

**Effects of Fenofibrate on CYP2D6**  
**and**  
**Regulation of ANG1 and RNASE4 by the FXR Agonist Obeticholic Acid**

Rebecca Kent

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**THESIS**

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Defense Committee:  
Hyunyoung Jeong, Chair and Advisor  
Hyunwoo Lee  
Maria Barbolina

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## LIST OF ABBREVIATIONS

CYP2D6	Cytochrome P450 2D6
SHP	Small heterodimer partner
HNF4 $\alpha$	Hepatocyte nuclear factor 4 alpha
PPAR $\alpha$	Peroxisome proliferator-activated receptor alpha
AMP	Antimicrobial peptide
MMP7	Matrix metalloproteinase-7 (also known as matrilysin)
MAT	Matrilysin
FXR	Farnesoid X receptor
ANG1	Angiogenin 1
Rnase4	Ribonuclease 4
OCA	Obeticholic Acid
HEK293T	Human embryonic kidney 293T cells
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
TNF $\alpha$	Tumor necrosis factor alpha
NF- $\kappa$ B	Nuclear factor kappa-light-chain-enhancer of activated B cells

## SUMMARY

### Chapter 1

CYP2D6 is a major drug metabolizing enzyme that exhibits large interindividual variability. It is important to understand the factors that lead to CYP2D6 variability, which can affect the efficacy and toxicity of drugs metabolized by CYP2D6. SHP is a corepressor that represses CYP2D6 expression by binding and inhibiting HNF4 $\alpha$ , and modulators of SHP have been shown to alter CYP2D6 expression. Fenofibrate, a drug commonly used to reduce triglyceride levels and total cholesterol levels in hypertriglycerideamic and hypercholesterolaemic patients, has been shown to induce SHP mRNA. Examination of the effect of fenofibrate on SHP and CYP2D6 can provide insight into the factors that control transcriptional regulation of CYP2D6.

In chapter one, we investigated the effect of fenofibrate treatment on the mRNA and protein expression of SHP and CYP2D6 in mice and primary human hepatocytes by using real-time polymerase chain reaction (qRT-PCR) and western blot. We also examined the role of PPAR $\alpha$ , a nuclear receptor induced by fenofibrate, in contributing to the regulation of CYP2D6 by transactivating the CYP2D6 promoter.

The results showed that mice treatment with fenofibrate for 5 days had a 2-fold increase in SHP protein level. Importantly, despite the repressive role of SHP in CYP2D6 promoter activity, the mRNA and protein levels of CYP2D6 did not differ between the fenofibrate-treated and control mice. Similar findings were obtained in fenofibrate-treated human hepatocytes. To explain the lack of changes in CYP2D6 mRNA level despite the increase in SHP protein, we examined whether fenofibrate-activated PPAR $\alpha$  transactivates the CYP2D6 promoter. In a transient transfection assay, PPAR $\alpha$  transfection alone led to a 4-fold increase in CYP2D6 promoter activity. In conclusion, these results indicate that PPAR $\alpha$  transactivates the CYP2D6 promoter and suggest that activation of the CYP2D6 promoter by PPAR $\alpha$  may counteract the inhibitory effects of increased SHP protein on CYP2D6 expression upon fenofibrate treatment.



## SUMMARY (continued)

### Chapter 2

Antimicrobial peptides (AMPs) are innate antibiotic effector molecules that contribute to the control of commensal bacteria load as well as defense against intestinal pathogens in the small intestine. Understanding the factors that regulate AMP expression is important because they have potential as therapeutic agents against pathogenic bacteria. Accumulating evidence suggests that the farnesoid X receptor (FXR) has a critical role in protecting against bacterial overgrowth in the small intestine. It is possible that FXR mediates regulation of AMP expression and their subsequent action on gut bacteria. To date, whether intestinal FXR regulates AMP expression remains unknown.

In chapter two, we investigated whether FXR is capable of regulating AMP expression by treating mice and human hepatocytes with an FXR agonist. qRT-PCR was then used to measure the mRNA expression of a range of AMPs. We also investigated whether FXR agonists can confer protection against pathogenic enteric bacterial infection by upregulating certain AMPs. To this end, we examined whether the FXR agonist obeticholic acid (OCA) effects the survival time of mice treated with *Salmonella enterica*.

The results showed a 2.5-fold increase in the ileal mRNA expression level of AMPs angiogenin 1 (ANG1) and ribonuclease 4 (RNASE4) in mice treated with two doses of OCA. Similar results were found in primary human hepatocytes treated with the FXR agonist GW4064. A transient transfection assay determined that FXR directly transactivates ANG1 and RNASE4 expression. Survival assays were conducted in which OCA was administered to mice either concurrently with *S. enterica* infection or one week prior to *S. enterica* transfection. There was not a significant difference in survival time between the groups administered OCA and vehicle.

In conclusion, FXR activation increased Ang1 and Rnase4 ileal mRNA levels in mice and primary human hepatocytes, likely due to FXR directly enhancing the transcription of Ang1 and Rnase4 mRNA. However, treatment with the FXR agonist OCA was not sufficient to confer

protection against *S. enterica* infection in mice and may not be a potential therapy for enteric pathogen infection.

## **1. Effects of fenofibrate on the expression of small heterodimer partner (SHP) and cytochrome P450 (CYP) 2D6**

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### **1.1 Introduction**

Cytochrome P450 (CYP) 2D6 is a major drug-metabolizing enzyme, responsible for eliminating 25% of marketed drugs. CYP2D6 exhibits large interindividual variability, in part due to differential transcriptional regulation of CYP2D6 (1, 2); however, the factors controlling transcriptional regulation of CYP2D6 remain poorly understood. The nuclear receptor small heterodimer partner (SHP) is a corepressor of many transcription factors, including hepatocyte nuclear factor (HNF) 4 $\alpha$  (3). SHP represses CYP2D6 expression by binding and inhibiting HNF4 $\alpha$ , and modulators of SHP have been shown to alter CYP2D6 expression (4). For example, CYP2D6 expression was increased in CYP2D6-humanized (Tg-CYP2D6) mice upon siRNA-induced SHP knock-down (4), and was repressed by a chemical inducer of SHP expression (i.e., GW4064) in Tg-CYP2D6 mice and human hepatocytes (5).

Fenofibrate is a fibric acid derivative commonly used to reduce triglyceride levels and total cholesterol levels in hypertriglycerideamic and hypercholesterolaemic patients (6). Pharmacological action of fenofibrate is mediated by peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ), the endogenous ligand of which is unsaturated fatty acids and eicosanoids (7). Fenofibrate is a synthetic ligand of the PPAR $\alpha$ , which partners with retinoid receptor  $\alpha$  (RXR $\alpha$ ) upon ligand binding (8). The PPAR $\alpha$ /RXR $\alpha$  heterodimer complex binds to the peroxisome proliferator response element (PPRE) in the promoter of target genes, which consists of a direct repeat 1 (DR1) binding motif (9). Activation of PPAR $\alpha$  induces the expression of CYP4A enzymes,

which catalyze the  $\omega$ -hydroxylation of long-chain fatty acids. This contributes to a reduction of fatty acid synthesis (10).

Fenofibrate has been shown to induce SHP mRNA and protein expression through activation of the AMP-activated protein kinase (AMPK) signaling pathway in HepG2 cells and mice (11). Whether the induction of SHP by fenofibrate in turn leads to repressed expression of hepatic CYP2D6 remains unknown. The goal of this study is to examine the effects of fenofibrate on hepatic CYP2D6 expression, and thus provide a mechanistic basis to predict potential drug-drug interactions between fenofibrate and CYP2D6 substrates.

## 1.2 **Materials and methods**

### 1.2.1 **Chemicals and reagents**

Fenofibrate was purchased from Sigma-Aldrich (St. Louis, MO).

### 1.2.2 **Animals**

Tg-CYP2D6 mice were previously described (12). Adult male mice (10 weeks of age and weighing 20-25 g) were used for the experiments. Mice were treated by intraperitoneal (IP) injection with 100mg/kg/day fenofibrate or vehicle (DMSO in olive oil) once per day for five days. On the sixth day, mice were sacrificed, and liver tissues were collected. All procedures were approved by the Institutional Animal Care and Use Committee in the University of Illinois at Chicago.

### 1.2.3 **Western blot**

Western blot was performed as described previously (4). CYP2D6 and SHP protein expression levels were determined by using the respective antibodies (CYP2D6, Corning, Corning, NY; SHP, H-160, Santa Cruz Biotechnology, Dallas, TX).

### 1.2.4 **Primary human hepatocytes**

Freshly isolated human hepatocytes from three donors were obtained from the Liver Tissue Cell Distribution System (Pittsburgh, PA; funded by the National Institutes of Health

Contract #HHSN276201200017C). Upon receiving the cells that were shipped overnight, the media used to preserve the cells was replaced with Williams' E media (without phenol red) containing 50 g/mL streptomycin, 50 U/mL penicillin, 100 nM dexamethasone, 1% insulin-transferrin-sodium selenite (ITS), 2 mM L-glutamine, and 15 mM HEPES. Cells were acclimated in the media overnight at 37°C with 5% CO<sub>2</sub>. The cells were treated with vehicle control (DMSO) or 200 µM fenofibrate for 48 hours. RNA was isolated from the hepatocytes from all three donors, while protein was isolated from hepatocytes from two of the donors.

#### 1.2.5 **Plasmids**

Luciferase reporter vectors containing the upstream DR1 regulatory region (and mutated DR1) in the CYP2D6 promoter was provided by Dr. H. Hara (Gifu Pharmaceutical University, Gifu, Japan). The PPARα expression plasmid and negative control pCMX vector were provided by Dr. Yoon-kwang Lee (Northeast Ohio Medical University, Rootstown, OH). The RXRα expression plasmid was provided by Dr. Alan Mclachlan (University of Illinois at Chicago, Chicago, IL).

#### 1.2.6 **Luciferase reporter assay**

HEK293T cells were seeded in 24-well plates at a density of 1x10<sup>5</sup> cells/ml. Twenty-four hours later, the cells were transfected with 0.8 ng of luciferase construct, 0.4 ng of PPARα and RXRα or negative control plasmids, and 0.04 ng of Renilla vector (Promega, Madison, WI) using Eugene HD transfection reagent (Promega) according to the manufacturer's protocol. The transfected cells were treated with fenofibrate or the PPARα inhibitor MK886 (Fisher Scientific, Pittsburgh, PA) 24 hours after transfection. The cells were harvested for determination of luciferase activity using a dual-luciferase assay kit (Promega) 24 hours after treatment. At least two independent experiments were performed in triplicate.

#### 1.2.7 **RNA isolation and quantitative real time-PCR (qRT-PCR)**

Total RNA was isolated from mouse liver tissues using Trizol (Life Technologies) and converted to cDNA using High-Capacity cDNA Reverse Transcription Kit (Life Technologies). Using the cDNA as a template, qRT-PCR was performed using StepOnePlus Real-Time PCR

System and the following primers from Integrated DNA Technologies (Coralville, Iowa): CYP2D6 (Hs.PT.49a.205234723), CYP3A4 (Hs.PT.58.1272782), human SHP (Hs.PT.58.38586840), mouse SHP (Mm.PT.58.41588826), human HNF4 $\alpha$  (Hs.PT.58.22303533), mouse HNF4 $\alpha$  (Mm.PT.58.6428917), and mouse CYP4A14 (Mm.PT.58.5965362). The results were expressed as fold changes under treatment using the gene expression levels normalized to those of GAPDH ( $2^{-\Delta\Delta C_t}$  method).

### 1.2.8 **Statistical analysis**

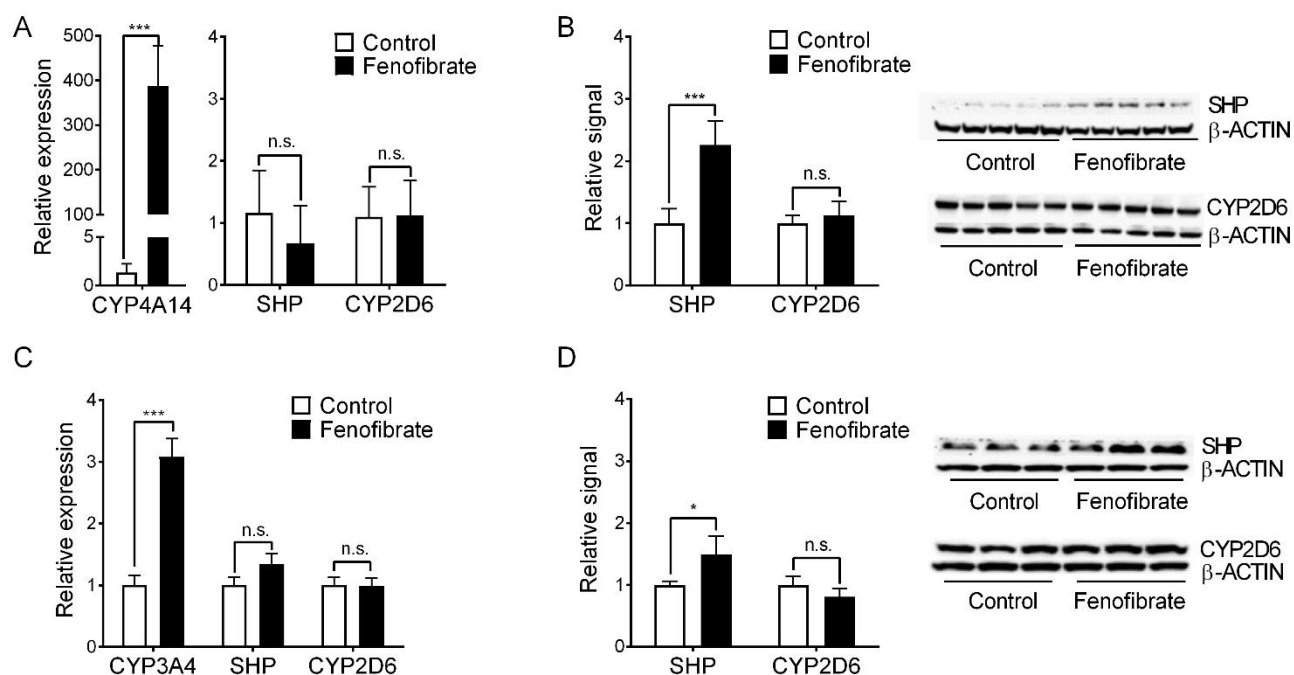
Values were reported as mean  $\pm$  standard deviation (S.D.). The control and fenofibrate-treated groups were compared using the Student's t-test.

## 1.3 **Results**

### 1.3.1 **Fenofibrate treatment increases small heterodimer partner (SHP) protein expression in mice and human hepatocytes.**

To determine the effects of fenofibrate on CYP2D6 mRNA and protein expression, fenofibrate or vehicle was administered by intraperitoneal injection for five days to Tg-CYP2D6 mice. In parallel, human hepatocytes were treated with fenofibrate or vehicle for 48 hours. mRNA and protein levels of CYP2D6 and SHP were determined by qRT-PCR and western blot in fenofibrate-treated mice or human hepatocytes. CYP4A14 and CYP3A4, representative target genes of PPAR $\alpha$  in mice and humans respectively (13, 14), were included as a positive control. In mice, while fenofibrate treatment led to a ~400-fold increase in CYP4A14 mRNA expression, there was no difference in the mRNA levels of SHP (Fig. 1A) and HNF4 $\alpha$  (data not shown) between the fenofibrate-treated and control groups. Interestingly, the western blot results showed an increase in SHP protein levels in fenofibrate-treated mice (Fig. 1B), with a magnitude of induction comparable to that by GW4064 (5). Importantly, despite the repressive role of SHP in CYP2D6 promoter activity (4), the mRNA (and protein) levels of CYP2D6 did not differ between the fenofibrate-treated and control mice (Fig. 1A and 1B). Similar findings (i.e., increased SHP

protein levels but no difference in CYP2D6 expression) were obtained in fenofibrate-treated human hepatocytes (Fig. 1C and D).



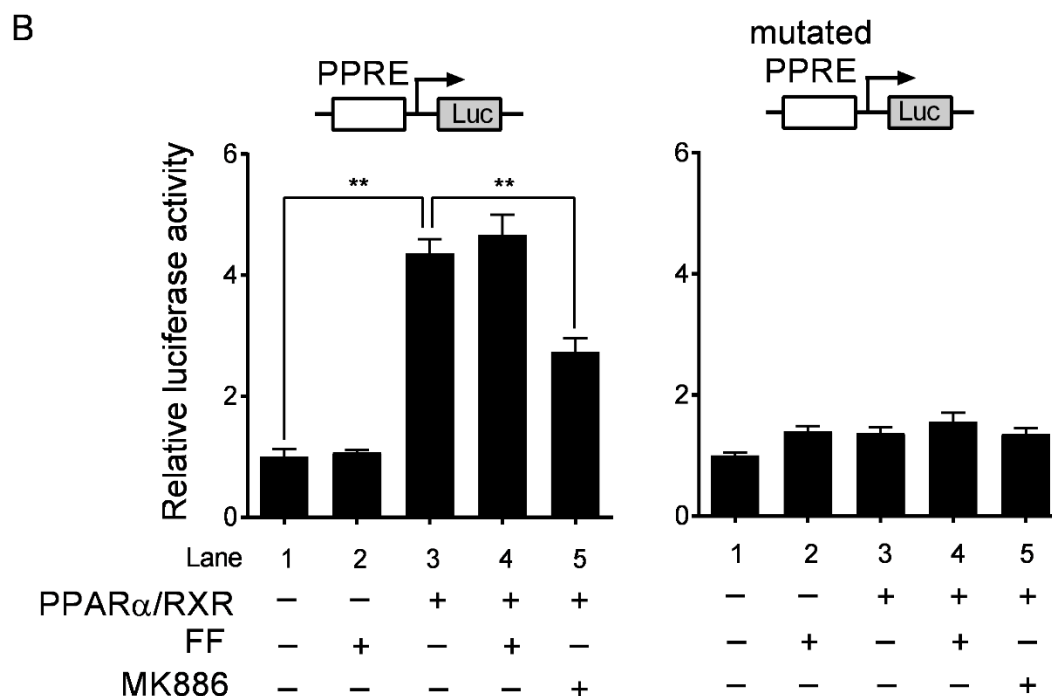
**Figure 1. SHP protein expression is increased with fenofibrate treatment in Tg-CYP2D6 mice and primary human hepatocytes.** A and B, Tg-CYP2D6 mice treated with fenofibrate (100 mg/kg/day) or vehicle control (olive oil) for five days (n=5 mice/group). C and D, Primary human hepatocytes were treated in triplicate with fenofibrate (200  $\mu$ M) for 48 hours. Data shown are representative results from one of three donors. Hepatic mRNA expression levels (A, C) and protein expression (B, D) were determined by qRT-PCR and western blot, respectively. Values are presented as mean  $\pm$  standard deviation. \*  $p < 0.05$ , \*\*\*  $p < 0.001$ ; n.s., not statistically significant.

### 1.3.2 **Peroxisome proliferator-activated receptor $\alpha$ (PPAR $\alpha$ ) positively regulates cytochrome P450 (CYP) 2D6 expression in HEK293T cells.**

To explain the lack of changes in CYP2D6 mRNA level despite the increase in SHP protein, we examined whether fenofibrate-activated PPAR $\alpha$  transactivates the CYP2D6 promoter. Examination of CYP2D6 gene revealed a putative PPRE (i.e., DR1) in the proximal region of the CYP2D6 promoter (Fig 2A). To determine whether PPAR $\alpha$  induces CYP2D6 promoter activity, HEK293T cells were co-transfected with the following vectors: PPAR $\alpha$  and RXR; a luciferase reporter vector containing the putative PPRE of the CYP2D6 promoter; and a *Renilla* expression vector. A CYP2D6 promoter vector harboring a mutated PPRE was included as a negative control. The cells were treated with fenofibrate, MK886 (a synthetic PPAR $\alpha$  inhibitor), or vehicle, and luciferase activity was measured. Interestingly, PPAR $\alpha$  transfection alone led to a significant increase in CYP2D6 promoter activity (Fig. 2B, left, lane 1 vs. 3), suggesting the presence of an endogenous ligand(s) in the cells. MK886 repressed the PPAR $\alpha$ -mediated induction of the CYP2D6 promoter (Fig 2B, left, lane 3 vs. 5). The changes in CYP2D6 promoter activity brought by PPAR $\alpha$  transfection and MK886 treatment were abrogated when the CYP2D6 promoter vector with a mutated PPRE was used (Fig. 2B, right). Together, these results indicate that PPAR $\alpha$  transactivates the CYP2D6 promoter and suggest that activation of the CYP2D6 promoter by PPAR $\alpha$  may counteract the inhibitory effects of increased SHP protein on CYP2D6 expression upon fenofibrate treatment. SHP is a known corepressor of multiple nuclear receptors (3).



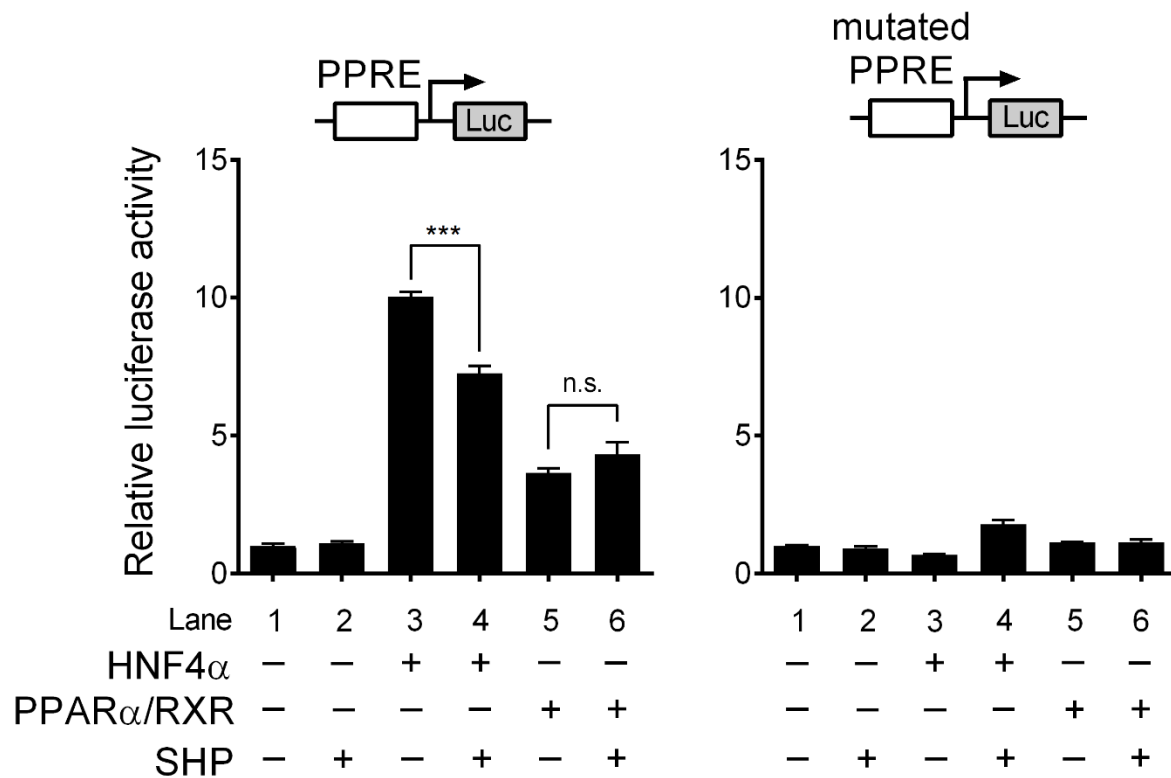
A -100 CTCTGCCACTGGCAGCACAGTCAACACAGCAGGTTCACTCA  
 -59 CAGCAGAGGGCAAAGGCCATCATCAGCTCCCTTTATAAGGG  
 -16 AAGGGTCACGCGCTCGGTGTGCTGAGAGTGT



**Figure 2. CYP2D6 promoter is positively regulated by PPAR $\alpha$  in HEK293T cells.** A, The direct repeat 1 (DR1) motif (underlined) in the upstream regulatory region of the CYP2D6 gene is a PPAR $\alpha$  response element (PPRE). B, HEK293T cells were transfected in triplicate with luciferase reporter vector containing CYP2D6 promoter PPRE (left) or mutated PPRE (right), renilla expression vector, PPAR $\alpha$  expression vector, and RXR expression vector. The cells were treated with vehicle (control), fenofibrate (200  $\mu$ M), or MK886 (2  $\mu$ M). Dual luciferase reporter assays were performed. \*\*  $p < 0.01$ .

To examine whether SHP represses PPAR $\alpha$  transactivation of CYP2D6 promoter, a promoter reporter assay was performed. HEK293T cells were co-transfected with expression vectors (SHP and PPAR $\alpha$ /RXR) and CYP2D6 promoter vector harboring the putative PPRE of

the CYP2D6 promoter. SHP repression of HNF4 $\alpha$  action on CYP2D6 promoter was examined as a positive control. SHP repressed HNF4 $\alpha$  transactivation of CYP2D6 promoter as expected (Fig. 3). However, co-transfection with SHP and PPAR $\alpha$ /RXR did not affect CYP2D6 promoter activation (Fig. 3), indicating that SHP does not repress PPAR $\alpha$  activation of the CYP2D6 promoter.



**Figure 3. Transactivation of CYP2D6 promoter by PPAR $\alpha$  is not inhibited by SHP.** HEK293T cells were transfected in triplicate with a luciferase reporter vector containing CYP2D6 promoter PPRE (left) or mutated PPRE (right), renilla expression vector, and respective expression vectors. Dual luciferase reporter assays were performed. \*\*\*  $p < 0.001$ .

#### 1.4 **Discussion**

To determine the effects of SHP modulator fenofibrate on CYP2D6 expression, we examined SHP and CYP2D6 expression levels in fenofibrate-treated Tg-CYP2D6 mice and human hepatocytes. The results revealed insignificant changes in CYP2D6 expression upon fenofibrate treatment.

Our results showed that fenofibrate treatment increased SHP protein expression without changing the mRNA level. This is inconsistent with the previous report where both mRNA and protein levels of SHP were enhanced by fenofibrate in mice (11). This could be potentially due to differences in study design. While fenofibrate was administered intraperitoneally in this study, it was included in diet of the mice in the previous study, subsequently leading to different amounts and frequency of fenofibrate dosage between the two studies. Regardless of this apparent discrepancy, both studies demonstrated increased protein expression of SHP.

In this study, an apparent disconnect between mRNA and protein levels of SHP was noted. miRNA is a small non-coding RNA that binds to mRNA and causes mRNA destabilization/inefficient translation (15), often causing such a disconnect between mRNA and protein levels of target genes. Of note, SHP has previously been reported to be regulated by miR-141-3p; however, the hepatic expression of miR-141-3p did not differ between the fenofibrate and vehicle-treated mice (data not shown). Whether fenofibrate modulates expression of as-yet-unknown miRNAs that can regulated SHP expression remains unknown. Another explanation for the disconnect between SHP mRNA and protein levels is altered degradation of SHP protein by fenofibrate. It has been shown that SHP protein is rapidly degraded via the ubiquitin-proteasome pathway (SHP protein half-life shorter than 30 min) (16). It remains unknown whether fenofibrate also inhibits the ubiquitin pathway in the liver, resulting in increased SHP protein expression.

Despite the role of SHP as a transcriptional repressor of CYP2D6 and increased hepatic SHP protein level in fenofibrate-treated mice, CYP2D6 expression did not differ between the fenofibrate- and vehicle-treated mice. Fenofibrate is an agonist of PPAR $\alpha$  that, upon ligand

activation, transactivates multiple gene promoters by binding the PPRE (17). Our results from the promoter reporter assay indicate that PPAR $\alpha$  activates the CYP2D6 promoter, likely via the DR1 sequence located within the proximal promoter region. We also demonstrated that SHP does not inhibit PPAR $\alpha$  activity on the CYP2D6 promoter, which can be attributed to significant differences in activator function-2 (AF2) domains between HNF4 $\alpha$  and PPAR $\alpha$ . SHP is known to inhibit HNF4 $\alpha$  activity in part by competing with coactivator binding at the AF-2 domain of HNF4 $\alpha$  (18). Together, these results suggest the possibility that PPAR $\alpha$  transactivation of the CYP2D6 promoter compensates for the repressed CYP2D6 promoter activity by SHP (Graphical Abstract). Of note, another PPAR $\alpha$  agonist clofibrate was reported not to induce SHP expression in mice (11). It is expected that clofibrate would induce CYP2D6 expression (via PPAR $\alpha$  transactivation of CYP2D6 promoter without SHP upregulation), and this remains to be tested.

In conclusion, fenofibrate increased hepatic SHP protein levels in Tg-CYP2D6 mice, but unexpectedly, this did not lead to a change in CYP2D6 expression. PPAR $\alpha$  transactivation of the CYP2D6 promoter is potentially responsible for these results. Drug-drug interaction between fenofibrate and CYP2D6 substrates appears unlikely.

## 2. Effects of FXR activation on Angiogenin1 (Ang1) and Ribonuclease4 (Rnase4) expression in the small intestine

### 2.1 Introduction

Antimicrobial peptides (AMPs) are innate antibiotic effector molecules that contribute to the control of commensal bacteria load as well as defense against intestinal pathogens in the small intestine (19-21). They are released from Paneth cells located in the crypts of Lieberkühn in the ileum (22). Most antibacterial AMPs are cationic and amphipathic, and therefore can bind and disrupt the membranes of gram (-) and gram (+) bacteria (23-25). Over 100 human host AMPs have been identified (26), many of which fall into the major categories of s100 proteins, defensins, cathelicidins, and ribonucleases. While AMPs are typically 30-100 amino acids in length, they can also be defined as antimicrobials that are encoded by genes and ribosomally synthesized regardless of length, as opposed to non-ribosomally synthesized antimicrobials found in bacteria and fungi (27, 28). The release of AMPs in granules from paneth cells to the intestinal lumen is stimulated by an increase in calcium and the subsequent activation of calcium-activated potassium channels (22). The increased calcium results from stimuli including cholinergic agonists, gram (-) and gram (+) bacteria, and bacterial products such as lipopolysaccharides and lipoteichoic acid (22, 29).

The release of AMP-containing granules from paneth cells is one way in which AMPs are regulated. They can also be transcriptionally regulated, as the expression of cathelicidins and defensins has been shown to be regulated by dietary nutrients such as vitamins A and D and products of microbial metabolism such as butyrate (30). Intestinal inflammation in diseases such as Crohn's disease and inflammatory bowel disease has also been shown to increase expression of defensins, lysozyme, and PLA<sub>2</sub> (31, 32). This may be due to increased differentiation of paneth cells in the colon that occurs with chronic inflammation (33). Activation of T-cells in the intestinal mucosa due to bacterial infection also increases the amount of paneth

cells and subsequently AMPs (34, 35). Certain AMPs, such as  $\alpha$ -defensin, are released as pro-peptides and need to be processed to a mature peptide by the luminal enzyme matrix metalloproteinase-7 (MMP7). In matrilysin (MAT) -null mice that do not express MMP7, small intestinal extracts had decreased antimicrobial activity against *Escherichia coli* and *Salmonella typhimurium* (19). MMP7 is also transcriptionally upregulated in response to pro-inflammatory cytokines (36).

Mice that do not produce a bioactive AMP ( $\alpha$ -defensin) were found to be more susceptible to infection by *Salmonella Typhimurium* (37), suggesting critical roles of AMPs in host defense against enteric pathogens and their potential as therapeutic agents against pathogenic bacteria. This study focuses on AMPs that are expressed in the mouse small intestine epithelium, many of which have been shown to contribute to pathogen defense or control of commensal bacteria.

The farnesoid X receptor (FXR) is a nuclear receptor that is known to play a key role in maintaining bile acid homeostasis (38, 39). FXR is expressed in the duodenum, jejunum, ileum, colon, and liver, with highest mRNA expression levels in the ileum (40). Accumulating evidence suggests critical roles of FXR in protecting against bacterial overgrowth in the small intestine (40, 41). In mice with bile duct ligation, a procedure that leads to intestinal bacterial overgrowth, a FXR agonist GW4064 abrogated bacterial overgrowth in the small intestine (18). The underlying mechanism was unclear. One potential possibility is FXR-mediated regulation of AMP expression and their subsequent action on bacteria. To date, whether intestinal FXR regulates AMP expression remains unknown.

The objective of this study is to examine whether FXR is capable of regulating AMP expression, and if so, elucidate the regulatory mechanisms. A concurrent goal is to investigate whether FXR agonists can confer protection against pathogenic enteric bacterial infection by upregulating certain AMPs.

## 2.2 **Materials and Methods**

### 2.2.1 **Chemicals and reagents**

Obeticholic acid (OCA) was purchased from ApexBio (Boston, MA). Methylcellulose (viscosity 400 CP) was purchased from Sigma-Aldrich (St. Louis, MO). GW4064 was purchased from Fisher Scientific (Waltham, MA).

### 2.2.2 **Animals**

Adult male C57BL/6 mice (8-11 weeks of age and weighing 20-25 g) were used for this study. *FXR-null* were provided by Dr. Grace Guo (Rutgers University, New Brunswick, NJ) and were bred at our institution. Tail snips were genotyped to confirm *FXR-null* status using the following probes for PCR: TCTCTTTAAGTGATGACGGGAATCT (forward 1), GCTCTAAGGAGAGTCACTTGTGCA (forward 2), GCATGCTCTGTTCATAAACGCCAT (reverse).

To screen for changes in ileal AMP mRNA levels, wild type mice (n=5; Jackson Laboratory, Bar Harbor, ME) and FXR-null mice (n=4) were treated with vehicle (1% methylcellulose, 1% triton-x in PBS) or GW4064 (50 mg/kg/dose) by oral gavage 14 and 2 hours before tissue collection.

To determine whether FXR activation protects against enteric pathogen infection, mice were either treated with vehicle (1% methylcellulose in PBS) or OCA (5 mg/kg/dose) one hour (n=10) or 7 days (then dosed every 12 hours) (n=7) before infection by oral gavage with  $1 \times 10^6$  *S. enterica* Typhimurium 14028s in 100  $\mu$ L PBS. *S. enterica* was provided by Dr. Hyunwoo Lee (University of Illinois at Chicago, Chicago, IL) and was prepared by Tamiko Oguri (University of Illinois at Chicago, Chicago, IL). Treatment with vehicle or OCA (5 mg/kg/dose) was then continued every 12 hours until the mice were moribund. Ciprofloxacin (10 mg/kg/dose; a positive control) was injected subcutaneously to treat the control group (n=5) every 12 hours. All

procedures were approved by the Institutional Animal Care and Use Committee in the University of Illinois at Chicago.

### 2.2.3 **RNA isolation and quantitative real time-PCR (qRT-PCR)**

Total RNA was isolated from mouse liver tissues using Trizol (Life Technologies) and converted to cDNA using High-Capacity cDNA Reverse Transcription Kit (Life Technologies). Using the cDNA as a template, qRT-PCR was performed using the StepOnePlus Real-Time PCR System. The primers, from Integrated DNA Technologies (Coralville, Iowa), are listed in Table 1. The results were expressed as fold changes under treatment using the gene expression levels normalized to those of  $\beta$ -actin ( $2^{-\Delta\Delta C_t}$  method).



**Table 1. Primers used to measure mRNA expression by qRT-PCR.**

<b>Gene Symbol</b>	<b>IDT Catalog Number</b>
<b>Mouse Genes</b>	
<i>Actb</i>	Mm.PT.58.33257376.gs
<i>Defa1</i>	Mm.PT.58.45876648.g
<i>Defa2</i>	Mm.PT.58.43738656
<i>Defa4</i>	Mm.PT.58.8548542
<i>Defa17</i>	Mm.PT.58.41364464.g
<i>Defa-rs2</i>	Mm.PT.58.32222123.g
<i>Defa-rs4</i>	Mm.PT.58.42812945
<i>Defa-rs10</i>	Mm.PT.58.11805105
<i>Defa-rs12</i>	Mm.PT.58.50500091.g
<i>Defb1</i>	Mm.PT.58.5676756
<i>Defb2</i>	Mm.PT.58.10515725
<i>Defb3</i>	Mm.PT.58.13402117
<i>Defb4</i>	Mm.PT.58.29993789
<i>Defb14</i>	Mm.PT.58.41499310
<i>Camp</i>	Mm.PT.58.21898136
<i>Lyz1</i>	Mm.PT.58.31099880
<i>Lyz2</i>	Mm.PT.58.7139960
<i>Pla2g2a</i>	Mm.PT.58.11921842
<i>Reg3g</i>	Mm.PT.58.29135869
<i>Ang4</i>	Mm.PT.56a.5355614
<i>Rnase4</i>	Mm.PT.58.43399772
<i>Lcn2</i>	Mm.PT.58.10167155
<i>Mmp7</i>	Mm.PT.58.12586478.g
<i>Ang1</i>	Mm.PT.58.21754583
<i>Shp</i>	Mm.PT.58.29701039
<b>Human Genes</b>	
<i>ACTB</i>	Hs.PT.39a.22214847
<i>CYP7A1</i>	Hs.PT.58.21408221
<i>ANG</i>	Hs.PT.56a.39009882
<i>RNASE4</i>	Hs.PT.58.22605109

#### 2.2.4 **Plasmids**

A luciferase reporter vector containing the inverted repeat 1 (IR-1) regulatory region in the ANG1/RNASE4 non-coding exon 264 base pairs downstream of the transcription start site was custom designed and purchased from Genscript (Piscataway Township, NJ). A sequence including the IR-1 and the surrounding 100 base pairs was inserted into a pGL3-basic backbone plasmid (Promega, Fitchburg, WI) driven by the thymidine kinase (TK) promoter. The sequence was inserted upstream of the promoter. The luciferase reporter vector driven by the SHP promoter, pGL2-SHP, was provided by Hueng-Sik Choi (Chonnam National University, Kwangju, Republic of Korea). The FXR expression plasmid, pcDNA3-FXR, was provided by Yoon-Kwang Lee (Northeast Ohio Medical University). The RXR $\alpha$  expression plasmid was provided by Dr. Alan McLachlan (University of Illinois at Chicago, Chicago, IL).

#### 2.2.5 **Luciferase reporter assay**

HEK293T cells were seeded in 24-well plates at a density of  $1 \times 10^5$  cells/ml. Twenty-four hours later, the cells were transfected with 0.8 ng of luciferase construct, 0.4 ng of FXR and RXR $\alpha$  or negative control plasmids, and 0.04 ng of Renilla vector (Promega, Madison, WI) using Fugene HD transfection reagent (Promega) according to the manufacturer's protocol. The transfected cells were treated with vehicle (DMSO) or OCA (1  $\mu$ M) 24 hours after transfection. The cells were harvested for determination of luciferase activity using a dual-luciferase assay kit (Promega) 24 hours after treatment. At least two independent experiments were performed in triplicate.

#### 2.2.6 **Statistical analysis**

Values were reported as mean  $\pm$  standard deviation (S.D.). The control and OCA-treated groups were compared using the Student's t-test. The Gehan Breslow Wilcoxon test was used to compare the survival curves of the control and OCA-treated mice.

## 2.3 **Results**

### 2.3.1 **Angiogenin 1 (Ang1) and ribonuclease 4 (Rnase4) expression is increased upon farnesoid X receptor (FXR) activation.**

To determine whether FXR in the small intestine regulates AMP expression, GW4064 (or vehicle) was administered by oral gavage to C57Bl/6 mice for two doses. Mice were sacrificed 2 hours after the second dose, and the ileum was collected. mRNA expression levels of 24 AMPs and MMP7, an enzyme that processes AMPs from a pro-peptide to a functional form (Table 1), were examined in the tissues (Fig. 4A). Nine of the 24 AMPs examined had nonexistent or minimal mRNA expression in the ileum of the mice we used in our study. Shp, a representative target gene of FXR in both mice and humans was included as a positive control (42). As expected, GW4064 led to a 70-fold increase in Shp expression (Fig. 4A). Of the AMPs screened, GW4064 treatment led to a 2.5-fold increase in Ang1 and Rnase4 mRNA expression (Fig. 4A), indicating that while the FXR agonist does not comprehensively induce AMP expression, it does upregulate Ang1 and Rnase4 in the mouse ileum.

To examine whether OCA activation of Ang1 and Rnase4 expression is mediated by FXR, its effects were examined in *Fxr-null* mice. The mice provided by Dr. Grace Guo (Rutgers University, New Brunswick, NJ) were bred at our institution, and tail snips of the offspring were genotyped to confirm the *Fxr-null* status. Genomic DNA was isolated from the tail snips of 2 male and 4 female *Fxr-null* mice, and the mutated FXR gene was amplified by PCR using three primer genotyping and run on an agarose gel (Genotyping protocol from Dr. Grace Guo). A tail-snip from a wild-type mouse was included as a positive control. As expected, the PCR product from the wild-type mouse DNA was 249 base pairs in size and the product from the *Fxr-null* mice tail snips were 291 base pairs in size (Fig 5), confirming the *Fxr-null* status of the mice.

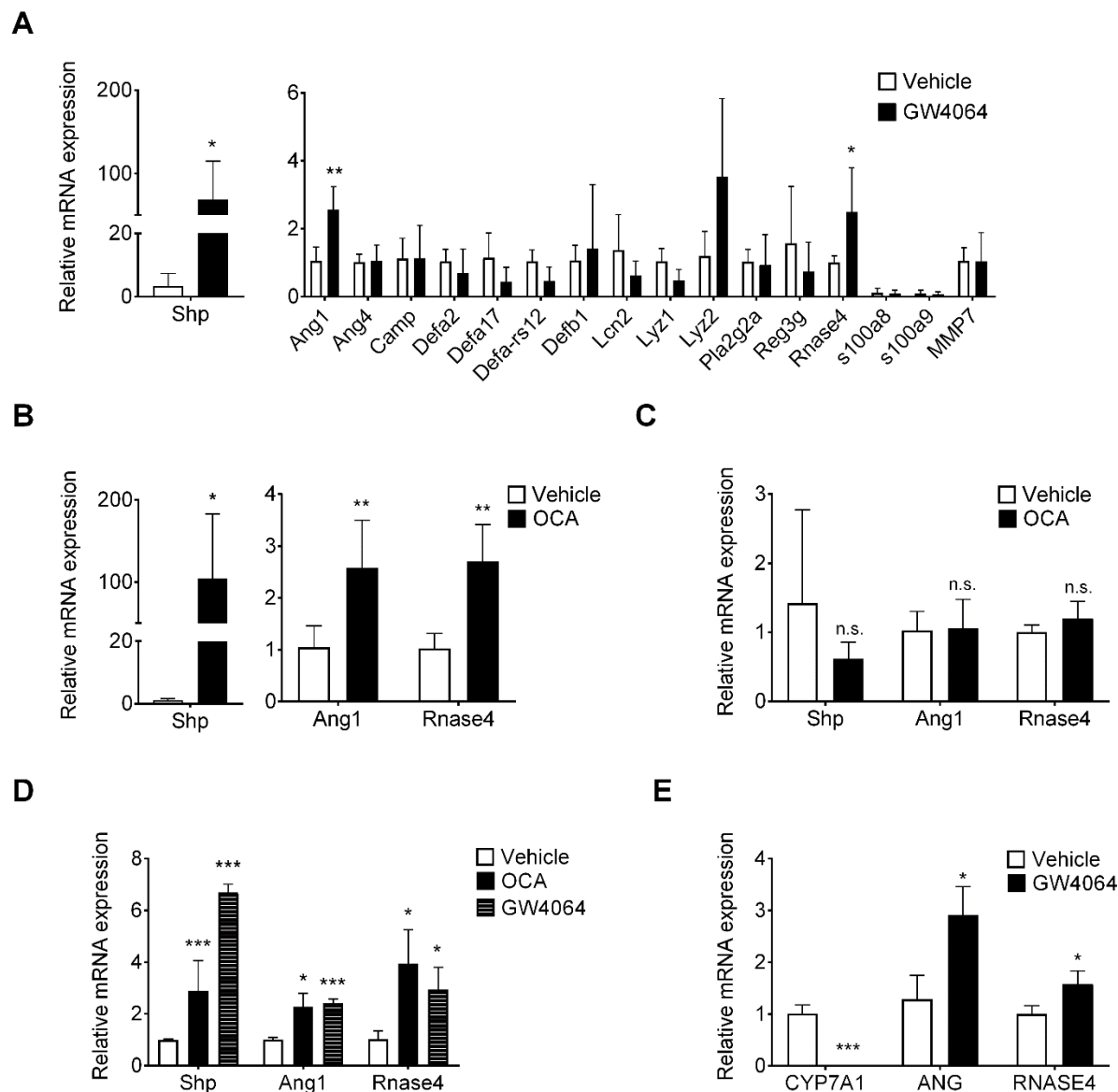
While GW4064 is often used as an FXR agonist in cell culture and animal studies, it has never made it to the clinic (43). Obeticholic acid (OCA) is a FXR agonist with similar binding affinity to GW4064 that is currently being used to treat primary biliary cholangitis (44). We were interested

in whether OCA will have similar effects on Ang1 and Rnase4 expression. Wild-type and FXR-null mice were administered with OCA (or vehicle) by oral gavage for two doses. Mice were sacrificed 2 hours after the second dose, the ileum was collected, and mRNA expression levels of Ang1 and Rnase4 were measured. Shp, a representative target gene of FXR, was included as a positive control. In wild-type mice, OCA led to a 100-fold increase in Shp expression (Fig. 4B). Similar to GW4064 treatment, OCA led to a 2.5-fold increase in Ang1 and Rnase4 mRNA expression (Fig. 4B). In *Fxr-null* mice, there was no change in the mRNA expression levels of Shp, Ang1, or Rnase4 upon OCA treatment (Fig. 4C). Ang1 and Rnase4 expression was enhanced by OCA in wild-type mice, but induction was abrogated in FXR-null mice, indicating that treatment with OCA is inducing Ang1 and Rnase4 expression through FXR activation.

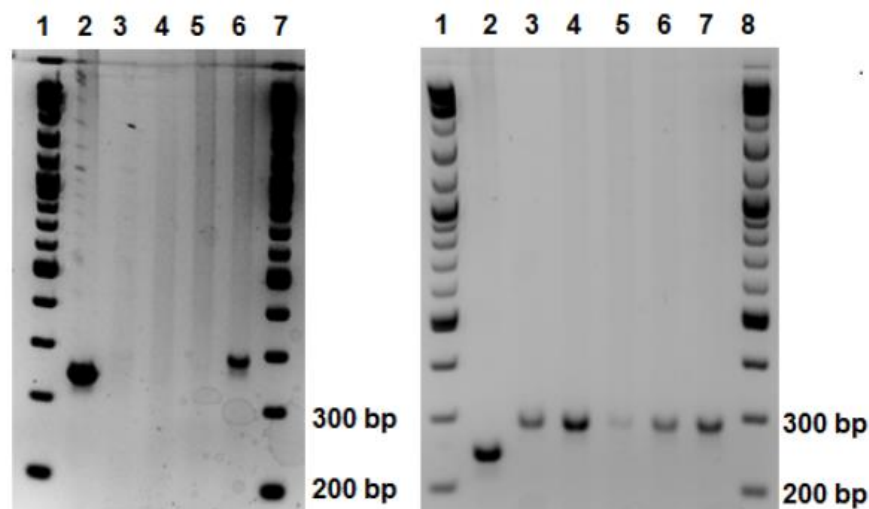
FXR activation is known to inhibit the expression of proinflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$  through nuclear factor-kappaB (NF- $\kappa$ B) in primary human immune cells (peripheral blood mononuclear cells, CD14 monocytes, and monocyte-derived dendritic cells), which can indirectly regulate AMP expression in the ileum (45). To examine whether FXR directly regulates Ang1 and Rnase4 expression, mouse intestinal organoids were treated with OCA or vehicle for 24 hours. Mouse intestinal organoids are established by isolating and culturing primary small intestinal epithelial stem cells which then differentiate and form organoids that are composed of the cells of the intestine epithelium (46-48). In the intestinal organoids, OCA also led to a 2.5-fold increase in Ang1 and a 4-fold increase in Rnase4 mRNA expression (Fig. 4C), indicating that FXR is directly inducing the expression of Ang1 and Rnase4 in the small intestine epithelium.

To determine whether FXR activation also leads to increased ANG (Ang1 homolog) and RNASE4 expression in humans, HCT-116, HT-29, and CACO-2 human intestinal cell lines were examined as a potential model. The basal levels of ANG and RNASE4 mRNA in these cells were low to non-existent, indicating that they may not serve as a model to test whether FXR activates ANG and RNASE4 in human cells. As an alternative, primary human hepatocytes were used as

a model. These cells were treated with GW4064 for 12 hours and mRNA expression levels were examined by qRT-PCR. As expected, GW4064 treatment led to significant decreases in CYP7A1 expression, a gene known to be downregulated upon FXR activation (42). This was accompanied by a 3-fold increase in ANG and a 1.5-fold increase in RNASE4 mRNA at 12 hours (Fig. 4D), indicating that FXR also upregulates the expression of human ANG and RNASE4.



**Figure 4. Ang1 and Rnase4 mRNA expression is increased upon FXR activation in mice and primary human hepatocytes.** A, Wild-type mice were treated with GW4064 (50 mg/kg) or vehicle control (1% methylcellulose, 1% triton-x in PBS) 14 and 2 hours before sacrifice and ileum collection (n=5 mice/group). B, Wild-type mice were treated with OCA (5 mg/kg) or vehicle control (1% methylcellulose in PBS) 14 and 2 hours before sacrifice and ileum collection (n=5 mice/group). C, FXR-null mice were treated with OCA (5 mg/kg) or vehicle control (1% methylcellulose in PBS) 14 and 2 hours before sacrifice (n=4 mice/group). D, Mouse intestinal organoids were treated in triplicate with 1  $\mu$ M OCA, 1  $\mu$ M GW4064, or DMSO vehicle for 24 hours. E, Primary human hepatocytes were treated in triplicate with 1  $\mu$ M GW4064 or vehicle (DMSO) for 12 hours (treated by Kyoung-Jae Won, University of Illinois at Chicago). A-E, mRNA expression levels were determined by qRT-PCR. Values are presented as mean  $\pm$  standard deviation. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  vs. vehicle; n.s., not statistically significant.



**Figure 5. Verification of *Fxr-null* mice.** Tail snips from four *Fxr-null* mice from Dr. Grace Guo (Rutgers University, New Brunswick, NJ) and bred in our institution and two wild-type C57Bl/6 mice were genotyped using PCR to amplify the mutated region of DNA. After amplification, the amplicons were run on 2% agarose gel and visualized with ethidium bromide. The amplicon from the wild-type mice is 249 base pairs (bp) in size, while the amplicon from the *Fxr-null* mice is 291 bp. Ladder: left, lane 1, 7; right, lane 1, 8. Wild-type mice amplicons: left, lane 2; right, lane 2. Male *Fxr-null* mice amplicons: left, lane 6; right, lane 7. Female *Fxr-null* mice amplicons: right, lanes 3-6.

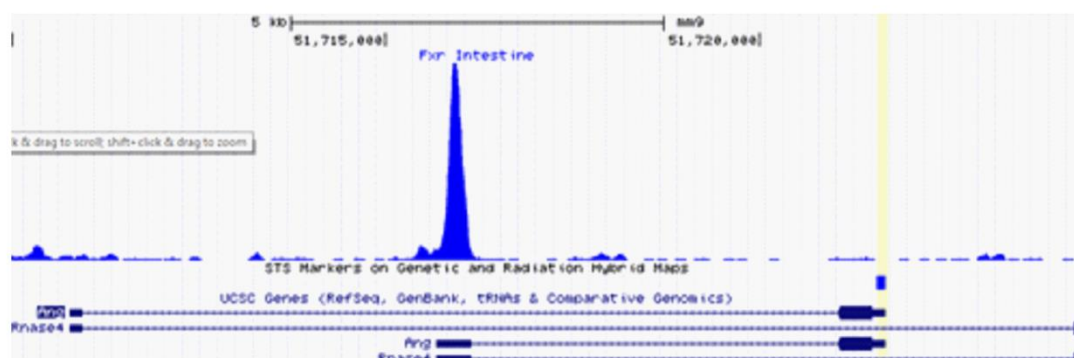
### 2.3.2 Farnesoid X receptor (FXR) transactivates Angiogenin1 (Ang1) and ribonuclease4 (Rnase4) promoters.

To examine the mechanism through which FXR induces Ang1 and Rnase4 mRNA expression, we investigated whether FXR transactivates ANG1 and RNASE4 promoters. Results from the UCSC Genome Browser database (49) revealed that FXR is recruited to a non-coding exon directly downstream of the promoter region of ANG1 and RNASE4 in the mouse small intestine (Fig 6A). The region harbored an inverted repeat 1 (IR1) sequence (Fig. 6B).

To test whether FXR indeed transactivates the promoter, a transient transfection reporter assay was carried out. HEK293T cells were co-transfected with the following vectors: FXR and RXR; a luciferase reporter vector containing the putative FXRE of the ANG1/RNASE4 promoter

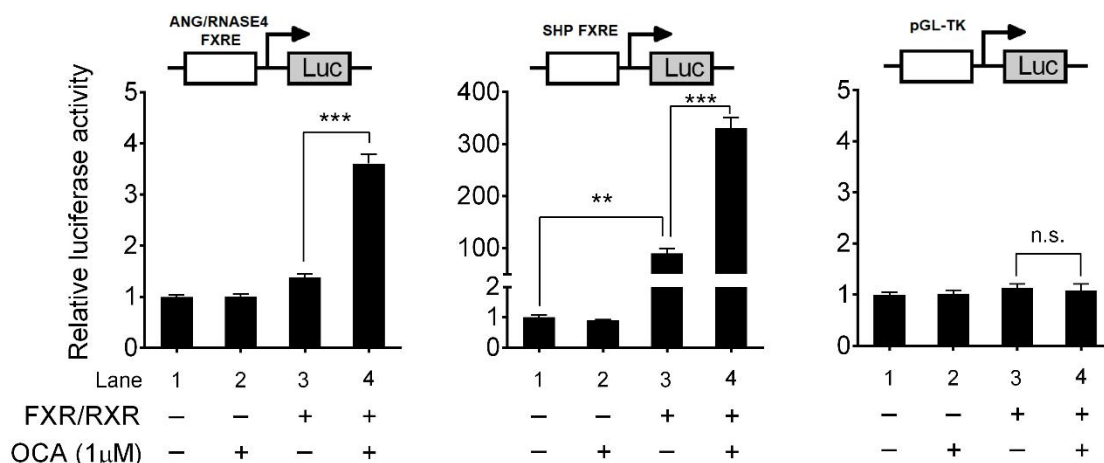
(or pGL3-TK/pGL2-SHP as negative/positive controls); and a *Renilla* expression vector. The cells were treated with 1 $\mu$ M OCA for 24 hours and luciferase activity was measured. Interestingly, FXR/RXR transfection alone led to a significant increase in SHP promoter activity (Fig. 6C, center, lane 1 vs. 3). OCA treatment led to a 3-fold increase in SHP promoter activity as compared to FXR/RXR transfected cells (Fig. 6C, center, lane 3 vs. 4), indicating that in this system OCA induces the promoter of a known FXR target gene. OCA treatment led to a 3.5-fold increase in ANG1/RNASE4 promoter activity (Fig. 6C, left, lane 3 vs. 4). Such induction was not observed in cells transfected with pGL3-TK (Fig. 6C, right, lane 3 vs. 4). Together, these results indicate that FXR transactivates ANG1/RNASE4 promoter.



**A****B**

204 GAAAGAGATGTGTGGTCCACCAGGTGGTTAATATTTGACCCAGGCATGCT

255 CAGGGGAAAGGTGAATGACTCCTTGTTTGCCCATGATTAGTGGCCTTGGT

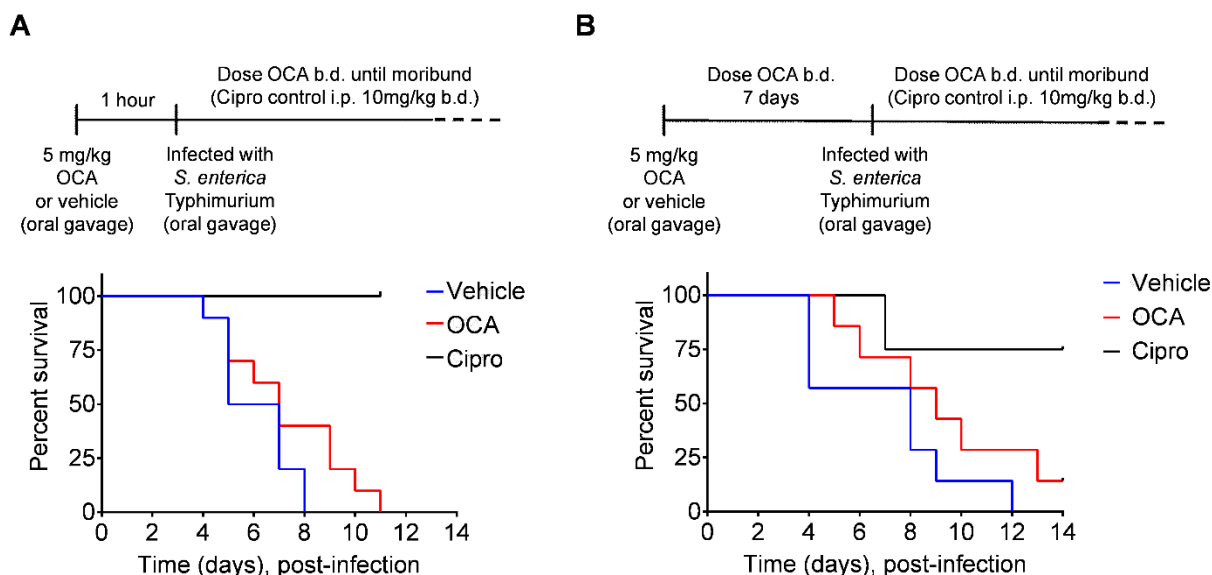
**C**

**Figure 6. FXR directly enhances transcription of ANG1 and RNASE4.** A, Chip-seq results from the UCSC Genome Browser Database showing that FXR is recruited to the Rnase4 and Ang1 gene in the mouse intestine (49). B, The portion of the ANG1/RNASE4 genome where the Chip-seq results show peak binding. The IR1 motif (underlined) in a non-coding exon of the ANG1/RNASE4 gene is a FXR response element (FXRE), beginning 264 bases after the transcription start site. C, HEK293T cells were transfected in triplicate with a luciferase reporter vector containing the ANG1/RNASE4 promoter FXRE (left), a reporter vector containing the SHP promoter FXRE (middle), or the pGL-TK backbone reporter vector used to construct the ANG1/RNASE4 promoter vector (right). The cells were also transfected with *Renilla* expression vector, FXR expression vector, and RXR expression vector. The cells were treated with OCA (1  $\mu$ M) or vehicle (DMSO). Dual luciferase reporter assays were performed. \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; n.s., not significant.

### 2.3.3 **Farnesoid X receptor (FXR) activation by obeticholic acid (OCA) treatment does not significantly increase survival of Salmonella-infected mice.**

To explore whether Ang1 and Rnase4 upregulation by FXR confers protection against pathogenic infection, the effects of OCA on survival after salmonella infection were examined. Mice were treated with OCA (or vehicle control) by oral gavage one hour prior to infection with *Salmonella enterica* Typhimurium (n=10). The mice were then dosed with OCA (or vehicle) every 12 hours until moribund, at which point they were sacrificed. Ciprofloxacin, an antibiotic known to control *S. enterica* infection in mice and sustain survival (50, 51), was included as a positive control, and a group of mice were injected with ciprofloxacin subcutaneously every 12 hours (n=5). All mice in the ciprofloxacin-treated group survived up to day 11 when the study was terminated and all mice in the vehicle control group failed to survive by day 8 (Fig 7A). While mice in the OCA treatment group survived for a longer time, the difference did not reach statistical significance (Fig 7A).

Considering that it is possible that induction of Ang1 and Rnase4 will promote changes in the small intestine that lead to a stronger defense against pathogenic bacteria over time, mice were pre-treated with OCA (or vehicle) for one week and its effects on salmonella infection were examined (n=7). After infection, treatment was continued every 12 hours until the mice became moribund. Ciprofloxacin was again used as a positive control, and the mice were treated subcutaneously every 12 hours beginning one hour before *S. enterica* infection (n=4). One mouse in the ciprofloxacin-treated group was moribund at day eight, while the rest survived until the study was terminated at day 14. All mice in the vehicle control group failed to survive by day 12, and all but one mouse in the OCA treatment group were moribund by day 13 (Fig 7B). Although one mouse in the group treated with OCA did survive the duration of the study, there was not a significant difference in survival time between the OCA and vehicle-treated groups (Fig. 7B). Together, these experiments indicate that FXR may not play a significant role in pathogen defense against enteric gram-negative bacteria.



**Figure 7. FXR activation by OCA treatment does not significantly increase survival of Salmonella-infected mice.** C57BL/6 mice were treated by oral gavage with 5 mg/kg OCA, vehicle (1% methylcellulose PBS) one hour (A) or one week (B) before infection with  $1 \times 10^6$  *S. enterica* Typhimurium in 100  $\mu$ L PBS. After infection, mice were dosed twice a day with either 5 mg/kg OCA (n=10), vehicle (n=10), or 10 mg/kg ciprofloxacin (Cipro) positive control (n=5) until moribund. Survival time (days) is shown below the treatment schemes. A and B, The difference in survival between the OCA and vehicle treated groups is not significant (A, p-value = 0.2627; B, p-value = 0.1670). The difference in survival between the ciprofloxacin and vehicle treated groups is significant (A, p-value = 0.0021; B, p-value = 0.0289).

## 2.4 Discussion

To determine the effects of the FXR agonist OCA on AMP expression, we examined AMP expression levels in OCA-treated wild type and FXR-null mice. The results revealed significant increases in ileal Ang1 and Rnase4 expression upon OCA treatment.

These results were confirmed in primary human hepatocytes with the FXR agonist GW4064. OCA and GW4064 exhibited similar effects on the induction of Ang1 and Rnase4 mRNA both *in vivo* in mice and *in vitro* in mouse intestinal organoids. Human intestinal cell lines HCT-116, HT-29, and CACO-2 were considered to test whether the results seen in mice are applicable to human ANG and RNASE4. We found that the cell lines expressed nonexistent or minimal FXR, ANG, or RNASE4 mRNA and were therefore insufficient models to test FXR agonists in human

cells. ANG and RNASE4 are expressed in the human small intestine (52), but this expression was not reflected in the cell lines we tested. Testing the effects of FXR agonists on primary human hepatocytes suggests that FXR activation does induce human ANG and RNASE4 mRNA expression.

Mouse and human Ang1 and Rnase4 mRNA share 5'-UTRs, and the two mRNAs are the result of differential splicing (53). There are also two variants of Ang1 and Rnase4 mRNA resulting from two promoters and two non-coding exons at the gene level. The promoters and non-coding exons (transcribed to 5'-UTRs) are shared by Ang1 and Rnase4. For Ang1 and Rnase4, both mRNA variants encode the same protein. Based on analysis of a ChIP-seq assay examining FXR targets in the mouse intestine through the UCSC Genome Browser (49), the peak FXR binding site on the Ang1/Rnase4 gene is an IR-1 site in the non-coding exon of the gene that encodes variant two of Ang1 and variant one of Rnase4 mRNA. This FXRE was included in the promoter reporter assay to test whether FXR directly induces expression of the Ang1 and Rnase4 genes. The results from this assay indicate that FXR activates the Ang1/Rnase4 promoter, likely via the IR1 sequence located within the non-coding exon directly downstream of the Ang1/Rnase4 promoter. A ChIP-seq assay of FXR binding sites in the liver and intestine shows that 61% of FXR binding sites in the intestine are directly associated with a gene rather than located in the upstream promoter region, with 1% of these overlapping with 5'-UTRs (49). Our results indicate that this is the case for FXR regulation of the Ang1 and Rnase4 genes.

Ang1 (also called Rnase5) and Rnase4 are both ribonucleases in the Rnase A gene superfamily. Rnase A ribonucleases often have broad antimicrobial activity. For example, Rnase6 and Rnase7 have antimicrobial activity against both gram negative and positive bacteria (54, 55). However, little is known about the antimicrobial activity of Ang1 or Rnase4. To explore the possible implications of Ang1 and Rnase4 induction upon treatment with an FXR agonist, we tested whether OCA treatment is sufficient to treat or protect against *S. enterica* Typhimurium infection in mice. In humans, *S. enterica* colonizes the intestinal lumen and leads to enterocolitis

but usually does lead to systemic infection (56). In mice, *S. enterica* also colonizes in the intestine, but infection spreads to lymphatic tissues associated with the gut. This leads to a systemic infection that eventually leads to the death of the mice (2). *S. enterica* infection of mice is therefore used as a model to study systemic typhoid-like infections that occur in humans. Our results showed that neither OCA treatment nor pre-treatment was sufficient to increase survival time after *S. enterica* Typhimurium infection. Since little is known about the antimicrobial activity of ANG1 and RNASE4, there are several possible directions for future study. For example, it is possible that the level of mRNA induction by FXR activation is not sufficient to protect against systemic infection by a gram-negative bacteria such as *Salmonella*. It is also possible that ANG1 and RNASE4 contribute more to maintaining the homeostasis of commensal bacteria rather than protection against pathogens. To this end, it would be interesting to determine whether FXR activation can be used to treat the clinical problem of commensal bacteria overgrowth in the small intestine.

In conclusion, FXR activation increased Ang1 and Rnase4 ileal mRNA levels in mice and primary human hepatocytes, likely due to FXR directly enhancing the transcription of Ang1 and Rnase4 mRNA. However, treatment with the FXR agonist OCA was not sufficient to confer protection against *S. enterica* infection in mice and may not be a potential therapy for enteric pathogen infection.

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## VITA

<b>Education</b>	2014-present	M.S. in Biopharmaceutical sciences University of Illinois at Chicago, College of Pharmacy Advisor: Hyunyoung Jeong, Pharm.D., Ph.D
	2009-2013	B.A. in Biology, <i>cum laude</i> Carthage College, Kenosha, WI
<b>Positions</b>	2014-present	Teaching Assistant Department of Biopharmaceutical Sciences College of Pharmacy, University of Illinois at Chicago
	2013-2014	Research and Development Laboratory Technician Rustoleum, Pleasant Prairie, WI

### Publications

1. Pan, X., R. Kent, Kyoung-Jae Won, and Hyunyoung Jeong. CYP2D6 induction by cholic acid feeding. *Drug Metabolism and Disposition*. 45(4): 346-352 (2017).
2. Kent, R. and Hyunyoung Jeong. Effects of Fenofibrate on the Expression of Small Heterodimer Partner (SHP) and Cytochrome P450 (CYP) 2D6. *Drug Metabolism Letters*. 11 (2017).

### Abstracts

1. Rebecca Kent, Rebecca Kent, Rong Lu, Jun Sun, Grace Guo, Hyunwoo Lee, and Hyunyoung Jeong. Farnesoid X receptor (FXR) activation induces antimicrobial peptide expression but does not confer protection against *Salmonella enterica* Typhimurium in mice. UIC College of Pharmacy Research Day, Chicago, IL, February 10, 2017.
2. Rebecca Kent, Xian Pan, and Hyunyoung Jeong. CYP2D6 activity is increased with cholic acid feeding in CYP2D6-humanized mice. Great Lakes Drug Metabolism and Disposition Meeting, Rosemont, IL, May 5, 2016.
3. Rebecca Kent and Hyunyoung Jeong. Fenofibrate upregulates SHP expression but does not alter CYP2D6 expression. Experimental Biology meeting, San Diego, CA, April 2, 2016.
4. Rebecca Kent, Xian Pan, and Hyunyoung Jeong. Cholic acid feeding increases CYP2D6 expression in CYP2D6-humanized mice. Experimental Biology meeting, San Diego, CA, April 2, 2016.
5. Rebecca Kent and Kristopher Koudelka. Characterization and Chemical Modification of Cowpea Mosaic Virus (CPMV). Exposition of Student and Faculty Research, Carthage College, Kenosha, WI, April 20, 2012.

### Awards


2012 Biology Department Service Fellow, Carthage College  
 2012 Departmental Scholarship for academic excellence in biology, Carthage College

### Professional Society Memberships

American Association of Pharmaceutical Scientists (AAPS)  
 American Society for Pharmacology and Experimental Therapeutics (ASPET)

## Drug metabolism letters

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