULT1C2, SULT1C3, and SULT2B1 mRNA levels, with the largest reduction seen for SULT1C3 (81%). The PPARγ agonist rosiglitazone (10 μM) had little effect on SULT expression, other than to decrease SULT1C3 mRNA content (72% decrease). Treatment with $VitD_3$ (VDR agonist, 0.1 μM) significantly increased the amounts of SULT1C2, SULT1C4, and SULT2B1 mRNA by 1.4- to 1.8-fold and decreased SULT1A1 TV1, SULT1B1, SULT1C3, SULT1E1, and SULT2A1 mRNA levels, with the largest reduction seen for SULT1C3 (68% decrease).

Fig. 2.9 shows the effects of the treatments on SULT expression in differentiated HepaRG cells. Many of the effects were comparable to those seen in the confluent cultures, including: (1) TCDD treatment decreased the expression of most SULTs; (2) CITCO treatment had relatively little effect on SULT expression; (3) rifampicin treatment significantly increased SULT1C4 mRNA content (by 6.3-fold) and decreased SULT1B1, SULT1C3, and SULT2A1 expression; (4) GW3965 treatment significantly increased SULT1A1 TV5 and SULT1C4 mRNA levels and markedly reduced (by >90%) SULT1C3 mRNA content; levels of SULT2A1 and SULT2B1 mRNA were also decreased; (5) GW4064 and CDCA treatments significantly decreased the mRNA levels of several SULTs, including SULT1C2, SULT1C3, SULT1E1, and SULT2A1; (6) GW7647 treatment

modestly but significantly increased SULT1B1 mRNA content and decreased the amount of SULT1C3 mRNA; (7) rosiglitazone treatment decreased SULT1C3 expression; and (8) VitD3 treatment significantly increased SULT1C2 and SULT2B1 mRNA levels (SULT1C4 mRNA content was also ~2-fold higher on average, although this effect was not significant in the differentiated cells) and decreased SULT1A1 TV1, SULT1B1, SULT1C3, and SULT2A1 mRNA levels. Differences that were noted between the differentiated and confluent HepaRG cells were: (1) TCDD treatment significantly decreased SULT1C2 mRNA content only in confluent cells and increased SULT1C3 mRNA only in differentiated cells; (2) rifampicin treatment significantly decreased SULT1E1 expression only in confluent cells and induced SULT2B1 in differentiated cells; and (3) $VitD_3$ treatment significantly decreased SULT1E1 expression only in confluent cells.

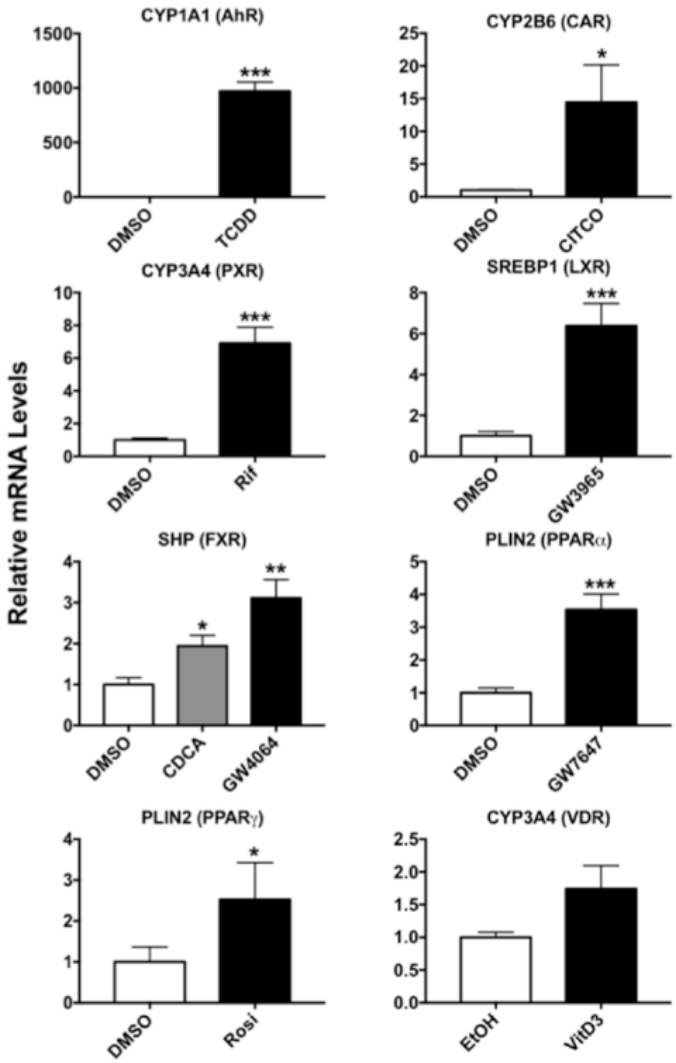


Figure 2.6: Effects of lipid- and xenobiotic-sensing receptor agonists on target gene expression in confluent HepaRG cells.
Ten days after plating, confluent HepaRG cells were incubated in treatment medium containing 0.1% DMSO, 0.1% ethanol (EtOH), 0.01 μM TCDD (AhR agonist), 1 μM CITCO (CAR), 10 μM rifampicin (Rif, PXR), 10 μM GW3965 (LXR), 1 μM GW4064 (FXR), 50 μM CDCA (FXR), 10 μM GW7647 (PPARα), 10 μM rosiglitazone (Rosi, PPARγ), or 0.1 μM VitD3 (VDR) for 48 hr, after which cells were harvested and the mRNA levels of a known target gene for each agonist and TATA-box binding protein (used as normalization gene) were measured. Each bar represents the mean relative mRNA level ± SEM compared to control (0.1% ethanol for VitD3; 0.1% DMSO for all other agonists) for three independent experiments. Significantly different from control, *p<0.05, **p<0.01, ***p<001. SREBP1, sterol regulatory element binding protein 1; SHP, short heterodimer partner; PLIN2, perilipin 2.
Figure taken with permission from (Dubaisi et al., 2018).

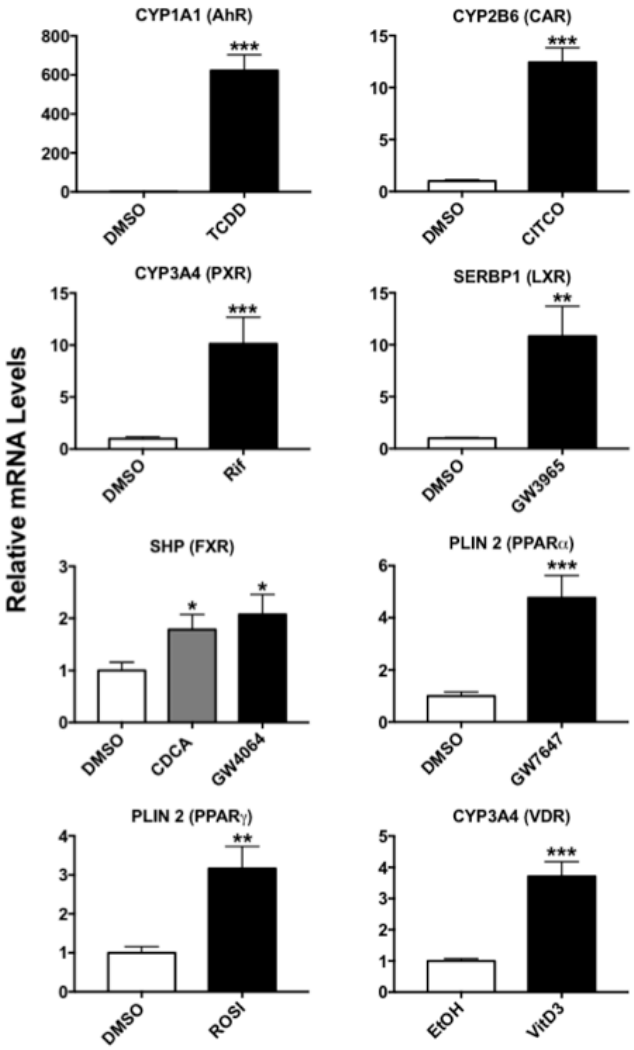


Figure 2.7: Effects of lipid- and xenobiotic-sensing receptor agonists on target gene expression in differentiated HepaRG cells.

Four weeks after plating, differentiated HepaRG cells were incubated with treatment medium alone for 72 hour and then treatment medium containing 0.1% DMSO, 0.1% ethanol (EtOH), 0.01 μM TCDD (AhR agonist), 1 μM CITCO (CAR), 10 μM rifampicin (Rif, PXR), 10 μM GW3965 (LXR), 1 μM GW4064 (FXR), 50 μM CDCA (FXR), 10 μM GW7647 (PPARα), 10 μM rosiglitazone (Rosi, PPARγ), or 0.1 μM VitD3 (VDR) for 48 hr, after which cells were harvested and the mRNA levels of a known target gene for each agonist and TATA-box binding protein (used as normalization gene) were measured. Each bar represents the mean relative mRNA level ± SEM compared to control (0.1% ethanol for VitD3; 0.1% DMSO for all other agonists) for four independent experiments. Significantly different from control, *p<0.05, **p<0.01, ***p<001. SREBP1, sterol regulatory element binding protein 1; SHP, short heterodimer partner; PLIN2, perilipin.

Figure taken with permission from (Dubaisi et al., 2018).

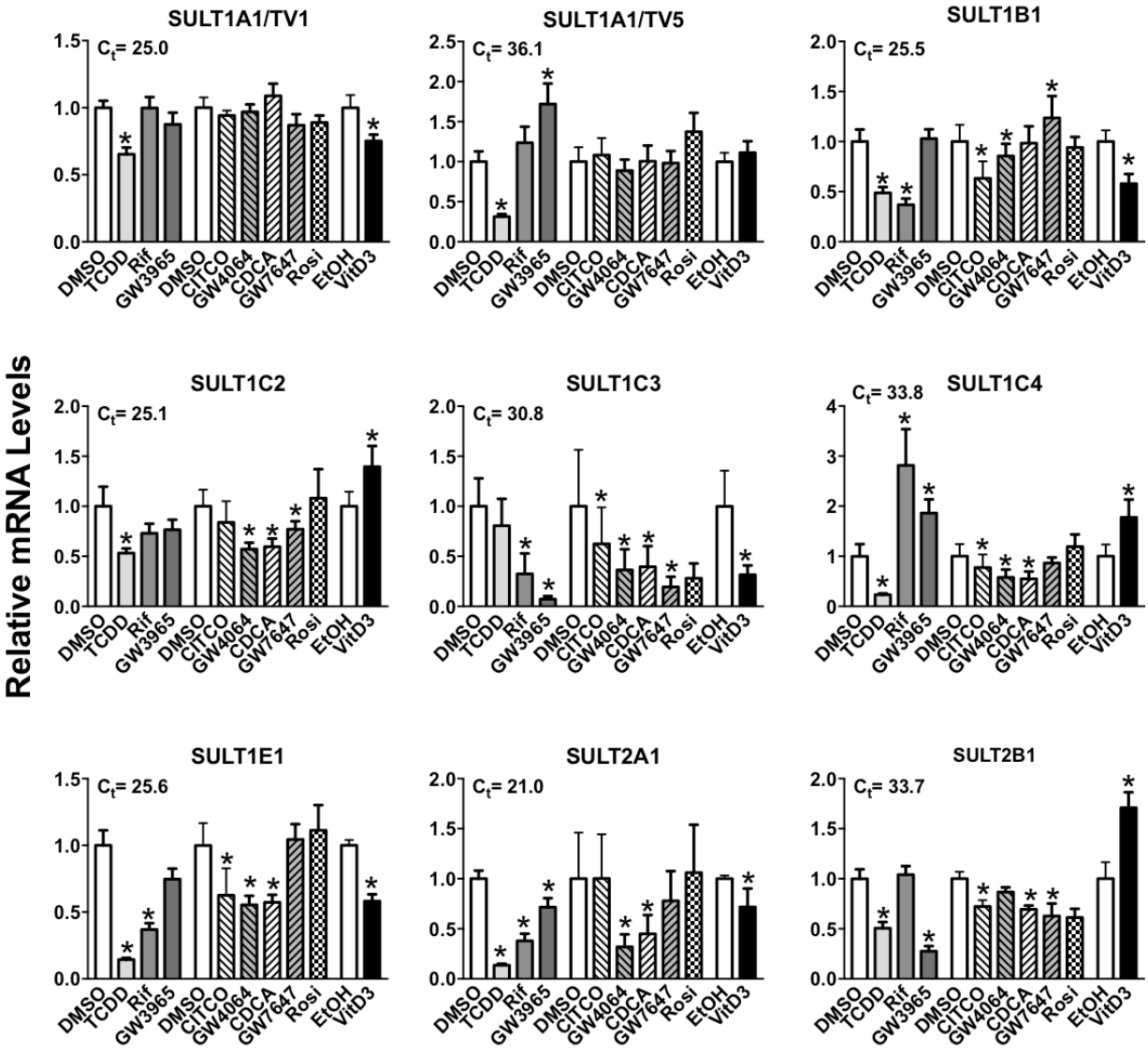


Figure 2.8: Effects of lipid- and xenobiotic-sensing receptor activators on SULT mRNA levels in confluent HepaRG cells.
Ten days after plating, confluent HepaRG cells were incubated in treatment medium containing 0.1% DMSO, 0.1% ethanol, 0.01 μM TCDD, 1 μM CITCO, 10 μM rifampicin (Rif), 10 μM GW3965, 1 μM GW4064, 50 μM CDCA, 10 μM GW7647, 10 μM rosiglitazone (Rosi), or 0.1 μM VitD3 for 48 hr, after which cells were harvested and SULT and TATA-box binding protein (used as normalization gene) mRNA levels were measured. Each bar represents the mean relative mRNA level ± range (for rosiglitazone treatment only) or SEM compared to control (0.1% ethanol for VitD3; 0.1% DMSO for all other agonists) for two (for rosiglitazone) or three independent experiments. *Significantly different from control, $p<0.05$.
Figure taken with permission from (Dubaisi et al., 2018).

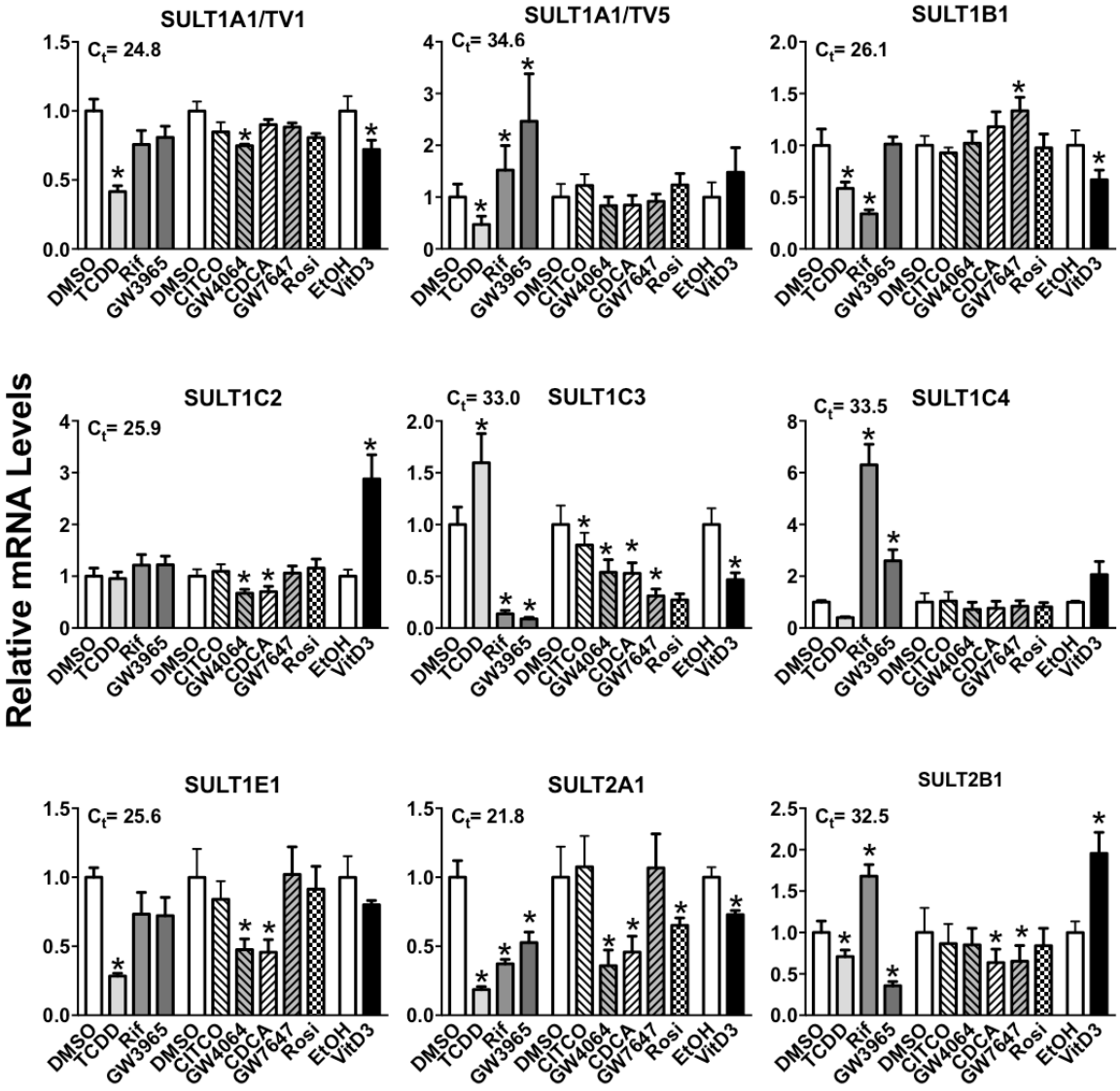


Figure 2.9: Effects of lipid- and xenobiotic-sensing receptor activators on SULT mRNA levels in differentiated HepaRG cells.

Four weeks after plating, differentiated HepaRG cells were incubated with treatment medium alone for 72 hour and then treatment medium containing 0.1% DMSO, 0.1% ethanol, 0.01 μM TCDD, 1 μM CITCO, 10 μM rifampicin (Rif), 10 μM GW3965, 1 μM GW4064, 50 μM CDCA, 10 μM GW7647, 10 μM rosiglitazone (Rosi), or 0.1 μM $VitD_3$ for 48 hr, after which cells were harvested and SULT and TATA-box binding protein (used as normalization gene) mRNA levels were measured. Each bar represents the mean relative mRNA level ± SEM relative to control (0.1% ethanol for $VitD_3$; 0.1% DMSO for all other agonists) from three independent experiments (except for SULT1A1/TV1) for the CITCO, GW4064, CDCA, GW7647, and Rosi treatment groups, where each bar represents mean ± range from two independent experiments).
*Significantly different from control, $p<0.05$.
Figure taken with permission from (Dubaisi et al., 2018).

2.3.5 Examining the role of AhR in the regulation of SULTs, CYP3A4, and CYP3A7 mRNA in differentiating HepaRG cells. Over the past two decades several studies reported that ligand-independent AhR activity regulates physiological processes during development in multiple tissues, including liver (Mitchell and Elferink, 2009). Our data shown in figures 2.8 and 2.9 indicated that activation of AhR by TCDD suppresses the expression of most SULT1 and SULT2 enzymes in differentiating HepaRG cells. To investigate the role of AhR in the regulation of SULT expression during liver cell differentiation without any stimulation by exogenous ligand, we defined the temporal expression profile of several SULTs and CYPs in an AhR KO HepaRG cell line, where AhR activity is completely absent. We evaluated the expression of SULTs and CYPs that are primarily expressed in prenatal liver specimens (will be presented in chapter 3) and confluent HepaRG cells (i.e., SULT1C2, SULT1C4, SULT1E1, CYP3A7) or differentiated cells (i.e., SULT2A1 and CYP3A4) as well as CYP1A1, which is a well-known target of AhR. The temporal expression patterns of SULT1C2, SULT1E1, SULT2A1, CYP3A4, and CYP3A7 in WT and AhR KO HepaRG cells were comparable (Fig. 2.10). However, the mRNA levels of these genes were considerably lower in the absence of AhR in the differentiating HepaRG cells, particularly in the proliferative and confluent stages (between day 5 and 14). Abolishing AhR signaling reduced the expression of SULT1C2, SULT1C4, SULT1E1, and SULT2A1 by 72-86%, 34-60%, 24-57%, and 69-92%, respectively in the proliferating and confluent cells. CYP3A4 and CYP3A7 mRNA also decreased by 30-96% and 67-96%, respectively, between days 5 and 14 in the absence of AhR (Fig. 2.10). The expression of CYP1A1 was relatively the same in AhR KO and WT HepaRG cells (Fig. 2.10).

2.3.6 The mechanism underlying the transcriptional regulation of SULT1C4 by LXR, PXR, and VDR. There are currently no known regulators of SULT1C4 expression in human cells. To examine the mechanism underlying the induction of SULT1C4 mRNA by LXR, PXR, and VDR (as shown in Fig 2.8 and 2.9), we evaluated fragments from the 5'-flanking region of SULT1C4 for their responsiveness to LXR, PXR, and VDR activation. To determine the transcription start

site of the SULT1C4 gene, we performed 5'-RACE analysis using RACE-ready cDNA from Caco-2 and HepaRG cells and a SULT1C4-specific reverse primer placed within exon 1 (listed in Appendix G). Using sequence and alignment analysis of 23 5'-RACE clones prepared from HepaRG and Caco-2, we determined that the 5'-end of most clones started at approximately the same site. Our analysis indicated that the transcription start site of 5'-RACE clones was 46 bp downstream of the reported transcription site for NM_006588.3 and NM_001321770.1. The translation start site was located in exon 1, as previously indicated by Freimuth et al. (Freimuth et al., 2000). The translation start site was 393 and 191 bp downstream of the transcription start site for 18 and 4 clones, respectively.

We then transfected a series of constructs containing ~1 or 2 Kb fragments (2.2 Kb and fragments A to I) from the region upstream of the transcription start site that were attached to the SULT1C4 core promoter (shown in figure 2.11A) into HepG2 cells, which are relatively easier to transfect compared to HepaRG cells. To compensate for the low expression of PXR and VDR in HepG2 cells, we cotransfected the cells with expression plasmids that express one of the two transcription factors (PXR-pSG5 and VDR-pcDNA3.1, respectively) as well as empty expression plasmids that were used as controls. LXR activation by 10 μM GW3965 upregulated the luciferase activity of the positive control SREBP1c-LXRE reporter in HepG2 cells (Fig. 2.11B and 2.12), but it did not have any effect on the activity of any of the SULT1C4 reporter constructs. Similarly, treatment with Rif (10μM) or $VitD_3$ (0.1μM) also induced the luciferase activity of the control CYP3A4-PXRE and SULT1C2-VDRE reporter constructs in HepG2 cells cotransfected with a PXR and VDR expression plasmid, respectively, without altering the luciferase activity of any of the SULT1C4 fragments (Fig. 2.11C, 2.11D, 2.13, and 2.14).

Because the effects of LXR and PXR agonist treatments, which produced the most notable upregulation of SULT1C4 mRNA, were observed in HepaRG cells (Fig. 2.8 and 2.9), we transfected the SULT1C4 reporter plasmids into HepaRG cells and examined their

responsiveness to the LXR and PXR agonists. Because SULT1C4 is primarily expressed in confluent HepaRG cells (Fig. 2.4), the transfections and treatments were done during confluency (day 9-12). Treatment with 10 μM GW3965 or Rif upregulated the luciferase activity of the control reporters, SREBP1c-LXRE and CYP3A4-XREM, respectively (Fig. 2.15 and 2.16). However, neither of the treatments had any effect on the luciferase activity of the SULT1C4 reporter plasmids (Fig. 2.15 and 2.16).

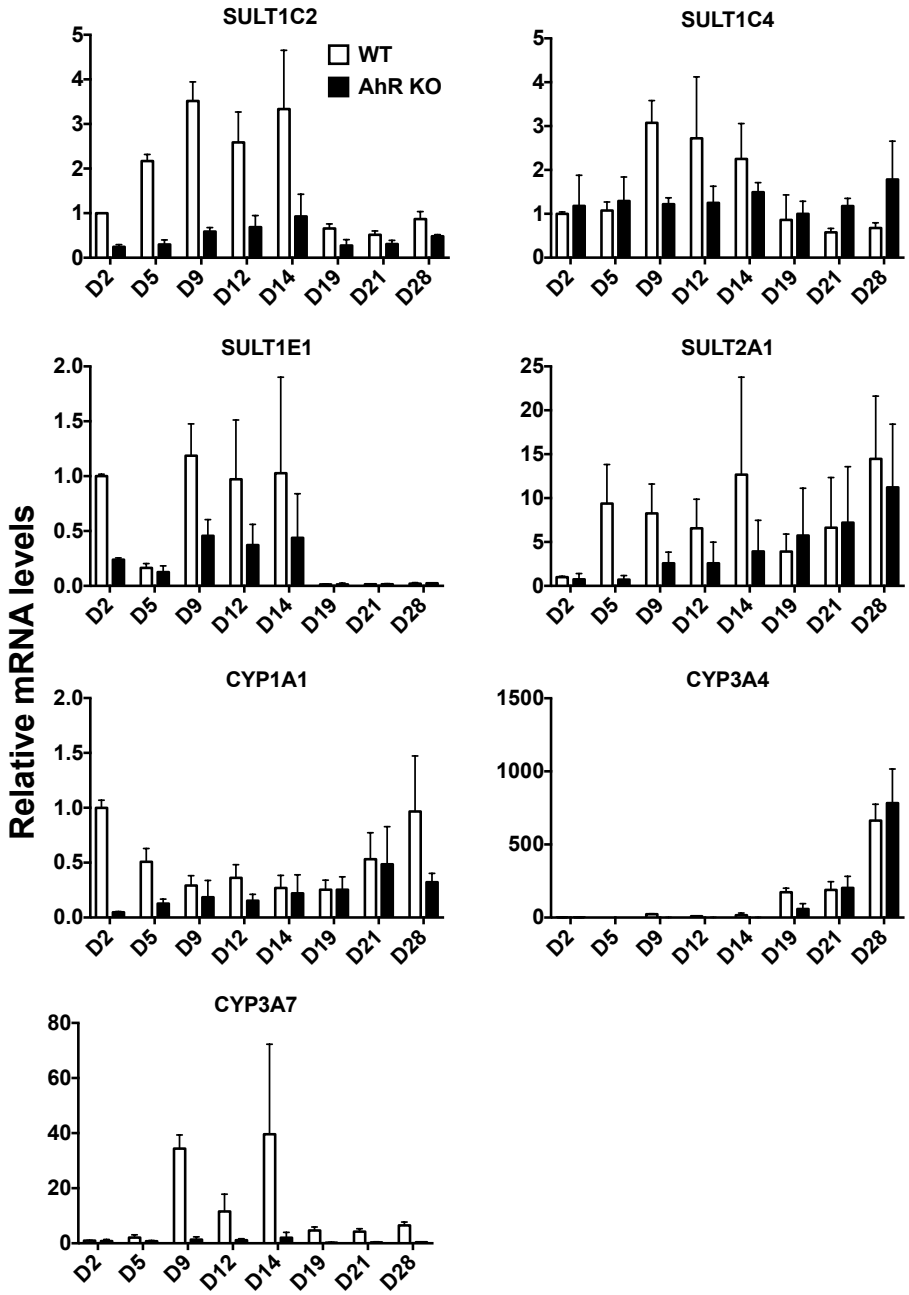


Figure 2.10: Temporal expression profiles of SULTs, CYP1A1, CYP3A4, and CYP3A7 in the absence of AhR signaling in HepaRG cells.
WT and AhR KO HepaRG cells were plated (day 0) and harvested on the days indicated in the figure for measurement of mRNA levels. mRNA levels were normalized to the levels measured on day two of the WT cells. The data show the expression patterns of SULT1C2, SULT1C4, SULT1E1, and SULT2A1 as well as CYP1A1, CYP3A4, and CYP3A7 from the proliferative to the confluent phase (open bars) and then through the differentiation phase (black bars). Data were normalized to TBP and are shown as mean ± range from two independent experiments that were each performed in duplicates (n=4).

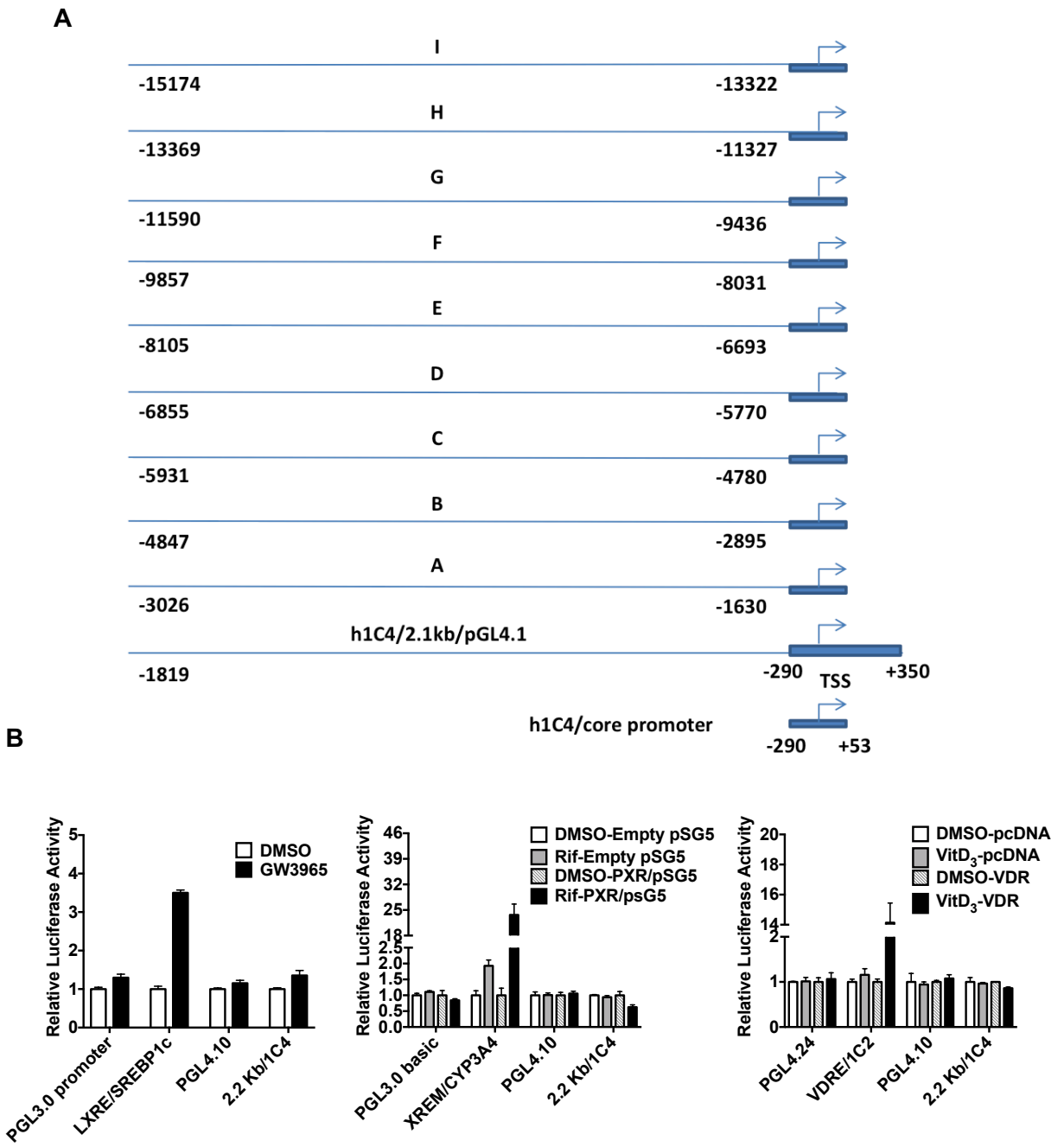


Figure 2.11: The responsiveness of the SULT1C4 2.2Kb reporter construct to LXR, PXR, and VDR agonist in HepG2 cells.
were used in the transfection analysis. HepG2 cells were transiently transfected with the SULT1C4 2.2 Kb (nt -1819:+350) luciferase reporter construct or an empty pGL4.10 [luc2]. Cells were also transfected with LXRE/SREBP1c (B), XREM/CYP3A4 (C), and VDRE/SULT1C2 (D) that were used as positive controls. 24 hours after transfection cells were treated with DMSO (0.1%), or the nuclear receptor agonist (B) GW3965 (10 μM), (C) rifampicin (Rif; 10 μM), and (D) $VitD_3$ (0.1 μM) for 48 hours. Cells treated with Rif or $VitD_3$ were cotransfected with a (C) PXR-pSG5 expression plasmid or empty pSG5 plasmid and (D) VDR-pcDNA3.1 expression plasmid or empty pcDNA3.1 plasmid. The cells were then harvested for measurement of luciferase activities. Each column represents the mean± S.D. of normalized (Firefly/Renilla) luciferase measurements relative to DMSO control (n = 3 wells per treatment) from one cell culture experiment.

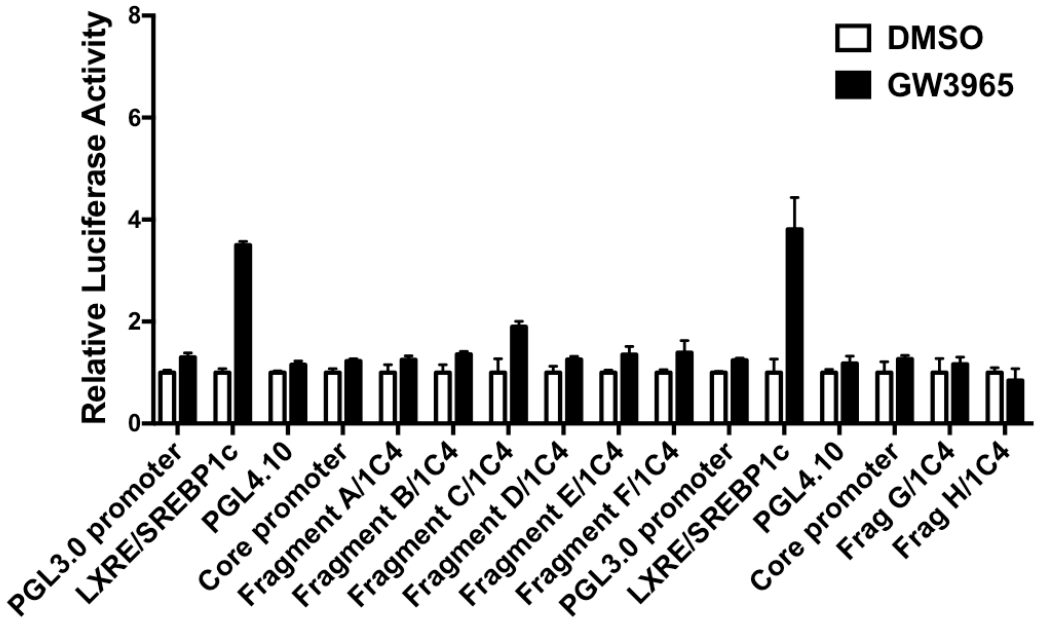


Figure 2.12: The responsiveness of SULT1C4 reporter constructs to LXR activation in HepG2 cells.

Cells were transiently transfected with the series of reporter plasmids that contain fragments A through H that span ~13 Kb region upstream the transcription start site of SULT1C4. Cells were also transfected with an empty pGL4.10 [luc2], a core promoter, or LXRE/SREBP1c reporter constructs (used as controls). 24 hours after transfection, cells were treated with GW3965 (10 μM) for 48 hours and then harvested for measurement of luciferase activities. Each column represents the mean± S.D. of normalized (Firefly/Renilla) luciferase measurements relative to DMSO control (n = 3 wells per treatment) from one cell culture experiment.

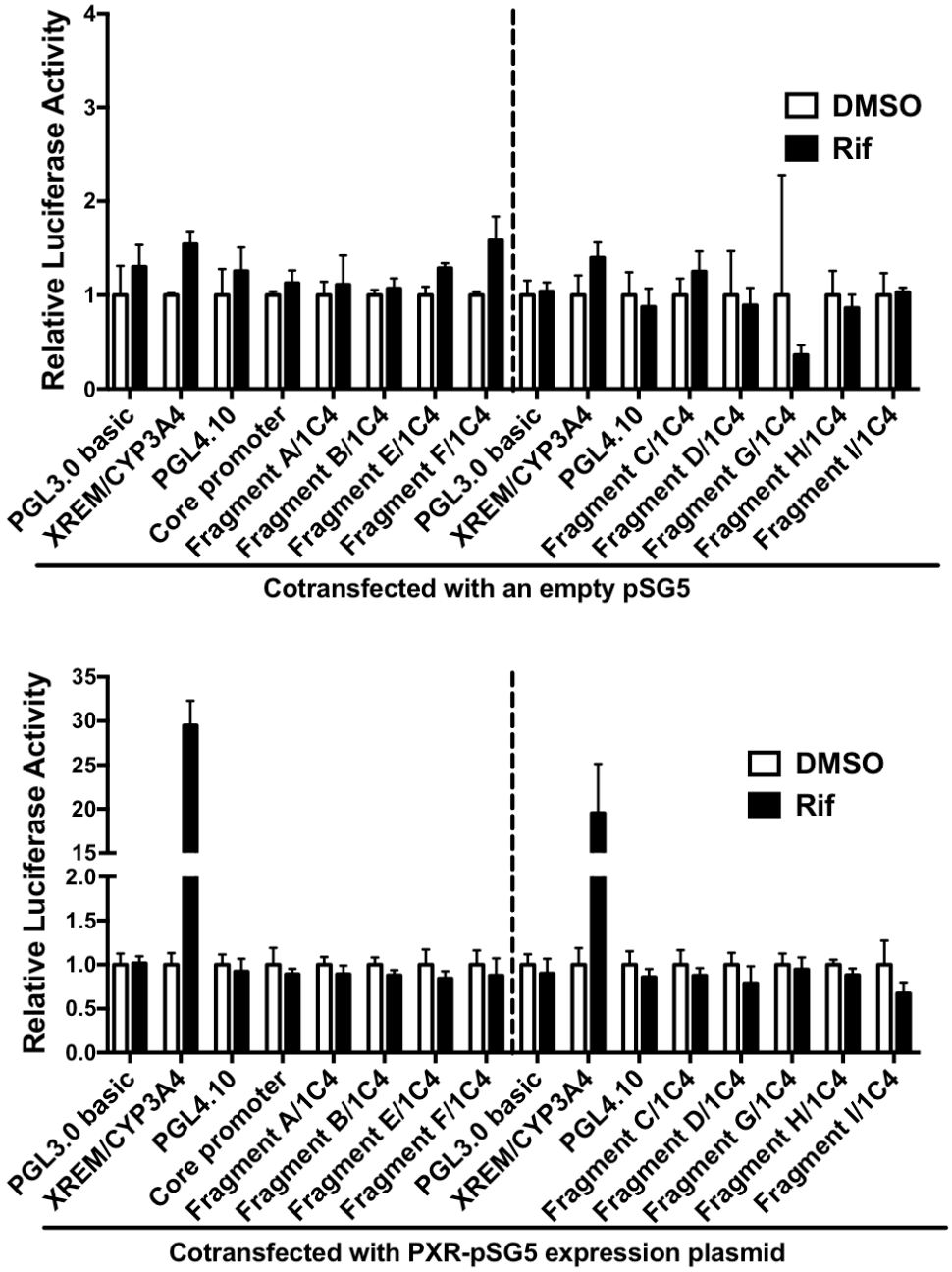


Figure 2.13: The responsiveness of a series of SULT1C4 reporter constructs to PXR activation in HepG2 cells.

Cells were transiently transfected with a series of reporter constructs containing fragments A through I that span ~15 Kb region upstream the transcription start site of SULT1C4. Cells were also transfected with an empty pGL4.10 [luc2], a core promoter, or XREM/CYP3A4 reporter constructs (used as controls) and PXR-pSG5 or an empty pSG5 expression plasmid. 24 hours after transfection, cells were treated with Rif (10 μM) and then harvested for measurement of luciferase activities. Each column represents the mean± S.D. of normalized (Firefly/Renilla) luciferase measurements relative to DMSO control (n = 3 wells per treatment) from two cell culture experiment.

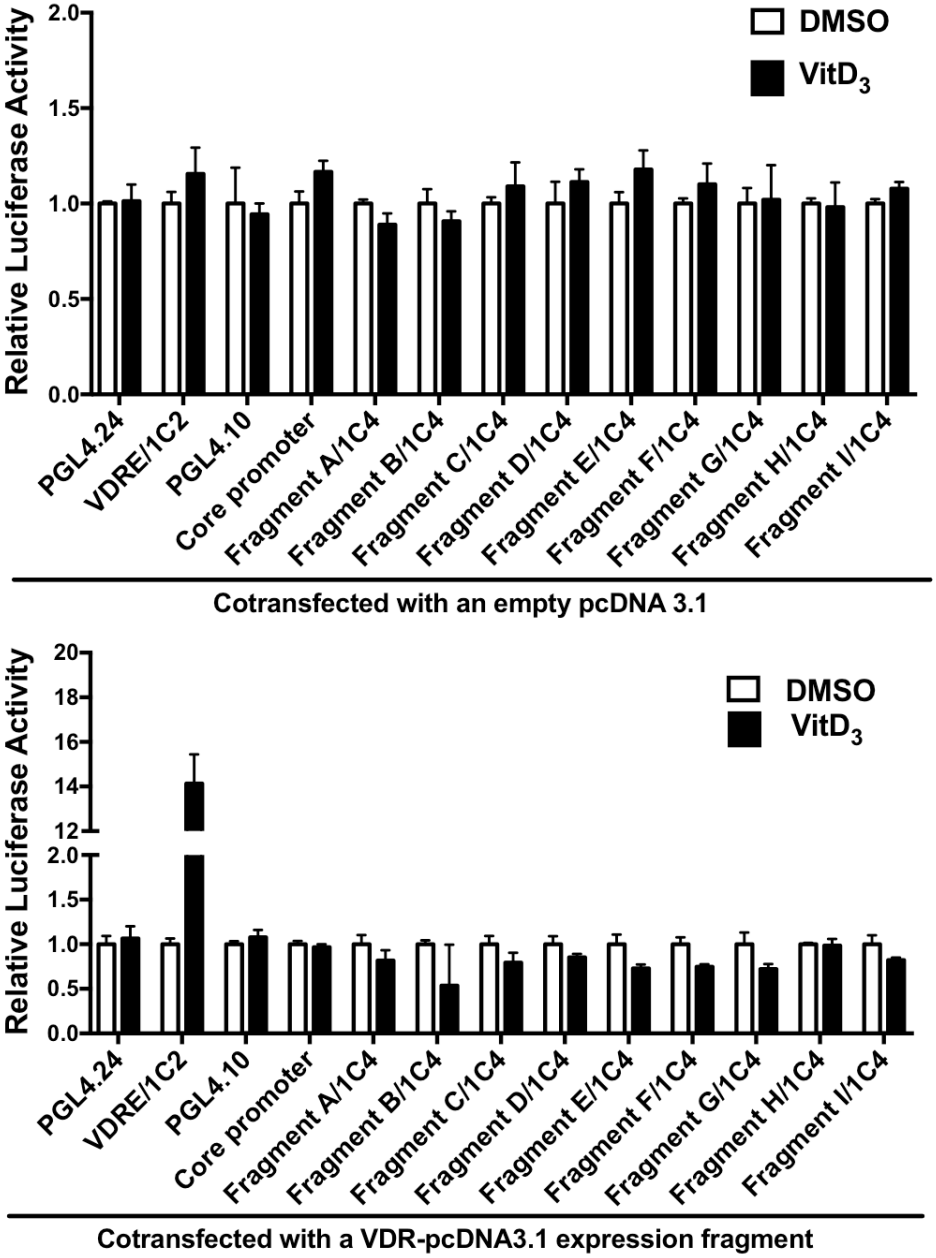


Figure 2.14: The responsiveness of a series of SULT1C4 reporter constructs to VDR activation in HepG2 cells.

Cells were transiently transfected with a series of reporter plasmids that contain fragments A through I that span ~15 Kb region upstream the transcription start site of SULT1C4. Cells were also transfected with an empty pGL4.10 [luc2], a core promoter, or VDRE/SULT1C2 reporter constructs (used as controls) and cotransfected with a VDR-pcDNA3.1 or an empty pcDNA3.1 expression plasmid. 24 hours after transfection cells were treated with $VitD_3$ (0.1 μM) and then harvested for measurement of luciferase activities. Each column represents the mean± S.D. of

normalized (Firefly/Renilla) luciferase measurements relative to DMSO control (n = 3 wells per treatment) from one cell culture experiment.

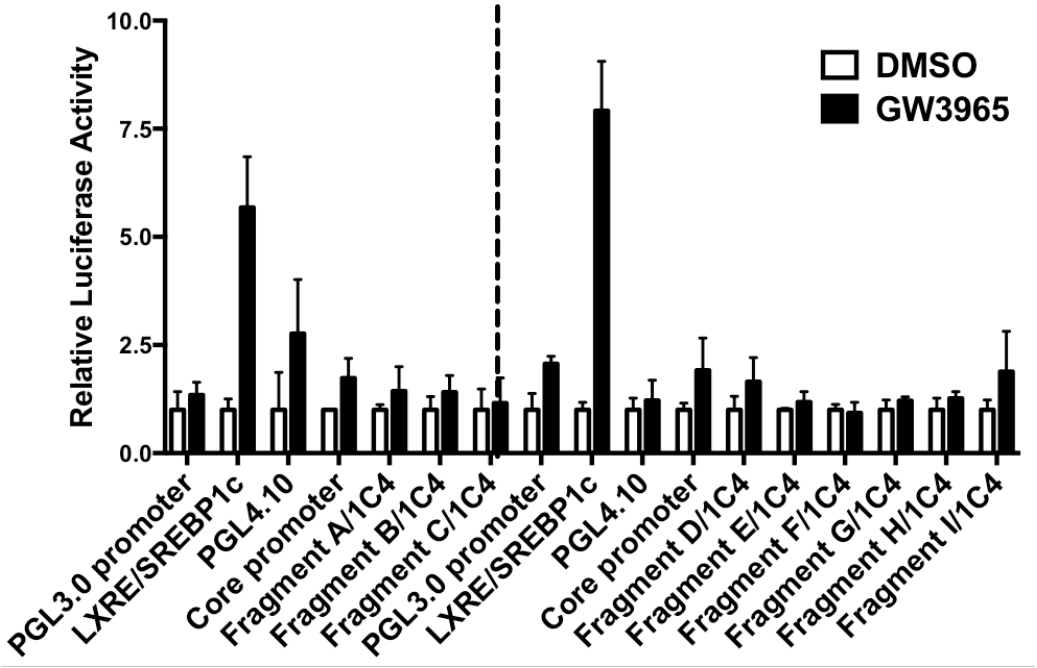


Figure 2.15: The responsiveness of a series of SULT1C4 reporter constructs to LXR activation in HepaRG cells.

Confluent cells (day 9) were transiently transfected with the series of reporter constructs that contain fragments A through I, spanning ~15 Kb region upstream from the transcription start site of SULT1C4. Cells were also transfected with an empty pGL4.10 [luc2], core promoter, or LXRE/SREBP1c reporter construct (used as controls). 24 hours after transfection, cells were treated with GW3965 (10 μM) for 48 hours and then harvested for measurement of luciferase activities. Each column represents the mean± S.D. of normalized (Firefly/Renilla) luciferase measurements relative to DMSO control (n = 3 wells per treatment) from two cell culture experiment.

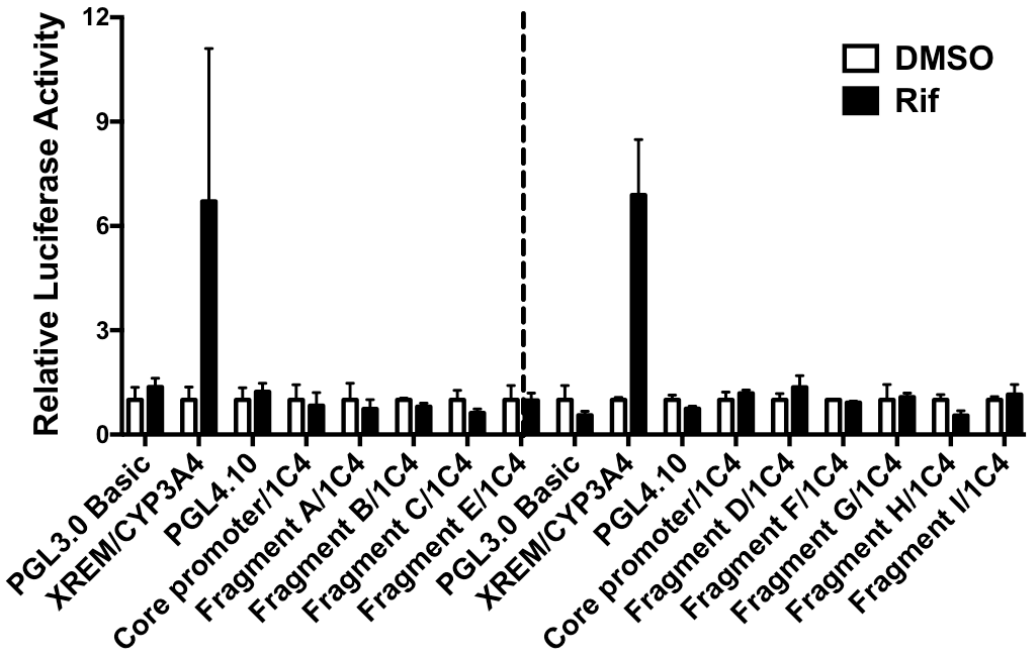


Figure 2.16: The responsiveness of a series of SULT1C4 reporter constructs to PXR activation in HepaRG cells.
Confluent cells (day 9) were transiently transfected with the series of reporter plasmids that contain fragments A through I, spanning ~15 Kb region upstream from the transcription start site of SULT1C4. Cells were also transfected with an empty pGL4.10 [luc2], core promoter, or XREM/CYP3A4 reporter construct (used as controls). 24 hours after transfection, cells were treated with Rif (10 μM) for 48 hours and then harvested for measurement of luciferase activities. Each column represents the mean± S.D. of normalized (Firefly/Renilla) luciferase measurements relative to DMSO control (n = 3 wells per treatment) from two cell culture experiment.

2.4 Discussion

Xenobiotic-metabolizing enzymes exhibit distinct patterns of developmental expression (Hines, 2008). Although some detoxification enzymes, such as CYP3A5, CYP3A7, FMO1, and SULT1E1, were reported to be expressed during early stages of gestation (Lacroix et al., 1997; Koukouritaki et al., 2002; Stevens et al., 2003; Duanmu et al., 2006), the expression of many other enzymes matures during later stages of development, including neonatal and postnatal periods. Previous studies showed that the expression of various phase I, such as CYP1A2, CYP3A4, CYP2C9, CYP2C19, and phase II enzymes, such as UGT1A4, UGT1A6, and UGT2B7, increased gradually and reached maximum abundancy during neonatal, infancy, childhood, or adulthood periods (Sonnier and Cresteil, 1998; Strassburg et al., 2002; Stevens et al., 2003; Koukouritaki et al., 2004; Bhatt et al., 2018). In this project , we examined the ontogeny of SULT1 and SULT2 enzymes that are expressed in the human liver and are among the most abundant conjugating enzymes in fetal tissues (Coughtrie, 2015).

There is currently no published information about SULT expression or regulation using *in vitro* models of human liver development. In the current investigation, we used cultures of primary fetal hepatocytes to identify regulators of SULT expression during early stages of liver development. Placement of fetal hepatocytes into primary culture decreased the expression of several SULTs. A notable exception was SULT1B1, which is reported to be the third most highly expressed SULT in adult human liver (Riches et al., 2009). SULT1B1 mRNA content increased 14-fold after the fetal hepatocytes were placed into culture, implying a difference in the mechanism(s) controlling basal expression of SULT1B1 relative to other SULTs.

To identify mechanisms that regulate SULT expression in human fetal hepatocytes, primary cultures were treated with several activators of lipid- and xenobiotic-sensing receptors. Several significant effects and non-significant trends were observed and are discussed in the context of previous findings. VDR activation significantly increased SULT1C2 as well as CYP3A7 and CYP3A4 mRNA levels. Although liver is not a classical $VitD_3$-responsive organ, VDR is

expressed in liver (Berger et al., 1988) and its activation has been shown to increase expression of several cytochromes P450 enzymes, including CYP3A4, in primary cultured adult human hepatocytes (Drocourt et al., 2002). VDR activation was found to increase SULT1C2 expression in LS180 colorectal adenocarcinoma cells (Rondini et al., 2014; Barrett et al., 2016). Also, treatment of fetal hepatocyte cultures with an agonist of PPARα (GW7647) or PPARγ (rosiglitazone) increased expression of SULT2A1 by approximately 2-fold, whereas treatment with the FXR agonist GW4064 suppressed SULT2A1 in the cultured fetal hepatocytes. PPARα and FXR were both previously reported to regulate SULT2A1 expression in primary human hepatocytes and HepG2 cells, respectively (discussed in chapter 1.5) (Fang et al., 2005; Miyata et al., 2006).

A novel finding in the cultured fetal hepatocytes was that treatment with the LXR agonist GW3965 significantly increased expression of SULT1A1 TV5. TV5 is reported to be a rare variant that contains a distinct 5'-untranslated region and lacks part of the 5'-coding region, and its transcription start site is located more than 10 Kb upstream from that of other SULT1A1 transcripts (NCBI information for NM_177536 and SULT1A1 gene). The distinct location of the TV5 promoter provides a plausible explanation for the unique regulation of this SULT1A1 variant by LXR, which prompts speculation that SULT1A1 TV5 could play a role in sterol metabolism.

Most studies using HepaRG cells have used differentiated cells as a model to complement the use of primary cultured human hepatocytes (Josse et al., 2008; Lubberstedt et al., 2011; Gerets et al., 2012; Klein et al., 2015). However, few studies have evaluated the changes in xenobiotic-metabolizing enzyme expression that occur as HepaRG cells pass through the stages of the differentiation process (Aninat et al., 2006; Hart et al., 2010; Ceelen et al., 2011; Tsuji et al., 2014; Bucher et al., 2016), and no studies have determined expression of the individual SULTs. We found that SULT1B1, SULT1C2, SULT1C3, SULT1C4, and SULT1E1 mRNA levels were highest in confluent HepaRG cells, whereas SULT2A1 RNA levels increased throughout the differentiation process. The temporal trends of gene expression, whereby the SULTs that are

preferentially expressed in fetal livers and hepatocytes are also preferentially expressed in confluent HepaRG cells while the SULT that is preferentially expressed in adult liver and hepatocytes is expressed at the highest level in differentiated HepaRG cells, provide additional support to the suggestion that HepaRG cells at these stages of the differentiation protocol can serve as experimental models of human hepatocyte development.

We also evaluated the effects of activators of lipid- and xenobiotic-sensing transcription factors on SULT expression in HepaRG cells, comparing effects at the confluent and differentiated stages. More activator-mediated changes in SULT expression were observed in the HepaRG cells than in the primary cultured fetal hepatocytes. However, while there were marked temporal changes in SULT expression as the cells underwent differentiation (noted above), most of the transcription factor activator-mediated changes were comparable in the confluent and differentiated HepaRG cells, indicating that the evaluated nuclear signaling pathways were already functional in the confluent cells. As seen in the human fetal hepatocytes, GW3965 and $VitD_3$ treatment increased SULT1A1 TV5 and SULT1C2 mRNA levels, respectively, while FXR agonists GW4064 and CDCA suppressed SULT2A1 expression. Treatment with GW4064 or CDCA also significantly suppressed SULT1E1 expression in confluent HepaRG cells, but GW4064 only produced a slight reduction of SULT1E1 mRNA content in primary cultured fetal hepatocytes. These data are consistent with the findings of a recent study that reported the suppressive effect of FXR on SULT1E1 expression in HepG2 cells (discussed in chapter 1.5) (Wang et al., 2017).

The effects of the PXR agonist rifampicin differed between HepaRG cells and fetal hepatocytes. Rifampicin treatment increased SULT1C4 expression and suppressed SULT1E1 and SULT2A1 in HepaRG cells but not in the fetal hepatocytes. We previously reported that rifampicin-mediated PXR activation suppresses hepatic SULT2A1 expression (Fang et al., 2007), while the mechanism of PXR-mediated suppression of SULT1E1 was described by Kodama et al. (2011). Rifampicin treatment also did not increase CYP3A4 or CYP3A7 expression in the

cultured fetal hepatocytes, suggesting that PXR was not functional in these cells. Our findings agree with a previous report by Maruyama et al. (2007), who evaluated cytochrome P450 expression in human fetal liver cells prepared from a pool of six normal human fetal livers (average 13 weeks of gestation). These cells expressed CYP3A4 and CYP3A7, and treatment with dexamethasone, which is an effective agonist of rodent but not human PXR, significantly increased CYP3A4 and CYP3A7 mRNA levels (Maruyama et al., 2007). However, treatment of the fetal liver cells with rifampicin did not increase CYP3A4 or CYP3A7 expression, and PXR mRNA was not detected by RT-PCR (Maruyama et al., 2007). Vyhlidal et al. (Vyhlidal et al., 2006) have also reported that PXR expression is lower in fetal relative to postnatal livers. These findings demonstrate that although confluent HepaRG cells model some aspects of the fetal hepatocyte, the presence of PXR signaling in confluent HepaRG cells and its absence in fetal hepatocytes indicates that confluent HepaRG cells do not fully recapitulate all aspects of the fetal hepatocyte, at least at the culture and gestation times that were evaluated.

To date, SULT1C3 mRNA has only been detected in human intestinal tissue and cells (Duniec-Dmuchowski et al., 2014). In this study, we detected SULT1C3 mRNA in HepaRG cells, mainly at the confluent stage where the mRNA levels were approximately the same as those for SULT1C4. SULT1C3 expression was significantly reduced in the HepaRG cells by most of the treatments that were evaluated, with almost complete suppression by the LXR agonist GW3965. It seems possible that these suppressive effects contribute to the lack of SULT1C3 expression that has generally been seen in human liver samples.

TCDD treatment was also found to suppress the expression of most SULTs in HepaRG cells. This finding is consistent with the suppressive effects of AhR agonist treatments on SULT expression that we have previously reported in rat hepatocytes (Runge-Morris, 1998) and MCF10A human breast epithelial cells (Fu et al., 2011). Fu et al. previously showed that the transcription of SULT1E1 is regulated by confluency in MCF10A cells through a suppressive action of AhR, which is more active in the preconfluent cells (Fu et al., 2011). Our preliminary data

presented in the current study we showed that the absence of AhR activity in differentiating HepaRG cells suppressed, to some extent, SULT1C2, SULT1E1, SULT2A1, CYP3A4, CYP3A7 mRNA, primarily in the proliferating and confluent cells. We are in the process of developing our own AhR KO HepaRG cell model to further examine the role of AhR in regulating SULT, CYP3A4, and CYP3A7 expression in the differentiating hepaRG. These results suggest that AhR activity could be required to achieve maximal expression of some SULTs and CYPs during the early stages of hepatocyte differentiation.

LXR, PXR, and VDR activation did not have any effect on the luciferase activity of reporter constructs covering ~15 Kb of the SULT1C4 5'-flanking region, suggesting that response regions for these receptors are located elsewhere in the SULT1C4 gene.

Several SULTs are expressed in human fetal liver, and thus these enzymes play important roles during early life, likely in the metabolism of both endogenous and xenobiotic substrates. This study represents the first effort to define patterns of SULT expression in cell culture models of human fetal liver and liver cell differentiation and identify signaling pathways that regulate SULT transcription in these cells. Further studies are warranted to understand the regulation of the SULTs during human development.

CHAPTER 3: DEVELOPMENTAL EXPRESSION OF THE CYTOSOLIC SULFOTRANSFERASES IN HUMAN LIVER

3.1 Introduction

Numerous xenobiotic-metabolizing enzymes that mediate the metabolism of many endogenous and foreign chemicals are expressed in the liver. The expression profiles of these enzymes vary markedly during liver development, thereby altering the ability of the liver to detoxify xenobiotics, bioactivate procarcinogens, and regulate the activity of estrogen and thyroid hormones as well as other endogenous compounds. The currently available information about the ontogeny of SULT1 and SULT2 enzymes in the liver is derived from a few studies that examined the expression of several SULTs using conventional methods, such as non-quantitative PCR, Western blots, and multi-tissue blots (Her et al., 1997; Sakakibara et al., 1998; Dooley et al., 2000; Stanley et al., 2005; Duanmu et al., 2006), a recent report by Ekstrom and Rane that evaluated SULT2A1 expression in fetal and adult liver specimens (Ekstrom and Rane, 2015), and the limited data that are contained in transcriptomic profiling studies of various adult and fetal tissues (e.g., GEO DataSet Accession numbers GDS181, GDS833, GDS1096, GDS3113, GDS3834). To expand our knowledge about the role of SULTs during development, we characterized the expression patterns of these enzymes at the RNA and protein levels using human liver specimens and cytosolic fractions from different life stages. To achieve a high level of rigor in our assessment, we evaluated SULT expression in three independent sets of human liver specimens using three different methods of measurement, two for mRNA and one for protein.

3.2 Materials and Methods

Materials: Human prenatal (18-19 weeks of gestation, n=10), infant (1-12 months old, n=10), and adult (18-50 years old, n=10) liver specimens analyzed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) were obtained from the University of Maryland National Institute of Child Health and Human Development Brain and Tissue Bank for Developmental Disorders (NICHD-BTB, National Institutes of Health Contract Number HHSN275200900011C,

Ref. No. #N01-HD-9-0011). A separate set of prenatal (weeks 14-16, n=10) and pediatric (0 days to 17 years, n=52) human liver specimens was analyzed by RNA sequencing (RNA-seq). The prenatal specimens were obtained from the Central Laboratory for Human Embryology at the University of Washington (Seattle, WA). The pediatric specimens were obtained from the NICHD-BTB and the Liver Tissue Cell Distribution System (National Institutes of Health Contract Number N01-DK-7-0004/HHSN267200700004C). In addition, 6 samples were generously provided by Xenotech, LLC (Kansas City, KS). All tissues were maintained at -80ºC prior to use. The use of these tissues was reviewed and declared non-human subjects research by the University of Missouri-Kansas City Pediatric Health Sciences Review Board at Children's Mercy Kansas City and the institutional review board at Wayne State University. Donor information (age, sex, and postmortem interval) for the human liver specimens is provided in Appendix I and J.

The library of human liver cytosolic fractions used for measurement of SULT protein levels (obtained from our collaborator Dr. Hines from the U.S. Environmental Protection Agency, Research Triangle Park, NC) was previously described (Duanmu et al., 2006). 193 samples from this library were available for the current analysis. The cytosolic fractions were prepared from livers at different developmental stages, starting from week 8 of gestation until 18 years, and include prenatal samples from first trimester (n= 15), second trimester (n=34), and third trimester (n=13); infants (0-1 years old, n=76); and children (1-18 years old, n= 55). Gender information was provided for 183 samples with 111 being male and 72 female. Only information about major diseases, cause of death, and ethnicity as well as the postmortem interval for the prenatal samples were available. Samples from individuals with disease processes that potentially would involve liver damage were excluded from the study. Tissues were stored at -80°C and approval was obtained from the Children's Hospital of Wisconsin and the Medical College of Wisconsin Institutional Review Boards. To prepare the liver cytosols, the liver tissues were homogenized in a buffer containing 100 mM Tris-HCl, pH 7.4, 250 mM sucrose, 5 mM EDTA, 0.1 mM DTT, and

0.25 mM phenylmethylsulfonyl fluoride. The samples were centrifuged at 105,000 g for 1 hour at 4°C to obtain the cytosolic fractions.

RNA Isolation and Gene Expression Analysis: For the samples analyzed by RT-qPCR, liver specimens were thawed on ice, and 30-50 mg pieces were dissected and homogenized in QIAzol Lysis Reagent using a TissueRuptor (Qiagen Inc., Germantown, MD). Total RNA was prepared using the RNeasy Plus Universal Mini Kit (Qiagen), and RNA quality was determined using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA integrity number (RIN) was used to assess the quality of the RNA. All prenatal RNA samples had RIN values > 8, whereas most of the infant and adult RNA samples had RIN values > 5. Three infant and 3 adult liver specimens yielded RNA with RIN values < 4. These samples were considered extensively degraded and were excluded from the analysis. RNA samples (3 µg) were reversed transcribed using the High Capacity cDNA Reverse Transcription Kit, according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA). RNA levels of 9 SULTs, CYP3A4, and CYP3A7 were measured as described in chapter 2.2. Data were normalized to 18S and to the median ΔCT in the prenatal group to calculate ΔCT and ΔΔCT, respectively, and then the $2^{-\Delta\Delta CT}$ method was used to quantify the relative changes in gene expression between the three developmental stages (Livak and Schmittgen, 2001).

The RNA-seq analysis was performed by our collaborators Drs. Vyhlidal and Gaedigk (from the Children's Mercy Kansas City in Kansas City, MO). For these samples, frozen liver specimens (20-30 mg) were homogenized and total RNA extracted according to the RNeasy protocol (Qiagen) with on-column DNase I treatment. The quality of the isolated RNA was assessed using an Experion Automated Electrophoresis Station (Bio-Rad, Hercules, CA) and was evaluated by the RNA quality indicator (RQI) value. All samples analyzed by RNA-seq had RQIs > 4.9, with 39 (out of 62) samples having RQIs >8. Libraries were then prepared from 1 µg total RNA of each sample using the TruSeq Stranded Total RNA Sample Prep Kit (Illumina, San Diego, CA). Paired-end sequencing (2 x 101 bp) of high output run mode was performed using the HiSeq

1500 instrument (Illumina, San Diego, CA). The resulting base calling (.bcl) files were converted to FASTQ files and trimmed RNA-seq reads were mapped to the human genome (GRCh37/hg19). Transcript assembly and abundance estimation were conducted using the Tuxedo Suite pipeline and reported in transcripts per kilobase of exon per million fragments mapped (TPM). TPM values were log-transformed and differences in mRNA expression were compared between age groups. Age groups were defined as follows: Group 0 (prenatal samples); Group 1 (infants <1 year of age), Group 2 (children 1-5 years of age), Group 3 (children 6-11 years of age), and Group 4 (adolescents, 12-17 years of age).

SULT protein quantification**:** Protein concentrations of liver cytosolic fractions were estimated using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). For each sample, 100 µg of protein was resolved on a 4-12% Bis-Tris gel (Thermo Fisher Scientific) in duplicate. The region between 30-40 kDa was excised and proteins were reduced, alkylated, and digested with trypsin in-gel. Peptides were eluted from the gel pieces and dried. Heavy AQUA peptides, with labels on C-terminal lysine or arginine residues, were purchased from Thermo Fisher Scientific. Aliquots of 10X stock heavy proteotypic peptides for eight SULTS at 40 fmol/µL were kept frozen at -80°C. On the day of the assay, peptides were resuspended in 30 µL of 1X heavy peptides in 5% acetonitrile, 0.1% formic acid, and 0.005% trifluoroacetic acid buffer. Peptides (5 µL) were separated by reversed-phase chromatography and introduced into a TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific). Transition settings were optimized for collision energies and peak retention times. Analysis time for each peptide spanned over a 2 minute window during the 20 minute gradient. Instrument settings included full width at half maximum of 0.7 and cycle time of 1.2 sec. Multiple reaction monitoring (MRM) settings are found in Appendix K.

Data were imported into Skyline (version 4.1.0; MacCoss Lab, University of Washington). Integrated peaks were manually validated and those with signal-noise ratios < 3 were excluded.

Areas-under-the-curve (AUCs) for each transition peak were summed per peptide, and final calculations for absolute quantitation were as follows:

$$\text{fmol SULT per mg cytosol} = 20 \text{ fmol} \times \frac{(\Sigma \text{ light transition AUCs})}{(\Sigma \text{ heavy transition AUCs})} \times \frac{6}{0.1 \text{ mg}}$$

Samples were analyzed in duplicate; therefore, results represent the average of two samples. For the purposes of plotting data and performing statistical analyses, the level of a SULT protein in a sample with an undetectable level of that protein was given a value of 0. The SULT protein quantification in liver cytosols was performed in collaboration with Dr. Joseph Caruso at the Proteomics Core Center at Wayne State University.

Data analysis: Samples were grouped according to age, and scatter and box-and-whisker plots were prepared for the mRNA and protein data, respectively. Statistical comparisons among groups were performed using the Kruskal-Wallis non-parametric analysis of variance (ANOVA) and Dunn's multiple comparison test, using Prism version 6 (GraphPad Software, La Jolla, CA). Correlation analyses to perform pairwise comparisons of gene expression were also performed using Prism.

3.3 Results

3.3.1 Developmental expression of SULT mRNA in human liver. To address the gap in our knowledge about the developmental expression profiles of ten SULTs, we obtained specimens of pre- and postnatal human liver and characterized the developmental expression of SULT1 and SULT2 family genes using RT-qPCR and RNA-seq. While CYP3A7 is established as a gene that is preferentially expressed during fetal life and remains detectable in many individuals until 2 years of age, CYP3A4 is known to be primarily expressed in postnatal liver (Lacroix et al., 1997; Stevens et al., 2003). Therefore, CYP3A7 and CYP3A4 mRNA levels were measured in our tissue sets to demonstrate that these expected patterns of expression were observed (Fig. 3.1 and 3.2).

Fig. 3.1 shows the levels of SULT mRNA in prenatal, infant, and adult liver, as measured by RT-qPCR. As indicated in chapter 2, SULT1A1 mRNA was measured using two different TaqMan Gene Expression Assays. SULT1A1 (TV1), 1A2, 1B1, 1C2, 1C4, 1E1, and 2A1 mRNAs were readily detectable (as determined by Ct values <30) in at least one of the developmental stages, but SULT1A1 (TV5), 1C3, and 2B1 mRNAs were minimally present at all stages (Ct ≥ 33) (Fig. 3.1). Although not statistically significant, SULT1E1 appeared to be preferentially expressed in the prenatal liver while SULT2A1 expression was significantly higher in the postnatal specimens, in agreement with a previous analysis of SULT1E1 and 2A1 immunoreactive protein in human liver cytosolic fractions that was published by Dr. Runge-Morris's lab (Duanmu et al., 2006). Nevertheless, SULT2A1 mRNA levels were relatively high in the prenatal specimens, as estimated by a median Ct value of 25.5, which was the third lowest (approximately same as SULT1A1 TV1) after SULT1C4 and 1E1. Like SULT2A1, SULT1A2 mRNA levels were higher in the postnatal specimens than in the prenatal specimens, while for SULT1B1, mRNA levels were higher in the adult than either the prenatal or infant specimens. SULT1C2 and 1C4 were preferentially expressed in the prenatal and infant liver specimens. However, the SULT1C2 and SULT1C4 expression patterns were not identical, as SULT1C2 mRNA content was highest in the infant livers, with a median level that was ~ 3-fold and 310-fold higher than it was in the prenatal and adult specimens, respectively. By contrast, SULT1C4 mRNA content was highest in the prenatal specimens, with a median level that was ~91 and ~192-fold higher than it was in the infant and adult specimens, respectively.

A separate set of pre- and postnatal human liver specimens was analyzed by RNA-seq, and the SULT transcript levels from this dataset were compared to the RT-qPCR findings. While both tissue sets included prenatal and infant liver specimens, the set used for RNA-seq included specimens from children 1-17 years of age (evaluated as four age groups: infants <1 year of age, children 1-5 years, children 6-11 years, and children 12-17 years) but did not include adult specimens. As for the RT-qPCR data, CYP3A7 and 3A4 displayed the expected patterns of

predominantly prenatal and postnatal expression, respectively (Fig. 3.2). The SULT1A1, 1A2, 1C2, 1C4, 1E1, and 2A1 developmental expression profiles determined by RNA-seq (Fig. 3.2) were generally consistent with those observed by RT-qPCR (Fig. 3.1). SULT1A1 was expressed at a relatively constant level throughout development, with SULT1A1 TV1/2 being the most abundant transcripts (Table 3.1); SULT1A2 expression did not vary significantly during development but was generally higher in the postnatal periods (particularly in infants and children 1-11 years old); SULT1C2, SULT1C4, and SULT1E1 were primarily expressed in the prenatal and infant specimens; and SULT2A1 expression increased after birth (Fig. 3.2). SULT1A1 TVs 3/4 and 5, 1C3, and 2B1 mRNA levels were low throughout development (Table 3.1). SULT1B1 mRNA levels in prenatal liver specimens were not significantly different from those in infant liver, which is also consistent with the RT-qPCR data, but were higher than they were in the specimens from children ages 1-5 and 6-11. The higher SULT1B1 mRNA levels that were present in the adult liver specimens analyzed by RT-qPCR could not be confirmed since the tissue set analyzed by RNA-seq did not include adult specimens. SULT1A3 mRNA, which was not analyzed by RT-qPCR, was also detected in prenatal and postnatal liver by RNA-seq, with highest expression in the prenatal and infant specimens. Two differences between the tissue sets analyzed by RT-qPCR and RNA-seq were that SULT1B1 and 1C4 were the seventh and first most abundant transcripts in the prenatal samples in Fig. 3.1 (as estimated by Ct values) but were the second and seventh most abundant transcripts in prenatal samples in Table 3.1 (as estimated by TPM values).

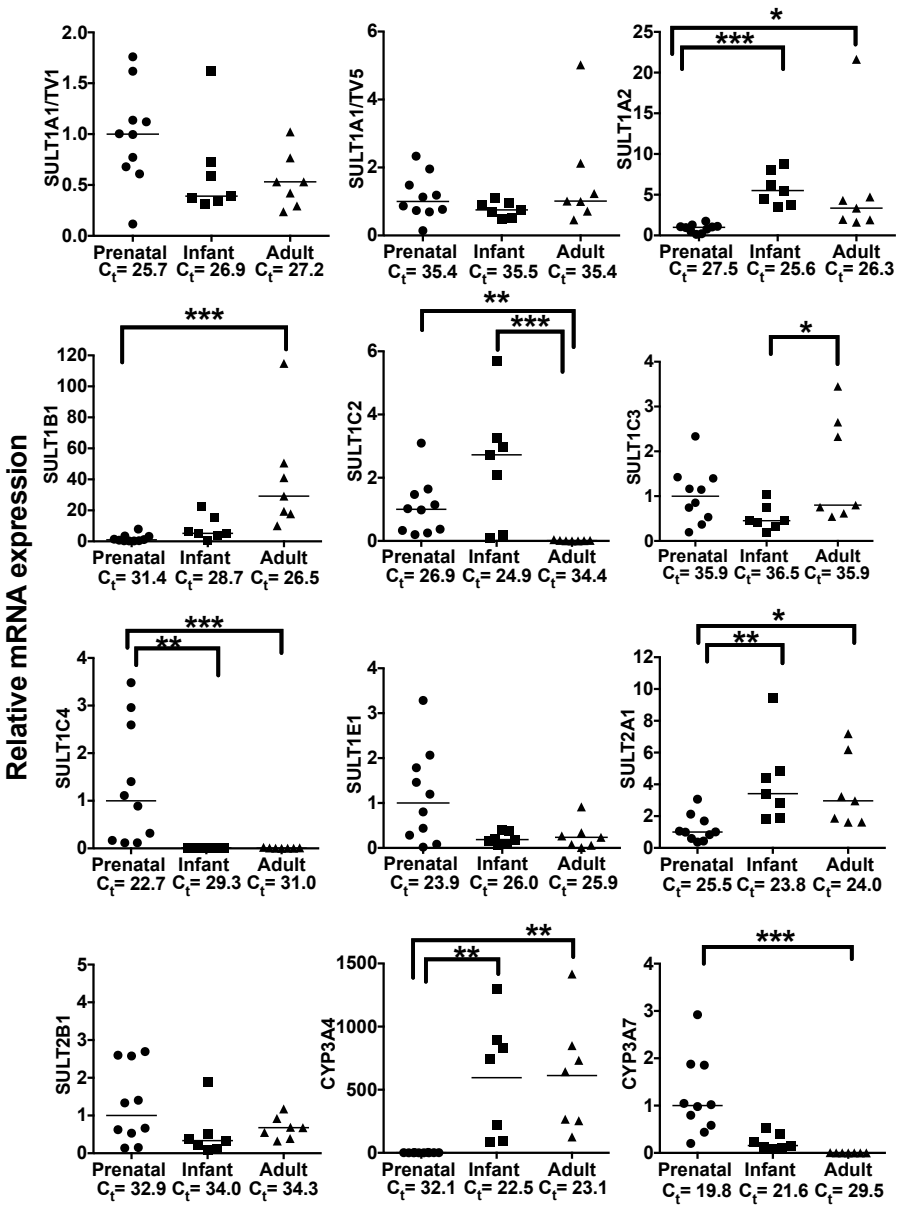


Figure 3.1: SULT mRNA Developmental expression profiles in human liver specimens analyzed by RT-qPCR.

RNA was isolated from human liver specimens from prenatal, infant, and adult donors, and levels of SULT1A1 (TV1 or TV5), 1B1, 1C2, 1C3, 1C4, 1E1, 2A1, and 2B1 were measured using TaqMan Gene Expression assays, as described in Materials and Methods. CYP3A4 and CYP3A7 mRNA levels were measured for comparison. The median cycle threshold (C_t) value for each transcript and developmental stage is shown as an estimation of abundance. For each transcript, data are normalized to the median mRNA level (C_t value) in the prenatal group and presented as scatter plots, with the horizontal lines representing the median values. *Significantly different, P< 0.05, **P<0.01, and ***P<0.001.

Table 3.1: Median Transcript per million (TPM) values for SULTs analyzed by RNA-seq

SULT Transcript	Prenatal	Infant	1-5 years	6-11 years	12-18 years
SULT1A1 (TV1/2)	11.01	14.41	7.12	10.98	17.87
SULT1A1 (TV3/4)	0.06	0.00	0.12	0.00	0.22
SULT1A1 (TV5)	0.62	0.59	0.43	0.74	1.44
SULT1A2	1.66	4.11	4.08	4.81	2.03
SULT1A3	5.40	2.91	2.40	1.44	2.18
SULT1B1	24.07	17.23	13.10	13.13	16.61
SULT1C2	8.58	2.63	0.42	0.13	0.21
SULT1C3	0.05	0.00	0.00	0.00	0.00
SULT1C4	4.44	0.83	0.31	0.22	0.21
SULT1E1	34.63	3.63	0.95	0.60	1.31
SULT2A1	10.03	68.42	84.41	40.07	150.80
SULT2B1	0.05	0.02	0.00	0.00	0.02

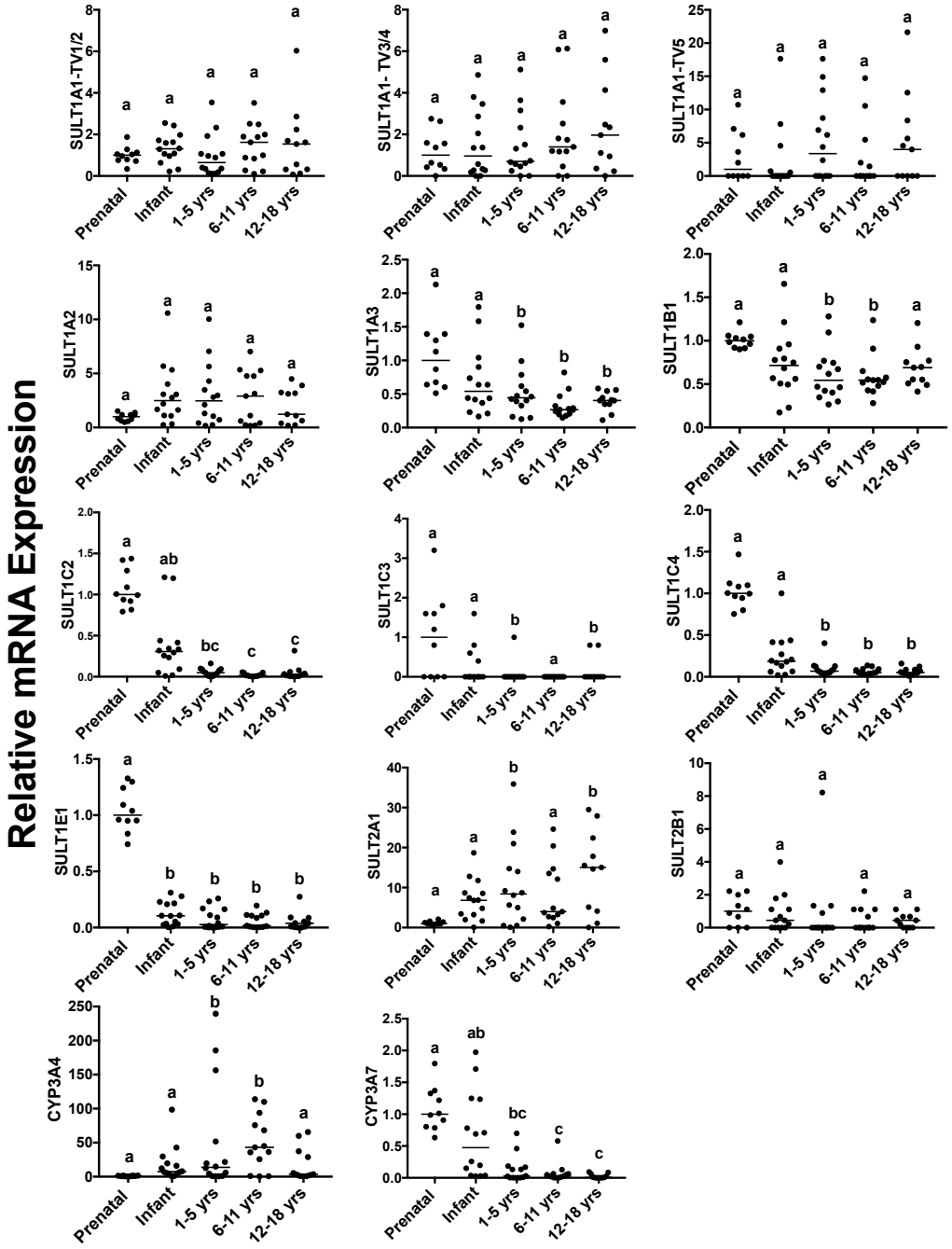


Figure 3.2: SULT mRNA Developmental expression profiles in human liver specimens analyzed by RNA-seq.

RNA-seq was performed on human liver specimens from prenatal and pediatric (divided into infant, 1-5 year-old, 6-11 year-old, and 12-18 year-old groups) donors, and TPM values for SULT1A1 (TV1/2, TV3/4, or TV5), 1A2, 1A3, 1B1, 1C2, 1C3, 1C4, 1E1, 2A1, and 2B1 and CYP3A4 and 3A7 were determined, as described in Materials and Methods. For each transcript, data are normalized to the median mRNA level in the prenatal group and presented as scatter plots, with the horizontal lines representing the median values. Groups not sharing a letter are significantly different from each other, $P < 0.05$.

Because the RT-qPCR and RNA-seq data indicated that several SULTs are expressed in prenatal liver, we explored the extent to which these genes were coordinately expressed in individual prenatal liver specimens as a potential clue to shared regulatory mechanisms. We, therefore, performed pairwise correlation analysis on the RT-qPCR and RNA-seq data for the genes that were relatively abundant in the prenatal specimens; SULT1A1 (TV 1 to 4), SULT1B1, SULT1C2, SULT1C4, SULT1E1, SULT2A1, and CYP3A7. In the specimens analyzed by RT-qPCR, SULT1C2, SULT1E1, SULT2A1, and CYP3A7 mRNA levels were highly correlated with each other (Fig. 3.3), but SULT1A1 (TV 1 to 4), SULT1B1, and SULT1C4 mRNA levels did not significantly correlate with any of the other genes that were examined (Appendix L). For the specimens analyzed by RNA-seq, SULT1A3 expression correlated with that of SULT1B1, SULT1C2 expression correlated with that of SULT1C4, and SULT1E1 expression correlated with that of SULT1A1 (TV1/2) and CYP3A7 (Fig. 3.4).

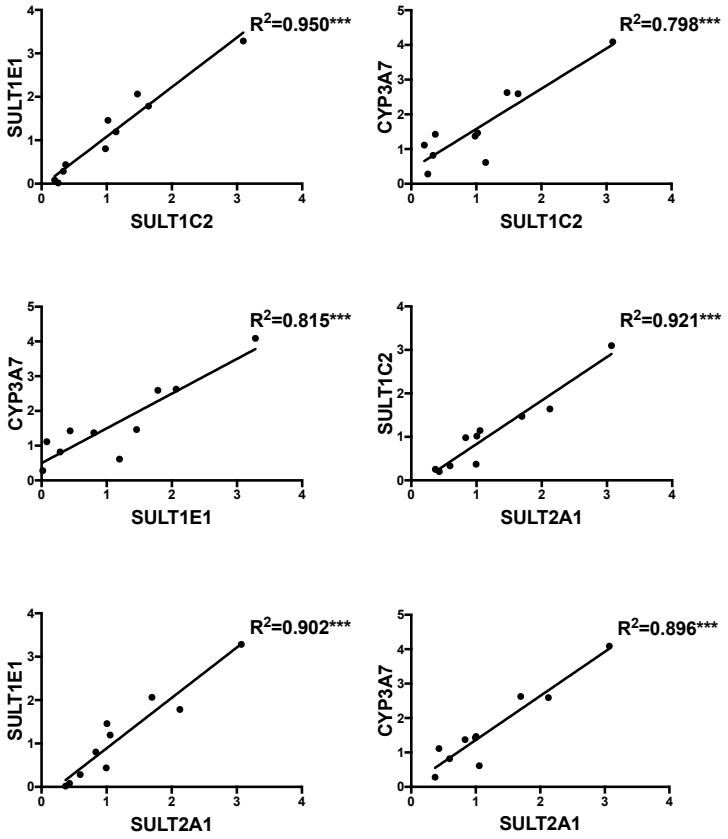


Figure 3.3: Correlation among the various SULTs and CYP3A7 mRNA measured by RT-qPCR in prenatal liver.

Pairwise correlation analyses were performed on SULT expression data from prenatal samples (n=10). Each scatter plot shows the expression data of one gene against another gene. The R^2 values from the correlation analysis are indicated. Significantly correlated, *p<0.05, ***p<0.005.

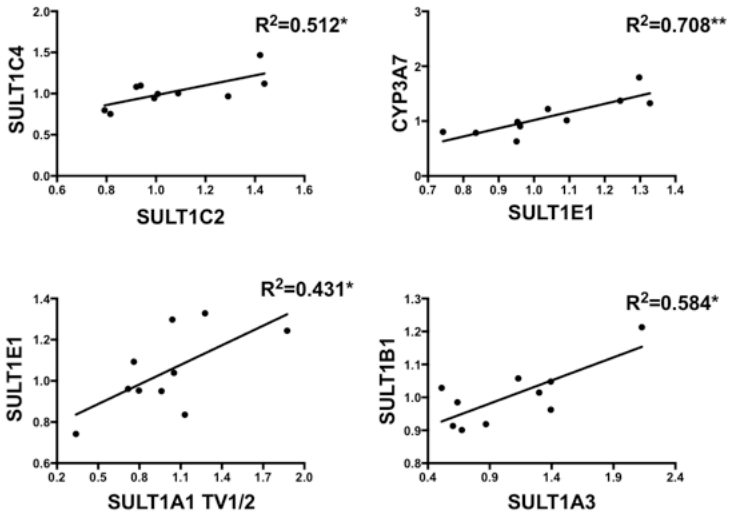


Figure 3.4: Correlation among the various SULTs and CYP3A7 mRNA measured by RNA-seq in prenatal liver.
Pairwise correlation analysis was performed on SULT1A1 (TV1/2), SULT1A3, SULT1B1, SULT1C2, SULT1C4, SULT1E1, SULT2A1, and CYP3A7 expression data in the prenatal liver (n=10). Each scatter plot shows the expression data of one gene against another. The R^2 values from the correlation analysis are indicated. Significantly correlated, *p<0.05, **p<0.01

3.3.2 SULT protein developmental expression in human liver. Because gene expression at the mRNA level does not always reflect protein abundancy, we used MRM to measure the protein levels of SULT1A1, 1A2, 1A3, 1B1, 1C2, 1C4, 1E1, and 2A1 in human liver cytosols isolated from prenatal, infant (0-12 months-old), and child (1-5, 6-11, and 12-18 years-old) donors. Half of these SULTs, SULT1A1, 1C2, 1E1, and 2A1, were detected in at least 90% of the samples that were evaluated, while the others were detected in smaller percentages of the samples (11-69%). Most SULT proteins were detected in the prenatal period, some as early as 9-10 weeks of gestation, and their prenatal levels were generally highest at the beginning of the second trimester (14-17 weeks) (Appendix M).

SULT1A1 was detected in all but two of the cytosolic fractions that were analyzed (both were prenatal samples) and was the most abundant SULT protein regardless of developmental stage (median levels of 1,130 to 2,681 fmol/mg cytosolic protein among the age brackets). SULT1A1 protein levels were relatively constant throughout the age groups that were evaluated, although the levels appeared to trend upward somewhat during later childhood (6-11 and 12-18 years) (Fig. 3.6).

SULT1A2 was detected in most (69%) of the cytosolic fractions that were analyzed, with the highest frequency of detection in children 12-18 years of age (88%). The median levels of SULT1A2 protein were highest in the two oldest age groups (195 fmol/mg in children 6-11 years and 145 fmol/mg in children 12-18 years).

SULT1A3 was detected in only 34% of the samples and was detected more frequently in samples from some of the earlier age groups [prenatal (60%) and children 1-5 years of age (48%), although not infant (16%)] than it was in samples from older children (13-19%). The median level of SULT1A3 in the prenatal samples was 144 fmol/mg.

SULT1B1 was detected in a relatively small percentage of the samples that were analyzed (38%). The percentage of detection was especially low in the prenatal samples (21%). Detection

then trended upward, from 36% of infant samples to 75% of children 12-18 years. The median level of SULT1B1 in children 12-18 was 331 fmol/mg.

SULT1C2 was detected in 95% of the samples that were evaluated. The median levels of protein were significantly higher in the prenatal samples (61 fmol/mg) than they were in the other age groups (except infants). Unlike SULT1C2 protein, and unlike the findings for SULT1C4 mRNA, SULT1C4 protein was detected in a small percentage of the samples (11%). The largest percent detection of SULT1C4 protein occurred in the prenatal samples (27%), and the majority of this was seen in the earliest ages that were evaluated (~9-16 weeks) (Fig. 3.5). No SULT1C4 protein was detected in children 6-18 years.

SULT1E1 was detected in all samples that were evaluated. SULT1E1 was the second most abundant SULT protein in prenatal liver cytosolic fractions (873 fmol/mg) and its levels were lower in the postnatal groups (104-156 fmol/mg).

SULT2A1 was detected in 90% of the samples and was detected at approximately this frequency in all age groups (87 to 92%). The median levels of SULT2A1 in cytosols from prenatal donors and children 12-18 were 45 and 158 fmol/mg, respectively.

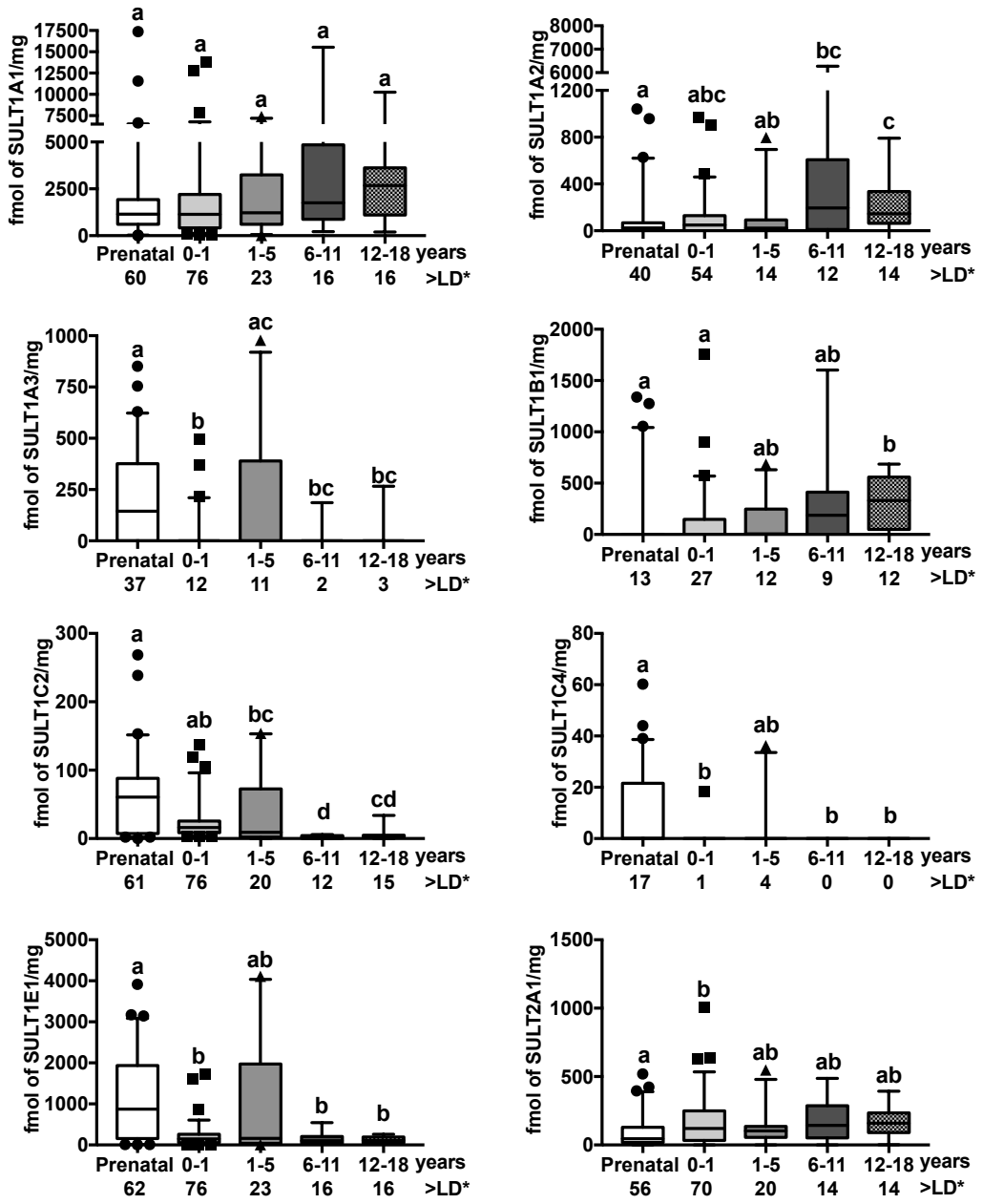


Figure 3.5: SULT protein developmental expression profiles in human liver cytosolic fractions analyzed by MRM.

SULT1A1, 1A2, 1A3, 1B1, 1C2, 1C4, 1E1, and 2A1 protein levels were measured by MRM in a library of 193 human liver cytosolic fractions from prenatal and pediatric (divided into infant, 1-5 year-old, 6-11 year-old, and 12-18 year-old groups) donors. The data for each developmental stage are presented as box and whisker plots of femtomole SULT/mg cytosolic protein. *>LD, greater than the limit of detection = the number of samples in each group with detectable levels of SULT protein. Groups not sharing a letter are significantly different from each other, $P < 0.05$.

3.4 Discussion

Exposure to drugs and other synthetic chemicals during fetal life can have serious consequences on the health of the exposed individuals as most of the xenobiotic-metabolizing enzymes that can protect against such insults are not well-developed during the prenatal period. Because the metabolic capacity of humans varies during development, the impact of foreign substances is affected by the combination of the xenobiotic-metabolizing enzymes that are expressed at the time of exposure by the fetus as well as by the mother and fetus. For example, the gastroprokinetic drug cisapride was once prescribed to treat gastroesophageal reflux and other gastrointestinal disorders in neonates and infants. However, due to low CYP3A4 activity, cisapride treatment of neonates was associated with significant adverse effects on heart rhythm (Kearns et al., 2003). Therefore, identifying the enzymes that determine the xenobiotic-metabolizing capacity of the liver during early stages of development is important, both to understand the physiological roles of these enzymes in human development and the risks that are associated with xenobiotic exposures during critical life windows of susceptibility.

To determine the developmental expression patterns of the major SULTs that have been detected in human liver, we used liver specimens that were mostly obtained from National Institutes of Health-supported tissue repositories. That human tissue RNA is susceptible to degradation due to postmortem processes as well as the handling and storage procedures that are utilized is well known (Holland et al., 2003). Although some of the liver RNA samples we used for RT-qPCR were partially degraded, we do not believe that this negatively affected our results. Previous studies have indicated that the effect of RNA quality is minimal when (1) C_t values are normalized to a reference gene, (2) PCR products are short (<200 bp), and (3) PCR reaction efficiency is high (Antonov et al., 2005; Fleige et al., 2006; Weis et al., 2007; Gonzalez-Herrera et al., 2013). In this study, we used TaqMan Gene Expression assays that produce relatively short amplicons (<200 bp) and have essentially 100% amplification efficiency, and we normalized mRNA levels to 18S RNA levels, which were relatively stable among the samples that were

included in the analysis. As for the RNA-seq data, the RQI values and total reads did not significantly correlate, suggesting that partial degradation of some RNA samples did not adversely affect the analysis. Additionally, the expression profiles of most SULTs that were determined by RT-qPCR, RNA-seq, and mass spectrometry were qualitatively similar, suggesting that RNA quality did not affect our overall conclusions.

Our analyses indicate that SULT1A1 (TV1), 1B1, 1A3, 1C2, 1C4, 1E1, and 2A1 transcripts are relatively abundant in prenatal human liver. The RT-qPCR and RNA-seq data were generally consistent, although some discrepancies were noted, such as the above-described differences in SULT1B1 and 1C4 RNA abundance in the prenatal specimens that were determined by the two methods. Additionally, the correlation analyses using the data analyzed by RT-PCR and RNA-seq we performed, suggested that the expression of several SULTs and CYP3A7 could be coregulated in prenatal liver. However, the genes that were found to be correlated by analyzing RT-qPCR data were different from these that were identified from the RNA-seq data. To some extent, these discrepancies could reflect the small differences in developmental ages of the prenatal specimens in the two datasets, as the specimens analyzed by RNA-seq were from subjects at 14-16 weeks of gestation while those analyzed by RT-qPCR were from donors at 18-19 weeks of gestation. Differences could also reflect the nature of the two RNA quantification approaches, where one specifically targets a particular region of a targeted transcript sequence and the other assembles and aggregates counts of sequence reads that map onto the human genome. However, the overall consistency of the findings, whereby generally comparable results were obtained in two independent sets of tissue specimens analyzed by two different mRNA measurement techniques, supports the validity of our findings.

In chapter 2, we showed that the most abundant SULT mRNAs (as determined by RT-qPCR) in primary cultured fetal human hepatocytes (in approximate order of abundancy) were SULT1C4 > 1E1 > 1A1 ≈ 2A1 > 1C2, which was the same order (as estimated by median Ct values) that was seen in the prenatal sample set analyzed by RT-qPCR (i.e., using the same

assays). In the HepaRG model, SULT1B1, 1C2, 1C3, 1C4, and 1E1 temporal expression patterns are consistent with the developmental expression patterns observed in the liver tissue specimens, assuming that confluent HepaRG cells represent an early differentiation stage akin to fetal liver, while differentiated HepaRG cells represent a stage that is more like adult liver.

We also determined the expression profiles of SULT proteins during liver development using a library of human liver subcellular fractions that has been previously utilized by us and others to characterize the developmental expression profiles of xenobiotic-metabolizing enzymes, including flavin-containing monooxygenase 1 and 3 (Koukouritaki et al., 2002), CYP2C9 and 2C19 (Koukouritaki et al., 2004), CYP2E1 (Johnsrud et al., 2003), CYP3A (Stevens et al., 2003), and SULT1A1, 1E1, and 2A1 (Duanmu et al., 2006). These previous studies used Western blot and enzymatic activity analyses to demonstrate that xenobiotic-metabolizing enzymes display three basic patterns of developmental expression (Hines, 2013), and that enzymes belonging to the same subfamily can have markedly different expression profiles.

In the current investigation, we used MRM to measure SULT protein contents. Mass spectrometry-based approaches have been used to detect and quantify enzymes and transporters involved in drug disposition (Groer et al., 2014; Cieslak et al., 2016; Bhatt et al., 2018). As confirmation of the approach, SULT1A1, 1E1, and 2A1 were found to exhibit the same expression profiles that we previously reported when the liver cytosolic fractions were analyzed by Western blot (Duanmu et al., 2006).

SULT1A2 mRNA and protein were detected in the liver specimens, and their levels were higher in the postnatal liver samples from donors more than 6 years of age than they were in prenatal or infant samples. These observations are at variance with a previous report that did not detect SULT1A2 protein in human hepatic or extra-hepatic normal tissue or tumor samples that were analyzed by Western blot (Nowell et al., 2005). In other earlier studies ,SULT1A2 mRNA was detected by conventional RT-PCR in some tissues including liver (Zhu et al., 1996; Dooley et al., 2000), and it was suggested that SULT1A2 mRNA could not be translated into protein

because of a splicing defect. However, two studies did detect low levels of SULT1A2 protein in adult human liver (Meinl et al., 2006; Teubner et al., 2007)

Unlike SULT1A2, SULT1A3 was preferentially expressed in prenatal specimens. In agreement with our analyses, previous studies demonstrated that SULT1A3 mRNA, protein, and enzymatic activity were detectable in fetal liver, but protein and activity were very low or undetectable in adult liver (Cappiello et al., 1991; Richard et al., 2001; Stanley et al., 2005; Riches et al., 2009), although SULT1A3 mRNA was reported to be detectable in adult liver (Wood et al., 1994; Dooley et al., 2000) and was observed by RNA-seq in the current study with samples from 12-18 year old donors.

As indicated in chapter 1.4.2, SULT1B1 is primarily expressed in the adult liver and it has been detected in the fetal liver at the mRNA level (Wang et al., 1998; Stanley et al., 2005; Meinl et al., 2006; Riches et al., 2009). In the current analysis, we determined that SULT1B1 protein levels increased during development. These findings were consistent with the observed SULT1B1 RNA expression pattern obtained by qPCR, but not RNA-seq. SULT1C2 mRNA and protein were preferentially expressed during early development, in agreement with previous studies reported in chapter 1.4.2 (Her et al., 1997; Stanley et al., 2005); one study did report detectable, but very low levels of mRNA in adult liver (Dooley et al., 2000).

Although SULT1C4 mRNA was relatively abundant in the prenatal liver specimens, its protein was present at very low levels. This discrepancy appears to be at least partially attributable to the expression of multiple SULT1C4 transcript variants in liver, some of which do not give rise to stable protein (to be discussed in chapter 4).

In this report we demonstrated that most of the SULT1 and SULT2 family members are expressed in the liver during early development, suggesting that SULTs could be involved in the regulation of physiological processes in the fetus, as well as metabolism of xenobiotics that pass through the placenta. Further studies are needed to clarify the roles of the SULTs as determinants of health and disease during gestation and throughout the human life-course.

CHAPTER 4: DEVELOPMENTAL EXPRESSION OF SULT1C4 TRANSCRIPT VARIANTS IN HUMAN LIVER

4.1 Introduction

The SULT1Cs are an understudied subfamily of enzymes that were identified and cloned using extrahepatic tissues (Her et al., 1997; Sakakibara et al., 1998; Duniec-Dmuchowski et al., 2014). Very little is known about the SULT1Cs in the liver, which is the major site of expression for most SULTs (Stanley et al., 2005; Riches et al., 2009). Data presented in chapter 2 and 3 and other previous reports indicated that SULT1C2 and SULT1C4 mRNA is preferentially expressed during early life stages. SULT1C3 expression was minimal in the hepatic tissues. While the abundance of SULT1C2 mRNA was reflected at the protein level, the expression of SULT1C4 mRNA and protein did not correlate with each other (as discussed in chapter 3). There are several transcript variants (TVs) of SULT1C4 indexed in the GenBank database, including the full-length mRNA containing seven exons (TV1, NM_006588), a variant mRNA lacking exons 3 and 4 (TV2, NM_001321770), two non-coding RNA variants (TV3, NR_135776 and TV4, NR_135779), and a predicted transcript variant (TVX1, XM_017003807). The purpose of this study was to identify the TVs that are expressed in the developing human liver and to determine the TVs that are translated into protein to gain more insight into the cause of the lack of correlation between SULT1C4 mRNA and protein expression.

4.2 Materials and Methods

Human Tissues: The human liver specimens that were used in this study are the same tissues that were analyzed by RT-qPCR and RNA-seq in chapter 3 (described in the methods section of chapter 3).

RNA isolation and gene expression analysis: Total RNA was isolated from Caco-2 colorectal adenocarcinoma cells or HepaRG cells using the Purelink RNA Mini Kit (Thermo Fisher Scientific). RNA was isolated from liver specimens analyzed by qRT-PCR and RNA-seq as described in chapter 3. RNA was reverse transcribed to cDNA and a primer set that was predicted

to amplify a 1.2 Kb fragment of the full-length SULT1C4 sequence (NM_006588.3) was used to evaluate SULT1C4 expression in Caco-2 and HepaRG cells by standard RT-PCR. The 3 resulting PCR fragments, representing different transcript variants (TVs) were ligated into pGL4.10 (Promega), sequenced, and used as synthetic standards for subsequent RT-qPCR analysis. RT-qPCR was performed using SYBR Green, a common forward primer, and reverse primers (Table 4.1) spanning an exon-exon junction that were designed to amplify one of the TVs shown in Fig. 4.2A. A standard curve of Ct versus attomol (amol) plasmid DNA was prepared for each SULT1C4 TV (Fig. 4.2B) by spiking varying amounts of the standard (100 ag to 100 pg) that were prepared by cloning the fragments shown in Fig. 4.1. For the purpose of plotting the data and calculating the amounts of each SULT1C4 TV detected in the liver specimens the amounts of each standard were converted from g to moles. The RNA content of each TV was then calculated using the following least squares line equations below generated from the standard curve for each TV and expressed as amol/µg RNA.

Equation 1 (TV1): $y = -3.639x + 13.22$

Equation 2 (TV2): $y = -4.229x + 16.04$

Equation 3 (E3DEL): $y = -3.77x + 14.91$

SULT1C4 TV expression in transfected HEK293 cells was determined using a TaqMan Gene Expression Assay (Thermo Fisher Scientific). The procedure for RNA-seq analysis was previously described in chapter 3. Transcript variant information was obtained by analyzing the RNA-seq data using StringTie analysis software (Pertea et al., 2015).

Western blot analysis: HEK293 cells were plated into 100 mm dishes and transfected with a complex consisting of a DDK-tagged pcDNA3.1 expression plasmid containing one of the SULT1C4 TVs (4 µg), pBluescript II KS+ (16 µg), and Lipofectamine 2000 (50 µl) for 72 hours. Whole cell lysates were prepared and quantified as previously described in the methods of chapter 2. Proteins (amounts indicated on the figure) were then resolved on 12.5% sodium dodecyl sulfate-polyacrylamide gels, transferred to polyvinylidene difluoride membranes, and

incubated for one hour with blocking buffer [2.5% non-fat dry milk in Tris-buffered saline with Tween 20 (Sigma-Aldrich)]. The membranes were then incubated overnight at 4°C with mouse monoclonal anti-DDK antibody (Clone OTI4C5; Origene, Rockville, MD) diluted 1:2000 followed by a two-hour incubation with horseradish peroxidase-conjugated goat anti-mouse IgG (sc-2005; Santa Cruz Biotechnology) diluted 1:20,000. The blot was stripped and reprobed with β-actin (as described in the methods of chapter 2).

Quantification of SULT1C4 TV protein by mass spectrometry: HEK293 cells were plated and transfected as described in the paragraph above. Whole cell lysates were prepared and quantified as described in methods of chapter 2. For each sample, 100 µg of protein was resolved on a 4-12% Bis-Tris gel (Thermo Fisher Scientific) in duplicate. The region between 20-43 kDa was excised and proteins were reduced, alkylated, and digested with trypsin in-gel. Peptides were eluted from the gel pieces and dried. The mass spectrometry analysis and quantification of SULT1C4 transcript variant content in the whole cell lysates was performed as described in chapter 3. Samples were analyzed in triplicate; therefore, results represent the average of three samples.

Statistical analysis: Data were analyzed as described in chapter 3.

Table 4.1: Primers used in the RT-qPCR analysis

Primer Name	Sequence
SULT1C4- 1.2 Kb forward	5'-TAG AGG GCT GGA TAG TGT GGT AGT G-3'
SULT1C4- 1.2 Kb reverse	5'-GAC ATG GAG AGA GGG AAG CTC AAT-3'
SULT1C4- forward	5'-CCT ATC CTA AAG CAG GAA CAA CA-3'
SULT1C4- TV1 reverse	5'-ATG AGC TTG TTC CAA ACC AG-3'
SULT1C4- TV2 reverse	5'-CAG GAG CCC CAG CAC ACA G -3'
SULT1C4- E3DEL reverse	5'-GGA TTT CTT GCT ACA TAG ATT ATC AG -3'

4.3 Results

4.3.1 Identification of SULT1C4 TVs expressed in human intestinal and hepatic cell lines.

Freimuth et al. is the only report that examined human SULT1C4 cDNA using fetal lung cDNA and 5'-RACE analysis (Freimuth et al., 2000). In the current analysis we used HepaRG and Caco-2 cDNA to investigate the SULT1C4 transcript variants that are expressed in human hepatic and intestinal cells. Using primers predicted to amplify an approximately 1.2Kb fragment of the full-length SULT1C4 (TV1) coding region, 1.2 Kb (upper band), 1.1 Kb (middle band), and 1 Kb (lower band) fragments were detected for both HepaRG and Caco-2 cells (Fig. 4.1). Sequencing of the fragments and alignment analysis revealed that the middle band represents a transcript that is missing exon 3 (E3DEL) and that the lower band is missing exons 3 and 4 and aligns with the TV2 that is reported in GenBank. These data indicated the presence of three potential transcripts that are co-expressed in human intestinal and liver cells.

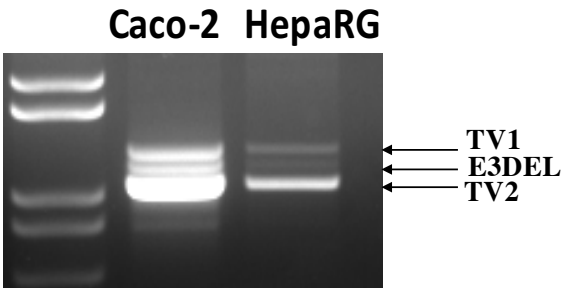


Figure 4.1: Amplification of three SULT1C4 TVs from Caco-2 and HepaRG cells.
RNA was isolated from Caco-2 and HepaRG cells and reverse transcribed to cDNA. PCR was performed using primers predicted to amplify an approximately 1.2Kb fragment of the full-length SULT1C4 (TV1) coding region. PCR products were resolved on a 1% agarose gel.

4.3.2 Expression of SULT1C4 TVs in the developing liver. To characterize the developmental expression patterns of SULT1C4 TVs expressed in the human liver, we used two sets of human liver specimens that were isolated from prenatal and postnatal donors. Each set of liver specimens was analyzed separately using either RT-qPCR or RNA-seq analysis.

As shown in figure 4.2C, prenatal, infant, and adult liver specimens analyzed by RT-qPCR expressed the three SULT1C4 TVs. All three TVs were primarily expressed in prenatal liver and their expression decreased markedly in the postnatal periods. TV2 was the most abundant transcript in prenatal, infant, and adult specimens. TV2 mRNA in prenatal liver (6.0 fmol/μg) was ~5-fold higher than that of TV1 (1.3 fmol/ug). Expression of the E3DEL transcript was minimal in all liver specimens. SULT1C4 transcript variant information generated by analyzing a library of prenatal and pediatric (i.e., infant and children 1-18 years-old) liver specimens using RNA-seq identified three TVs expressed in the liver, TV1, TV2, and non-coding TV. These TVs were also preferentially expressed in the prenatal livers and TV2 was the most abundantly expressed TV whereas the levels of expression of TV1 and the non-coding RNAs were much lower.

4.3.3 SULT1C4 TV protein abundance. To investigate the reason for the lack of correlation between SULT1C4 RNA and protein in the developing liver (reported in chapter 3), we examined the SULT1C4 TV protein content in whole cell lysates prepared from HEK293 cells transfected with DDK-tagged TV1, TV2, or E3DEL expression plasmid. The results of the Western blot analysis indicated that only TV1 and TV2 were expressed at the protein level (Fig. 4.3A). Contrary to our observation at the RNA level, TV1 protein was more abundant that of TV2 (Fig. 4.3A). When the E3DEL amino acid sequence was computationally examined using SnapGene software (GSL Biotech, Chicago, IL), we found that deletion of exon 3 causes a frameshift mutation that introduces a premature stop codon, which explains the absence of E3DEL protein. We also measured the mRNA levels of the three SULT1C4 transcripts using RT-qPCR to confirm that the differences in protein levels were not caused by variation in the transfection or transcription

efficiency. In Fig. 4.3B, we showed that the mRNA levels of TV1, TV2, and E3DEL were relatively the same (Fig. 4.3B),.

As some factors, such as the binding affinity of the protein to the membrane during transfer in Western blot analysis, can influence the results obtained from Western blot, we used MRM to quantify TV1 and TV2 protein in the transfected HEK293 cells. Our results demonstrated that TV1 and TV2 were both expressed in the whole cell lysates prepared from transfected HEK293 cells, but only TV1 protein level was quantifiable [5.31 fmol/mg] (Table 4.2).

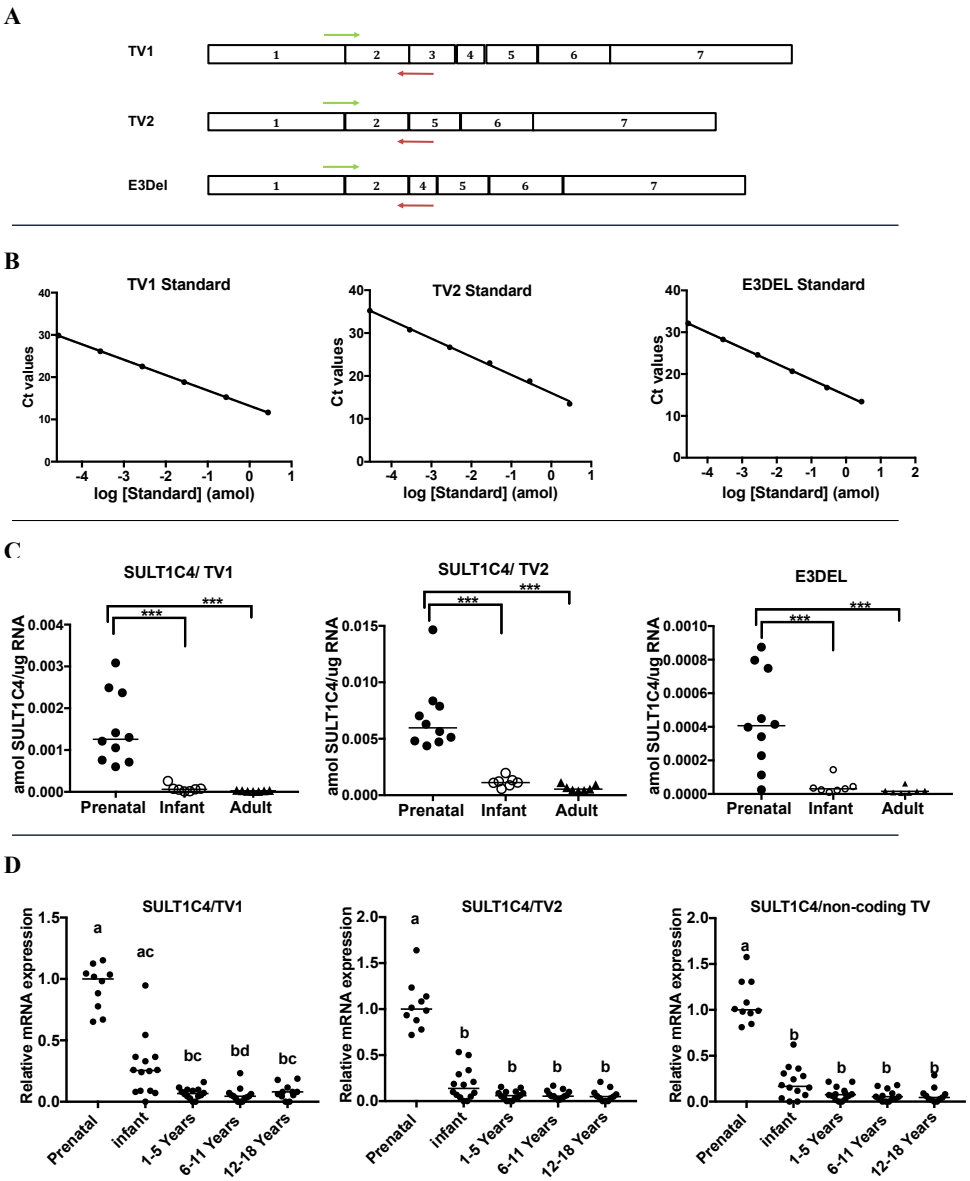


Figure 4.2: Developmental expression of SULT1C4 TVs in human liver.
(A) Schematic representation of the SULT1C4 TVs that were identified in Caco-2 and HepaRG cells and the primers designed to detect these transcripts individually. (B) A standard was prepared for each SULT1C4 transcript by cloning the 3 fragments shown in Fig. 4.1, and standard curves were prepared to permit quantification of transcript amounts. (C) RNA was isolated from specimens of prenatal (n=10), infant (n=7), and adult (n=7) human liver, and SULT1C4 TV levels were measured using RT-qPCR. For each TV, data were grouped according to developmental stage and are expressed as amol SULT1C4 transcript/µg of RNA. Data are shown as scatter plots with the horizontal lines representing the median values. ***Significantly different P< 0.001. (D) RNA was isolated from prenatal (n=10) and pediatric (n=52) human liver specimen samples and analyzed by RNA-seq. Data are shown as scatter plots with the horizontal lines representing the median values. Groups not sharing a letter are significantly different from otherone an, p<0.05.

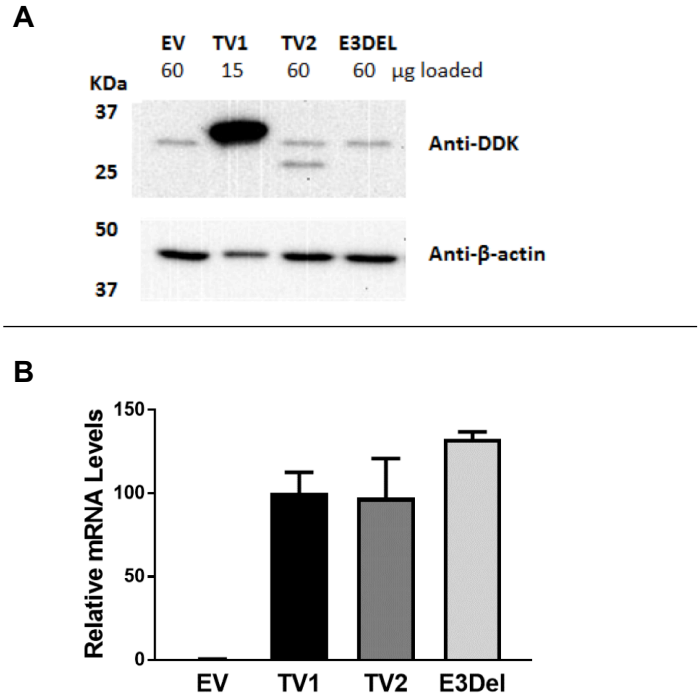


Figure 4.3: SULT1C4 protein and mRNA levels after transfection of expression plasmids for individual TVs into HEK293 Cells.

Whole cell lysates (A) and RNA (B) were prepared from HEK293 cells that were transfected with DDK-tagged TV1, TV2, or E3DEL expression plasmid and analyzed by RT-qPCR and Western blot, respectively. The results shown are from one representative experiment. Similar results were obtained from two additional experiments. EV, empty vector.

Table 4.2: SULT1C4 TVs' protein quantification in whole cell lysates of transfected HEK293 cells by MRM.

Sample	Amount (fmol/mg)
EV	N/A
TV1	5.31± 0.27
TV2	<LD

<LD stands for below limit of detection

4.4 Discussion

Xenobiotic-metabolizing enzymes that are expressed during early developmental periods are crucial in determining the influences of xenobiotic exposures on the developing fetus. Although they generally play a protective role, detoxification enzymes can increase the mutagenicity of many compounds that enter the body, and could therefore enhance the susceptibility of the fetuses to cancers in tissues that are exposed to bioactivated procarcinogens (Banoglu, 2000). Many studies have reported the detection of SULT mRNA, protein, and enzyme activity in various human tissues isolated from prenatal donors (Hines, 2008), indicating that these enzymes are involved in xenobiotic metabolism and the regulation of physiological functions during gestation.

SULT1C4 mRNA was reported to be abundantly expressed in fetal lung and kidney (Sakakibara et al., 1998). Using two separate sets of human liver specimens isolated from prenatal and postnatal donors and *in vitro* models of human liver development (i.e., HepaRG cells and primary cultures of fetal hepatocytes), we demonstrated that SULT1C4 mRNA is primarily expressed in prenatal period or undifferentiated hepatocytes, respectively (presented in chapters 2 and 3). Recent studies indicated that SULT1C4 has high sulfonation capacity towards a wide range of drugs, environmental pollutants, and procarcinogens (Table 1.1). Guidry et al. recently reported that SULT1C4 can activate various estrogenic compounds, including dietary flavonoids and environmental estrogens (Guidry et al., 2017). These findings suggested that SULT1C4 could metabolize exogenous substrates and regulate hormone signaling pathways in human fetuses. Therefore, it is important to determine the tissue-specific and developmental expression patterns of SULT1C4 to improve our understanding of its role in modulating the susceptibility of human tissues to chemical exposures and regulating physiological functions, including hormone activity.

In the current analysis we identified at least four SULT1C4 transcript variants that were co-expressed in human hepatic and intestinal cells and human liver specimens that were analyzed

by RT-qPCR and RNA-seq. The four SULT1C4 transcripts were preferentially expressed in the prenatal livers in agreement with our findings in chapter 3, where we determined that SULT1C4 mRNA is preferentially expressed in the prenatal liver using the same two sets of samples that were analyzed in this study.

Our RT-qPCR and RNA-seq analyses indicated that TV2 is the most abundant transcript in human livers. By aligning the amino acid sequences of the TV1- and TV2-encoded proteins, also referred to as isoforms a (NP_006579.2) and b (NP_001308699.1) in the NCBI database, we determined that isoform b has a 75-amino acid deletion. The deletion does not cause a frameshift mutation, and thus the remaining amino acid sequence of isoform b aligns perfectly with that of isoform a (except for a single amino acid change). However, because the active site of SULT1C4 (catalytic histidine residue) is located within exon 3 (personal communication from collaborator, Dr. Charles Falany, University of Alabama at Birmingham), TV2, which lacks exons 3 and 4, cannot encode an active sulfotransferase enzyme.

Unlike TV1, the protein encoded by TV1 is more abundant than that of the TV2-encoded protein. This finding could explain the lack of correlation between SULT1C4 mRNA and protein in the human liver specimens and cytosolsic fraction, respectively, that was reported in chapter 3. Sequence changes at the amino acid level can impact enzyme function, activity, stability, binding, and dissociation (Yue et al., 2005; Capriotti et al., 2012; Bhattacharya et al., 2017). Therefore, it is plausible that the discrepancy in the abundancy between TV2 mRNA and protein is because of the decreased stability of the TV2 protein, which could be caused by the deletion of sequences within exon 3 and 4 that are essential for maintaining the stability of the protein.

The findings of this study suggest that SULT1C4 might not play a major role in the developing liver, but it is possible that this enzyme could be involved in metabolizing xenobiotics and endogenous molecules in other tissues, including kidney and lung, where it was previously detected (Sakakibara et al., 1998). Further investigation is required to understand the functional implications of SULT1C4 TV expression in hepatic and extra-hepatic human tissues, primarily

during early developmental periods, and to determine the reason behind the discrepancy between the expression of TV2 at the mRNA and protein levels.

CHAPTER 5: TRANSCRIPTIONAL REGULATION OF HUMAN CYTOSOLIC SULFOTRANSFERASE 1C3 BY PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORγ IN LS180 HUMAN COLORECTAL ADENOCARCINOMA CELLS

5.1 Introduction

SULT1C3 is one of the least characterized human SULTs. The computationally predicted SULT1C3 gene consisted of seven protein coding-exons that span 18 Kb and an initial coding exon (exon 2) that has 3 in-frame ATG translation start sites (Freimuth et al., 2004). SULT1C3 gene was also predicted to have a duplication of exons 7 and 8 that could theoretically be transcribed and processed into four splice variants containing exons 7a/8a, 7a/8b, 7b/8a, or 7b/8b (the first TV to be indexed in the NCBI database) that encode isoforms SULT1C3a-d, respectively (Freimuth et al., 2004). In a later study, SULT1C3d putative cDNA was identified using computational analysis, suggesting that the this TV is the most favorably expressed SULT1C3 TV (Meinl et al., 2008a). Substrates of the SULT1C3d enzyme, including benzylic alcohols, cholesterol, and lithocholic acid, were identified by expressing the recombinant enzyme in bacteria (Allali-Hassani et al., 2007; Meinl et al., 2008a). Also, a recent study reported that SULT1C3 had the highest sulfonation capacity of twelve human SULTs tested towards tolvaptan, which is a selective vasopressin V_2-receptor antagonist that possesses a benzylic hydroxy group (Fang et al., 2015). Previous studies did not detect SULT1C3d, which is encoded by SULT1C3 mRNA reference sequence (NM_001008743), in any of the twenty human tissues that were used for expression profiling (Freimuth et al., 2004; Meinl et al., 2008a). However, using reverse transcription-polymerase chain reaction and rapid amplification of cDNA ends (RACE) analysis, Dr. Kocarek's lab characterized and detected SULT1C3 mRNA containing exons 7a/8a, encoding SULT1C3a, in human small intestine and colon and in LS180 colorectal adenocarcinoma cells (Duniec-Dmuchowski et al., 2014). They also found that SULT1C3 mRNA is up-regulated by activation of several nuclear receptors, including PPAR α and γ agonists (Rondini et al., 2014). The purpose of this study was to determine the mechanism responsible for the PPAR-mediated

transcriptional up-regulation of SULT1C3 in LS180 cells by identifying functional PPRE(s) in a polymorphic region of the SULT1C3 promoter.

5.2 Materials and Methods

Materials: Ciprofibrate was provided by Sterling Winthrop Pharmaceuticals Research Division (Rennselaer, NY). 4-[[[2-[3-fluoro-4-(trifluoromethyl)phenyl]-4-methyl-5-thiazolyl]methyl]thio]-2-methylphenoxyacetic acid (GW0742) was obtained from Tocris. Sources of the following materials were all listed in the methods section of chapter 2: DMSO, GW3965, GW4064, GW7647, rosiglitazone, rifampicin, TCDD, cell culture media and supplements, Lipofectamine 2000, and oligonucleotides. Targets of the agonists used is listed in Appendix E.

Cell culture: LS180 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, MEM non-essential amino acids, 100 U/ml penicillin, and 100 µg/ml streptomycin. HEK293 cells were provided by Dr. Ye-Shih Ho (Wayne State University) and cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. Cells were maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

Preparation of SULT1C3 reporter plasmids: Genomic DNA was isolated from MCF10A and LS180 cells using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Genomic DNA (100 ng), Herculase II Fusion DNA Polymerase (Agilent Technologies, Santa Clara, CA), and a primer set that was predicted to amplify a 2.8 Kb fragment of the SULT1C3 5'-flanking region (nt -2780:+38), as identified by our previous 5'- RACE analysis, were used for PCR (Duniec-Dmuchowski et al., 2014). The resulting PCR fragments were ligated into the KpnI and XhoI sites of the promoterless pGL4.10[luc2] firefly luciferase reporter plasmid (Promega Corporation, Madison, WI). The plasmid containing the 2.8 Kb insert was used as a template to prepare a construct containing ~1 Kb of the SULT1C3 5'-flanking sequence (nucleotides -1008:+38), which was subsequently used

as a template to prepare a series of deletion constructs that were designed based on the positions of three PPRE motifs, at nucleotides -769, -446, and -383, that were predicted by computational analysis using MatInspector (Genomatix, Ann Arbor, MI) (Quandt et al., 1995; Cartharius et al., 2005). All primer sequences are shown in Table 5.1. The sequences of all SULT1C3 clones were confirmed using the services of the Applied Genomics Technology Center at Wayne State University.

Site-directed mutagenesis of PPRE motifs: Mutations were introduced into the three predicted PPREs using the wild-type or singly mutated (at the -446 PPRE) SULT1C3 1 Kb construct as template and the QuikChange II Site-Directed Mutagenesis Kit according to the manufacturer's instructions (Agilent Technologies, Santa Clara, CA). The mutagenic primers are listed in Table 5.1.

Transient transfection analysis and treatments: Approximately 250,000 LS180 cells/well in 1 ml of supplemented MEM were plated into 12-well plates. Cells were transfected 48 to 72 hours after seeding with a complex containing 4 µl Lipofectamine 2000, 1.6 µg of a firefly luciferase reporter plasmid, and 1 ng pRL-CMV (Promega) per well diluted in 400 µl Opti-MEM (Life Technologies). 24 hours after transfection, fresh supplemented MEM was added containing either DMSO (0.1% final concentration) or a transcription factor activator (at concentrations indicated in the text below and figure legends). Treatment medium was changed after 24 hours. Cells were lysed, collected, and analyzed as described in chapter 2.2.

PPARγ *in vitro* binding assay: A PPARγ expression plasmid (pTR151) was provided by Dr. Todd Leff (Wayne State University). HEK293 cells were plated into 100 -mm dishes and transiently transfected with a complex containing 50 µl Lipofectamine 2000, 4 µg of PPARγ expression plasmid, 0.8 µg of a Tet-off plasmid, and 15.2 µg of pBluescript II KS^+ (Agilent Technologies). Forty-eight hours after transfection, cells were harvested and nuclear extracts were prepared using the NucBuster Protein Extraction Kit (Millipore, Billerica, MA). Competitive binding experiments were performed using the TransAM PPARγ Kit (Active Motif, Carlsbad, CA), an

ELISA-based assay. Each well in the 96-well plate contained an immobilized biotinylated oligonucleotide probe that included a consensus PPRE sequence. Competitor oligonucleotides containing the wild-type or mutated SULT1C3 PPRE (at nt -769) or CYP4A1 PPRE as positive control (Aldridge et al., 1995) were designed (sequences are shown in Table 5.1), purchased, and annealed by heating at 95ºC for 5 minutes followed by slowly cooling to room temperature. Binding assays were performed according to the manufacturer's instructions. Incubations included 5 µg of nuclear protein extract and 0, 30, or 100 pmol of competitor oligonucleotide, and were performed for one hour. PPARγ binding to the biotinylated probe was determined by colorimetric analysis using a microplate reader (CLARIOstar, BMG LABTECH, Cary, NC) at wavelengths of 450 and 655 nm.

RNA interference: LS180 cells were plated into 12-well plates and cotransfected with 1.6 µg of the SULT1C3 1 Kb reporter plasmid and 20 pmol of an siRNA pool targeting PPARα, PPARδ, or PPARγ mRNA (ON-TARGETplus SMARTpool siRNA; Dharmacon, Lafayette, CO) or a negative control siRNA pool (ON-TARGETplus non-targeting control siRNA, Dharmacon). Cells were transfected using 4 µl Lipofectamine 2000 and 1 ng pRL-CMV per well. Twenty-four hours after transfection, cells were treated with DMSO (final concentration of 0.1%), rosiglitazone (1 µM), GW7647 (10 µM), or GW0742 (10 µM). Treatment medium was changed after 24 hours. After 48-hour treatments, cells were collected to measure firefly and *Renilla* luciferase activities as described above. A luciferase reporter containing the PPRE from the promoter of the CYP4A1 gene was used as a positive control for detection of PPAR knockdown (Kocarek and Mercer-Haines, 2002).

Genotyping analysis: Genomic DNA used for the genotyping analysis was isolated from human liver samples (n = 77). 100 ng of genomic DNA, HotStarTaq Plus DNA Polymerase, and two primer sets (listed in table 5.1 and shown in Fig. 5.6A) were used to PCR amplify the SULT1C3 alleles in the liver samples. The primer set A and B amplifies the full-length allele (1174 bp) or the deleted allele (328 bp) whereas the primer set B and C amplifies only the full-length allele (645

bp). The PCR products were sequenced and aligned to the 2.8 Kb fragment from the promoter region of the SULT1C3.

Statistical analysis: Statistical analysis was performed using GraphPad Prism (version 6; GraphPad, La Jolla, CA). Data were analyzed using one-way analysis of variance (ANOVA) followed by the Neuman-Keuls post-hoc test. $p<0.05$ was considered significantly different. Data are presented as means ± SD relative to DMSO-treated control. In each experiment, all treatments were performed in triplicate. Each experiment was repeated three times.

Table 5.1: Primers used to prepare reporter constructs and for genotyping analysis and oligonucleotides used in the PPARγ *in vitro* binding assay

Primer Name	Sequence
SULT1C3- TSS Reverse*	5'-GGG CTC GAG GCT CCA GGA CAC TGT GCA AGC AA-3'
SULT1C3-2.8Kb Forward	5'-GGG GGT ACC TCT GGT CCT CCT TCA TTC CCG SUCAA-3'
SULT1C3- 1Kb Forward	5'-GGG GGT ACC ATG CTC TAC ATA ATT CAC GTC-3'
SULT1C3-DEL1 Forward	5'-GGG GGT ACC ACA GAG GAC AGA CAA TGT AAA T-3'
SULT1C3-DEL2 Forward	5'-GGG GGT ACC TTT TAT TAC AGG CCT TGT GGT-3'
SULT1C3-DEL3 Forward	5'-GGG GGT ACC TTT CTA CAG GGT CAA AGG GA-3'
SULT1C3-DEL4 Forward	5'-GGG GGT ACC AAC AGG ATG AAA TAA TTG TGC-3'
SULT1C3- MUT#1 Sense	5'-GGA GTT AAG TAA ATA TTG TAC AGA AGG TAT TGT TAA AAT TCC ATA TAT TTA CAT TGTT CTG TCC TCT GTT TTG CAA-3'
SULT1C3- MUT#2 Sense	5'-CCG TAG TTA AAA TTG GTG TAG AAG AAA AAG CTT TTT AGG AAA CCA CAA GGC CTGT TAA AAC-3'
SULT1C3- MUT#3 Sense	5'-ACT TGC ACA ATT ATT TCA TCC TGT TCC CTG GAT CCC TGT AGA AAA TAT ATT CTA TTG CCT CT-3'
SULT1C3- WT PPRE Sense	5'-AAC AAT GAA CTC TGT ACA ATA TTT -3'
SULT1C3- MUT PPRE Sense	5'-AAC AAT ACC TTC TGT ACA ATA TTT -3'
CYP4A1- WT PPRE Sense	5'-GAA ACT AGG GTA AAG TTC AGT GAG -3'
CYP4A1- MUT PPRE Sense	5'-GAA ACT CGG AGC ACG TTA AGT GAG -3'
SULT1C3- Forward primer A	5'-AGC CAA GTG TAA TGA TGA TAT GAA CC-3'
SULT1C3- Reverse primer B	5'-TTG CTG CCT TTA GTC AAA CTG CT-3'
SULT1C3- Forward primer C	5'-CGA CAT TCT TGC CCT GAA ATA CAC A-3'

*The same reverse primer was used to prepare the 2.8Kb, 1.9Kb, 1Kb, DEL1, DEL2, and DEL3 SULT1C3 fragments.

5.3 Results

5.3.1 Evaluation of the SULT1C3 5'-flanking region for responsiveness to transcription factor activators. Dr. Kocarek's lab previously reported that SULT1C3 mRNA levels in LS180 cells were increased by treatments with several transcription factor activators, including ciprofibrate (PPARα), rosiglitazone (PPARγ), GW4064 (FXR), GW3965 (LXR), rifampicin (PXR), and TCDD (AhR) (Rondini et al., 2014). To determine the mechanisms underlying the regulation of SULT1C3 by these transcription factor activators, luciferase reporter constructs containing portions of the 5'-flanking region of SULT1C3 were prepared. While attempting to amplify a 2.8 Kb fragment (-2789: +36) using human genomic DNA from two different sources, LS180 cells and the MCF10A mammary epithelial cell line, both the expected 2.8 Kb fragment and a 1.9 Kb fragment were generated from LS180 cells, while only the 1.9 Kb fragment was amplified from MCF10A cells (Fig. 5.1A). Sequencing of the two fragments revealed that the 1.9 Kb fragment had an internal deletion of 863 nt (-1008: -146) relative to the 2.8 Kb fragment (Fig. 5.1A). LS180 cells were transiently transfected with a reporter plasmid containing the 2.8 Kb or 1.9 Kb fragment and then treated for 48 hourhours with 100 μM ciprofibrate, 10 μM rosiglitazone, 1 μM GW4064, 10 μM GW3965, 30 μM rifampicin, or 0.01 μM TCDD. Of these treatments, ciprofibrate, rosiglitazone, GW3965, and GW4064 significantly increased luciferase activity from the 2.8 Kb reporter construct, while rifampicin and TCDD had no effect. However, the GW3965 and GW4064 treatments also increased luciferase activity from the empty reporter plasmid, indicating that these treatments did not activate elements within the 2.8 Kb SULT1C3 5'-flanking region. Ciprofibrate and rosiglitazone did not increase the activity of the 1.9 Kb reporter construct (Fig. 5.1B), suggesting that the 863 nt deletion region contained essential elements for PPAR-mediated activation of SULT1C3 transcription. Rosiglitazone treatment also significantly activated a reporter construct containing only the 863 nt deletion and more proximal promoter region (1 Kb construct; nt -1008: +36), further supporting the importance of the 863 nt deletion region for PPAR-mediated SULT1C3 transcriptional activation (Fig. 5.2A).

5.3.2 Concentration-dependent effects of PPARα, PPARδ, and PPARγ agonists on SULT1C3 transcriptional activation. To evaluate the SULT1C3 863 nt deletion region further for its responsiveness to PPAR activation, we determined the concentration-dependent effects of agonists for the three PPARs on SULT1C3 reporter activity. For these studies, LS180 cells were transfected with the 1 Kb reporter construct and then treated with varying concentrations (0.1-10 μM) of rosiglitazone (PPARγ), GW7647 (PPARα), or GW0742 (PPARδ). Rosiglitazone treatment maximally increased reporter activity at the lowest concentration tested, which is consistent with its high potency for PPARγ (Fig. 5.2B). While GW7647 and GW0742 also increased reporter activity, the sub-micromolar concentrations that would reflect the high reported potencies of these compounds for their respective receptors had little or no effect, while the higher concentrations that might reflect cross-activation of another PPAR produced concentration-dependent increases that reached ~3-fold at 10 μM, which is comparable to the magnitude of increase produced by rosiglitazone (Fig. 5.2B). Treatment with 10 μM GW7647 and GW0742 also induced luciferase activity from the reporter containing the longer 2.8 KB region but not the 1.9 Kb construct lacking the 863 nt fragment (Fig. 5.2C), as was seen for ciprofibrate and rosiglitazone (Fig. 5.1B).

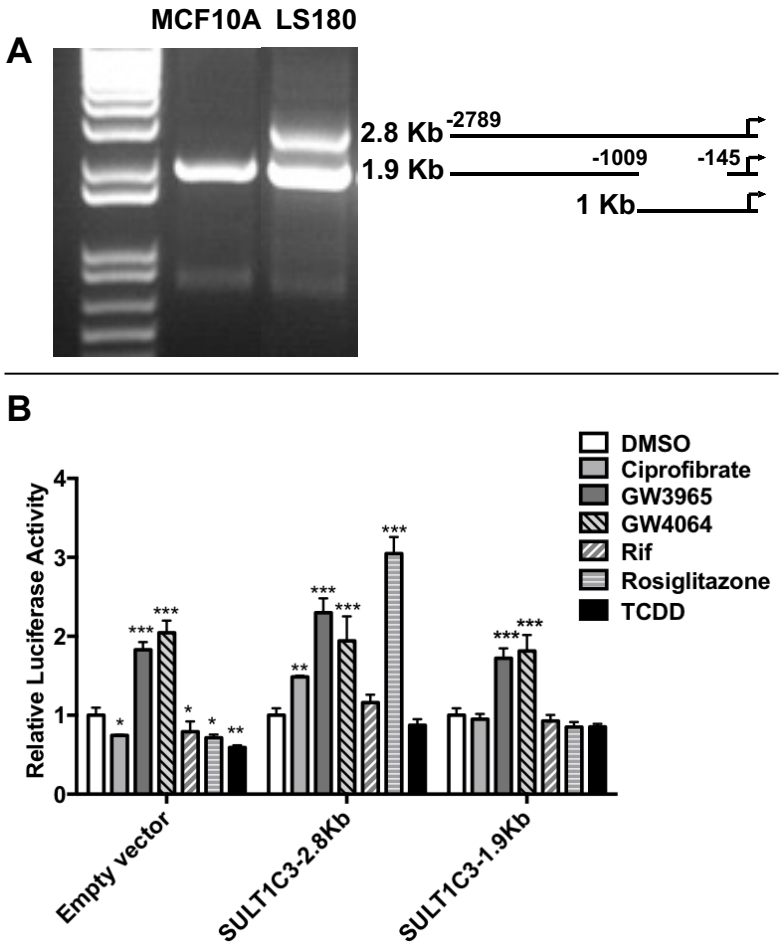


Figure 5.1: Impact of a deletion in the 5'-flanking region of the SULT1C3 gene on its regulation by transcription factor activators.
(A) PCR was performed with primers designed to amplify a 2.8 Kb fragment of the SULT1C3 5'-flanking region using genomic DNA from MCF10A or LS180 cells. The PCR products were resolved on a 1% agarose gel. A schematic representation of the 2.8, 1.9, and 1 Kb SULT1C3 5'-flanking fragments is shown adjacent to the gel image. (B) LS180 cells were transiently transfected with the SULT1C3-2.8 Kb or 1.9 Kb luciferase reporter plasmid or with the pGL4.10 [luc2] empty reporter vector and then treated with DMSO (0.1%), ciprofibrate (PPARα agonist, 100 μM), GW3965 (LXR, 10 μM), GW4064 (FXR, 1 μM), rifampicin (PXR, 30 μM), rosiglitazone (PPARγ, 10 μM), or TCDD (AhR, 0.01 μM) for 48 hours. The cells were then harvested for measurement of luciferase activities. Each column represents the mean ± S.D. of normalized (Firefly/*Renilla*) luciferase measurements relative to DMSO control (n=3 wells per treatment) from one cell culture experiment. Similar data were obtained in two additional independent experiments. *, **, ***Significantly different from DMSO-treated controls at P< 0.05, 0.01, and 0.001, respectively.
Figure taken with permission from (Dubaisi et al., 2016)

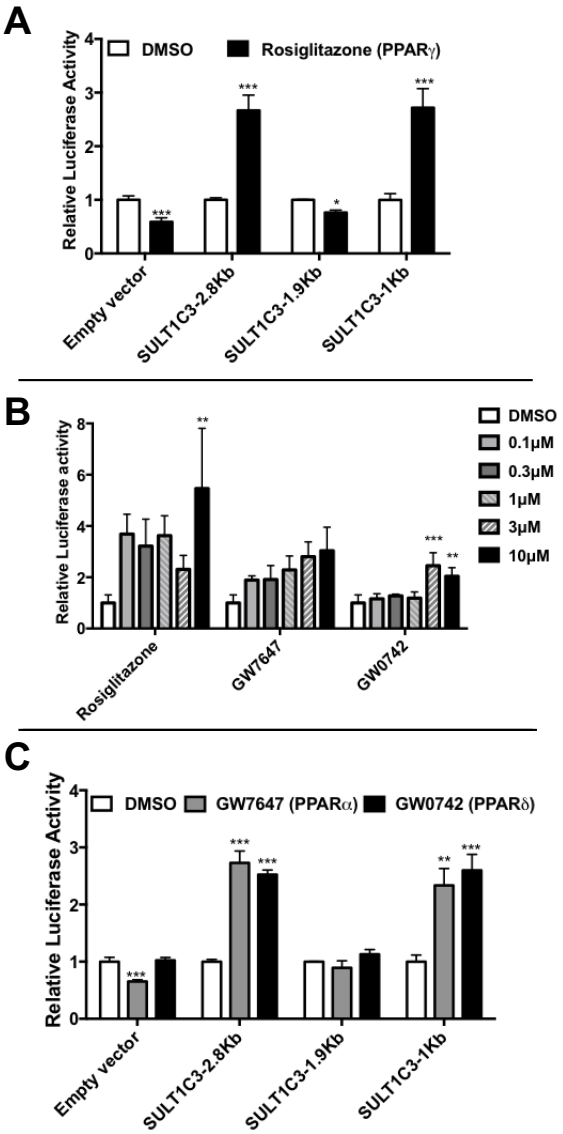


Figure 5.2: Effects of PPAR agonists on transcription of SULT1C3 reporter constructs. (A) LS180 cells were transiently transfected with a luciferase reporter plasmid containing either the 2.8 Kb or 1.9 Kb SULT1C3 5'-flanking region fragment, with the 1Kb reporter containing the deleted region, or with control empty vector. 24 Hours after transfection the cells were treated with DMSO (0.1%) or rosiglitazone (10 μM) for 48 hours. (B) LS180 cells transfected with the 1 Kb reporter were treated with DMSO (0.1%) or with 0.1 to 10 μM rosiglitazone (PPARγ agonist), GW7647 (PPARα), or GW0742 (PPARδ) for 48 hours. (C) LS180 cells transfected with the 2.8 Kb, 1.9 Kb, or 1 Kb reporter or with control empty vector were treated with DMSO (0.1%), GW7647 (10 μM), or GW0742 (10 μM). The cells were then harvested for measurement of luciferase activities. Each column represents the mean ± S.D. of normalized (Firefly/*Renilla*) luciferase measurements relative to the corresponding DMSO control (n=3 wells per treatment) from one cell culture experiment. Similar data were obtained in two additional independent experiments. *, **, ***Significantly different from DMSO-treated control at P< 0.05, 0.01, and 0.001, respectively.
Figure taken with permission from (Dubaisi et al., 2016)

5.3.3 Identification of a functional PPRE within the 863 nt deletion region of the SULT1C3 gene.

Computational analysis identified three putative PPREs within the 1 Kb reporter fragment (-1008: +36) located -769, -446, and -383 nt upstream from the transcription start site. To determine the functionality of these predicted PPREs, we first prepared four reporter constructs that progressively deleted the three PPREs from the 1 Kb fragment and transfected them into LS180 cells. Treatment with rosiglitazone (1 μM), GW7647 (10 μM), or GW0742 (10 μM) for 48 hours increased the luciferase activity of the 1 Kb construct and deletion construct containing all three PPREs (-808:+36) by 3- to 5- fold relative to DMSO-treated controls, but did not increase the activity of the three reporters that lacked the first PPRE at -769 (Fig. 5.3A). These data implicate the PPRE at nt -769 as an essential element for obtaining PPAR-mediated SULT1C3 transactivation.

To confirm the importance of the PPRE at -769 and determine the involvement of the other two predicted PPREs in the transcriptional activation of SULT1C3, mutations were introduced into each of the PPREs. LS180 cells were transfected with reporters containing either the wild-type 1 Kb fragment or the 1Kb fragment with one or two mutated PPREs. Mutation of the distal PPRE (at nt -769) eliminated the response of the 1 Kb reporter to rosiglitazone, GW7647, and GW0742 treatments (Fig. 5.3B). However, the PPAR agonists were all able to produce significant activation of reporters in which one or both of the more proximal PPREs (at nt -446 and -383) were mutated (Fig. 5.3B). These data indicate that only the PPRE at -769 is essential for obtaining PPAR-mediated activation of SULT1C3 transcription.

A competitive ELISA-based *in vitro* DNA-binding assay was used to determine the ability of PPARγ to bind directly to the distal PPRE. As shown in Fig 5.4, addition of 30 or 100 pmol of a double-stranded competitor oligonucleotide containing the wild-type SULT1C3 PPRE, but not the mutated SULT1C3 PPRE, significantly decreased the amount of PPARγ that bound to a biotinylated capture probe. This level of inhibition was approximately the same as that produced by a competitor containing the CYP4A1 PPRE

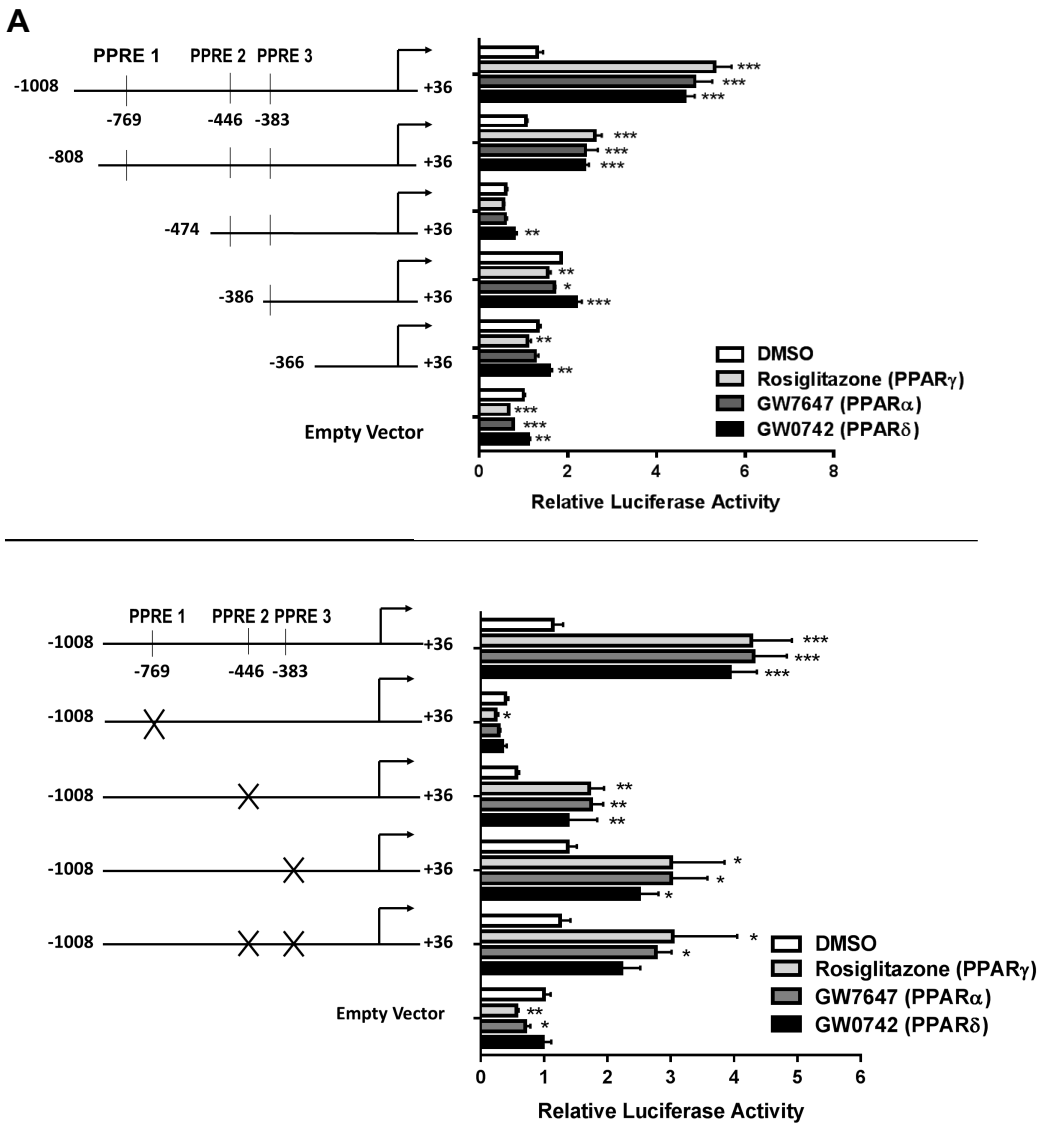


Figure 5.3: Evaluation of three computationally predicted PPREs within the deleted region of the SULT1C3 5'-flanking region.

LS180 cells were transiently transfected with a series of reporter plasmids with progressive 5'-deletions from the 1 Kb SULT1C3 reporter plasmid that were designed based on the positions of computationally predicted PPREs (A) or with the 1 Kb SULT1C3 reporter plasmid containing either the wild-type sequence or site-directed mutations at one or two of the predicted PPREs (B). Transfected cells were treated with DMSO (0.1%), rosiglitazone (1 μM), GW7647 (10 μM), or GW0742 (10 μM) for 48 hours and then harvested for measurement of luciferase activities. Each column represents the mean ± S.D. of normalized (Firefly/*Renilla*) luciferase measurements relative to the DMSO-treated, empty vector-transfected group (n=3 wells per treatment) from one cell culture experiment. Similar data were obtained in two additional independent experiments. *, **, ***Significantly different from DMSO-treated controls at P< 0.05, 0.01, and 0.001, respectively.

Figure taken with permission from (Dubaisi et al., 2016)

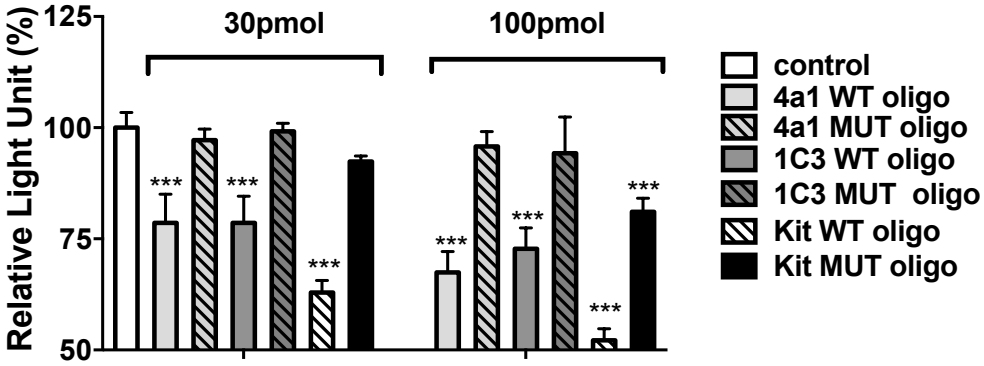


Figure 5.4: In vitro binding of PPARγ to the predicted SULT1C3 PPRE at nt -769.
In vitro binding was determined by incubating a biotinylated oligonucleotide containing a consensus PPRE with PPARγ-containing nuclear extract in the absence or presence of unbiotinylated competitor oligonucleotide (30 or 100 pm) containing wild-type or mutated CYP4A1 or SULT1C3 PPRE, as described in Methods. Each column represents the mean ± S.D. absorbance relative to the control absorbance (no competitor added; n=4). ***Significantly different from control at P<0.001.
Figure taken with permission from (Dubaisi et al., 2016)

5.3.4 Predominance of PPARγ in the transcriptional regulation of SULT1C3. Rosiglitazone, GW7647, and GW0742 are potent and selective agonists of PPARγ, PPARα, and PPARδ, respectively, although each agonist can cross-activate non-target PPAR receptors at sufficiently high concentrations. As shown in Fig. 5.2, while rosiglitazone increased SULT1C3 reporter expression at low concentrations consistent with PPARγ activation, the effects of GW7647 and GW0742 were only evident at relatively high concentrations, suggesting that these compounds might have increased SULT1C3 transcription by cross-activating PPARγ rather than by activating their target receptors. To test this possibility, we assessed the impact of siRNA-mediated knockdown of each PPAR on activation of the 1Kb reporter construct by 1 μM rosiglitazone, 10 μM GW7647, and 10 μM GW0742. The knockdown of PPARγ decreased activation of the positive control CYP4A1-PPRE reporter and the SULT1C3 1 Kb reporter by almost 90%, not only by rosiglitazone, but also by GW7647 and GW0742 (Fig 5.5A). However, knockdown of PPARα or PPARδ had little to no effect on reporter activation by rosiglitazone, GW7647, or GW0742 (Fig 5.5B and 5.5C). These data indicate that the effects of all three PPAR agonists on SULT1C3 transcription can mainly be attributed to the activation of PPARγ.

5.3.5 Genotyping for the full-length and variant SULT1C3 alleles in the human genome. To verify the existence of the deletion (863 nt) in the genome of normal individuals, we genotyped for the SULT1C3 alleles in human liver specimens (in collaboration with Dr. Erin Scheutz at the St. Jude Children's Research Hospital). Our preliminary experiments showed that primer set A and B preferentially amplified a fragment from the variant allele (328 bp) over a fragment from the full-length allele (1174 bp), and thus the individuals that carry both alleles could not be identified using this primer set. To identify heterozygotes, we designed a forward primer that was placed within the deleted region (primer C). The primer set C and B exclusively amplified a fragment from the full-length allele (645 bp). Fig. 5.6B is a representative gel image that shows the genotype for the SULT1C3 gene in the genome of some individuals. The 1174 bp (upper panel) and 645 bp (lower panel) were detected in homozygotes for the full-length allele, as shown in specimens with ID

numbers 443, 637, 675, and 765. Only a 328 bp band (upper panel) was detected in homozygotes for the variant SULT1C3 allele, as shown in specimens with ID numbers 350, 432, 482, 663, and 740. Two bands were amplified in heterozygotes, the 385 bp fragment (upper panel) and the 645 bp fragment (lower panel), as shown in specimen ID numbers 433, 619, 623, 638, 656, 674, 684, 686, 727, 744, and 769.

Overall, there were 18 homozygotes for the full-length allele, 15 homozygotes for the variant allele, and 44 heterozygotes and the calculated frequency for the deleted allele was 0.48 (Fig. 5.6C). The sequencing of the PCR products and alignment of these products to the full-length fragment indicated that the variant allele has 863 nt deleted and an additional 18 nt inserted at the site of the deletion.

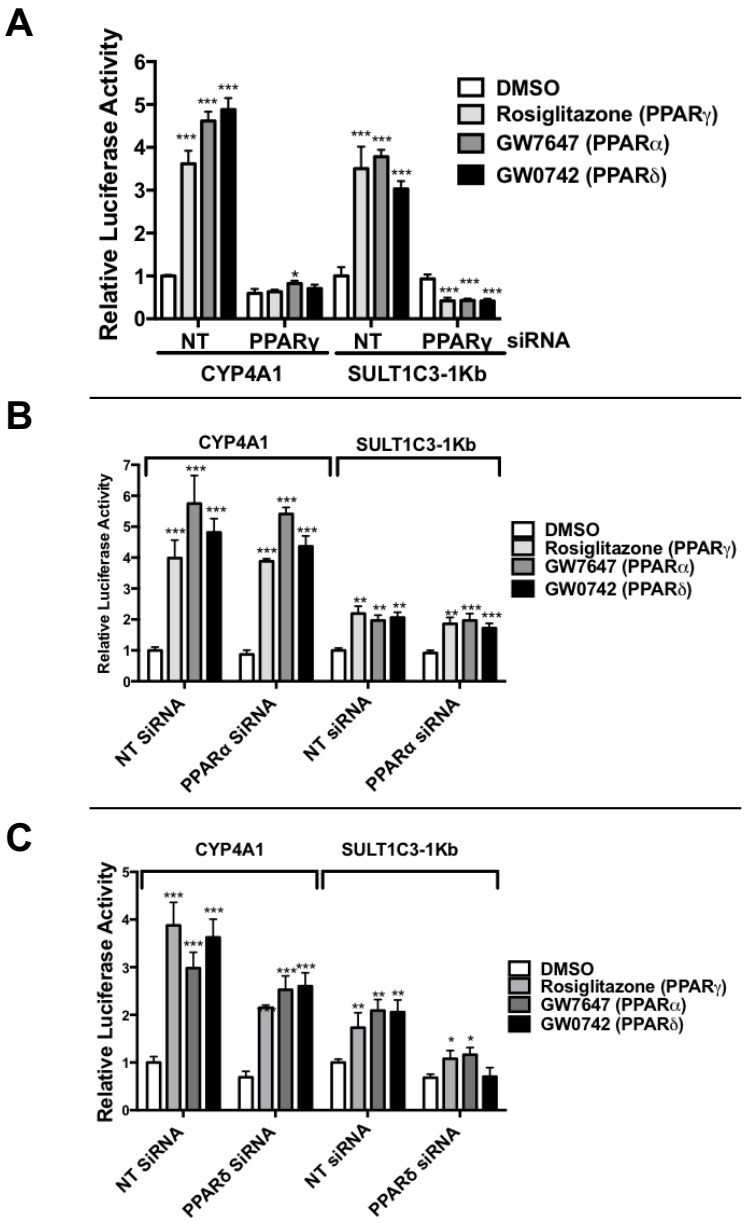
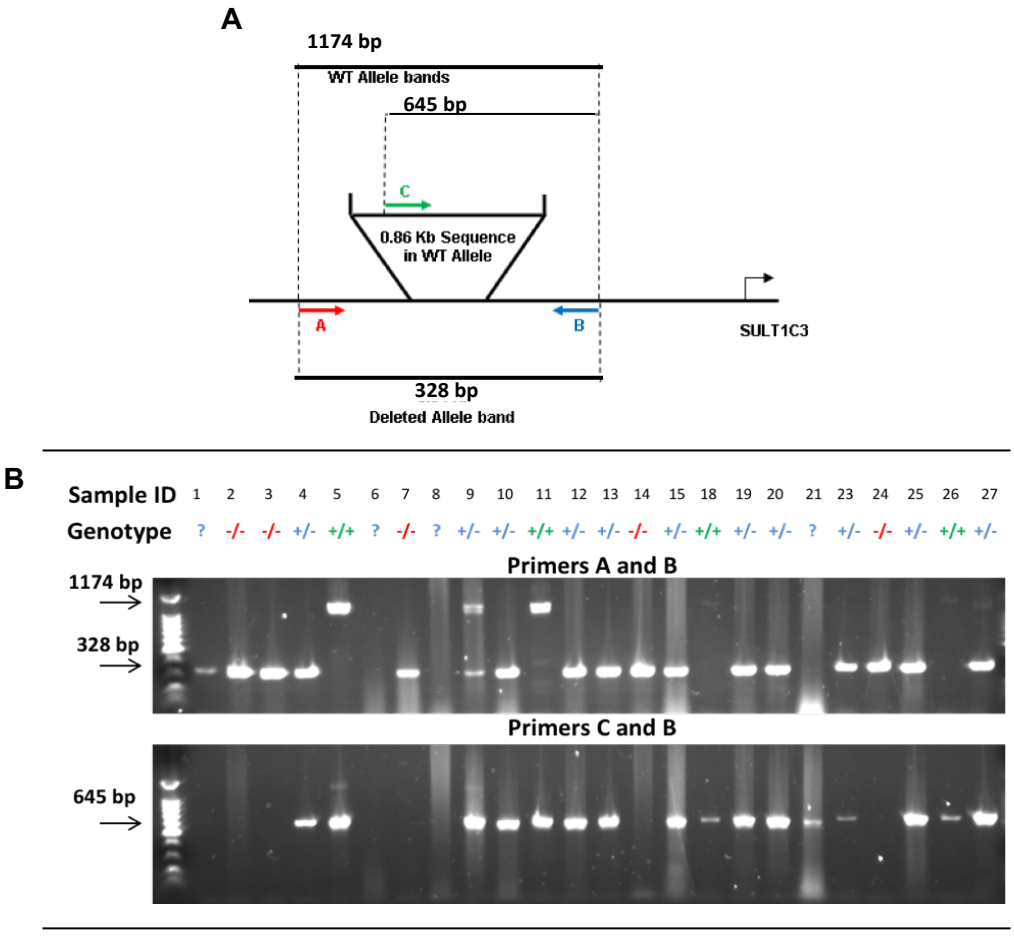


Figure 5.5: Effect of PPAR knockdowns on SULT1C3 transcriptional activation by different classes of PPAR agonist.
LS180 cells were transiently cotransfected with either the SULT1C3 1Kb or the CYP4A1 PPRE reporter plasmid (positive control) and 20 pmol of either non-targeting (NT) siRNA or siRNA targeting PPARγ (A), PPARα (B), or PPARδ (C). 24 Hours after transfection cells were treated with DMSO (0.1%), rosiglitazone (1 μM), GW7647 (10 μM), or GW0742 (10 μM) for 48 hours and then harvested for measurement of luciferase activities. Each column represents the mean ± S.D. of normalized (Firefly/*Renilla*) luciferase measurements (3 wells per treatment). Similar data were obtained in two additional independent experiments. *, **, ***Significantly different from DMSO-treated controls at P< 0.05, 0.01, and 0.001, respectively.
Figure taken with permission from (Dubaisi et al., 2016)



C

Genotypes	Observed #
Homozygote reference	18
Heterozygote	44
Homozygote variant	15
Variant allele frequency	0.48*

Figure 5.6: Genotyping analysis for full-length and variant alleles in the 5'-flanking region of SULT1C3 gene in human liver.
(A) Schematic representation demonstrating the locations of the primers used for the genotyping analysis and the expected sizes of the PCR products. (B) PCR was performed with primers designed to genotype SULT1C3 5'-flanking region using genomic DNA prepared from human liver specimens (n=77). The PCR products were resolved on a 1% agarose gel. (C) A table presenting the number of observed homozygotes for full-length and variant alleles and heterozygotes as well as the overall frequency of the variant allele.

5.4 Discussion

The intestine is a major portal of entry for many drugs, environmental chemicals, and other xenobiotics, and the intestine is therefore equipped with many xenobiotic-metabolizing enzymes, including several SULTs. The intestine is the major site of expression for SULT1A3, SULT1B1, and SULTE1, relative to the kidney, liver, and lung (Riches et al., 2009). SULT1A1, SULT1A3, and SULT1B1 are abundantly expressed throughout the gastrointestinal tract, while SULT1E1 and SULT2A1 are expressed in the jejunum, ileum, and cecum (Teubner et al., 2007; Riches et al., 2009). SULT1C enzymes are also expressed in the gastrointestinal tract (as shown in Table 1.2) as well as several human intestinal cell lines, including LS180 and Caco-2 cells (Sakakibara et al., 1998; Dooley et al., 2000; Meinl et al., 2008b; Rondini et al., 2014).

Rondini et al. previously reported that SULT1C3 expression in LS180 cells is induced by AhR, FXR, LXR, PPARα, PPARγ, and PXR agonists (Rondini et al., 2014). However, we report here that only PPAR agonists activated SULT1C3 transcription through sequence information contained within a 2.8 Kb fragment (-2789: +36) of the gene's 5'-flanking region. This finding implies that the *cis*-elements controlling SULT1C3 expression by the other transcription factors are located in other regions of the SULT1C3 gene, possibly further upstream or within the 7.1 Kb intron that separates non-coding exon 1 from exon 2 (Duniec-Dmuchowski et al., 2014).

While attempting to amplify a 2.8 Kb fragment of the SULT1C3 5'-flanking region, we found that amplicons of two different sizes were generated from LS180 genomic DNA; the expected 2.8 Kb fragment and a smaller fragment with an internal 863 nt deletion, from -1008 to -146 relative to the transcription start site. However, only the smaller fragment was detected in MCF10A cells indicating that there was variability in the SULT1C3 5'-flanking sequence among sources of genomic DNA. We also detected a variant allele that has the same 863 nt deleted in the genome of 59 individuals. This sequence variability appears to be attributable to a deletion polymorphism because the 1000 Genomes Project includes this structural variant in its database (esv3591922) (Genomes Project et al., 2012). Since this deletion region contains a functional PPARγ binding

site, it is plausible that SULT1C3 expression may vary among individuals depending on whether they carry the variant allele.

The three PPARs are expressed in fetal and adult human intestine (Abbott et al., 2010), and we have shown that PPARα and PPARγ are expressed in LS180 cells (PPARδ was not evaluated) (Rondini et al., 2014). In the current investigation we found that rosiglitazone, a potent and selective PPARγ agonist, significantly increased the activities of luciferase reporter plasmids containing the deletion region of the SULT1C3 5'-flanking region (i.e., the 2.8 Kb and 1 Kb reporters). Although the luciferase activity of these reporters was also increased by PPARα and PPARδ agonist treatments, these effects were probably attributable to cross-activation of PPARγ since (1) relatively high concentrations of the PPARα and PPARδ agonists were needed to induce the reporter activity and (2) siRNA targeting PPARγ abolished reporter activation by all PPAR agonists while siRNA targeting PPARα or PPARδ had little effect. These data indicate that PPARγ is the predominant PPAR that regulates SULT1C3 transcription in LS180 cells.

PPARγ is highly expressed in the various regions of human intestine, at levels that are comparable to those detected in adipocytes, and this transcription factor could play a role in gastrointestinal morphogenesis during fetal development (Fajas et al., 1997; Huin et al., 2000; Abbott, 2009; Abbott et al., 2010). In human intestine, PPARγ signaling has been linked to growth arrest, apoptosis, and differentiation (Gupta et al., 2001; Gupta et al., 2003; Thompson, 2007). In human colon cancer cells, PPARγ-regulated genes have been classified into three functional categories: regulation of lipid metabolism, signal transduction, and motility and adhesion (Chen et al., 2006; Bush et al., 2007; Su et al., 2007). SULT1C3 could play a role in intestinal physiology by metabolizing one or more endogenous molecules that function in the regulation of these PPARγ-regulated processes.

In summary, we have identified a functional PPRE in the 5'-flanking region of the SULT1C3 gene, thereby establishing SULT1C3 as a direct PPARγ target in intestinal cells. This finding implies that SULT1C3 could play a role in PPARγ-regulated processes associated with intestinal

development and function. Since the PPRE is located within a polymorphic region of the SULT1C3 gene, our findings also provide a mechanistic rationale to hypothesize that there could be considerable differences among individuals in the intestinal expression of SULT1C3. Further studies are needed to establish the genotype-phenotype relationship between the presence of the PPRE and intestinal SULT1C3 expression. The findings of our genotyping analysis and the reports in 1000 Genomes Database indicated that the structural variant lacking the PPRE appears to be a fairly common polymorphism (the overall allele frequency of esv3591922 in the 1000 Genomes Database is 0.3329). The data from the 1000 Genome Database demonstrated that the frequency of this polymorphism varied among the different populations (frequencies of esv3591922 for African, Ad Mixed American, East Asian, European, and South Asian super populations of 0.2852, 0.3329, 0.1091, 0.4394, and 0.5184, respectively). It is possible that inter-individual differences in intestinal SULT1C3 expression could have pharmacological and toxicological implications, for example by modifying the risk for intestinal bioactivation of procarcinogenic molecules.

CHAPTER 6: FINAL CONCLUSIONS AND FUTURE DIRECTIONS

Eugen Baumann was the first to discover the process of sulfonation in 1876 (Baumann, 1876), and since then a large number of xenobiotics and endogenous molecules have been found to be conjugated with a sulfonate group, thereby modulating the activity of these molecules. SULT1 and SULT2 expression was detected in prenatal hepatic and extrahepatic tissues, suggesting that these enzymes are responsible for the biotransformation of xenobiotics during fetal life (Barker et al., 1994; Stanley et al., 2005; Duanmu et al., 2006). However, none of these studies performed a comprehensive analysis of SULT expression that includes the understudied SULTs, such as the SULT1Cs, or identified signaling pathways that regulate the expression of these enzymes in differentiating hepatocytes or intestinal cells. For this reason, we (1) examined the role of lipid- and xenobiotic-sensing transcription factors in the regulation of SULT expression using *in vitro* models of human liver development, (2) determined the expression profiles of SULT1 and SULT2 mRNA and protein in prenatal and postnatal liver specimens and cytosolic fractions, (3) examined the expression profile of SULT1C4 TVs in the developing human liver, and (4) determined the mechanism involved in the transcriptional regulation of SULT1C3 by PPARγ.

Based on previous studies, we hypothesized that (1) most SULT1 and SULT2 mRNA and protein are expressed in the immature liver and that these enzymes are expressed with class I, II, and III expression patterns (described in chapter 1) and (2) SULT1 and SULT2 expression is regulated by lipid- and xenobiotic-sensing transcription factors. Our data demonstrated that SULTs, primarily SULT1A1, SULT1C2, SULT1C4, SULT1E1, and SULT2A1, were abundantly expressed in primary cultures of fetal hepatocytes, confluent HepaRG cells, and prenatal liver specimens. We also classified the expression of individual SULTs into the three classes of developmental expression patterns. Our results also indicated that SULT1 and SULT2 expression was regulated by lipid- and xenobiotic-sensing nuclear receptors as well as AhR. These findings suggest that SULT1 and SULT2 enzymes are part of regulatory networks that are involved in mediating the effects of xenobiotics and endogenous molecules during early development, and

further implicate SULT1 and SULT2 enzymes in the regulation of essential cellular functions in the immature liver. To further confirm these observations, future work should examine the regulation of the individual SULTs in models of human liver development that undergo a complex differentiation process resembling that of human liver cells in vivo. Induced pluripotent stem cells that can be induced to differentiate into hepatocyte-like cells and humanized mouse models that express human SULT genes are models that can be used to investigate the regulatory mechanisms controlling SULT expression and to study their role in hepatocyte differentiation. It will also be interesting to determine some of the substrates that are metabolized by SULT1 and SULT2 enzymes in the differentiating liver cells to help us understand the physiological importance of these enzymes.

Current evidence suggests that SULT1C4 is primarily expressed in fetal tissues (Sakakibara et al., 1998). In this project we found that SULT1C4 mRNA is abundantly expressed in primary fetal hepatocytes, confluent HepaRG cells, and prenatal liver specimens, but the protein levels are very low throughout development. Based on our data, we believe that the inconsistency between SULT1C4 mRNA and protein levels is due to the low translation efficiency or decreased stability of the protein made from SULT1C4 TV2, which is the most abundant TV at the mRNA level. Future experiments should include examining the stability of the SULT1C4 TV1 and TV2 protein that could explain the lack of correlation in the abundance of TV1 and TV2 mRNA and protein. We are currently working on developing an ELISA assay that will allow us to examine TV1 and TV2 protein stability by pulse labeling with a non-radiolabeled modified amino acid. Since our findings suggest that SULT1C4 does not play an important metabolic role in the liver, further studies using extrahepatic tissues is needed to examine the role of SULT1C4 in other tissues.

Finally, our work on SULT1C3 suggests that this enzyme is involved in regulating intestinal processes that are controlled by PPARγ. Our data also indicate that a genetic variation in the SULT1C3 promoter region affects the regulation of SULT1C3 by PPARγ. To identify the biological pathways that are regulated by SULT1C3, future studies should focus on identifying target genes

and substrates that are regulated by SULT1C3 expression using human intestinal *in vitro* models, tissue specimens, and humanized mouse models. Experiments examining the correlation between SULT1C3 genotype and phenotype in human intestinal tissues could also elucidate the impact of this allelic variation on SULT1C3 expression.

APPENDIX A: PERMISSION TO REPUBLISH FIGURE 1.1

ELSEVIER LICENSE
TERMS AND CONDITIONS

Oct 24, 2018

This Agreement between Sarah Dubaisi ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	4455170455152
License date	Oct 24, 2018
Licensed Content Publisher	Elsevier
Licensed Content Publication	Drug Metabolism and Pharmacokinetics
Licensed Content Title	Structural plasticity in the human cytosolic sulfotransferase dimer and its role in substrate selectivity and catalysis
Licensed Content Author	Zachary E. Tibbs,Katie Jo Rohn-Glowacki,Frank Crittenden,Amber L. Guidry,Charles N. Falany
Licensed Content Date	Feb 1, 2015
Licensed Content Volume	30
Licensed Content Issue	1
Licensed Content Pages	18
Start Page	3
End Page	20
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Original figure numbers	Fig. 2
Title of your thesis/dissertation	Characterization of Cytosolic Sulfotransferase Expression and Regulation in Human Liver and Intestine
Expected completion date	Jan 2019
Estimated size (number of pages)	195
Requestor Location	Sarah Dubaisi 6135 woodward ave DETROIT, MI 48202 United States Attn: Sarah Dubaisi
Publisher Tax ID	98-0397604
Total	0.00 USD
Terms and Conditions	

APPENDIX B: PERMISSION TO REPUBLISH FIGURE 1.2

Hello Sarah,

Permission is granted for the use of Fig 1, p.1 in Signaling by Nuclear Receptors in your PhD thesis only. Please include complete reference and copyright to Cold Spring Harbor Laboratory Press.

Best wishes for success with your thesis,

Carol C. Brown
Books Development, Marketing and Sales
Cold Spring Harbor Laboratory Press
500 Sunnyside Blvd
Woodbury, New York 11797
516 422 4038 ph.
516 422 4095 fx.
brown@cshl.edu

APPENDIX C: PERMISSION TO REPUBLISH FIGURES AND TABLES IN CHAPTER 2

October 11, 2018

Sarah Dubaisi
Pharmacology
Wayne State University
6135 Woodward Ave.
Detroit, MI 48202

Email: ek9363@wayne.edu

Dear Sarah Dubaisi:

This is to grant you permission to reproduce the following figures and tables in your dissertation titled "Characterization of Phenol and Hydroxysteroid Cytosolic Sulfotransferases in Human Liver and Intestine" for Wayne State University:

> Figures 1, 2, 3, 4, 5, and 6, Supplemental Tables 1 and 2, and Supplemental Figures 1, 2, and 3 from S Dubaisi, KG Barrett, H Fang, J Guzman-Lepe, A Soto-Gutierrez, TA Kocarek, and M Runge-Morris (2018) Regulation of Cytosolic Sulfotransferases in Models of Human Hepatocyte Development, *Drug Metab Dispos*, 46(8): 1146-1156; DOI: https://doi.org/10.1124/dmd.118.081398

Permission to reproduce the figures and tables is granted for worldwide use in all languages, translations, and editions, and in any format or medium including print and electronic. The authors and the source of the materials must be cited in full, including the article title, journal title, volume, year, and page numbers.

Sincerely yours,

Richard Dodenhoff
Journals Director

APPENDIX D: CHARACTERISTICS OF FETAL HEPATOCYTES DONORS

Tissue ID	Gestational Age	Sex	Hepatocyte Viability at Isolation
HFet#100	12 Weeks	Female	90%
HFet#101	14 Weeks	Female	90%
HFet#104	18 Weeks	Female	87%
HFet#105	18 Weeks	Female	90%
HFet#106	22 Weeks	Male	88%

Table taken with permission from (Dubaisi et al., 2018)

APPENDIX E: TARGETS AND TREATMENT CONSENTRATIONS OF AGONISTS USED IN CHAPTERS 2 AND 5

Agonist	Target Pathway
TCDD	AhR
CITCO	CAR
GW3965	LXR
Rifampicin	PXR
CDCA	FXR
GW4064	FXR
Ciprofibrate	PPARα
GW7647	PPARα
GW0742	PPARδ
Rosiglitazone	PPARγ
$VitD_3$	VDR

TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; AhR, aryl hydrocarbon receptor; CITCO, 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime; CAR, constitutive androstane receptor; GW3965, 3-[3-[[[2-chloro-3-(trifluoromethyl)phenyl]methyl](2,2-diphenylethyl)amino]propoxy]benzeneacetic acid hydrochloride; LXR, liver X receptor; PXR, pregnane X receptor; CDCA, chenodeoxycholate; GW4064, 3-[2-[2-chloro-4-[[3-(2,6-dichlorophenyl)-5-(1-methylethyl)-4-isoxazolyl]methoxy]phenyl]ethenyl]benzoic acid; FXR, farnesoid X receptor; GW7647, 2-[[4-[2-[[(cyclohexylamino)carbonyl](4-cyclohexylbutyl)amino]ethyl]phenyl]thio]-2-methylpropanoic acid; GW0742, 4-[[[2-[3-fluoro-4-(trifluoromethyl)phenyl]-4-methyl-5-thiazolyl]methyl]-thio]-2-methylphenoxyacetic acid; PPAR, peroxisome proliferator-activated receptor; VDR, vitamin D receptor; VitD3, 1α,25-dihydroxyvitamin D3

APPENDIX F: TAQMAN GENE EXPRESSION ASSAYS USED IN CHAPTERS 2 AND 3.

Gene	TaqMan Assay ID
CYP1A1	Hs00153120_m1
CYP2B6	Hs04183483_g1
CYP3A4	Hs00604506_m1
CYP3A7	Hs00426361_m1
PLIN2	Hs00605340_m1
SHP	Hs00222677_m1
SREBP1	Hs01088679_g1
SULT1A1- TV5	Hs00738644_m1
SULT1A1- TV1	APFVK4A[1]
SULT1A1- TV1 to 4	Hs00742033_mH
SULT1B1	Hs00234899_m1
SULT1C2	Hs00602560_m1
SULT1C3	Hs01371045_m1
SULT1C4	Hs00923769_m1
SULT1E1	Hs00193690_m1
SULT2A1	Hs00234219_m1
SULT2B1	Hs00190268_m1
18S	4319413E
GAPDH	4326317E
TBP	4310891E

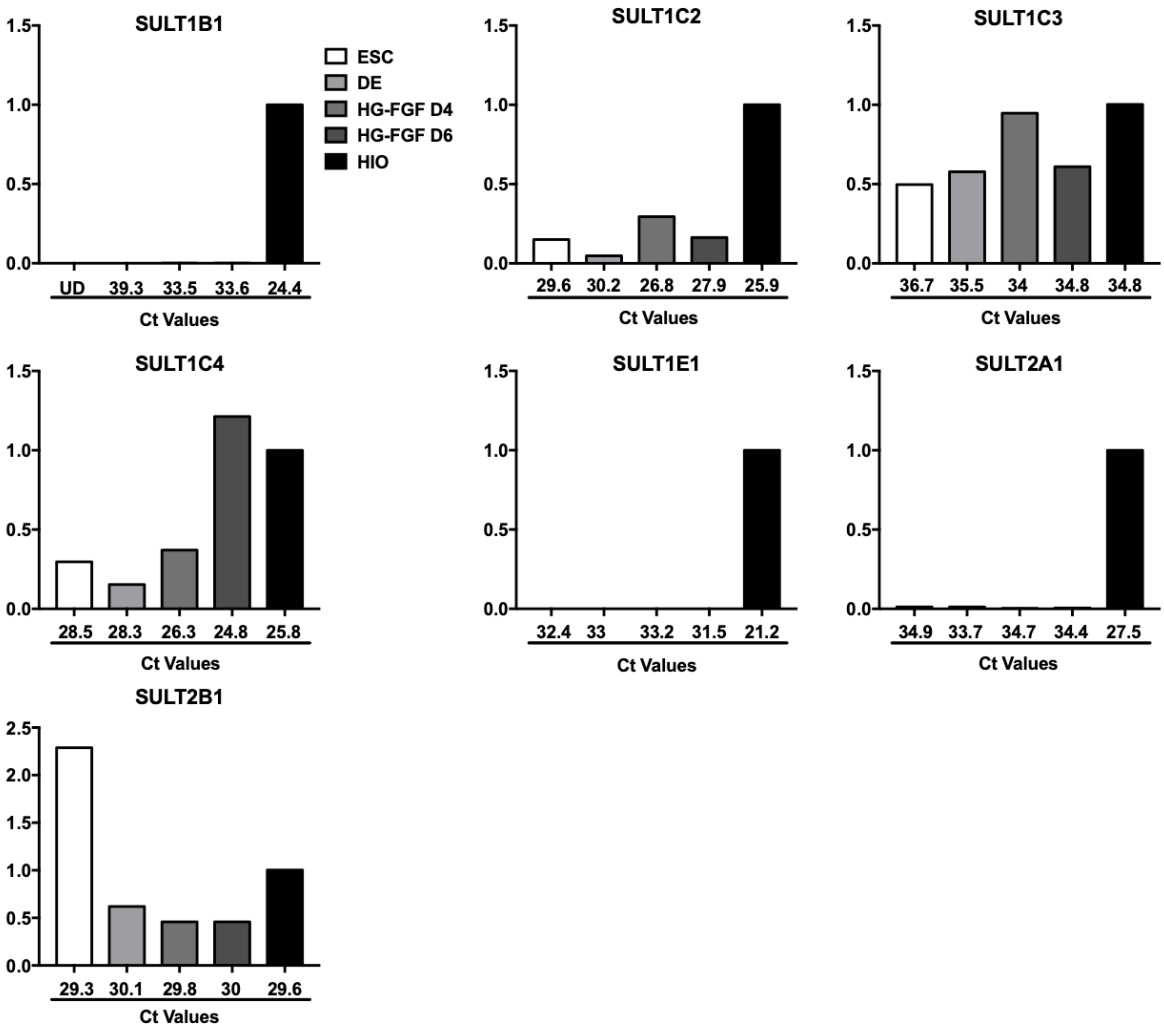
Table taken with permission from (Dubaisi et al., 2018)

APPENDIX G: PRIMERS USED FOR THE RACE ANALYSIS AND TO PREPARE SULT1C4 REPORTER CONSTRUCTS

Primer name	Sequence
RACE Reverse	5'-TCC AAG CCA AGC CTG ATG ACC TGC T-3'
2.2 Kb Forward	5'-GGC AGT TTA AAT TCA AAC CCA-3'
2.2 Kb Reverse	5'-GTG GTA GTG TGG TGG ATA GAG TGC T-3'
Fragment A Forward	5'-**GAG CTC GCT AGC CTC GAG** CTT CCT CTT GCT TCG GTT TCA AGT -3'
Fragment A Reverse	5'-**GGA TCT GAA TCT CTC GAG** GTG ATC TTG ACT ACA AAC ACT GCT C-3'
Fragment B Forward	5'-**GAG CTC GCT AGC CTC GAG** CAC AGT GAG AAG CCT GTA CTA AGG GA-3'
Fragment B Reverse	5'-**GGA TCT GAA TCT CTC GAG** GGA GAG TGA GCT AAG CCT GTG T-3'
Fragment C Forward	5'-**GAG CTC GCT AGC CTC GAG** ACC CAT GTG GTT CCT TGT AAC ACT -3'
Fragment C Reverse	5'-**GGA TCT GAA TCT CTC GAG** GTG CTG CCT GTC TAT CAT GGG TC-3'
Fragment D Forward	5'-**GAG CTC GCT AGC CTC GAG** AAT CCG AAC ACC AGA CTC TTC TGA-3'
Fragment D Reverse	5'-**GAG CTC GCT AGC CTC GAG** GGG AAA TGG TCC TGG GTA TGT GT -3'
Fragment E Forward	5'-**GAG CTC GCT AGC CTC GAG** AGT GAG ATC ACA CCC CAT GAA G -3'
Fragment E Reverse	5'-**GGA TCT GAA TCT CTC GAG** TGG AGA TTC AAA GTG TCT CAA AGT-3'
Fragment F Forward	5'-**GAG CTC GCT AGC CTC GAG** GGC ATG TCT TTC TAT ATG GAT GTG G-3'
Fragment F Reverse	5'-**GGA TCT GAA TCT CTC GAG** AGC CAG GTA ATT GGG AAT TGG T -3'
Fragment G Forward	5'-**GAG CTC GCT AGC CTC GAG** TGT GAT AAA TGC AAG TGA GGT TGG-3'
Fragment G Reverse	5'-**GAG CTC GCT AGC CTC GAG** GGG AGA GGC AAT GCT TTA AAT TTG T-3'
Fragment H Forward	5'-**GAG CTC GCT AGC CTC GAG** TCT AAA GGA GAG GAC AGA GGA AGA -3'
Fragment H Reverse	5'-**GAG CTC GCT AGC CTC GAG** GCT TCC TTA GAG AAG GGG ATT TCA-3'
Fragment I Forward	5'-**GAG CTC GCT AGC CTC GAG** TGG CAC TAC AAT GGC TTC TAA TCA-3'
Fragment I Reverse	5'-**GAG CTC GCT AGC CTC GAG** AGA TCT GAG ATT CCC CTT GCT TTT-3'

Note: The sequences in bold were added to all primers that were used with the In-Fusion HD Cloning Kit to PCR amplify and fuse the fragments with the linearized PGL4.10 reporter plasmid containing the core promoter.

APPENDIX H: EXPRESSION OF SULT mRNA IN THE DIFFERENTIATING HIOs



Temporal expression of SULTs during the differentiation of ESCs into HIOs. Cells were harvested on the indicated time points for measurement of mRNA levels. mRNA levels were normalized to the levels measured in the HIOs. The data show the expression patterns of seven cytosolic SULTs from the ESCs, DE, HG (treated with FGF for 4 and 6 days), and HIOs. The cycle threshold (Ct) values for the various genes are shown below the x-axis. 3 wells were pooled together for the ESCs, DE, and HG samples and multiple HIOs were pooled in 1 sample. Data were normalized to GAPDH. Similar results were obtained from a second experiment.

APPENDIX I: DONOR INFORMATION FOR SAMPLES ANALYZED BY RT-QPCR

Developmental Stage	Sample ID	Sex	Prenatal Age	Postnatal Age	Postmortem Interval (hours)
Prenatal	34	Female	18 weeks		1
	40	Female	18 weeks		1
	42	Female	18 weeks		1
	235	Female	19 weeks		1
	246	Male	19 weeks		1
	276	Male	18 weeks		1
	317	Male	19 weeks		1
	893	Female	19 weeks		2
	1330	Female	18 weeks		2
	1390	Female	18 weeks		1
Infant	75	Male		96 days	36
	82	Male		137 days	37
	83	Male		69 days	27
	326	Female		66 days	19
	1102	Male		119 days	22
	1472	Female		118 days	19
	1490	Female		70 days	23
Adult	289	Male		24 years, 362 days	5
	602	Male		27 years, 42 days	15
	819	Male		18 years, 217 days	28
	1021	Male		19 years, 242 days	14
	1028	Male		39 years, 11 days	14
	1539	Female		33 years, 177 days	23
	5611	Female		50 years, 183 days	15

APPENDIX J: DONOR INFORMATION FOR SAMPLES ANALYZED BY RNA-SEQ

Developmental Stage	Sample ID	Sex	Prenatal Age	Postnatal Age	Postmortem Interval (hours)
Prenatal	20636	Male	14.7 weeks		<2
	21248	Female	14.7 weeks		<2
	21251	Male	14.7 weeks		<2
	21432	Male	16.1 weeks		<2
	21601	Male	16.4 weeks		<2
	21605	Male	14.7 weeks		<2
	21806	Female	16.4 weeks		<2
	21883	Male	16.4 weeks		<2
	21949	Female	16.1 weeks		<2
	21978	Female	15.6 weeks		<2
Infant	86	Male		56 days	11
	432	Male		4 days	2
	435	Male		274 days	10
	569	Male		133 days	16
	759	Male		35 days	7
	774	Male		273 days	10
	780	Male		0 days	13
	825	Male		334 days	11
	1055	Male		96 days	12
	1157	Female		20 days	14
	1281	Male		206 days	6
	1296	Male		98 days	16
	1325	Female		182 days	18
	1547	Male		259 days	10
Child	64	Male		15 years	13
	346	Male		3 years	11.17
	617	Female		1 year, 347 days	9
	677	Male		1 year, 353 days	13
	689	Female		5 years	19.5
	792	Male		4 years	14.5
	872	Male		2 years	14.5
	885	Male		17 years	12.5
	1860	Male		8 years, 2 days	5
	8902	Male		7 years	Surgical specimen
	8906	Male		12 years	Surgical specimen
	8910	Male		14 years	Surgical specimen
	8917	Female		6 years	Surgical specimen
	8920	Male		11 years	Surgical specimen
	8924	Female		9 years	Surgical specimen
	8925	Male		8 years	Surgical specimen
	8926	Female		1 year, 304 days	Surgical specimen
	8935	Male		17 years	Surgical specimen
	9003	Female		7 years	Surgical specimen
	9006	Male		10 years	Surgical specimen
	9011	Female		3 years, 183 days	Surgical specimen
	9013	Male		11 years	Surgical specimen
	9023	Female		2 years, 213 days	Surgical specimen
	9027	Male		12 years	Surgical specimen
	9032	Male		14 years	Surgical specimen

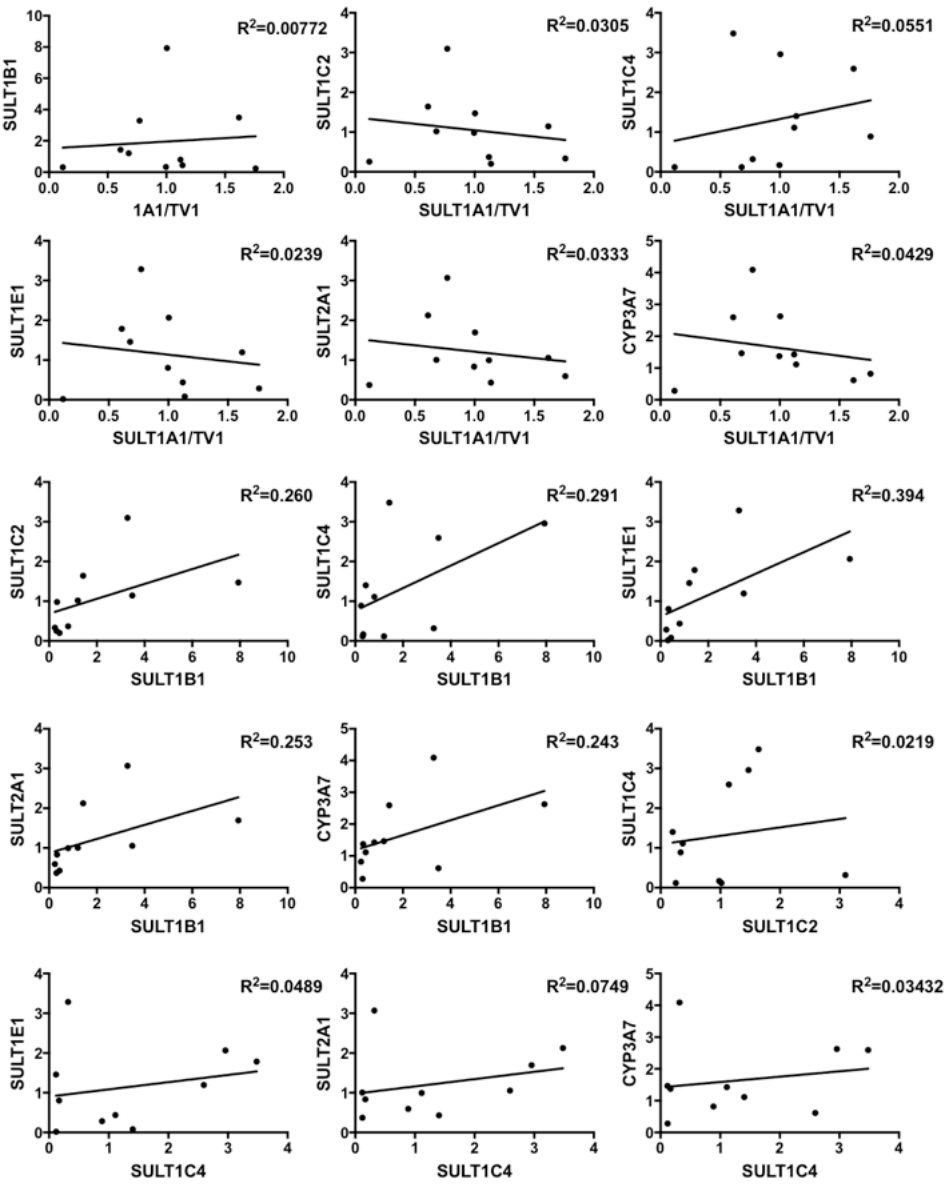
9036	Female	5 years	Surgical specimen
9101	Male	2 years	Surgical specimen
9127	Male	15 years	Surgical specimen
9608	Male	4 years	Surgical specimen
9609	Male	4 years	Surgical specimen
9611	Male	9 years	Surgical specimen
9612	Male	3 years	Surgical specimen
70898	Male	7 years	Not recorded
70994	Male	16 years	24
71000	Male	6 years	12
71008	Male	13 years	24
71058	Female	10 years	15
71281	Male	16 years	15

APPENDIX K: MULTIPLE REACTION MONITORING (MRM) SETTINGS FOR SULT PROTEINS

			Parent			Transition		
Protein	Peptide	Collision Energy (%)	Label	m/z	z	Fragment	m/z	z
SULT1A1	VHPEPGTWDSFLEK	23	light	547.935	3	b2	237.135	1
SULT1A1	VHPEPGTWDSFLEK	16	light	547.935	3	y6	738.367	1
SULT1A1	VHPEPGTWDSFLEK	23	heavy	550.606	3	b2	237.135	1
SULT1A1	VHPEPGTWDSFLEK	16	heavy	550.606	3	y6	746.381	1
SULT1A1	VHPEPGTWDSFLEK	35	light	821.399	2	b2	237.135	1
SULT1A1	VHPEPGTWDSFLEK	35	heavy	825.406	2	b2	237.135	1
SULT1A2	VYPHPGTWESFLEK	19	light	563.947	3	y5	623.340	1
SULT1A2	VYPHPGTWESFLEK	12	light	563.947	3	y12	714.351	1
SULT1A2	VYPHPGTWESFLEK	19	light	563.947	3	y10	1193.584	1
SULT1A2	VYPHPGTWESFLEK	19	heavy	566.619	3	y5	631.354	1
SULT1A2	VYPHPGTWESFLEK	12	heavy	566.619	3	y12	718.358	1
SULT1A2	VYPHPGTWESFLEK	19	heavy	566.619	3	y10	1201.598	1
SULT1A3	AHPEPGTWDSFLEK	24	light	538.591	3	b2	209.103	1
SULT1A3	AHPEPGTWDSFLEK	15	light	538.591	3	y5	623.340	1
SULT1A3	AHPEPGTWDSFLEK	15	light	538.591	3	b7	690.321	1
SULT1A3	AHPEPGTWDSFLEK	15	light	538.591	3	y6	738.367	1
SULT1A3	AHPEPGTWDSFLEK	24	heavy	541.263	3	b2	209.103	1
SULT1A3	AHPEPGTWDSFLEK	15	heavy	541.263	3	y5	631.354	1
SULT1A3	AHPEPGTWDSFLEK	15	heavy	541.263	3	b7	690.321	1
SULT1A3	AHPEPGTWDSFLEK	15	heavy	541.263	3	y6	746.381	1
SULT1B1	NLNDEILDR	19	light	551.280	2	b2	228.134	1
SULT1B1	NLNDEILDR	21	light	551.280	2	y3	403.230	1
SULT1B1	NLNDEILDR	19	light	551.280	2	y7	874.426	1
SULT1B1	NLNDEILDR	19	heavy	556.285	2	b2	228.134	1
SULT1B1	NLNDEILDR	21	heavy	556.285	2	y3	413.238	1
SULT1B1	NLNDEILDR	19	heavy	556.285	2	y7	884.435	1
SULT1C2	IVQETSFEK	18	light	540.782	2	y5	611.304	1
SULT1C2	IVQETSFEK	16	light	540.782	2	y6	740.346	1
SULT1C2	IVQETSFEK	14	light	540.782	2	y7	868.405	1
SULT1C2	IVQETSFEK	18	heavy	544.789	2	y5	619.318	1
SULT1C2	IVQETSFEK	16	heavy	544.789	2	y6	748.360	1
SULT1C2	IVQETSFEK	14	heavy	544.789	2	y7	876.419	1
SULT1C4	IVHYTSFDVMK	20	light	447.229	3	y2	278.153	1

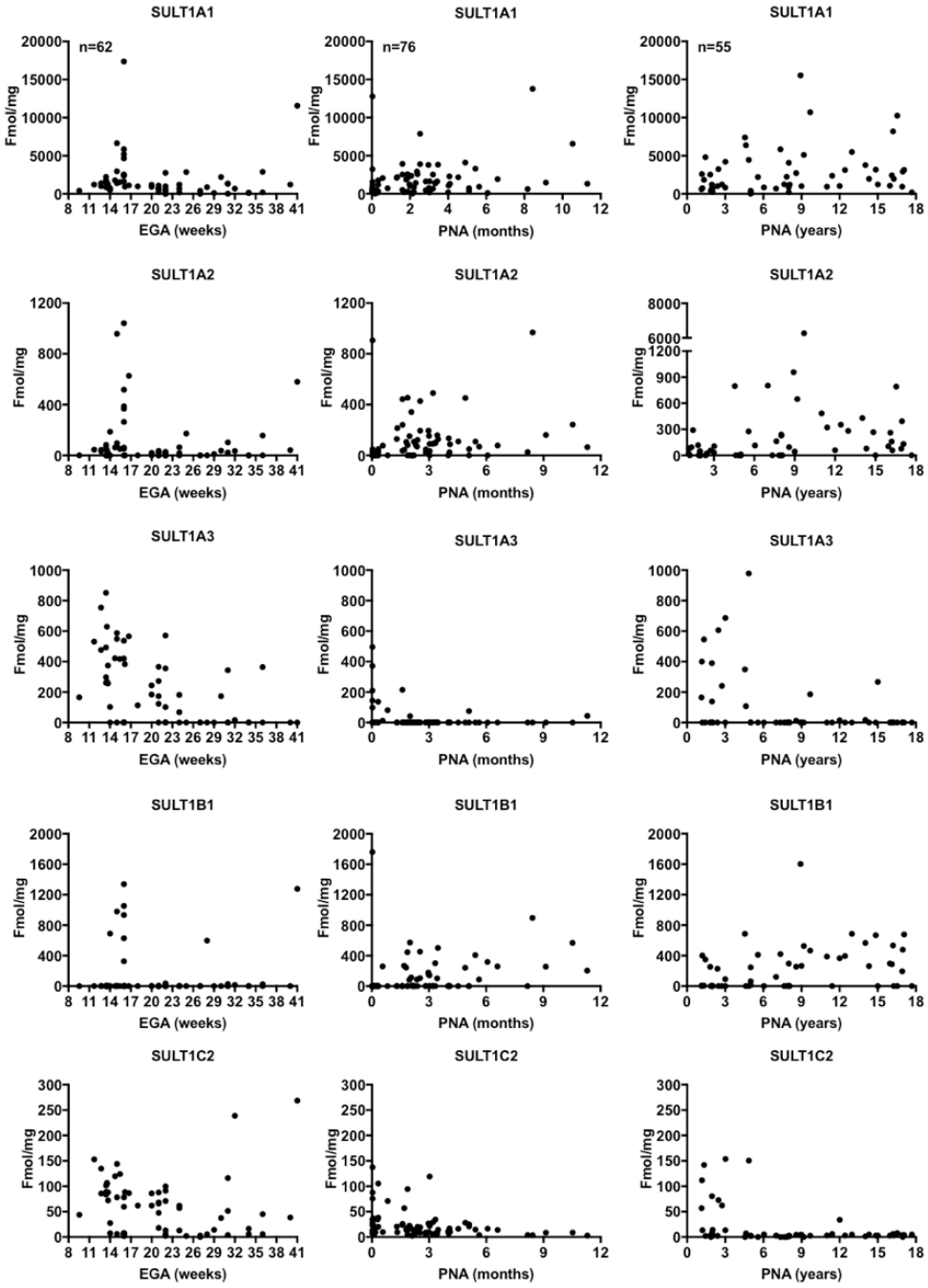
SULT1C4	IVHYTSFDVMK	23	light	447.229	3	b3	350.219	1
SULT1C4	IVHYTSFDVMK	13	light	447.229	3	y4	492.249	1
SULT1C4	IVHYTSFDVMK	20	heavy	449.900	3	y2	286.167	1
SULT1C4	IVHYTSFDVMK	23	heavy	449.900	3	b3	350.219	1
SULT1C4	IVHYTSFDVMK	13	heavy	449.900	3	y4	500.263	1
SULT1E1	KPSEELVDR	22	light	536.785	2	y8	472.738	2
SULT1E1	KPSEELVDR	26	light	536.785	2	y7	847.416	1
SULT1E1	KPSEELVDR	26	light	536.785	2	y8	944.468	1
SULT1E1	KPSEELVDR	22	heavy	541.789	2	y8	477.742	2
SULT1E1	KPSEELVDR	26	heavy	541.789	2	y7	857.424	1
SULT1E1	KPSEELVDR	26	heavy	541.789	2	y8	954.477	1
SULT2A1	DEDVIILTYPK	19	light	653.350	2	y6	734.445	1
SULT2A1	DEDVIILTYPK	18	light	653.350	2	y7	847.529	1
SULT2A1	DEDVIILTYPK	19	light	653.350	2	y8	946.597	1
SULT2A1	DEDVIILTYPK	19	heavy	657.358	2	y6	742.459	1
SULT2A1	DEDVIILTYPK	18	heavy	657.358	2	y7	855.543	1
SULT2A1	DEDVIILTYPK	19	heavy	657.358	2	y8	954.611	1

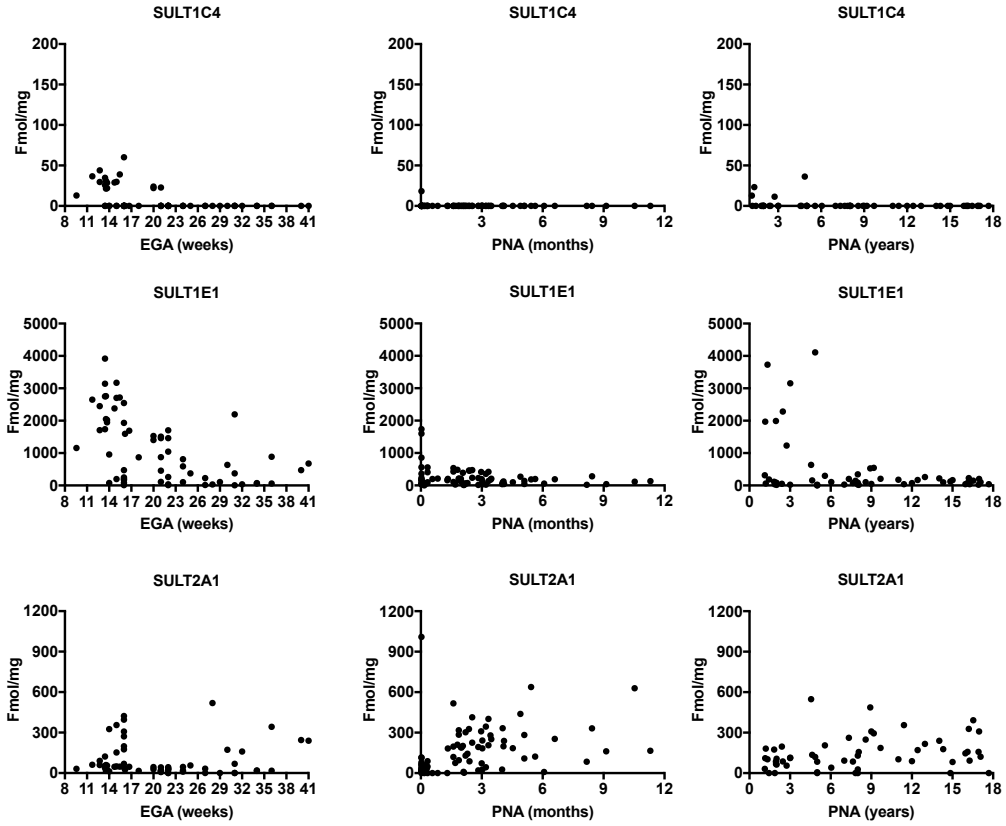
APPENDIX L: CORRELATION ANALYSES FOR RNA-SEQ DATA FROM CHAPTER 3



Correlation between SULT1A1, SULT1B1, and SULT1C4 expression measured by RT-PCR in prenatal liver. SULT1A1, SULT1B1, and SULT1C4 expression from the prenatal samples (n=10) were analyzed using using pairwaise correlation analysis. Each scatter plot shows the expression data of one gene against another. The R^2 values from the correlation analysis are indicated.

APPENDIX M: PROTEIN QUANTIFICATION DATA FROM CHAPTER 3 PRESENTED AS SCATTER PLOTS





Developmental expression of SULT protein in human liver cytosols analyzed by MRM. SULT protein contents (fmol/mg) are shown as scatter plots against age of the donor. Age was divided into three groups: prenatal [estimated gestational age (EGA) in weeks (left)], infant [postnatal age (PNA) in months (middle)], and 1-18 years-old (PNA in years, right).

APPENDIX N: PERMISSION TO REPUBLISH FIGURES AND TABLES IN CHAPTER 5

October 17, 2018

Sarah Talal Dubaisi
Wayne State University
6135 Woodward Ave.
Detroit, MI 48202

Email: ek9363@wayne.edu

Dear Sarah Talal Dubaisi:

This is to grant you permission to include the following article in your dissertation entitled "Characterization of Phenol and Hydroxysteroid Cytosolic Sulfotransferases in Human Liver and Intestine" for the Wayne State University:

> S Dubaisi, H Fang, TA Kocarek, and M Runge-Morris (2016) Transcriptional Regulation of Human Cytosolic Sulfotransferase 1C3 by Peroxisome Proliferator-Activated Receptor γ in LS180 Human Colorectal Adenocarcinoma Cells, *Mol Pharmacol*, 90(5): 562-569; DOI: https://doi.org/10.1124/mol.116.106005

On the first page of each copy of this article, please add the following:

> Reprinted with permission of the American Society for Pharmacology and Experimental Therapeutics. All rights reserved.

In addition, the original copyright line published with the paper must be shown on the copies included with your dissertation.

Sincerely yours,

Richard Dodenhoff
Journals Director

REFERENCES

Abbott BD (2009) Review of the expression of peroxisome proliferator-activated receptors alpha (PPAR alpha), beta (PPAR beta), and gamma (PPAR gamma) in rodent and human development. *Reproductive toxicology* **27:**246-257.

Abbott BD, Wood CR, Watkins AM, Das KP, and Lau CS (2010) Peroxisome proliferator-activated receptors alpha, Beta, and gamma mRNA and protein expression in human fetal tissues. *PPAR research* **2010**.

Abe T, Kakyo M, Tokui T, Nakagomi R, Nishio T, Nakai D, Nomura H, Unno M, Suzuki M, Naitoh T, Matsuno S, and Yawo H (1999) Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. *The Journal of biological chemistry* **274:**17159-17163.

Adjei AA and Weinshilboum RM (2002) Catecholestrogen sulfation: possible role in carcinogenesis. *Biochemical and biophysical research communications* **292:**402-408.

Aldridge TC, Tugwood JD, and Green S (1995) Identification and characterization of DNA elements implicated in the regulation of CYP4A1 transcription. *The Biochemical journal* **306 (Pt 2):**473-479.

Aleksunes LM, Yeager RL, Wen X, Cui JY, and Klaassen CD (2012) Repression of hepatobiliary transporters and differential regulation of classic and alternative bile acid pathways in mice during pregnancy. *Toxicological sciences : an official journal of the Society of Toxicology* **130:**257-268.

Allali-Hassani A, Pan PW, Dombrovski L, Najmanovich R, Tempel W, Dong A, Loppnau P, Martin F, Thornton J, Edwards AM, Bochkarev A, Plotnikov AN, Vedadi M, and Arrowsmith CH (2007) Structural and chemical profiling of the human cytosolic sulfotransferases. *PLoS biology* **5:**e97.

Alnouti Y and Klaassen CD (2008) Regulation of sulfotransferase enzymes by prototypical microsomal enzyme inducers in mice. *The Journal of pharmacology and experimental therapeutics* **324:**612-621.

Aninat C, Piton A, Glaise D, Le Charpentier T, Langouet S, Morel F, Guguen-Guillouzo C, and Guillouzo A (2006) Expression of cytochromes P450, conjugating enzymes and nuclear receptors in human hepatoma HepaRG cells. *Drug metabolism and disposition: the biological fate of chemicals* **34:**75-83.

Antonov J, Goldstein DR, Oberli A, Baltzer A, Pirotta M, Fleischmann A, Altermatt HJ, and Jaggi R (2005) Reliable gene expression measurements from degraded RNA by quantitative real-time PCR depend on short amplicons and a proper normalization. *Lab Invest* **85:**1040-1050.

Assem M, Schuetz EG, Leggas M, Sun D, Yasuda K, Reid G, Zelcer N, Adachi M, Strom S, Evans RM, Moore DD, Borst P, and Schuetz JD (2004) Interactions between hepatic Mrp4 and Sult2a as revealed by the constitutive androstane receptor and Mrp4 knockout mice. *The Journal of biological chemistry* **279:**22250-22257.

Bai Q, Xu L, Kakiyama G, Runge-Morris MA, Hylemon PB, Yin L, Pandak WM, and Ren S (2011) Sulfation of 25-hydroxycholesterol by SULT2B1b decreases cellular lipids via the LXR/SREBP-1c signaling pathway in human aortic endothelial cells. *Atherosclerosis* **214:**350-356.

Bairam AF, Rasool MI, Kurogi K, and Liu MC (2017) On the Molecular Basis Underlying the Metabolism of Tapentadol Through Sulfation. *Eur J Drug Metab Pharmacokinet* **42:**793-800.

Bamber DE, Fryer AA, Strange RC, Elder JB, Deakin M, Rajagopal R, Fawole A, Gilissen RA, Campbell FC, and Coughtrie MW (2001) Phenol sulphotransferase SULT1A1*1 genotype is associated with reduced risk of colorectal cancer. *Pharmacogenetics* **11:**679-685.

Banoglu E (2000) Current status of the cytosolic sulfotransferases in the metabolic activation of promutagens and procarcinogens. *Curr Drug Metab* **1:**1-30.

Banoglu E and King RS (2002) Sulfation of indoxyl by human and rat aryl (phenol) sulfotransferases to form indoxyl sulfate. *Eur J Drug Metab Pharmacokinet* **27:**135-140.

Barker EV, Hume R, Hallas A, and Coughtrie WH (1994) Dehydroepiandrosterone sulfotransferase in the developing human fetus: quantitative biochemical and immunological characterization of the hepatic, renal, and adrenal enzymes. *Endocrinology* **134:**982-989.

Barrett KG, Fang H, Gargano MD, Markovich D, Kocarek TA, and Runge-Morris M (2013) Regulation of murine hepatic hydroxysteroid sulfotransferase expression in hyposulfatemic mice and in a cell model of 3'-phosphoadenosine-5'-phosphosulfate deficiency. *Drug metabolism and disposition: the biological fate of chemicals* **41:**1505-1513.

Barrett KG, Fang H, Kocarek TA, and Runge-Morris M (2016) Transcriptional Regulation of Cytosolic Sulfotransferase 1C2 by Vitamin D Receptor in LS180 Human Colorectal Adenocarcinoma Cells. *Drug metabolism and disposition: the biological fate of chemicals* **44:**1431-1434.

Baulieu EE (1998) Neurosteroids: a novel function of the brain. *Psychoneuroendocrinology* **23:**963-987.

Berger U, Wilson P, McClelland RA, Colston K, Haussler MR, Pike JW, and Coombes RC (1988) Immunocytochemical detection of 1,25-dihydroxyvitamin D receptors in normal human tissues. *J Clin Endocrinol Metab* **67:**607-613.

Beyerle J, Frei E, Stiborova M, Habermann N, and Ulrich CM (2015) Biotransformation of xenobiotics in the human colon and rectum and its association with colorectal cancer. *Drug metabolism reviews* **47:**199-221.

Bhatt DK, Mehrotra A, Gaedigk A, Chapa R, Basit A, Zhang H, Choudhari P, Boberg M, Pearce RE, Gaedigk R, Broeckel U, Leeder JS, and Prasad B (2018) Age- and genotype-dependent variability in the protein abundance and activity of six major uridine diphosphate-glucuronosyltransferases in human liver. *Clin Pharmacol Ther*.

Bhattacharya R, Rose PW, Burley SK, and Prlic A (2017) Impact of genetic variation on three dimensional structure and function of proteins. *PloS one* **12:**e0171355.

Blanchard RL, Freimuth RR, Buck J, Weinshilboum RM, and Coughtrie MW (2004) A proposed nomenclature system for the cytosolic sulfotransferase (SULT) superfamily. *Pharmacogenetics* **14:**199-211.

Bourgine J, Billaut-Laden I, Happillon M, Lo-Guidice JM, Maunoury V, Imbenotte M, and Broly F (2012) Gene expression profiling of systems involved in the metabolism and the disposition of xenobiotics: comparison between human intestinal biopsy samples and colon cell lines. *Drug metabolism and disposition: the biological fate of chemicals* **40:**694-705.

Brennan MD and Condra J (2005) Transmission disequilibrium suggests a role for the sulfotransferase-4A1 gene in schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* **139B:**69-72.

Brix LA, Duggleby RG, Gaedigk A, and McManus ME (1999) Structural characterization of human aryl sulphotransferases. *The Biochemical journal* **337 (Pt 2):**337-343.

Bucher S, Jalili P, Le Guillou D, Begriche K, Rondel K, Martinais S, Zalko D, Corlu A, Robin MA, and Fromenty B (2016) Bisphenol a induces steatosis in HepaRG cells using a model of perinatal exposure. *Environ Toxicol*.

Bush CR, Havens JM, Necela BM, Su W, Chen L, Yanagisawa M, Anastasiadis PZ, Guerra R, Luxon BA, and Thompson EA (2007) Functional genomic analysis reveals cross-talk between peroxisome proliferator-activated receptor gamma and calcium signaling in human colorectal cancer cells. *The Journal of biological chemistry* **282:**23387-23401.

Capdevila J, Chacos N, Werringloer J, Prough RA, and Estabrook RW (1981) Liver microsomal cytochrome P-450 and the oxidative metabolism of arachidonic acid. *Proceedings of the National Academy of Sciences of the United States of America* **78:**5362-5366.

Cappiello M, Giuliani L, Rane A, and Pacifici GM (1991) Dopamine sulphotransferase is better developed than p-nitrophenol sulphotransferase in the human fetus. *Dev Pharmacol Ther* **16:**83-88.

Capriotti E, Nehrt NL, Kann MG, and Bromberg Y (2012) Bioinformatics for personal genome interpretation. *Brief Bioinform* **13:**495-512.

Carpenter SP, Lasker JM, and Raucy JL (1996) Expression, induction, and catalytic activity of the ethanol-inducible cytochrome P450 (CYP2E1) in human fetal liver and hepatocytes. *Molecular pharmacology* **49:**260-268.

Cartharius K, Frech K, Grote K, Klocke B, Haltmeier M, Klingenhoff A, Frisch M, Bayerlein M, and Werner T (2005) MatInspector and beyond: promoter analysis based on transcription factor binding sites. *Bioinformatics* **21:**2933-2942.

Ceelen L, De Spiegelaere W, David M, De Craene J, Vinken M, Vanhaecke T, and Rogiers V (2011) Critical selection of reliable reference genes for gene expression study in the HepaRG cell line. *Biochem Pharmacol* **81:**1255-1261.

Chen L, Bush CR, Necela BM, Su W, Yanagisawa M, Anastasiadis PZ, Fields AP, and Thompson EA (2006) RS5444, a novel PPARgamma agonist, regulates aspects of the differentiated phenotype in nontransformed intestinal epithelial cells. *Molecular and cellular endocrinology* **251:**17-32.

Chinnici CM, Timoneri F, Amico G, Pietrosi G, Vizzini G, Spada M, Pagano D, Gridelli B, and Conaldi PG (2015) Characterization of Liver-Specific Functions of Human Fetal Hepatocytes in Culture. *Cell Transplant* **24:**1139-1153.

Chou HC, Lang NP, and Kadlubar FF (1995) Metabolic activation of N-hydroxy arylamines and N-hydroxy heterocyclic amines by human sulfotransferase(s). *Cancer Res* **55:**525-529.

Choughule KV, Joswig-Jones CA, and Jones JP (2015) Interspecies differences in the metabolism of methotrexate: An insight into the active site differences between human and rabbit aldehyde oxidase. *Biochem Pharmacol* **96:**288-295.

Cieslak A, Kelly I, Trottier J, Verreault M, Wunsch E, Milkiewicz P, Poirier G, Droit A, and Barbier O (2016) Selective and sensitive quantification of the cytochrome P450 3A4 protein in human liver homogenates through multiple reaction monitoring mass spectrometry. *Proteomics* **16:**2827-2837.

Coughtrie MW (2002) Sulfation through the looking glass--recent advances in sulfotransferase research for the curious. *The pharmacogenomics journal* **2:**297-308.

Coughtrie MW (2015) Ontogeny of Human Conjugating Enzymes. *Drug Metab Lett* **9:**99-108.

Coughtrie MWH (2016) Function and organization of the human cytosolic sulfotransferase (SULT) family. *Chem Biol Interact* **259:**2-7.

Dajani R, Cleasby A, Neu M, Wonacott AJ, Jhoti H, Hood AM, Modi S, Hersey A, Taskinen J, Cooke RM, Manchee GR, and Coughtrie MW (1999) X-ray crystal structure of human dopamine sulfotransferase, SULT1A3. Molecular modeling and quantitative structure-activity relationship analysis demonstrate a molecular basis for sulfotransferase substrate specificity. *The Journal of biological chemistry* **274:**37862-37868.

de Waziers I, Cugnenc PH, Yang CS, Leroux JP, and Beaune PH (1990) Cytochrome P 450 isoenzymes, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues. *The Journal of pharmacology and experimental therapeutics* **253:**387-394.

Ding X, Lichti K, Kim I, Gonzalez FJ, and Staudinger JL (2006) Regulation of constitutive androstane receptor and its target genes by fasting, cAMP, hepatocyte nuclear factor alpha, and the coactivator peroxisome proliferator-activated receptor gamma coactivator-1alpha. *The Journal of biological chemistry* **281:**26540-26551.

Doherty MM and Charman WN (2002) The mucosa of the small intestine: how clinically relevant as an organ of drug metabolism? *Clin Pharmacokinet* **41:**235-253.

Dong D, Ako R, and Wu B (2012) Crystal structures of human sulfotransferases: insights into the mechanisms of action and substrate selectivity. *Expert Opin Drug Metab Toxicol* **8:**635-646.

Dooley TP, Haldeman-Cahill R, Joiner J, and Wilborn TW (2000) Expression profiling of human sulfotransferase and sulfatase gene superfamilies in epithelial tissues and cultured cells. *Biochemical and biophysical research communications* **277:**236-245.

Drake AJ, Raubenheimer PJ, Kerrigan D, McInnes KJ, Seckl JR, and Walker BR (2010) Prenatal dexamethasone programs expression of genes in liver and adipose tissue and increased hepatic lipid accumulation but not obesity on a high-fat diet. *Endocrinology* **151:**1581-1587.

Drocourt L, Ourlin JC, Pascussi JM, Maurel P, and Vilarem MJ (2002) Expression of CYP3A4, CYP2B6, and CYP2C9 is regulated by the vitamin D receptor pathway in primary human hepatocytes. *The Journal of biological chemistry* **277:**25125-25132.

Duanmu Z, Weckle A, Koukouritaki SB, Hines RN, Falany JL, Falany CN, Kocarek TA, and Runge-Morris M (2006) Developmental expression of aryl, estrogen, and hydroxysteroid sulfotransferases in pre- and postnatal human liver. *The Journal of pharmacology and experimental therapeutics* **316:**1310-1317.

Dubaisi S, Barrett KG, Fang H, Guzman-Lepe J, Soto-Gutierrez A, Kocarek TA, and Runge-Morris M (2018) Regulation of Cytosolic Sulfotransferases in Models of Human Hepatocyte Development. *Drug metabolism and disposition: the biological fate of chemicals*.

Dubaisi S, Fang H, Kocarek TA, and Runge-Morris M (2016) Transcriptional Regulation of Human Cytosolic Sulfotransferase 1C3 by Peroxisome Proliferator-Activated Receptor

gamma in LS180 Human Colorectal Adenocarcinoma Cells. *Molecular pharmacology* **90:**562-569.

Duniec-Dmuchowski Z, Rondini EA, Tibbs ZE, Falany CN, Runge-Morris M, and Kocarek TA (2014) Expression of the orphan cytosolic sulfotransferase SULT1C3 in human intestine: characterization of the transcript variant and implications for function. *Drug metabolism and disposition: the biological fate of chemicals* **42:**352-360.

Dunn RT, 2nd and Klaassen CD (1998) Tissue-specific expression of rat sulfotransferase messenger RNAs. *Drug metabolism and disposition: the biological fate of chemicals* **26:**598-604.

Dussault I, Yoo HD, Lin M, Wang E, Fan M, Batta AK, Salen G, Erickson SK, and Forman BM (2003) Identification of an endogenous ligand that activates pregnane X receptor-mediated sterol clearance. *Proceedings of the National Academy of Sciences of the United States of America* **100:**833-838.

Echchgadda I, Song CS, Oh T, Ahmed M, De La Cruz IJ, and Chatterjee B (2007) The xenobiotic-sensing nuclear receptors pregnane X receptor, constitutive androstane receptor, and orphan nuclear receptor hepatocyte nuclear factor 4alpha in the regulation of human steroid-/bile acid-sulfotransferase. *Molecular endocrinology* **21:**2099-2111.

Echchgadda I, Song CS, Oh TS, Cho SH, Rivera OJ, and Chatterjee B (2004a) Gene regulation for the senescence marker protein DHEA-sulfotransferase by the xenobiotic-activated nuclear pregnane X receptor (PXR). *Mech Ageing Dev* **125:**733-745.

Echchgadda I, Song CS, Roy AK, and Chatterjee B (2004b) Dehydroepiandrosterone sulfotransferase is a target for transcriptional induction by the vitamin D receptor. *Molecular pharmacology* **65:**720-729.

Ekstrom L and Rane A (2015) Genetic variation, expression and ontogeny of sulfotransferase SULT2A1 in humans. *The pharmacogenomics journal* **15:**293-297.

Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, and Auwerx J (1997) The organization, promoter analysis, and expression of the human PPARgamma gene. *The Journal of biological chemistry* **272:**18779-18789.

Falany CN, He D, Dumas N, Frost AR, and Falany JL (2006) Human cytosolic sulfotransferase 2B1: isoform expression, tissue specificity and subcellular localization. *The Journal of steroid biochemistry and molecular biology* **102:**214-221.

Falany CN, He D, Li L, Falany JL, Wilborn TW, Kocarek TA, and Runge-Morris M (2009) Regulation of hepatic sulfotransferase (SULT) 1E1 expression and effects on estrogenic activity in cystic fibrosis (CF). *The Journal of steroid biochemistry and molecular biology* **114:**113-119.

Falany CN, Krasnykh V, and Falany JL (1995) Bacterial expression and characterization of a cDNA for human liver estrogen sulfotransferase. *The Journal of steroid biochemistry and molecular biology* **52:**529-539.

Falany CN and Rohn-Glowacki KJ (2013) SULT2B1: unique properties and characteristics of a hydroxysteroid sulfotransferase family. *Drug metabolism reviews* **45:**388-400.

Falany CN, Vazquez ME, and Kalb JM (1989) Purification and characterization of human liver dehydroepiandrosterone sulphotransferase. *The Biochemical journal* **260:**641-646.

Falany CN, Xie X, Wang J, Ferrer J, and Falany JL (2000) Molecular cloning and expression of novel sulphotransferase-like cDNAs from human and rat brain. *The Biochemical journal* **346 Pt 3:**857-864.

Falany JL, Azziz R, and Falany CN (1998) Identification and characterization of cytosolic sulfotransferases in normal human endometrium. *Chem Biol Interact* **109:**329-339.

Falany JL, Macrina N, and Falany CN (2004) Sulfation of tibolone and tibolone metabolites by expressed human cytosolic sulfotransferases. *The Journal of steroid biochemistry and molecular biology* **88:**383-391.

Fan LQ, You L, Brown-Borg H, Brown S, Edwards RJ, and Corton JC (2004) Regulation of phase I and phase II steroid metabolism enzymes by PPAR alpha activators. *Toxicology* **204:**109-121.

Fang HL, Strom SC, Cai H, Falany CN, Kocarek TA, and Runge-Morris M (2005) Regulation of human hepatic hydroxysteroid sulfotransferase gene expression by the peroxisome proliferator-activated receptor alpha transcription factor. *Molecular pharmacology* **67:**1257-1267.

Fang HL, Strom SC, Ellis E, Duanmu Z, Fu J, Duniec-Dmuchowski Z, Falany CN, Falany JL, Kocarek TA, and Runge-Morris M (2007) Positive and negative regulation of human hepatic hydroxysteroid sulfotransferase (SULT2A1) gene transcription by rifampicin: roles of hepatocyte nuclear factor 4alpha and pregnane X receptor. *The Journal of pharmacology and experimental therapeutics* **323:**586-598.

Fang JL, Wu Y, da Costa GG, Chen S, Chitranshi P, and Beland FA (2015) Human sulfotransferases enhance the cytotoxicity of tolvaptan. *Toxicological sciences : an official journal of the Society of Toxicology*.

Fleige S, Walf V, Huch S, Prgomet C, Sehm J, and Pfaffl MW (2006) Comparison of relative mRNA quantification models and the impact of RNA integrity in quantitative real-time RT-PCR. *Biotechnol Lett* **28:**1601-1613.

Forbes KJ, Hagen M, Glatt H, Hume R, and Coughtrie MW (1995) Human fetal adrenal hydroxysteroid sulphotransferase: cDNA cloning, stable expression in V79 cells and functional characterisation of the expressed enzyme. *Molecular and cellular endocrinology* **112:**53-60.

Freimuth RR, Raftogianis RB, Wood TC, Moon E, Kim UJ, Xu J, Siciliano MJ, and Weinshilboum RM (2000) Human sulfotransferases SULT1C1 and SULT1C2: cDNA characterization, gene cloning, and chromosomal localization. *Genomics* **65:**157-165.

Freimuth RR, Wiepert M, Chute CG, Wieben ED, and Weinshilboum RM (2004) Human cytosolic sulfotransferase database mining: identification of seven novel genes and pseudogenes. *The pharmacogenomics journal* **4:**54-65.

Fromm MF, Kauffmann HM, Fritz P, Burk O, Kroemer HK, Warzok RW, Eichelbaum M, Siegmund W, and Schrenk D (2000) The effect of rifampin treatment on intestinal expression of human MRP transporters. *Am J Pathol* **157:**1575-1580.

Fu J, Fang H, Paulsen M, Ljungman M, Kocarek TA, and Runge-Morris M (2011) Regulation of estrogen sulfotransferase expression by confluence of MCF10A breast epithelial cells: role of the aryl hydrocarbon receptor. *The Journal of pharmacology and experimental therapeutics* **339:**597-606.

Fujita K, Nagata K, Yamazaki T, Watanabe E, Shimada M, and Yamazoe Y (1999) Enzymatic characterization of human cytosolic sulfotransferases; identification of ST1B2 as a thyroid hormone sulfotransferase. *Biol Pharm Bull* **22:**446-452.

Garcia PL, Hossain MI, Andrabi SA, and Falany CN (2018) Generation and Characterization of SULT4A1 Mutant Mouse Models. *Drug metabolism and disposition: the biological fate of chemicals* **46:**41-45.

Gebhardt R (1992) Metabolic zonation of the liver: regulation and implications for liver function. *Pharmacol Ther* **53:**275-354.

Genomes Project C, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, and McVean GA (2012) An integrated map of genetic variation from 1,092 human genomes. *Nature* **491:**56-65.

Gerets HH, Tilmant K, Gerin B, Chanteux H, Depelchin BO, Dhalluin S, and Atienzar FA (2012) Characterization of primary human hepatocytes, HepG2 cells, and HepaRG cells at the mRNA level and CYP activity in response to inducers and their predictivity for the detection of human hepatotoxins. *Cell Biol Toxicol* **28:**69-87.

Ghose R, Omoluabi O, Gandhi A, Shah P, Strohacker K, Carpenter KC, McFarlin B, and Guo T (2011) Role of high-fat diet in regulation of gene expression of drug metabolizing enzymes and transporters. *Life Sci* **89:**57-64.

Glatt H (2000) Sulfotransferases in the bioactivation of xenobiotics. *Chem Biol Interact* **129:**141-170.

Gong H, Guo P, Zhai Y, Zhou J, Uppal H, Jarzynka MJ, Song WC, Cheng SY, and Xie W (2007) Estrogen deprivation and inhibition of breast cancer growth in vivo through activation of the orphan nuclear receptor liver X receptor. *Molecular endocrinology* **21:**1781-1790.

Gonzalez-Herrera L, Valenzuela A, Marchal JA, Lorente JA, and Villanueva E (2013) Studies on RNA integrity and gene expression in human myocardial tissue, pericardial fluid and blood, and its postmortem stability. *Forensic Sci Int* **232:**218-228.

Goodwin B, Hodgson E, and Liddle C (1999) The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module. *Molecular pharmacology* **56:**1329-1339.

Groer C, Busch D, Patrzyk M, Beyer K, Busemann A, Heidecke CD, Drozdzik M, Siegmund W, and Oswald S (2014) Absolute protein quantification of clinically relevant cytochrome P450 enzymes and UDP-glucuronosyltransferases by mass spectrometry-based targeted proteomics. *J Pharm Biomed Anal* **100:**393-401.

Guidry AL, Tibbs ZE, Runge-Morris M, and Falany CN (2017) Expression, purification and characterization of human cytosolic sulfotransferase (SULT) 1C4. *Horm Mol Biol Clin Investig* **29:**27-36.

Gupta RA, Brockman JA, Sarraf P, Willson TM, and DuBois RN (2001) Target genes of peroxisome proliferator-activated receptor gamma in colorectal cancer cells. *The Journal of biological chemistry* **276:**29681-29687.

Gupta RA, Sarraf P, Brockman JA, Shappell SB, Raftery LA, Willson TM, and DuBois RN (2003) Peroxisome proliferator-activated receptor gamma and transforming growth

factor-beta pathways inhibit intestinal epithelial cell growth by regulating levels of TSC-22. *The Journal of biological chemistry* **278:**7431-7438.

Hardwick RN, Ferreira DW, More VR, Lake AD, Lu Z, Manautou JE, Slitt AL, and Cherrington NJ (2013) Altered UDP-glucuronosyltransferase and sulfotransferase expression and function during progressive stages of human nonalcoholic fatty liver disease. *Drug metabolism and disposition: the biological fate of chemicals* **41:**554-561.

Hart SN, Li Y, Nakamoto K, Subileau EA, Steen D, and Zhong XB (2010) A comparison of whole genome gene expression profiles of HepaRG cells and HepG2 cells to primary human hepatocytes and human liver tissues. *Drug metabolism and disposition: the biological fate of chemicals* **38:**988-994.

Hashiguchi T, Shindo S, Chen SH, Hong JS, and Negishi M (2018) Sulfotransferase 4A1 Increases Its Expression in Mouse Neurons as They Mature. *Drug metabolism and disposition: the biological fate of chemicals* **46:**860-864.

He D, Frost AR, and Falany CN (2005) Identification and immunohistochemical localization of Sulfotransferase 2B1b (SULT2B1b) in human lung. *Biochim Biophys Acta* **1724:**119-126.

Her C, Kaur GP, Athwal RS, and Weinshilboum RM (1997) Human sulfotransferase SULT1C1: cDNA cloning, tissue-specific expression, and chromosomal localization. *Genomics* **41:**467-470.

Her C, Wood TC, Eichler EE, Mohrenweiser HW, Ramagli LS, Siciliano MJ, and Weinshilboum RM (1998) Human hydroxysteroid sulfotransferase SULT2B1: two enzymes encoded by a single chromosome 19 gene. *Genomics* **53:**284-295.

Higashi Y, Fuda H, Yanai H, Lee Y, Fukushige T, Kanzaki T, and Strott CA (2004) Expression of cholesterol sulfotransferase (SULT2B1b) in human skin and primary cultures of human epidermal keratinocytes. *J Invest Dermatol* **122:**1207-1213.

Hines RN (2007) Ontogeny of human hepatic cytochromes P450. *J Biochem Mol Toxicol* **21:**169-175.

Hines RN (2008) The ontogeny of drug metabolism enzymes and implications for adverse drug events. *Pharmacol Ther* **118:**250-267.

Hines RN (2013) Developmental expression of drug metabolizing enzymes: impact on disposition in neonates and young children. *Int J Pharm* **452:**3-7.

Hino H, Tateno C, Sato H, Yamasaki C, Katayama S, Kohashi T, Aratani A, Asahara T, Dohi K, and Yoshizato K (1999) A long-term culture of human hepatocytes which show a high growth potential and express their differentiated phenotypes. *Biochemical and biophysical research communications* **256:**184-191.

Hoekstra R, Nibourg GA, van der Hoeven TV, Plomer G, Seppen J, Ackermans MT, Camus S, Kulik W, van Gulik TM, Elferink RP, and Chamuleau RA (2013) Phase 1 and phase 2 drug metabolism and bile acid production of HepaRG cells in a bioartificial liver in absence of dimethyl sulfoxide. *Drug metabolism and disposition: the biological fate of chemicals* **41:**562-567.

Holland NT, Smith MT, Eskenazi B, and Bastaki M (2003) Biological sample collection and processing for molecular epidemiological studies. *Mutation research* **543:**217-234.

Honkakoski P and Negishi M (1998) Protein serine/threonine phosphatase inhibitors suppress phenobarbital-induced Cyp2b10 gene transcription in mouse primary hepatocytes. *The Biochemical journal* **330 (Pt 2):**889-895.

Honkakoski P, Zelko I, Sueyoshi T, and Negishi M (1998) The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene. *Mol Cell Biol* **18:**5652-5658.

Honma W, Kamiyama Y, Yoshinari K, Sasano H, Shimada M, Nagata K, and Yamazoe Y (2001) Enzymatic characterization and interspecies difference of phenol sulfotransferases,

ST1A forms. *Drug metabolism and disposition: the biological fate of chemicals* **29:**274-281.

Honma W, Shimada M, Sasano H, Ozawa S, Miyata M, Nagata K, Ikeda T, and Yamazoe Y (2002) Phenol sulfotransferase, ST1A3, as the main enzyme catalyzing sulfation of troglitazone in human liver. *Drug metabolism and disposition: the biological fate of chemicals* **30:**944-949.

Hui Y, Luo L, Zhang L, Kurogi K, Zhou C, Sakakibara Y, Suiko M, and Liu MC (2015) Sulfation of afimoxifene, endoxifen, raloxifene, and fulvestrant by the human cytosolic sulfotransferases (SULTs): A systematic analysis. *J Pharmacol Sci* **128:**144-149.

Hui Y, Yasuda S, Liu MY, Wu YY, and Liu MC (2008) On the sulfation and methylation of catecholestrogens in human mammary epithelial cells and breast cancer cells. *Biol Pharm Bull* **31:**769-773.

Huin C, Corriveau L, Bianchi A, Keller JM, Collet P, Kremarik-Bouillaud P, Domenjoud L, Becuwe P, Schohn H, Menard D, and Dauca M (2000) Differential expression of peroxisome proliferator-activated receptors (PPARs) in the developing human fetal digestive tract. *J Histochem Cytochem* **48:**603-611.

Ihunnah CA, Jiang M, and Xie W (2011) Nuclear receptor PXR, transcriptional circuits and metabolic relevance. *Biochim Biophys Acta* **1812:**956-963.

Iida A, Sekine A, Saito S, Kitamura Y, Kitamoto T, Osawa S, Mishima C, and Nakamura Y (2001) Catalog of 320 single nucleotide polymorphisms (SNPs) in 20 quinone oxidoreductase and sulfotransferase genes. *J Hum Genet* **46:**225-240.

Ilett KF, Tee LB, Reeves PT, and Minchin RF (1990) Metabolism of drugs and other xenobiotics in the gut lumen and wall. *Pharmacol Ther* **46:**67-93.

Iwatsubo T, Hirota N, Ooie T, Suzuki H, Shimada N, Chiba K, Ishizaki T, Green CE, Tyson CA, and Sugiyama Y (1997) Prediction of in vivo drug metabolism in the human liver from in vitro metabolism data. *Pharmacol Ther* **73:**147-171.

Jancova P, Anzenbacher P, and Anzenbacherova E (2010) Phase II drug metabolizing enzymes. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* **154:**103-116.

Jiang YJ, Kim P, Elias PM, and Feingold KR (2005) LXR and PPAR activators stimulate cholesterol sulfotransferase type 2 isoform 1b in human keratinocytes. *Journal of lipid research* **46:**2657-2666.

Johnsrud EK, Koukouritaki SB, Divakaran K, Brunengraber LL, Hines RN, and McCarver DG (2003) Human hepatic CYP2E1 expression during development. *The Journal of pharmacology and experimental therapeutics* **307:**402-407.

Josse R, Aninat C, Glaise D, Dumont J, Fessard V, Morel F, Poul JM, Guguen-Guillouzo C, and Guillouzo A (2008) Long-term functional stability of human HepaRG hepatocytes and use for chronic toxicity and genotoxicity studies. *Drug metabolism and disposition: the biological fate of chemicals* **36:**1111-1118.

Kalaany NY and Mangelsdorf DJ (2006) LXRS and FXR: the yin and yang of cholesterol and fat metabolism. *Annu Rev Physiol* **68:**159-191.

Kearns GL, Robinson PK, Wilson JT, Wilson-Costello D, Knight GR, Ward RM, van den Anker JN, and Pediatric Pharmacology Research Unit N (2003) Cisapride disposition in neonates and infants: in vivo reflection of cytochrome P450 3A4 ontogeny. *Clin Pharmacol Ther* **74:**312-325.

Kester MH, Kaptein E, Roest TJ, van Dijk CH, Tibboel D, Meinl W, Glatt H, Coughtrie MW, and Visser TJ (1999) Characterization of human iodothyronine sulfotransferases. *J Clin Endocrinol Metab* **84:**1357-1364.

Klaassen CD and Boles JW (1997) Sulfation and sulfotransferases 5: the importance of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) in the regulation of sulfation. *FASEB J* **11:**404-418.

Klein M, Thomas M, Hofmann U, Seehofer D, Damm G, and Zanger UM (2015) A systematic comparison of the impact of inflammatory signaling on absorption, distribution,

metabolism, and excretion gene expression and activity in primary human hepatocytes and HepaRG cells. *Drug metabolism and disposition: the biological fate of chemicals* **43:**273-283.

Kocarek TA and Mercer-Haines NA (2002) Squalestatin 1-inducible expression of rat CYP2B: evidence that an endogenous isoprenoid is an activator of the constitutive androstane receptor. *Molecular pharmacology* **62:**1177-1186.

Kodama S, Hosseinpour F, Goldstein JA, and Negishi M (2011) Liganded pregnane X receptor represses the human sulfotransferase SULT1E1 promoter through disrupting its chromatin structure. *Nucleic acids research* **39:**8392-8403.

Konig J, Rost D, Cui Y, and Keppler D (1999) Characterization of the human multidrug resistance protein isoform MRP3 localized to the basolateral hepatocyte membrane. *Hepatology* **29:**1156-1163.

Koukouritaki SB, Manro JR, Marsh SA, Stevens JC, Rettie AE, McCarver DG, and Hines RN (2004) Developmental expression of human hepatic CYP2C9 and CYP2C19. *The Journal of pharmacology and experimental therapeutics* **308:**965-974.

Koukouritaki SB, Simpson P, Yeung CK, Rettie AE, and Hines RN (2002) Human hepatic flavin-containing monooxygenases 1 (FMO1) and 3 (FMO3) developmental expression. *Pediatr Res* **51:**236-243.

Kremers P, Goujon F, De Graeve J, Van Cantfort J, and Gielen JE (1981) Multiplicity of cytochrome P-450 in primary fetal hepatocytes in culture. *Eur J Biochem* **116:**67-72.

Kurogi K, Chen M, Lee Y, Shi B, Yan T, Liu MY, Sakakibara Y, Suiko M, and Liu MC (2012a) Sulfation of buprenorphine, pentazocine, and naloxone by human cytosolic sulfotransferases. *Drug Metab Lett* **6:**109-115.

Kurogi K, Chepak A, Hanrahan MT, Liu MY, Sakakibara Y, Suiko M, and Liu MC (2014) Sulfation of opioid drugs by human cytosolic sulfotransferases: metabolic labeling study and enzymatic analysis. *Eur J Pharm Sci* **62:**40-48.

Kurogi K, Davidson G, Mohammed YI, Williams FE, Liu MY, Sakakibara Y, Suiko M, and Liu MC (2012b) Ethanol sulfation by the human cytosolic sulfotransferases: a systematic analysis. *Biol Pharm Bull* **35:**2180-2185.

La Merrill M, Cirillo PM, Terry MB, Krigbaum NY, Flom JD, and Cohn BA (2013) Prenatal exposure to the pesticide DDT and hypertension diagnosed in women before age 50: a longitudinal birth cohort study. *Environ Health Perspect* **121:**594-599.

Labroo RB, Paine MF, Thummel KE, and Kharasch ED (1997) Fentanyl metabolism by human hepatic and intestinal cytochrome P450 3A4: implications for interindividual variability in disposition, efficacy, and drug interactions. *Drug metabolism and disposition: the biological fate of chemicals* **25:**1072-1080.

Lacroix D, Sonnier M, Moncion A, Cheron G, and Cresteil T (1997) Expression of CYP3A in the human liver--evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. *Eur J Biochem* **247:**625-634.

Laurent V, Glaise D, Nubel T, Gilot D, Corlu A, and Loyer P (2013) Highly efficient SiRNA and gene transfer into hepatocyte-like HepaRG cells and primary human hepatocytes: new means for drug metabolism and toxicity studies. *Methods in molecular biology* **987:**295-314.

Lazaro CA, Croager EJ, Mitchell C, Campbell JS, Yu C, Foraker J, Rhim JA, Yeoh GC, and Fausto N (2003) Establishment, characterization, and long-term maintenance of cultures of human fetal hepatocytes. *Hepatology* **38:**1095-1106.

Lee AJ, Cai MX, Thomas PE, Conney AH, and Zhu BT (2003) Characterization of the oxidative metabolites of 17beta-estradiol and estrone formed by 15 selectively expressed human cytochrome p450 isoforms. *Endocrinology* **144:**3382-3398.

Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, and Kliewer SA (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest* **102:**1016-1023.

Li L, He D, Wilborn TW, Falany JL, and Falany CN (2009) Increased SULT1E1 activity in HepG2 hepatocytes decreases growth hormone stimulation of STAT5b phosphorylation. *Steroids* **74:**20-29.

Li X, Clemens DL, and Anderson RJ (2000) Sulfation of iodothyronines by human sulfotransferase 1C1 (SULT1C1)*. *Biochem Pharmacol* **60:**1713-1716.

Lin JH, Chiba M, and Baillie TA (1999) Is the role of the small intestine in first-pass metabolism overemphasized? *Pharmacol Rev* **51:**135-158.

Lin R and White JH (2004) The pleiotropic actions of vitamin D. *Bioessays* **26:**21-28.

Lindros KO (1997) Zonation of cytochrome P450 expression, drug metabolism and toxicity in liver. *Gen Pharmacol* **28:**191-196.

Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25:**402-408.

Liyou NE, Buller KM, Tresillian MJ, Elvin CM, Scott HL, Dodd PR, Tannenberg AE, and McManus ME (2003) Localization of a brain sulfotransferase, SULT4A1, in the human and rat brain: an immunohistochemical study. *J Histochem Cytochem* **51:**1655-1664.

Lu R, Kanai N, Bao Y, and Schuster VL (1996) Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA(hPGT). *J Clin Invest* **98:**1142-1149.

Lu X, Jiang K, Han L, Zhang M, Zhou Y, Ma Y, Zhou Y, and Meng S (2015) Sulfonation of curcuminoids: characterization and contribution of individual SULT enzymes. *Molecular nutrition & food research* **59:**634-645.

Lubberstedt M, Muller-Vieira U, Mayer M, Biemel KM, Knospel F, Knobeloch D, Nussler AK, Gerlach JC, and Zeilinger K (2011) HepaRG human hepatic cell line utility as a surrogate for primary human hepatocytes in drug metabolism assessment in vitro. *J Pharmacol Toxicol Methods* **63:**59-68.

Luo L, Mei X, Xi Y, Zhou C, Hui Y, Kurogi K, Sakakibara Y, Suiko M, and Liu MC (2016a) Sulfation of 6-Gingerol by the Human Cytosolic Sulfotransferases: A Systematic Analysis. *Planta Med* **82:**238-243.

Luo L, Zhou C, Hui Y, Kurogi K, Sakakibara Y, Suiko M, and Liu MC (2016b) Human cytosolic sulfotransferase SULT1C4 mediates the sulfation of doxorubicin and epirubicin. *Drug Metab Pharmacokinet*.

Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, Haussler MR, and Mangelsdorf DJ (2002) Vitamin D receptor as an intestinal bile acid sensor. *Science* **296:**1313-1316.

Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, and Shan B (1999) Identification of a nuclear receptor for bile acids. *Science* **284:**1362-1365.

Mangelsdorf DJ and Evans RM (1995) The RXR heterodimers and orphan receptors. *Cell* **83:**841-850.

Maninger N, Wolkowitz OM, Reus VI, Epel ES, and Mellon SH (2009) Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). *Front Neuroendocrinol* **30:**65-91.

Marlowe JL and Puga A (2005) Aryl hydrocarbon receptor, cell cycle regulation, toxicity, and tumorigenesis. *J Cell Biochem* **96:**1174-1184.

Maruyama M, Matsunaga T, Harada E, and Ohmori S (2007) Comparison of basal gene expression and induction of CYP3As in HepG2 and human fetal liver cells. *Biol Pharm Bull* **30:**2091-2097.

Mathis JM, Prough RA, and Simpson ER (1986) Synergistic induction of monooxygenase activity by glucocorticoids and polycyclic aromatic hydrocarbons in human fetal hepatocytes in primary monolayer culture. *Arch Biochem Biophys* **244:**650-661.

Mauvais-Jarvis F, Clegg DJ, and Hevener AL (2013) The role of estrogens in control of energy balance and glucose homeostasis. *Endocrine reviews* **34:**309-338.

Mayer R, Kartenbeck J, Buchler M, Jedlitschky G, Leier I, and Keppler D (1995) Expression of the MRP gene-encoded conjugate export pump in liver and its selective absence from the canalicular membrane in transport-deficient mutant hepatocytes. *J Cell Biol* **131:**137-150.

McMillan BJ and Bradfield CA (2007a) The aryl hydrocarbon receptor is activated by modified low-density lipoprotein. *Proceedings of the National Academy of Sciences of the United States of America* **104:**1412-1417.

McMillan BJ and Bradfield CA (2007b) The aryl hydrocarbon receptor sans xenobiotics: endogenous function in genetic model systems. *Molecular pharmacology* **72:**487-498.

Meinl W, Donath C, Schneider H, Sommer Y, and Glatt H (2008a) SULT1C3, an orphan sequence of the human genome, encodes an enzyme activating various promutagens. *Food Chem Toxicol* **46:**1249-1256.

Meinl W, Ebert B, Glatt H, and Lampen A (2008b) Sulfotransferase forms expressed in human intestinal Caco-2 and TC7 cells at varying stages of differentiation and role in benzo[a]pyrene metabolism. *Drug metabolism and disposition: the biological fate of chemicals* **36:**276-283.

Meinl W, Pabel U, Osterloh-Quiroz M, Hengstler JG, and Glatt H (2006) Human sulphotransferases are involved in the activation of aristolochic acids and are expressed in renal target tissue. *Int J Cancer* **118:**1090-1097.

Meloche CA and Falany CN (2001) Expression and characterization of the human 3 beta-hydroxysteroid sulfotransferases (SULT2B1a and SULT2B1b). *The Journal of steroid biochemistry and molecular biology* **77:**261-269.

Meloche CA, Sharma V, Swedmark S, Andersson P, and Falany CN (2002) Sulfation of budesonide by human cytosolic sulfotransferase, dehydroepiandrosterone-

sulfotransferase (DHEA-ST). *Drug metabolism and disposition: the biological fate of chemicals* **30:**582-585.

Meltzer HY, Brennan MD, Woodward ND, and Jayathilake K (2008) Association of Sult4A1 SNPs with psychopathology and cognition in patients with schizophrenia or schizoaffective disorder. *Schizophr Res* **106:**258-264.

Merlo DF, Agramunt S, Anna L, Besselink H, Botsivali M, Brady NJ, Ceppi M, Chatzi L, Chen B, Decordier I, Farmer PB, Fleming S, Fontana V, Forsti A, Fthenou E, Gallo F, Georgiadis P, Gmuender H, Godschalk RW, Granum B, Hardie LJ, Hemminki K, Hochstenbach K, Knudsen LE, Kogevinas M, Kovacs K, Kyrtopoulos SA, Lovik M, Nielsen JK, Nygaard UC, Pedersen M, Rydberg P, Schoket B, Segerback D, Singh R, Sunyer J, Tornqvist M, van Loveren H, van Schooten FJ, Vande Loock K, von Stedingk H, Wright J, Kleinjans JC, Kirsch-Volders M, van Delft JH, and NewGeneris C (2014) Micronuclei in cord blood lymphocytes and associations with biomarkers of exposure to carcinogens and hormonally active factors, gene polymorphisms, and gene expression: the NewGeneris cohort. *Environ Health Perspect* **122:**193-200.

Miki Y, Nakata T, Suzuki T, Darnel AD, Moriya T, Kaneko C, Hidaka K, Shiotsu Y, Kusaka H, and Sasano H (2002) Systemic distribution of steroid sulfatase and estrogen sulfotransferase in human adult and fetal tissues. *J Clin Endocrinol Metab* **87:**5760-5768.

Mitchell KA and Elferink CJ (2009) Timing is everything: consequences of transient and sustained AhR activity. *Biochem Pharmacol* **77:**947-956.

Miyata M, Matsuda Y, Tsuchiya H, Kitada H, Akase T, Shimada M, Nagata K, Gonzalez FJ, and Yamazoe Y (2006) Chenodeoxycholic acid-mediated activation of the farnesoid X receptor negatively regulates hydroxysteroid sulfotransferase. *Drug Metab Pharmacokinet* **21:**315-323.

Moore LB, Goodwin B, Jones SA, Wisely GB, Serabjit-Singh CJ, Willson TM, Collins JL, and Kliewer SA (2000) St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proceedings of the National Academy of Sciences of the United States of America* **97:**7500-7502.

Mullur R, Liu YY, and Brent GA (2014) Thyroid hormone regulation of metabolism. *Physiol Rev* **94:**355-382.

Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, and Soto AM (2007) Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. *Reproductive toxicology* **23:**383-390.

Ning B, Nowell S, Sweeney C, Ambrosone CB, Williams S, Miao X, Liang G, Lin D, Stone A, Ratnasinghe DL, Manjanatha M, Lang NP, and Kadlubar FF (2005) Common genetic polymorphisms in the 5'-flanking region of the SULT1A1 gene: haplotypes and their association with platelet enzymatic activity. *Pharmacogenet Genomics* **15:**465-473.

Nowell S and Falany CN (2006) Pharmacogenetics of human cytosolic sulfotransferases. *Oncogene* **25:**1673-1678.

Nowell S, Green B, Tang YM, Wiese R, and Kadlubar FF (2005) Examination of human tissue cytosols for expression of sulfotransferase isoform 1A2 (SULT1A2) using a SULT1A2-specific antibody. *Molecular pharmacology* **67:**394-399.

Nunn AD, Scopigno T, Pediconi N, Levrero M, Hagman H, Kiskis J, and Enejder A (2016) The histone deacetylase inhibiting drug Entinostat induces lipid accumulation in differentiated HepaRG cells. *Sci Rep* **6:**28025.

Owen BM, Milona A, van Mil S, Clements P, Holder J, Boudjelal M, Cairns W, Parker M, White R, and Williamson C (2010) Intestinal detoxification limits the activation of hepatic pregnane X receptor by lithocholic acid. *Drug metabolism and disposition: the biological fate of chemicals* **38:**143-149.

Ozawa S, Chou HC, Kadlubar FF, Nagata K, Yamazoe Y, and Kato R (1994) Activation of 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b] pyridine by cDNA-expressed human and rat arylsulfotransferases. *Jpn J Cancer Res* **85:**1220-1228.

Ozawa S, Nagata K, Shimada M, Ueda M, Tsuzuki T, Yamazoe Y, and Kato R (1995) Primary structures and properties of two related forms of aryl sulfotransferases in human liver. *Pharmacogenetics* **5 Spec No:**S135-140.

Pai TG, Sugahara T, Suiko M, Sakakibara Y, Xu F, and Liu MC (2002) Differential xenoestrogen-sulfating activities of the human cytosolic sulfotransferases: molecular cloning, expression, and purification of human SULT2B1a and SULT2B1b sulfotransferases. *Biochim Biophys Acta* **1573:**165-170.

Pawlak M, Lefebvre P, and Staels B (2015) Molecular mechanism of PPARalpha action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *J Hepatol* **62:**720-733.

Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, and Salzberg SL (2015) StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol* **33:**290-295.

Puga A, Maier A, and Medvedovic M (2000) The transcriptional signature of dioxin in human hepatoma HepG2 cells. *Biochem Pharmacol* **60:**1129-1142.

Quandt K, Frech K, Karas H, Wingender E, and Werner T (1995) MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. *Nucleic acids research* **23:**4878-4884.

Radovic B, Hussong R, Gerhauser C, Meinl W, Frank N, Becker H, and Kohrle J (2010) Xanthohumol, a prenylated chalcone from hops, modulates hepatic expression of genes involved in thyroid hormone distribution and metabolism. *Molecular nutrition & food research* **54 Suppl 2:**S225-235.

Raftogianis RB, Wood TC, Otterness DM, Van Loon JA, and Weinshilboum RM (1997) Phenol sulfotransferase pharmacogenetics in humans: association of common SULT1A1 alleles with TS PST phenotype. *Biochemical and biophysical research communications* **239:**298-304.

Raftogianis RB, Wood TC, and Weinshilboum RM (1999) Human phenol sulfotransferases SULT1A2 and SULT1A1: genetic polymorphisms, allozyme properties, and human liver genotype-phenotype correlations. *Biochem Pharmacol* **58:**605-616.

Rainey WE, Carr BR, Sasano H, Suzuki T, and Mason JI (2002) Dissecting human adrenal androgen production. *Trends Endocrinol Metab* **13:**234-239.

Rasool MI, Bairam AF, Kurogi K, and Liu MC (2017) On the sulfation of O-desmethyltramadol by human cytosolic sulfotransferases. *Pharmacol Rep* **69:**953-958.

Reyes H, Reisz-Porszasz S, and Hankinson O (1992) Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science* **256:**1193-1195.

Richard K, Hume R, Kaptein E, Stanley EL, Visser TJ, and Coughtrie MW (2001) Sulfation of thyroid hormone and dopamine during human development: ontogeny of phenol sulfotransferases and arylsulfatase in liver, lung, and brain. *J Clin Endocrinol Metab* **86:**2734-2742.

Riches Z, Stanley EL, Bloomer JC, and Coughtrie MW (2009) Quantitative evaluation of the expression and activity of five major sulfotransferases (SULTs) in human tissues: the SULT "pie". *Drug metabolism and disposition: the biological fate of chemicals* **37:**2255-2261.

Rodrigues RM, Govaere O, Roskams T, Vanhaecke T, Rogiers V, and De Kock J (2016) Gene expression data from acetaminophen-induced toxicity in human hepatic in vitro systems and clinical liver samples. *Data Brief* **7:**1052-1057.

Rondini EA, Fang H, Runge-Morris M, and Kocarek TA (2014) Regulation of human cytosolic sulfotransferases 1C2 and 1C3 by nuclear signaling pathways in LS180 colorectal adenocarcinoma cells. *Drug metabolism and disposition: the biological fate of chemicals* **42:**361-368.

Rumin S, Berthillon P, Tanaka E, Kiyosawa K, Trabaud MA, Bizollon T, Gouillat C, Gripon P, Guguen-Guillouzo C, Inchauspe G, and Trepo C (1999) Dynamic analysis of hepatitis C virus replication and quasispecies selection in long-term cultures of adult human hepatocytes infected in vitro. *J Gen Virol* **80 (Pt 11):**3007-3018.

Runge-Morris M (1998) Regulation of sulfotransferase gene expression by glucocorticoid hormones and xenobiotics in primary rat hepatocyte culture. *Chem Biol Interact* **109:**315-327.

Runge-Morris M and Kocarek TA (2005) Regulation of sulfotransferases by xenobiotic receptors. *Curr Drug Metab* **6:**299-307.

Runge-Morris M and Kocarek TA (2009) Regulation of sulfotransferase and UDP-glucuronosyltransferase gene expression by the PPARs. *PPAR research* **2009:**728941.

Runge-Morris M and Kocarek TA (2013) Expression of the sulfotransferase 1C family: implications for xenobiotic toxicity. *Drug metabolism reviews* **45:**450-459.

Runge-Morris M, Kocarek TA, and Falany CN (2013) Regulation of the cytosolic sulfotransferases by nuclear receptors. *Drug metabolism reviews* **45:**15-33.

Runge-Morris MA (1997) Regulation of expression of the rodent cytosolic sulfotransferases. *FASEB J* **11:**109-117.

Sadler NC, Nandhikonda P, Webb-Robertson BJ, Ansong C, Anderson LN, Smith JN, Corley RA, and Wright AT (2016) Hepatic Cytochrome P450 Activity, Abundance, and Expression Throughout Human Development. *Drug metabolism and disposition: the biological fate of chemicals* **44:**984-991.

Saeki Y, Sakakibara Y, Araki Y, Yanagisawa K, Suiko M, Nakajima H, and Liu MC (1998) Molecular cloning, expression, and characterization of a novel mouse liver SULT1B1 sulfotransferase. *J Biochem* **124:**55-64.

Saini SP, Sonoda J, Xu L, Toma D, Uppal H, Mu Y, Ren S, Moore DD, Evans RM, and Xie W (2004) A novel constitutive androstane receptor-mediated and CYP3A-independent pathway of bile acid detoxification. *Molecular pharmacology* **65:**292-300.

Sakakibara Y, Suiko M, Pai TG, Nakayama T, Takami Y, Katafuchi J, and Liu MC (2002) Highly conserved mouse and human brain sulfotransferases: molecular cloning, expression, and functional characterization. *Gene* **285:**39-47.

Sakakibara Y, Yanagisawa K, Katafuchi J, Ringer DP, Takami Y, Nakayama T, Suiko M, and Liu MC (1998) Molecular cloning, expression, and characterization of novel human SULT1C sulfotransferases that catalyze the sulfonation of N-hydroxy-2-acetylaminofluorene. *The Journal of biological chemistry* **273:**33929-33935.

Sayyed K, Vee ML, Abdel-Razzak Z, Jouan E, Stieger B, Denizot C, Parmentier Y, and Fardel O (2016) Alteration of human hepatic drug transporter activity and expression by cigarette smoke condensate. *Toxicology* **363-364:**58-71.

Schmiedlin-Ren P, Thummel KE, Fisher JM, Paine MF, and Watkins PB (2001) Induction of CYP3A4 by 1 alpha,25-dihydroxyvitamin D3 is human cell line-specific and is unlikely to involve pregnane X receptor. *Drug metabolism and disposition: the biological fate of chemicals* **29:**1446-1453.

Schneider CA, Rasband WS, and Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* **9:**671-675.

Schwarz D, Kisselev P, Ericksen SS, Szklarz GD, Chernogolov A, Honeck H, Schunck WH, and Roots I (2004) Arachidonic and eicosapentaenoic acid metabolism by human CYP1A1: highly stereoselective formation of 17(R),18(S)-epoxyeicosatetraenoic acid. *Biochem Pharmacol* **67:**1445-1457.

Seedorf U and Aberle J (2007) Emerging roles of PPARdelta in metabolism. *Biochim Biophys Acta* **1771:**1125-1131.

Senggunprai L, Yoshinari K, and Yamazoe Y (2009) Selective role of sulfotransferase 2A1 (SULT2A1) in the N-sulfoconjugation of quinolone drugs in humans. *Drug metabolism and disposition: the biological fate of chemicals* **37:**1711-1717.

Sever R and Glass CK (2013) Signaling by nuclear receptors. *Cold Spring Harb Perspect Biol* **5:**a016709.

Shao J, Stapleton PL, Lin YS, and Gallagher EP (2007) Cytochrome p450 and glutathione s-transferase mRNA expression in human fetal liver hematopoietic stem cells. *Drug metabolism and disposition: the biological fate of chemicals* **35:**168-175.

Sharanek A, Burban A, Humbert L, Bachour-El Azzi P, Felix-Gomes N, Rainteau D, and Guillouzo A (2015) Cellular Accumulation and Toxic Effects of Bile Acids in Cyclosporine A-Treated HepaRG Hepatocytes. *Toxicological sciences : an official journal of the Society of Toxicology* **147:**573-587.

Shatalova EG, Loginov VI, Braga EA, Kazubskaia TP, Sudomoina MA, Blanchard RL, and Favorova OO (2006) [Association of polymorphisms in SULT1A1 and UGT1A1 Genes with breast cancer risk and phenotypes in Russian women]. *Mol Biol (Mosk)* **40:**263-270.

Shen DD, Kunze KL, and Thummel KE (1997) Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Adv Drug Deliv Rev* **27:**99-127.

Shen L, Liu Z, Gong J, Zhang L, Wang L, Magdalou J, Chen L, and Wang H (2014) Prenatal ethanol exposure programs an increased susceptibility of non-alcoholic fatty liver disease in female adult offspring rats. *Toxicol Appl Pharmacol* **274:**263-273.

Shiratani H, Katoh M, Nakajima M, and Yokoi T (2008) Species differences in UDP-glucuronosyltransferase activities in mice and rats. *Drug metabolism and disposition: the biological fate of chemicals* **36:**1745-1752.

Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, and Gonzalez FJ (2000) Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* **102:**731-744.

Soldatow VY, Lecluyse EL, Griffith LG, and Rusyn I (2013) In vitro models for liver toxicity testing. *Toxicol Res (Camb)* **2:**23-39.

Song CS, Echchgadda I, Baek BS, Ahn SC, Oh T, Roy AK, and Chatterjee B (2001) Dehydroepiandrosterone sulfotransferase gene induction by bile acid activated farnesoid X receptor. *The Journal of biological chemistry* **276:**42549-42556.

Song CS, Echchgadda I, Seo YK, Oh T, Kim S, Kim SA, Cho S, Shi L, and Chatterjee B (2006) An essential role of the CAAT/enhancer binding protein-alpha in the vitamin D-induced expression of the human steroid/bile acid-sulfotransferase (SULT2A1). *Molecular endocrinology* **20:**795-808.

Sonnier M and Cresteil T (1998) Delayed ontogenesis of CYP1A2 in the human liver. *Eur J Biochem* **251:**893-898.

Srivastava A, Maggs JL, Antoine DJ, Williams DP, Smith DA, and Park BK (2010) Role of reactive metabolites in drug-induced hepatotoxicity. *Handb Exp Pharmacol***:**165-194.

Stanley EL, Hume R, and Coughtrie MW (2005) Expression profiling of human fetal cytosolic sulfotransferases involved in steroid and thyroid hormone metabolism and in detoxification. *Molecular and cellular endocrinology* **240:**32-42.

Stevens JC, Hines RN, Gu C, Koukouritaki SB, Manro JR, Tandler PJ, and Zaya MJ (2003) Developmental expression of the major human hepatic CYP3A enzymes. *The Journal of pharmacology and experimental therapeutics* **307:**573-582.

Strassburg CP, Strassburg A, Kneip S, Barut A, Tukey RH, Rodeck B, and Manns MP (2002) Developmental aspects of human hepatic drug glucuronidation in young children and adults. *Gut* **50:**259-265.

Strott CA (2002) Sulfonation and molecular action. *Endocrine reviews* **23:**703-732.

Su W, Bush CR, Necela BM, Calcagno SR, Murray NR, Fields AP, and Thompson EA (2007) Differential expression, distribution, and function of PPAR-gamma in the proximal and distal colon. *Physiological genomics* **30:**342-353.

Sueyoshi T, Green WD, Vinal K, Woodrum TS, Moore R, and Negishi M (2011) Garlic extract diallyl sulfide (DAS) activates nuclear receptor CAR to induce the Sult1e1 gene in mouse liver. *PloS one* **6:**e21229.

Sueyoshi T, Kawamoto T, Zelko I, Honkakoski P, and Negishi M (1999) The repressed nuclear receptor CAR responds to phenobarbital in activating the human CYP2B6 gene. *The Journal of biological chemistry* **274:**6043-6046.

Sundaram RS, Szumlanski C, Otterness D, van Loon JA, and Weinshilboum RM (1989) Human intestinal phenol sulfotransferase: assay conditions, activity levels and partial purification of the thermolabile form. *Drug metabolism and disposition: the biological fate of chemicals* **17:**255-264.

Tashiro A, Sasano H, Nishikawa T, Yabuki N, Muramatsu Y, Coughtrie MW, Nagura H, and Hongo M (2000) Expression and activity of dehydroepiandrosterone sulfotransferase in human gastric mucosa. *The Journal of steroid biochemistry and molecular biology* **72:**149-154.

Teubner W, Meinl W, Florian S, Kretzschmar M, and Glatt H (2007) Identification and localization of soluble sulfotransferases in the human gastrointestinal tract. *The Biochemical journal* **404:**207-215.

Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, and Willingham MC (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proceedings of the National Academy of Sciences of the United States of America* **84:**7735-7738.

Thomae BA, Rifki OF, Theobald MA, Eckloff BW, Wieben ED, and Weinshilboum RM (2003) Human catecholamine sulfotransferase (SULT1A3) pharmacogenetics: functional genetic polymorphism. *J Neurochem* **87:**809-819.

Thomas M, Burk O, Klumpp B, Kandel BA, Damm G, Weiss TS, Klein K, Schwab M, and Zanger UM (2013) Direct transcriptional regulation of human hepatic cytochrome P450 3A4 (CYP3A4) by peroxisome proliferator-activated receptor alpha (PPARalpha). *Molecular pharmacology* **83:**709-718.

Thompson EA (2007) PPARgamma physiology and pathology in gastrointestinal epithelial cells. *Mol Cells* **24:**167-176.

Thompson PD, Jurutka PW, Whitfield GK, Myskowski SM, Eichhorst KR, Dominguez CE, Haussler CA, and Haussler MR (2002) Liganded VDR induces CYP3A4 in small intestinal and colon cancer cells via DR3 and ER6 vitamin D responsive elements. *Biochemical and biophysical research communications* **299:**730-738.

Thummel KE, Brimer C, Yasuda K, Thottassery J, Senn T, Lin Y, Ishizuka H, Kharasch E, Schuetz J, and Schuetz E (2001) Transcriptional control of intestinal cytochrome P-4503A by 1alpha,25-dihydroxy vitamin D3. *Molecular pharmacology* **60:**1399-1406.

Tibbs ZE, Rohn-Glowacki KJ, Crittenden F, Guidry AL, and Falany CN (2015) Structural plasticity in the human cytosolic sulfotransferase dimer and its role in substrate selectivity and catalysis. *Drug Metab Pharmacokinet* **30:**3-20.

Tobita T, Guzman-Lepe J, Takeishi K, Nakao T, Wang Y, Meng F, Deng CX, Collin de l'Hortet A, and Soto-Gutierrez A (2016) SIRT1 Disruption in Human Fetal Hepatocytes Leads to Increased Accumulation of Glucose and Lipids. *PloS one* **11:**e0149344.

Tsuji S, Kawamura F, Kubiura M, Hayashi A, Ohbayashi T, Kazuki Y, Chesne C, Oshimura M, and Tada M (2014) Dual-color fluorescence imaging to monitor CYP3A4 and CYP3A7 expression in human hepatic carcinoma HepG2 and HepaRG cells. *PloS one* **9:**e104123.

Uppal H, Saini SP, Moschetta A, Mu Y, Zhou J, Gong H, Zhai Y, Ren S, Michalopoulos GK, Mangelsdorf DJ, and Xie W (2007) Activation of LXRs prevents bile acid toxicity and cholestasis in female mice. *Hepatology* **45:**422-432.

Urquhart BL, Tirona RG, and Kim RB (2007) Nuclear receptors and the regulation of drug-metabolizing enzymes and drug transporters: implications for interindividual variability in response to drugs. *J Clin Pharmacol* **47:**566-578.

Visser TJ (1994) Role of sulfation in thyroid hormone metabolism. *Chem Biol Interact* **92:**293-303.

Visser TJ (1996) Role of sulfate in thyroid hormone sulfation. *Eur J Endocrinol* **134:**12-14.

Vyhlidal CA, Gaedigk R, and Leeder JS (2006) Nuclear receptor expression in fetal and pediatric liver: correlation with CYP3A expression. *Drug metabolism and disposition: the biological fate of chemicals* **34:**131-137.

Wada T, Ihunnah CA, Gao J, Chai X, Zeng S, Philips BJ, Rubin JP, Marra KG, and Xie W (2011) Estrogen sulfotransferase inhibits adipocyte differentiation. *Molecular endocrinology* **25:**1612-1623.

Wang J, Falany JL, and Falany CN (1998) Expression and characterization of a novel thyroid hormone-sulfating form of cytosolic sulfotransferase from human liver. *Molecular pharmacology* **53:**274-282.

Wang LQ, Falany CN, and James MO (2004) Triclosan as a substrate and inhibitor of 3'-phosphoadenosine 5'-phosphosulfate-sulfotransferase and UDP-glucuronosyl transferase in human liver fractions. *Drug metabolism and disposition: the biological fate of chemicals* **32:**1162-1169.

Wang LQ and James MO (2006) Inhibition of sulfotransferases by xenobiotics. *Curr Drug Metab* **7:**83-104.

Wang RW, Newton DJ, Scheri TD, and Lu AY (1997) Human cytochrome P450 3A4-catalyzed testosterone 6 beta-hydroxylation and erythromycin N-demethylation. Competition during catalysis. *Drug metabolism and disposition: the biological fate of chemicals* **25:**502-507.

Wang S, Yuan X, Lu D, Guo L, and Wu B (2017) Farnesoid X receptor regulates SULT1E1 expression through inhibition of PGC1alpha binding to HNF4alpha. *Biochem Pharmacol* **145:**202-209.

Watabe T, Ishizuka T, Isobe M, and Ozawa N (1982) A 7-hydroxymethyl sulfate ester as an active metabolite of 7,12-dimethylbenz[alpha]anthracene. *Science* **215:**403-405.

Waxman DJ (1999) P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. *Arch Biochem Biophys* **369:**11-23.

Weis S, Llenos IC, Dulay JR, Elashoff M, Martinez-Murillo F, and Miller CL (2007) Quality control for microarray analysis of human brain samples: The impact of postmortem factors, RNA characteristics, and histopathology. *J Neurosci Methods* **165:**198-209.

Wilborn TW, Comer KA, Dooley TP, Reardon IM, Heinrikson RL, and Falany CN (1993) Sequence analysis and expression of the cDNA for the phenol-sulfating form of human liver phenol sulfotransferase. *Molecular pharmacology* **43:**70-77.

Wilborn TW, Lang NP, Smith M, Meleth S, and Falany CN (2006) Association of SULT2A1 allelic variants with plasma adrenal androgens and prostate cancer in African American men. *The Journal of steroid biochemistry and molecular biology* **99:**209-214.

Wood TC, Aksoy IA, Aksoy S, and Weinshilboum RM (1994) Human liver thermolabile phenol sulfotransferase: cDNA cloning, expression and characterization. *Biochemical and biophysical research communications* **198:**1119-1127.

Xia Y, Stadler D, Lucifora J, Reisinger F, Webb D, Hosel M, Michler T, Wisskirchen K, Cheng X, Zhang K, Chou WM, Wettengel JM, Malo A, Bohne F, Hoffmann D, Eyer F, Thimme R, Falk CS, Thasler WE, Heikenwalder M, and Protzer U (2016) Interferon-gamma and

Tumor Necrosis Factor-alpha Produced by T Cells Reduce the HBV Persistence Form, cccDNA, Without Cytolysis. *Gastroenterology* **150:**194-205.

Yalcin EB, More V, Neira KL, Lu ZJ, Cherrington NJ, Slitt AL, and King RS (2013) Downregulation of sulfotransferase expression and activity in diseased human livers. *Drug metabolism and disposition: the biological fate of chemicals* **41:**1642-1650.

Yamamoto A, Debrah-Pinamang M, DiModica NJ, Kurogi K, Naqvi AA, Hui Y, Sakakibara Y, Suiko M, and Liu MC (2016) Identification and Characterization of the Human Cytosolic Sulfotransferases Mediating the Sulfation of Clioquinol and Iodoquinol. *Drug Metab Lett* **10:**200-205.

Yamamoto A, Liu MY, Kurogi K, Sakakibara Y, Saeki Y, Suiko M, and Liu MC (2015) Sulphation of acetaminophen by the human cytosolic sulfotransferases: a systematic analysis. *J Biochem* **158:**497-504.

Yang HY, Lee QP, Rettie AE, and Juchau MR (1994) Functional cytochrome P4503A isoforms in human embryonic tissues: expression during organogenesis. *Molecular pharmacology* **46:**922-928.

Yasuda S, Idell S, Fu J, Carter G, Snow R, and Liu MC (2007) Cigarette smoke toxicants as substrates and inhibitors for human cytosolic SULTs. *Toxicol Appl Pharmacol* **221:**13-20.

Yue P, Li Z, and Moult J (2005) Loss of protein structure stability as a major causative factor in monogenic disease. *Journal of molecular biology* **353:**459-473.

Zhu BT and Conney AH (1998) Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis* **19:**1-27.

Zhu X, Veronese ME, Iocco P, and McManus ME (1996) cDNA cloning and expression of a new form of human aryl sulfotransferase. *Int J Biochem Cell Biol* **28:**565-571.

Zorn AM (2008) Liver development, in: *StemBook*, Cambridge (MA).

ABSTRACT

CHARACTERIZATION OF PHENOL AND HYDROXYSTEROID CYTOSOLIC SULFOTRANSFERASES IN HUMAN LIVER AND INTESTINE

by

SARAH DUBAISI

May 2019

Advisor: Dr. Melissa Runge-Morris

Major: Pharmacology

Degree: Doctor of Philosophy

SULTs are conjugation enzymes that can modify the activity of a myriad of foreign and endogenous molecules. SULT expression was detected in various human tissues, including liver, small intestine, and colon. There are 13 human SULT genes that are classified into 4 families, SULT1, SULT2, SULT4, and SULT6. In humans, SULT1 and SULT2 families include 11 genes that are further divided into 6 subfamilies. In addition to their role in xenobiotic detoxification and regulation of physiological processes, SULT enzymes were implicated in the bioactivation of procarcinogens. Previous studies detected the expression of most SULT1 and SULT2 enzymes during early development, as early as the embryonic stage. There is limited information about the developmental expression profiles and regulation of SULT1 and SULT2 enzymes in the liver and intestine. The objective of this study was to gain more insight into the roles of SULT1 and SULT2 enzymes during prenatal and postnatal periods in the two main metabolic organs, liver and intestine. To learn more about the regulation of SULT mRNA in differentiating liver cells, we first characterized their expression in primary cultures of human fetal hepatocytes and the HepaRG model of liver cell differentiation, and then examined the effect of treatment with activators of lipid- and xenobiotic-sensing receptors on SULT expression in these *in vitro* models. Using RT-qPCR analysis we demonstrated that SULT1A1 (transcript variants 1, TV1), SULT1C2, SULT1C4, SULT1E1, and SULT2A1 mRNA was the most abundant in human fetal hepatocytes. In HepaRG

cells, SULT1C2 and SULT1E1 mRNA and protein increased during the transition from proliferation to confluency and then decreased as the cells underwent further differentiation whereas SULT2A1 mRNA and protein increased during differentiation. Like SULT1C2, SULT1C3, SULT1C4, and SULT1B1 mRNA levels were highest in the confluency stage. SULT1A1 and SULT2B1 mRNA levels remined relatively constant. Treatment of fetal hepatocytes as well as confluent and differentiating HepaRG cells with activators of aryl hydrocarbon receptor, constitutive androstane receptor, liver X receptor, peroxisome proliferator-activated receptors (PPARs), pregnane X receptor, and vitamin D receptor indicated that SULT1 and SULT2 mRNA is regulated by xenobiotic stimuli.

We also determined the developmental expression profiles of SULT expression in libraries of human liver specimens and cytosols that were collected from prenatal and postnatal (i.e. infants, children 1-18 years-old, and adults) donors using RT-PCR and RNA-seq analysis to measure SULT mRNA and multiple monitoring reaction (MRM) analysis for SULT protein quantification. In this dissertation we reported that SULT1A1 expression did not vary substantially during development; SULT1A3, SULT1C2, SULT1C4, and SULT1E1 expression was highest in prenatal and/or infant specimens; SULT1A2 and SULT2A1 expression was highest postnatally; and SULT1B1 mRNA, as determined by RT-qPCR analysis and protein appears to be highest in children and adults. SULT1A1 (TV5), SULT1C3, and SULT2B1 mRNA levels were low regardless of developmental stage. SULT1C4 mRNA was most abundant in the prenatal livers, but the protein levels were very low. To investigate the reason for this discrepancy we measured the mRNA levels of SULT1C4 TVs in the same human liver specimens described above and determined whether the individual variants can be translated into protein. Using RT-qPCR and RNA-seq analyses we detected at least four SULT1C4 transcript variants, including TV1, TV2, E3DEL, which were detected in the intestinal and hepatic cell lines we examined. These TVs were preferentially expressed in prenatal liver and TV2 was the most abundant of all. Using Western blot analysis we found that only TV1 and TV2 are translated into protein, but TV2 protein was

much lower than that of TV1. This finding suggests that TV2 is either less efficiently translated into protein than is TV1 or that the TV2 protein is more rapidly degraded, and thus could explain the lack of correlation between SULT1C4 mRNA and protein level. Therefore, we conclude that SULT1 and SULT2 expression is modulated by xenobiotics and that most of these enzymes play an important role in hepatic metabolism, especially during early life stages.

Lastly, we examined the mechanism underlying the transcriptional regulation of SULT1C3, which is one of the least studied SULTs, by PPARγ. While attempting to amplify a 2.8 Kb fragment from different sources of human genomic DNA, a 1.9 Kb fragment was sometimes co-amplified with the expected 2.8 Kb fragment. When aligning the 1.9 Kb fragment sequence to the published SULT1C3 5'-flanking sequence an 863 nt deletion (nt -146 to -1008 relative to the transcription start site) was revealed. Transfection of reporter plasmids containing the 2.8 and 1.9 Kb fragments into LS180 cells followed by treatment with PPARα, δ, and γ induced the luciferase expression of the 2.8 but not the 1.9 Kb construct and indicated that the 863 nt deletion region was sufficient to confer PPAR-inducible reporter expression. Three putative PPAR-response elements (PPRE) were identified by computational analysis. Serial deletions, site-directed mutations, and RNA interference analysis demonstrated that only the distal PPRE (at nt -769) was required to mediate PPARγ transcriptional activation of SULT1C3. Genotyping analysis revealed that a similar deletion exist in the human genome. These findings suggest that SULT1C3 play a role in the regulation of PPARγ-controlled pathways.

AUTOBIOGRAPHICAL STATEMENT

Sarah Dubaisi

Education

Ph.D. in Pharmacology **2013-2018**

Wayne State University School of Medicine, Detroit, MI

M.S. in Nutrition and Food Science **2010-2012**

Wayne State University, Detroit, MI

B.S in Nutrition and Dietetics **2007-2010**

American University of Beirut, Beirut, Lebanon

Peer-reviewed articles

1. **Dubaisi S,** Hailin F, Gaedigk R, Vyhlidal CA, Caruso JA, Kocarek TA, and Runge-Morris M, Expression of SULT1C4 Transcript Variants in the Developing Human Liver, in preparation
2. **Dubaisi S,** Caruso JA, Gaedigk R, Vyhlidal CA, Hines RN, Kocarek TA, and Runge-Morris M, Developmental Expression of the Cytosolic Sulfotransferases in Human Liver, in preparation.
3. **Dubaisi S,** Barrett KG, Fang H, Guzman-Lepe J, Soto-Gutierrez A, Kocarek TA, and Runge-Morris M (2018) Regulation of Cytosolic Sulfotransferases in Models of Human Hepatocyte Development. Drug metabolism and Disposition doi: 10.1124/dmd.118.081398
4. **Dubaisi S**, Fang H, Kocarek TA, and Runge-Morris M (2016) Transcriptional Regulation of Human Cytosolic Sulfotransferase 1C3 by Peroxisome Proliferator-Activated Receptor gamma in LS180 Human Colorectal Adenocarcinoma Cells. Molecular pharmacology 90:562-569.
5. Wu J, Shaw J, **Dubaisi S**, Valeriote F, and Li J (2014) In vitro metabolism and drug-drug interaction potential of UTL-5g, a novel chemo- and radioprotective agent. Drug metabolism and disposition: the biological fate of chemicals 42:2058-2067.