THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENT OF VETERINARY AND BIOMEDICAL SCIENCES

PROFILING THE EXPRESSION OF AMINO ACID TRANSPORTERS WITHIN THE CONTEXT OF THE ARYL HYDROCARBON RECEPTOR

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ABSTRACT

The aryl hydrocarbon receptor (AHR) is a transcription factor of the basic helix-loop-helix-PER-ARNT-SIM superfamily. Exogenous and endogenous ligands for the AHR mediate an array of physiologic and toxicological functions. This research hopes to identify the role of AHR dependent gene regulation on amino acid homeostasis which involves amino acid metabolism and transport. This equilibrium is usually perturbed in certain tumors, where elevated expression of transporters increases the flux of amino acids in order to supply proliferative cells and examining the relationship between AHR and amino acid homeostasis could give insights into the carcinogenic mechanism of AHR ligands as well as identify potential therapeutic targets. Quantitative real-time PCR showed that OSC-19 cells exposed to TCDD exhibited increased expression of *SLC7A5/SLC3A2*. These transporters were further expressed when cultured under amino acid deficiency (10%). Significant increased expression of *SLC16A14*, a monocarboxylic acid transporter was also observed within the amino acid deficient cells. Furthermore, AHR does not appear to affect expression of tRNA synthases (*GARS, QARS, and WARS*) or amino acid sensing (*GCN2/ATF4*) genes. Mouse jejunum samples were also used to examine the extent with which broccoli derived glucobrassicins mediated AHR induced transporter expression. Surprisingly, *SLC7A5/SLC3A2* expression was not significantly altered compared to control mice. Downregulation of *SLC43A3*, a purine nucleobase transporter was also observed which could potentially offer some protective effect against carcinogenesis in the gut. However, there is not enough evidence to conclude a relationship exist and the data indicates that amino acid transporters in the small intestine are not susceptible to AHR regulation.

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INTRODUCTION

The Aryl Hydrocarbon Receptor: An Overview

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that is recognized for its role in the metabolism of xenobiotics. Activation of AHR is characterized by the upregulation of phase I and phase II metabolic enzymes such as cytochrome P4501A1 (CYP1A1) and cytochrome P4501B1 (CYP1B1) that can detoxify or bioactivate xenobiotic compounds[2]. Exogenous ligands for AHR include halogenated aromatic hydrocarbons (HAHs) and polycyclic aromatic hydrocarbons (PAHs) such as *2,3,7,8-tetrachlorodibenzo-p-dioxin* (TCDD or dioxin) and Benzol[a]pyrene (BaP)[1,3]. TCDD is a potent agonist (picomolar binding affinity) [3] that is involved in tumor promotion[4] and can also cause chloracne, an inflammatory condition that results in painful skin lesions[5]. The latter of which was notably observed in the dioxin poisoning of Ukrainian president Viktor Yushchenko in 2004[1]. AHR agonists like BaP can also be metabolized by cytochrome P450s to electrophilic intermediates that generate genotoxic reactive oxygen species (ROS) and DNA adducts[6,7]. The known functions of AHR have also expanded to diverse physiological roles which were first observed through the use of *Ahr*$^{-/-}$ knockout mice. Initial attempts to create *Ahr*$^{-/-}$ knockout mice resulted in embryonic lethality[8], suggesting that AHR was involved in other functions besides xenobiotic metabolism. Subsequent knockout mice models survived, but still had problems with normal vasculture[9] and organ development[10], and immune challenge[11]. Further studies have also identified roles in endocrine function[12] gastrointestinal homeostasis[13], inflammatory signaling[10] and cell differentiation[14]. It is

clear that AHR mediates a wide range of both toxicological and physiological pathways and further research will only continue to improve our understanding of the nature and scope of the AHR. Also whether the AHR is a therapeutic target to treat chronic diseases is under investigation.

AHR Structure

AHR belongs to the basic helix-loop-helix-PER-ARNT-SIM family of transcription factors that includes HIF-1α among others and the AHR is the only member of this family that binds ligands[1,15]. The basic-helix-loop-helix domain (bHLH) is a structural motif that functions as the DNA binding domain, facilitates heterodimerization with the aryl hydrocarbon receptor nuclear transporter (ARNT), and is part of the chaperone binding site[16]. Two PAS repeats (PAS A and PAS B) are also involved with dimerization with ARNT while PAS B is involved in chaperone binding and contains the ligand binding domain[17]. The C-terminus contains a glutamine rich region (Q-rich) which is required for transactivation and coactivator recruitment[18]. A schematic of the functional domains of AHR is shown in figure 1.

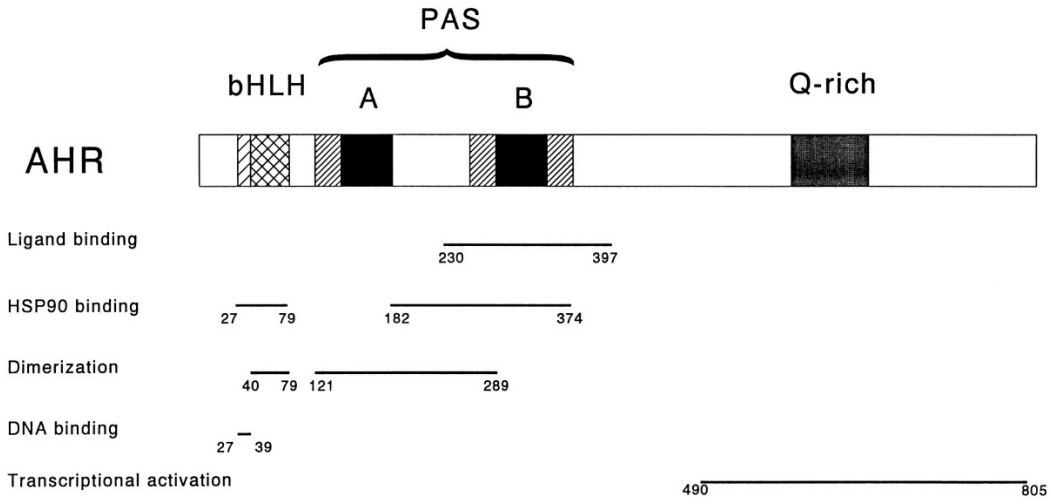

Figure 1: Functional Domains of AHR[18].

AHR Signaling Pathway

In its unliganded state, AHR is sequestered in the cytosol in a complex with heat shock protein 90 (HSP90) proteins, prostaglandin E synthase 3 (p23) and hepatitis B Virus X-associated protein 2 (XAP2)[1]. HSP90 interacts with the bHLH and PAS B domains on AHR, preventing heterodimerization with ARNT (which also utilizes those domains). In addition, HSP-90 maintains the competency of the ligand binding domain of AHR[19,20]. The phosphoprotein p23 also acts to prevent spontaneous AHR/ARNT dimerization and may recognize importin-β proteins that enhance nuclear translocation[1]. XAP2, which was first identified in the Perdew laboratory[21], prevents shuttling of unliganded AHR to the nucleus[22]. When an AHR ligand like TCDD binds to AHR, it induces a conformation change that exposes a nuclear localization sequence (NLS) resulting in nuclear shuttling[1]. Once inside the nucleus, the chaperone complex dissociates and AHR dimerizes with ARNT and can bind to the core sequence (5′-TNGCGTG-3′) of dioxin response elements (DREs) which are core nucleotide localization sequences found upstream of inducible AHR targets[23]. This facilitates coactivator recruitment to the transactivation domain of AHR which subsequently leads to chromatin remodeling and transcription of target genes[1,15]. The signaling pathway is illustrated in figure 2.

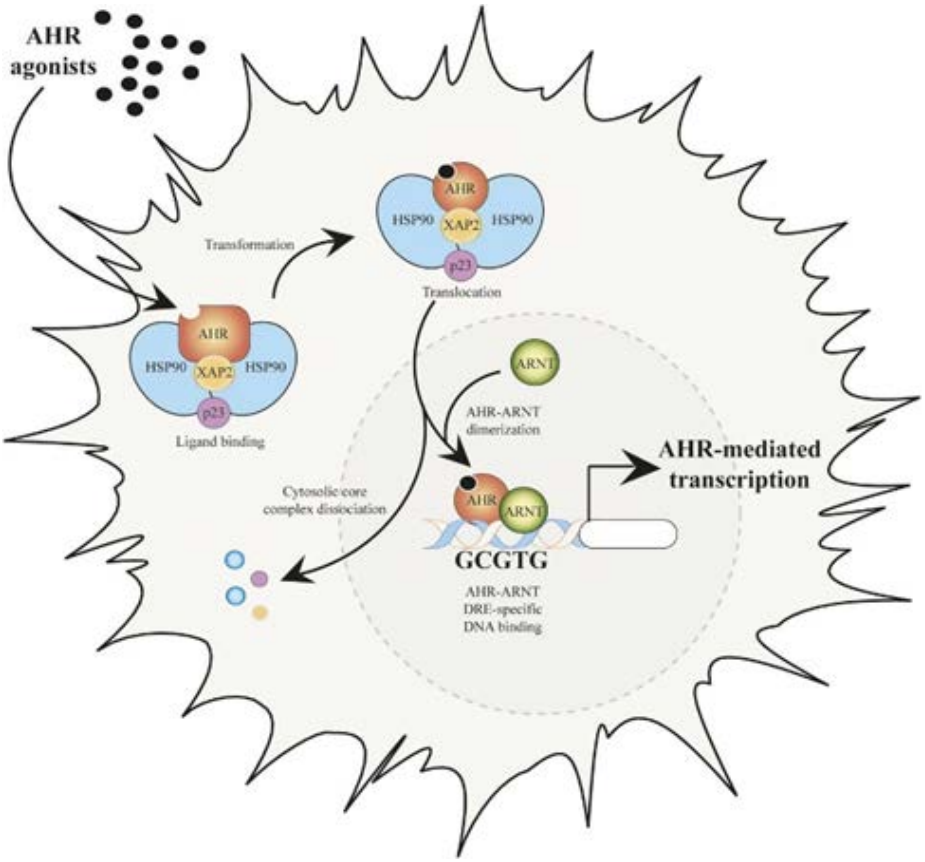

Figure 2: Signaling Pathway of AHR[26].

Sources of AHR Ligands

The AHR contains a promiscuous ligand binding pocket[3] that responds to a wide range of exogenous and endogenous compounds. Typically, exogenous ligands belong to two broad categories (HAHs and PAHs) which bind to AHR with relatively high affinity[1]. HAHs like dioxins, dibenzofurans, and biphenyls have binding affinities in the pM or nM range[3,24]. The more labile PAHs such as benzol[a]pyrene, benzoflavones, and benzanthracenes are less potent with affinities in the nM or µM range[24]. Many of these ligands are produced by the thermal decomposition and recycling of organic compounds from both natural and synthetic sources which include wildfires, volcanic eruptions, cigarette smoke, pesticides, and grilling food[25]. In addition to these high

affinity ligands, low affinity endogenous ligands can be derived from our diet. Flavonoids, tryptophan and indole metabolites, and carotenoids have all been identified to activate AHR, albeit weakly compared to TCDD with some having binding affinities in the micromolar range or higher[1,26]. Glucobrassicins are indole metabolites that are particularly common in cruciferous vegetables. These compounds are enzymatically cleaved by myrosinases upon mastication to produce AHR agonists such as indole-3-carbinol (I3C) and indole-3-acetonitrile (I3ACN). Furthermore, metabolites of myrosinase activity may undergo nonenzymatic condensation in the acidic environment of the stomach to form more potential AHR ligands such as 3,3′diindolylmethane, indolo[3,2-b]carbazole (ICZ), and 2-(indol-3-ylmethyl)-3,3′-diindolylmethane [26,27]. These agonists can activate AHR in the gastrointestinal tract to influence gene expression. These pathways are shown in figure 3.

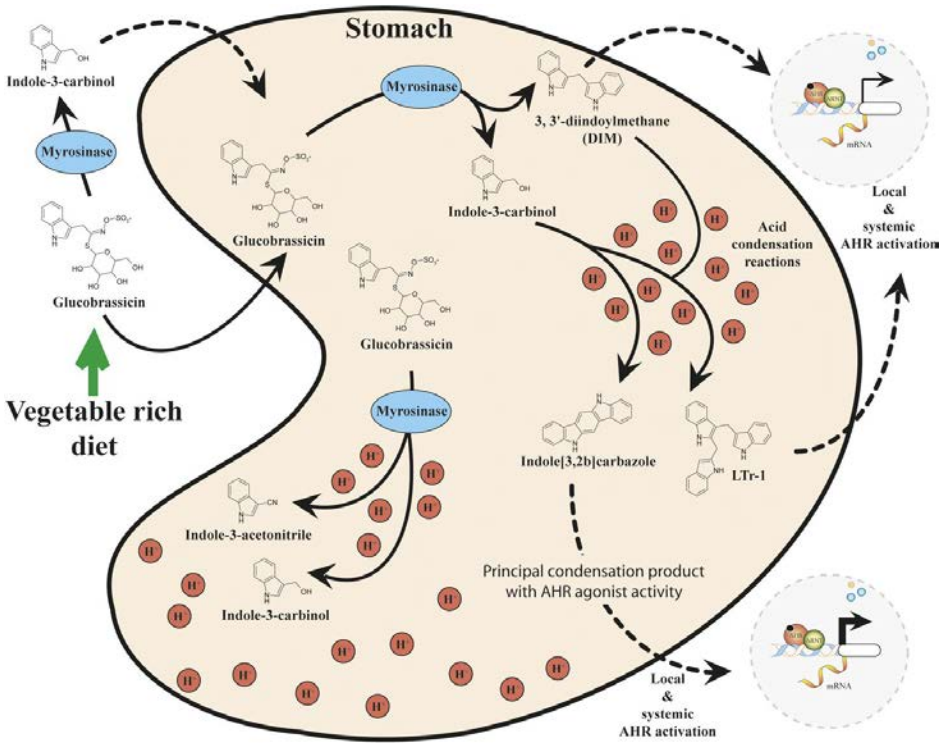

Figure 3: Production of AHR Ligands from Broccoli Rich Diet[26].

Amino Acid Homeostasis

Amino acids are organic compounds that function as monomers for protein synthesis, energy sources, or act as hormone and neurotransmitter precursors[28]. Proteins are composed of twenty proteinogenic amino acids, which can be further classified as essential or non-essential. Essential amino acids such as histidine, leucine, and phenylalanine must be obtained from nutrients while non-essential amino acids such as arginine and glycine can be synthesized de novo[29]. Since cells constantly require proteins to function properly, the pools of each amino acid are tightly regulated by controlling flux through amino acid transporters and signaling the biosynthesis and degradation of amino acids and proteins. This high turnover requires close surveillance of amino acid production by the mechanistic target of rapamycin (mTOR or mTORC1) signaling pathway[28,30] and the general control non-depressible 2 (GCN2) pathway[31]. Inactive mTORC1 is sequestered in the cytoplasm and is regulated by multiple proteins. When amino acids such as arginine and leucine are abundant, they bind to dimers Sestrin and Castor 1/2 which are found on GATOR 2 proteins. GATOR2 then moves in to inhibit GATOR1 which inactivates mTORC1 when amino acids are scarce. This allows RAG proteins to shift toward a GTP bound state as opposed to a GDP bound state, activating mTORC1 signaling cascades[28]. In addition, tuberculosis sclerosis complex 2 (TSC2) also regulates mTORC1 signaling by acting as a GTPase of mTORC1 activator Rheb. TSC2 is inhibited when it is phosphorylated by growth factors or mitogens. Typically, inactive mTORC1 will increase catabolic breakdown of proteins and amino acids while an active mTORC1 complex will target anabolic processes[28,30]. While mTORC1 reacts to an abundance of amino acids, the stress kinase GCN2 senses amino acid deficiency[28]. When any amino acid becomes depleted, it results in the buildup of uncharged tRNAs (unbound to an amino acid) that will bind to and activate GCN2. The kinase phosphorylates a serine residue on the

translation factor eIF2 subunit α[32]. When the cell is not undergoing any kind of stress, eIF2 bound to GTP delivers methionyl-tRNA to mRNA to initiate translation. During translation, GTP is hydrolyzed to GDP and the eIF2: GDP complex dissociates. In order to continue translation, the GDP must be re-phosphorylated to GTP by the guanine exchange activity of eIF2B. When the α subunit of eIF2 is phosphorylated, it can no longer act as a substrate for eIF2B which prevents translation[33].

The Role of AHR in Amino Acid Transporters

The activation of AHR by ligands such as TCDD has been implicated in the development of many cancers[4,34]. However, the mechanism by which AHR activity promotes tumorigenesis is poorly understood. Recent evidence demonstrates that the AHR may be involved in the regulation of amino acid transporters, specifically SLC7A5 and SLC3A2, better known as LAT1[34-37]. Studies in vitro have indicated that LAT1 was expressed highly in multiple cancer cell lines as well as in solid tumors in vivo[35,36]. In addition, unpublished evidence from the Perdew laboratory indicates that similar results are found in OSC-19 cells, a tongue squamous cell carcinoma cell line. LAT1 mediates the uptake of amino acids such as tryptophan[28]. Once inside the cell, 90% of dietary tryptophan has been reported to enter the indoleamine-2,3-dioxygenase/ Trp 2,3-dioxygenase (IDO/TDO) pathway[38], where it is metabolized to kynurenine, an AHR ligand. Additionally, uptake of tryptophan requires simultaneous export of kynurenine[26]. Further stimulation of AHR can upregulate IDO and IL-6 (which also increases expression of IDO)[39-41]. LAT1 also cotransports tryptophan and leucine into the cell which can stimulate mTORC1 signaling pathways, promoting survival and proliferation while inhibiting apoptosis and autophagy[28].

Membrane Transporter Functions

Gene	Function	Gene	Function
EMMPRIN	Transmembrane glycoprotein that is involved in angiogenesis and directs monocarboxylate transporters to the plasma membrane[42]	***SLC16A5***	Transports butanide, neteglinde, and probenecid and is involved in glucose homeostasis
SLC1A4	Antiporter for alanine, serine, and cysteine	***SLC16A6***	Transports ketone bodies
SLC1A5	Antiporter for alanine, serine, cysteine, threonine, and glutamine	***SLC16A9***	Transports carnitine
SLC3A1	Heterodimeric partner with *SLC7A9*	***SLC16A10***	Transports aromatic amino acids (tryptophan, phenylalanine, and tyrosine).
SLC3A2	Heterodimeric partner with *SLC7A5*	***SLC16A11***	Unknown
SLC6A14	Sodium and chloride dependent transporter of all neutral and cationic amino acids	***SLC16A12***	Transports creatine
SLC6A19	Sodium dependent transporter of all neutral amino acids.	***SLC16A13***	Unknown
SLC7A1	Uniporter for lysine, arginine, and ornithine	***SLC16A14***	Unknown
SLC7A2	Uniporter for lysine, arginine, and ornithine	***SLC36A4***	Transporter for proline and tryptophan, and Also functions as an amino acid sensor
SLC7A5	Antiporter for histidine, methionine, leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan.	***SLC38A1***	Sodium dependent transporter for glycine, alanine, asparagine, cysteine, glutamine, histidine, and methionine
SLC7A8	Antiporter for all neutral amino acids except for proline.	***SLC38A2***	Sodium dependent transporter for glycine, proline, alanine, asparagine, cysteine, glutamine, histidine, methionine, and serine
SLC7A9	Antiporter for lysine, arginine, ornithine, and cysteine. Mutations in this transporter are often associated with cystinuria, an autosomal recessive condition that causes increased cysteine levels in the urine[43]	***SLC38A3***	Sodium coupled transporter for glutamine, asparagine, and histidine
SLC7A11	Antiporter for aspartic acid, glutamic acid, and cystine	***SLC43A3***	Purine-selective nucleobase transporter[44]
SLC16A4	Unknown		

Table 1: Function of Membrane Transporters[28, 45, 46]

Aims of Research

The purpose of this research is to characterize the expression of amino acid transporters in response to induced AHR activity in two setting: in OSC-19 cancer cells and within the jejunum of the gastrointestinal tract. Amino acid transporters such as LAT1 are found to be expressed highly within certain tumors, likely to keep up with the demands of the proliferative cells and enhance survival signals[35]. Additionally, dioxin response elements have been mapped to the promoter of *LAT1*[37] and studies have shown that the transporter responds to TCDD induced AHR[37]. Moreover, amino acid deficiency, a state common to tumors lacking vascularization, may also increase expression of amino acid transporters such as *LAT1*[47]. This evidence provides insight into the carcinogenic potential of TCDD mediated AHR. To take that one step further, this research will analyze the expression of a range of amino acid and monocarboxylate transporters (listed in Table 1) to determine if any are transcriptionally upregulated by AHR. Close crosstalk with mTORC1 and GCN2 amino acid sensing pathways may cause amino acid transporter exchange to change as well[28,48]. GCN2 activation leads to stimulation of Activating Transcription Factor 4 (ATF4) mediated angiogenesis in order to increase nutrient supplies[48]. Therefore, it is expected that amino acid deprivation and potentially TCDD treatment would increase expression of *GCN2*.

While these transporters are often scrutinized in the context of cancer, these is little evidence of their role in the gastrointestinal tract. Endogenous activation of AHR has been linked to inflammatory signaling and immune modulation[10], and may also regulate the absorption of amino acids[37]. Broccoli, which has been recognized as a prominent source of dietary derived AHR ligands[26], will be fed to a group of mice for two weeks alongside a control group given purified chow. Following the treatment phase, the mice will be euthanized and the jejunum, where the majority of amino acid uptake occurs, will be collected.

It is hypothesized that AHR increases expression of amino acid transporters that would be beneficial to a tumor by increasing intracellular uptake of amino acids and activating GCN/ATF4. In addition, it is hypothesized that AHR induced amino acid transporter expression will increase the absorption of tryptophan and subsequently increase production of AHR agonists in a positive feedback loop. Evidence supporting this mechanism of AHR activation would provide insight into the role of AHR in nutrient absorption in the gut which could fuel both beneficial and detrimental outcomes.

METHODS AND MATERIALS

Animals

C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). *Ahr*$^{-/-}$ knockout mice were provided as a gift from Dr. Christopher Bradfield (University of Wisconsin, Madison, WI). Mice were housed and fed in accordance with the Pennsylvania State University IACUC protocols.

Cell Culture

OSC-19 cell lines were maintained in α-modified essential media (Sigma, St. Louis, MO) or Dulbecco's modified eagle media/nutrient mixture F-12 (DMEM F-12) (Sigma, St. Louis, MO) supplemented with 8% fetal bovine serum (Hyclone Laboratories, Logan, UT) and 1% penicillin (Sigma, St. Louis, MO) and streptomycin (Sigma, St. Louis, MO). Cell culture plates were kept at 37ºC under 5% CO_2. TCDD was provided by Dr. Stephen Safe (Texas A&M University).

RNA Isolation and Quantitative Real Time PCR

RNA was isolated from mouse jejunum samples or OSC-19 cells using TRI Reagent (Sigma, St. Louis MO). cDNA was prepared using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) from isolated RNA. Quantitative real-time PCR (qPCR) was run on a CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA) using PerfeCTa SYBR Green Supermix from iQ (Quanta Biosciences, Beverly, MA). Primers were purchased (Integrative

DNA Technologies, Coralville, Iowa) for real-time PCR and are listed in the appendix. Gene expression was normalized using ribosomal protein Rpll3a

Statistical Analysis

Normalized expression of target genes was analyzed in GraphPad Prism for significance using two tail t-tests or 1-way ANOVA followed by Tukey's multiple comparison test. Histograms were plotted as the mean of the replicates with error bars denoting the standard deviation between the replicates. Statistical significance is expressed by p-value ≤ 0.05 (*), p-value ≤ 0.01 (**), and p-value ≤ 0.001(***).

DATA AND RESULTS

Transporter Expression in OSC-19 Cells

Validating the Integrity of Rpl13a as a Reference Gene

Ribosomal protein L13a (*Rpl13a*) has been identified as a suitable reference gene in multiple cancer cell lines and with various treatments (variable oxygen levels, etc.)[49,50]. However, there are no studies that indicate whether or not the same would hold true when subjected to amino acid starvation, which is known to affect protein synthesis pathways in the cytosol[51]. In addition, amino acid starvation has been shown to regulate autophagic processes through mTORC1 signaling[52] which could lead to degradation of ribosomal proteins. With this in mind, we compared the mRNA levels of *Rpl13a* across all treatment groups and to another reference gene, *GAPDH*. We found no significant differences among any of the treatment groups for either *Rpl13a* or *GAPDH*. In addition, the ratio and inverse ratio of *Rpl13a* to *GAPDH* showed no significant changes. This data suggests that *Rpl13a* remains stable under treatment with TCDD and/or amino acid deprivation, making it a suitable reference gene.

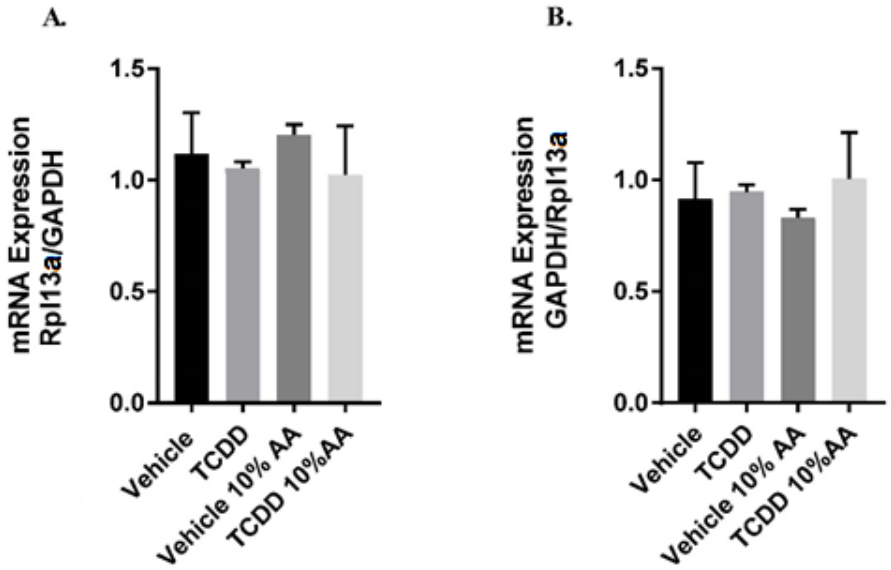

Figure 4: Rpl13a Remains a Suitable Reference Gene

OSC-19 cells were pretreated with TCDD (2nM) for 24 h. Cells were then incubated in normal media or DMEM/F-12 for 6 h. mRNA expression was analyzed via quantitative real-time PCR as ratios of *Rpl13a/GAPDH* (A), and *GAPDH/Rpl13a*. Statistical significance is indicated by an asterisk ($P < 0.05$ (*), $P < 0.01$ (**), and $P< 0.001$ (***)).

Induction of Known AHR Target Genes by TCDD

Treatment of OSC-19 cells with TCDD and/or amino acid restriction was conducted in cell culture to examine AHR mediated effects on target genes (*CYP1A1*, *SLC7A5*, and *SLC3A2).* AHR is involved in the regulation of several drug metabolizing enzymes such as *CYP1A1*[2]. Therefore, treatment with a potent agonist such as TCDD should result in upregulation of *CYP1A1*. Furthermore, the amino acid transporters *SLC7A5* and *SLC3A2* contain DREs and have been shown to respond to AHR in previous studies[37]. Amino acid deprivation may also affect metabolic flux by regulating amino acid transporter expression[53,54]. Transporters such as *SLC7A5* may be necessary to shuttle leucine to activate mTORC1 pathways and it has been suggested that

inhibition of mTORC1 in a nutrient deprived environment may in part be due to downregulation of cytoplasmic amino acid shuttling by their corresponding transporters[54]. The results show that TCDD induced significant expression of all three targets genes. Amino acid deprivation also increased *CYP1A1* and *SLC7A5* expression in cells treated with TCDD.

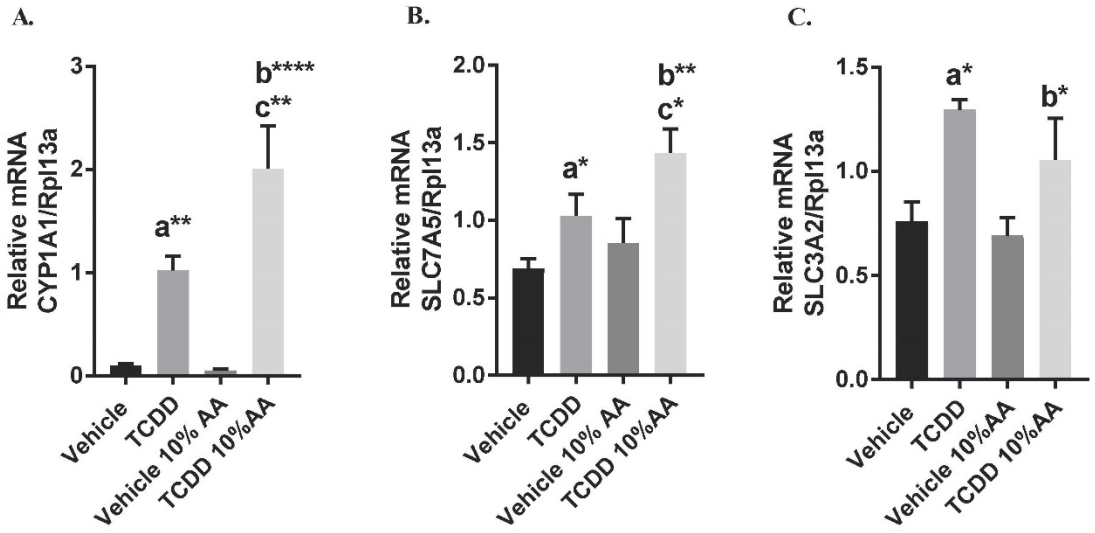

Figure 5: Known AHR Target Genes in OSC-19 Cells

OSC-19 cells were pretreated with TCDD (2nM) for 24 h. Cells were then incubated in normal media or DMEM/F-12 for 6 h. mRNA expression was normalized to *Rpl13a* for *CYP1A1* (A), *SLC7A5* (B), and *SLC3A2* (C). Statistical significance is indicated by an asterisk ($P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***)) compared to Vehicle (a), TCDD (b), and Vehicle 10% AA (c).

Amino Acid Transporter Expression

Amino acid and monocarboxylic acid transporters are an important regulator of amino acid homeostasis and energy metabolism. Patterns of increased expression of these transporters has been observed to be elevated in several types of tumors to support their rapid proliferation rate[35]. Specifically, *SLC1A5*, *SLC7A5*, *SLC7A11*, and *SLC6A14* have all been characterized with elevated expression in certain cancers[45]. Since AHR mediated expression of *SLC7A5* and *SLC3A2* has been

observed in OSC-19 and MCF-7 cell lines[37], other transporters were analyzed to determine the role of AHR in amino acid transporter regulation. It was predicted that transporters involved in the transport of tryptophan from the SLC6 and SLC7 families might be more likely affected, since tryptophan metabolites would serve as AHR agonist, increasing expression of these transporters in a positive feedback loop. Of the neutral amino acid transporters (Figure 6), there was no significant increase in response to TCDD treatment. However, *SLC38A1, SLC38A2,* and *SLC43A3* had reduced levels of expression when cultured under amino acid restricted media. This was likely a feedback mechanism to shut down amino acid transport and synthesis in order to conserve resources for vital cellular functions[28]. There were also no differences among the cationic amino acid transporters (Figure 7). Among the monocarboxylate transporters (Figure 8), *SLC16A14* expression increased when treated with TCDD, but only within the cells with restricted amino acid availability. While the function of *SLC16A14* has not been identified, it is most closely related *SLC16A2, SLC16A9,* and *SLC16A10*[55], suggesting that it may transport aromatic amino acids such as tryptophan. Ten transporters did not amplify, suggesting they were not expressed at any significant levels (*EMMPRIN, SLC3A1, SLC6A14, SLC6A19, SLC7A9, SLC16A4, SLC16A6 SLC16A11,* and *SLC38A3*).

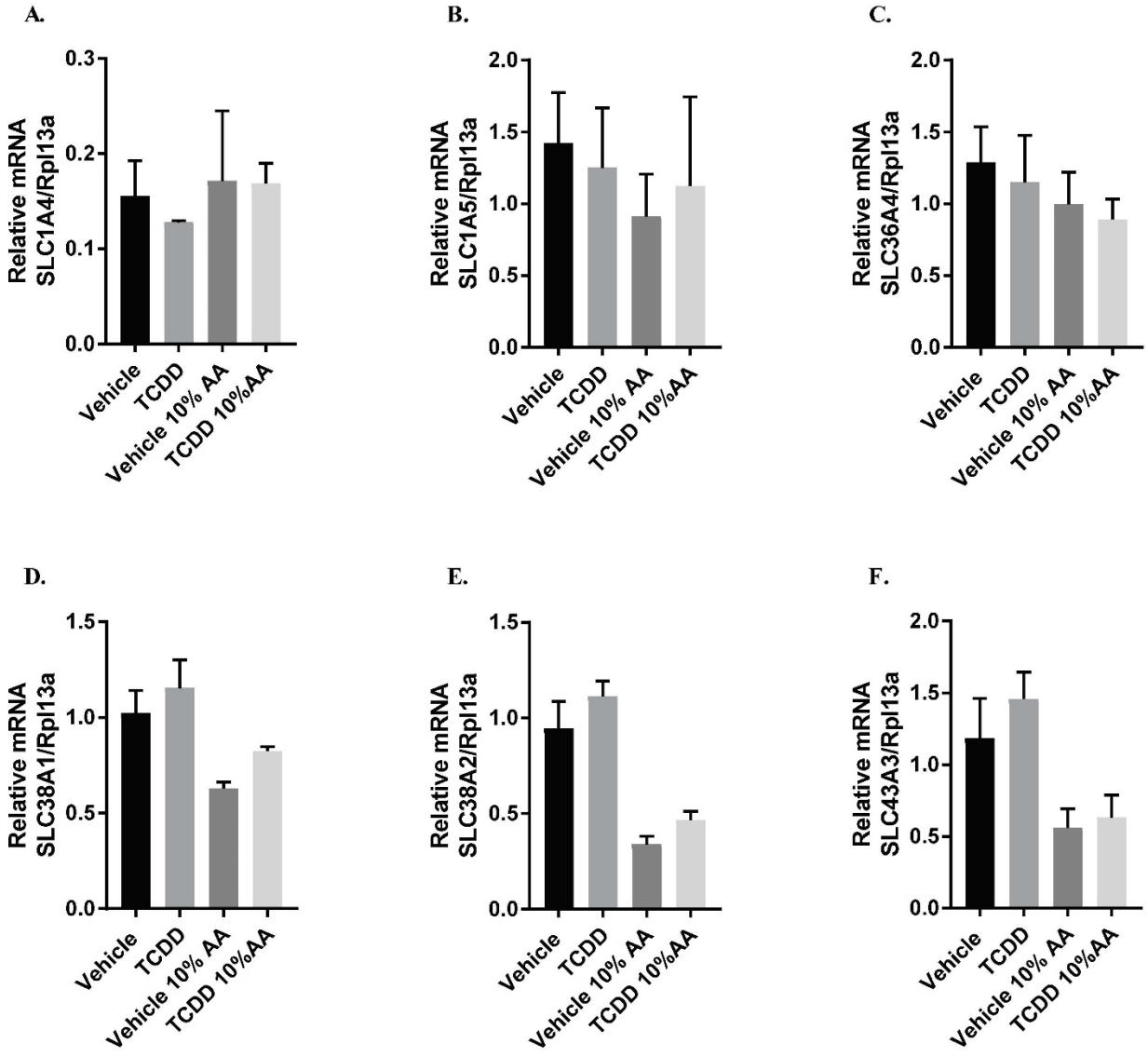

Figure 6: Neutral Amino Acid Transporter Expression in OSC-19 Cells

OSC-19 cells were pretreated with TCDD (2nM) for 24 h. Cells were then incubated in normal media or DMEM/F-12 for 6 h. mRNA expression was analyzed via quantitative real-time PCR for *SLC1A4* (A), *SLC1A5* (B) *SLC36A4* (C), *SLC38A1* (D), *SLC38A2* (E), *SLC43A3* (F) normalized to *Rpl13a*. Statistical significance is indicated by an asterisk ($P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***)).

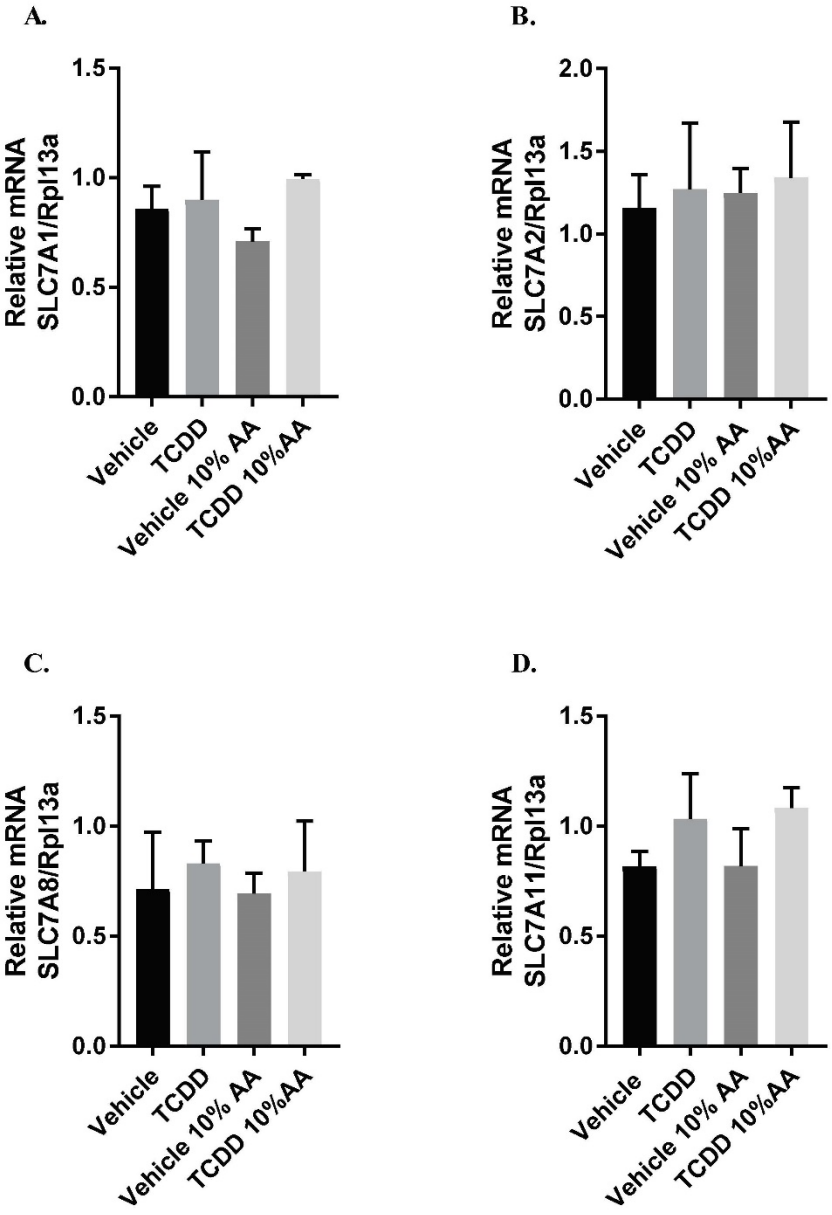

Figure 7: Cationic Amino Acid Transporter Expression in OSC-19 Cells

OSC-19 cell line was pretreated with TCDD (2nM) for 24 h. Cells were then incubated in normal media or DMEM/F-12 for 6 h. mRNA expression was analyzed via quantitative real-time PCR for *SLC7A1* (A), *SLC7A2* (B) *SLC7A8* (C), *SLC7A11* (D) normalized to *Rpl13a*. Statistical significance is indicated by an asterisk ($P < 0.05$ (*), $P < 0.01$ (**), and $P< 0.001$ (***)).

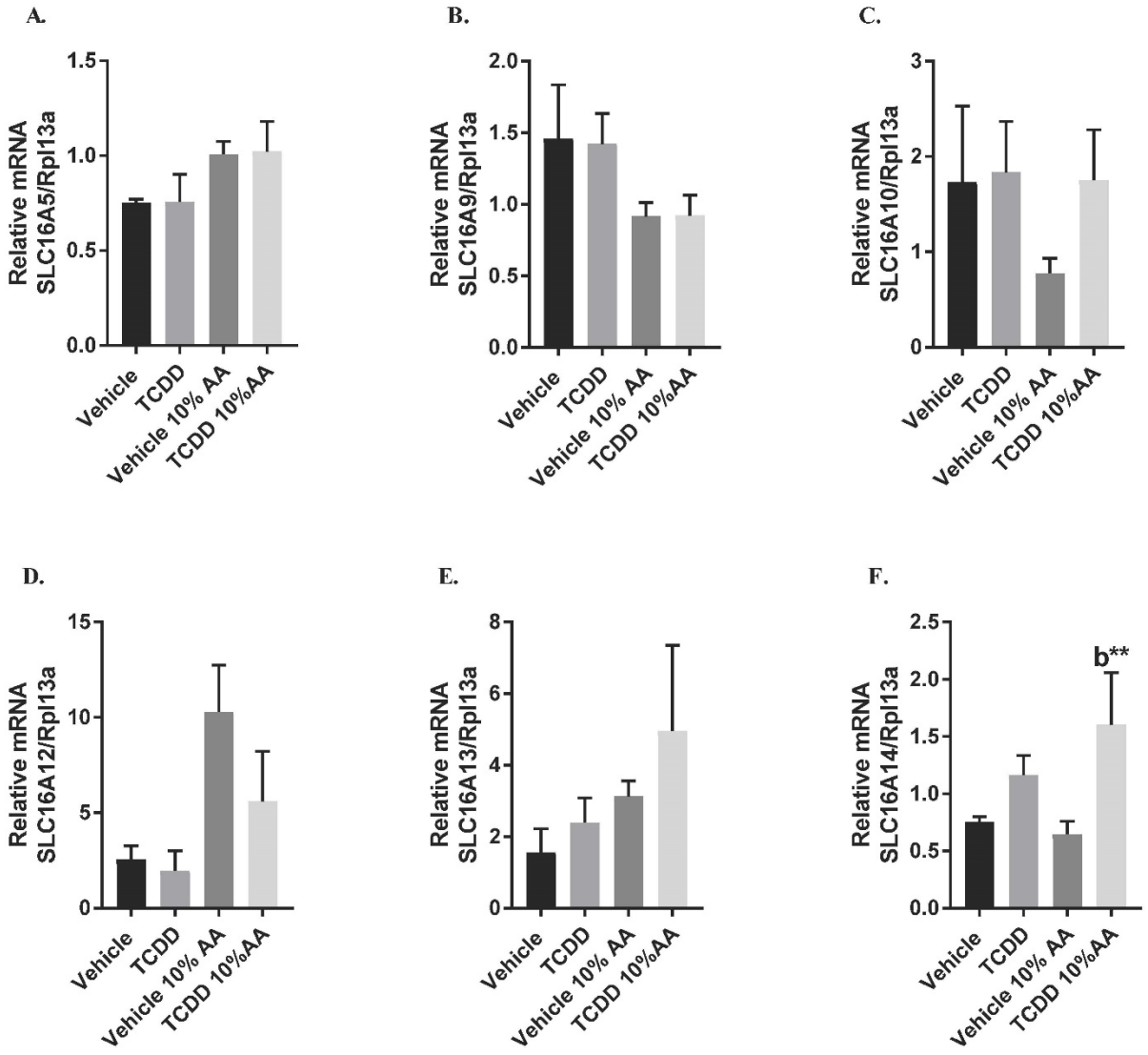

Figure 8: Monocarboxylate Transporter Expression in OSC-19 Cells

OSC-19 cells were pretreated with TCDD (2nM) for 24 h. Cells were then incubated in normal media or DMEM/F-12 for 6 h. mRNA expression was analyzed via quantitative real-time PCR for *SLC16A5* (A), *SLC16A9* (B) *SLC16A10* (C), *SLC16A12* (D), *SLC16A13* (E), *SLC16A14* (F) normalized to *Rpl13a*. Statistical significance is indicated by an asterisk ($P < 0.05$ (*), $P < 0.01$ (**), and $P< 0.001$ (***)) compared to Vehicle (a), TCDD (b), and Vehicle 10% AA (c).

tRNA Synthase and GCN2/ATF4 Expression

Transfer RNAs (tRNA) expression levels in response to TCDD treatment and amino acid starvation was also examined. tRNAs bind to specified amino acids in order to build peptides from mRNA coding instructions[56]. The pool of tRNA is thought to be dynamic in order to match the needs of the cells[56] which would suggest that tRNAs and by association tRNA synthases might be susceptible to a variety of cellular stresses. Studies have also indicated that tRNA synthases are found to be highly expressed in certain cancers[57.] Due to the demands placed by tumor cells, we thought that we might see upregulation in response to TCDD. GARS, QARS, and WARS are tRNA synthases for glycine, glutamine, and tryptophan respectively[57] and surprisingly, we did not see any change in tRNA synthase levels from either amino acid starvation or TCDD treatment (Figure 9).

GCN2 and ATF4 pathways were also examined. GCN2 is a serine/threonine kinase that senses nutrient deprivation by binding to uncharged tRNAs[31]. It translationally upregulates ATF4 which promotes amino acid biosynthesis and angiogenesis[48]. These genes regulate amino acid metabolism and have been found to be elevated in tumors[48]. Despite previous evidence, there was no significant difference in *GCN2/ATF4* genes.

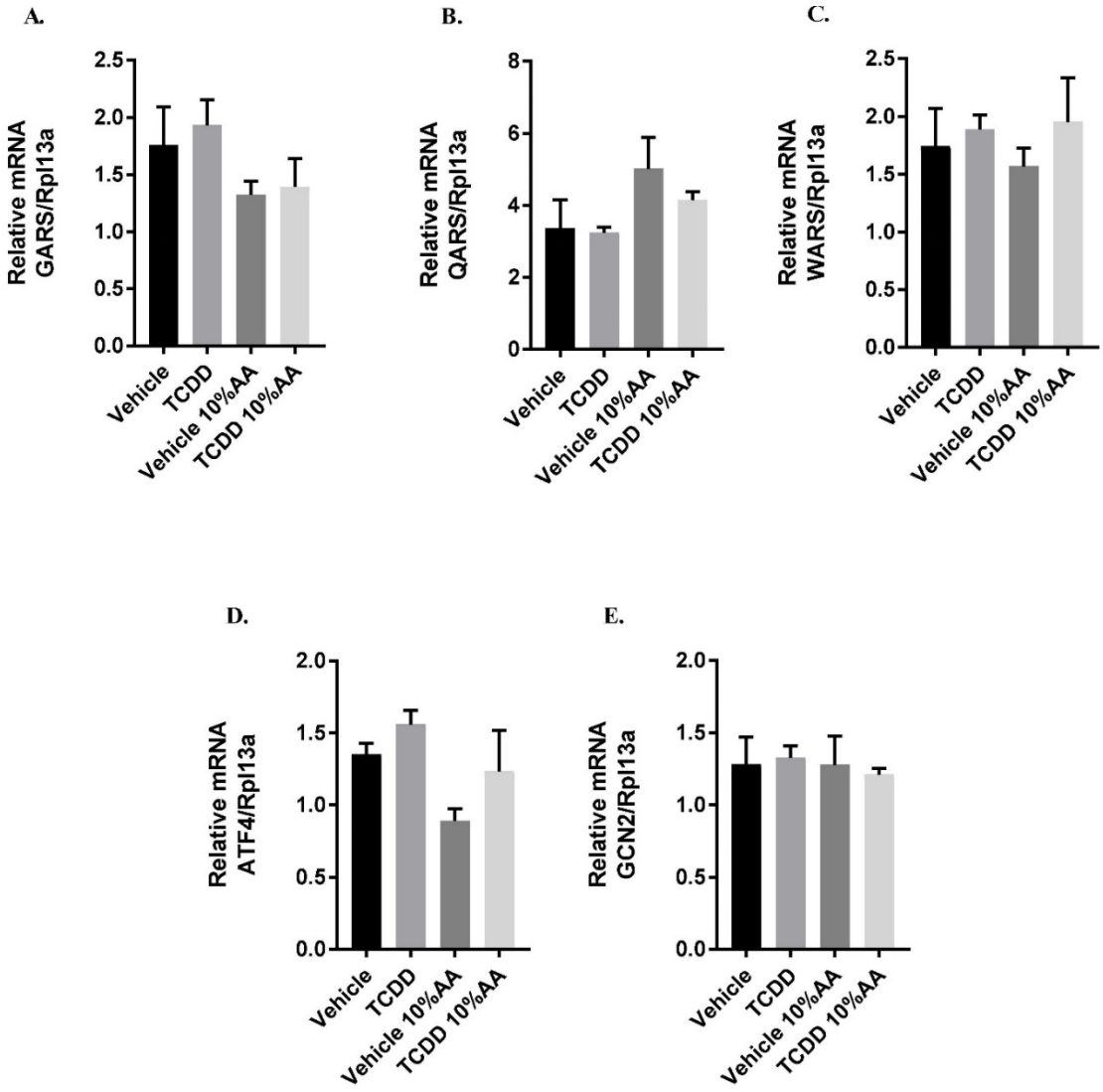

Figure 9: tRNA Synthases and GCN2/ATF4 Expression is Unaffected by AHR

OSC-19 cells were pretreated with TCDD (2nM) for 24 h. Cells were then incubated under normal media or DMEM/F-12 for 6h. mRNA expression was analyzed via quantitative real-time PCR. Expression was normalized to *Rpl13a* for *GARS* (A), *QARS* (B), *WARS* (C), *ATF4* (D), and *GCN2* (E). Statistical significance is indicated by an asterisk ($P < 0.05$ (*), $P < 0.01$ (**), and $P< 0.001$ (***)).

Transporter Expression in Mouse Jejunum

Induction of Known AHR target genes by Glucobrassicins

The production of endogenous ligands from our diet have expanded the physiological role of AHR. In the gastrointestinal tract, several studies have suggested that activation of AHR by dietary ligands is involved in anti-inflammatory signaling and immune surveillance[10,58]. In conjunction with data produced in the Perdew laboratory showing the interaction of AHR with SLC7A5/SLC3A2, it was hypothesized that regulation of these transporters may add to the understanding of the multifaceted role of AHR signaling in the GI tract. Wildtype *Ahr*$^{+/+}$ mice were fed a purified chow diet or one supplemented with 15% broccoli rich in glucobrassicins. After two weeks, the jejunum of the mice was isolated for qPCR analysis. The jejunum exhibits high expression of amino acid transporters in order to prevent downstream utilization by microbiota in the colon[59]. As expected, *CYP1A1* showed significant increase in gene expression. However, *SLC7A5/SLC3A2* expression was non-significant. Since broccoli derived agonists are significantly less potent than TCDD, it may be possible that an effect was too small to be observed.

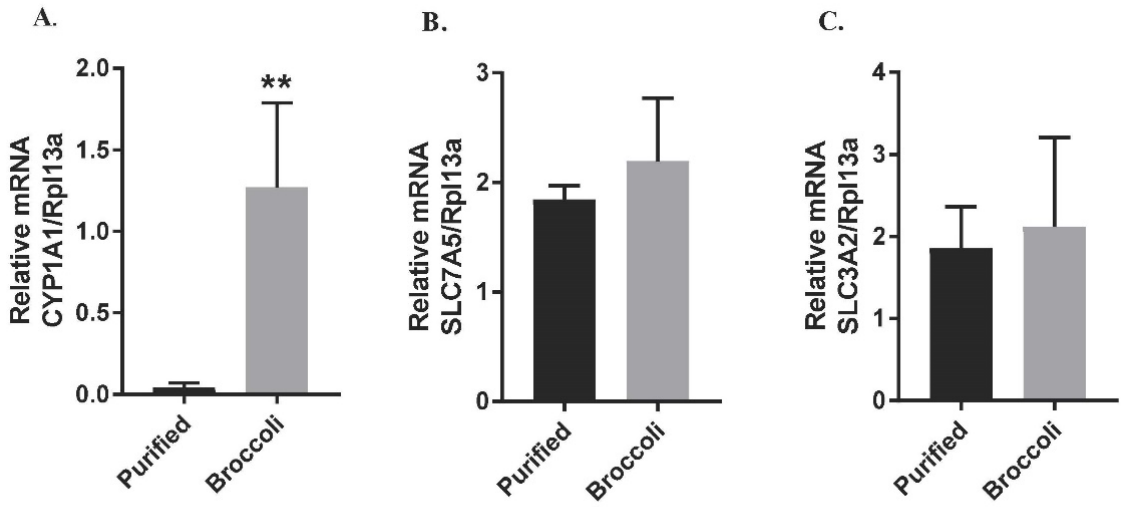

Figure 10: Known AHR Target Genes in Mouse Jejunum

Wildtype *Ahr*$^{+/+}$ mice were fed a purified chow diet (4) or one supplemented with broccoli (6) for two weeks. Both diets were isocalorically matched. RNA was isolated from the jejunum and analyzed by qPCR normalized to *Rpl13a* for *CYP1A1* (A), *SLC7A5* (B), and *SLC3A2* (C). Statistical significance is indicated by an asterisk ($P < 0.05$ (*), $P < 0.01$ (**), and $P< 0.001$ (***)).

Amino Acid Transporter Expression

Amino acid transporters were examined in the jejunum in order to gain insight into the role of AHR in the gastrointestinal tract. Similar effects (or lack of) were observed in jejunum samples and there were no significant changes in neutral or cationic amino acid transporter expression in mice fed with broccoli compared to controls. Additionally, there were no significant changes in the gene expression of monocarboxylic acid transporters. However, there was increased expression of *SLC7A9* that was borderline insignificant. It may worthwhile to investigate the interactions of this transporter further. Lastly, there was decreased expression of *SLC43A3* which is a nucleobase transporter[44].

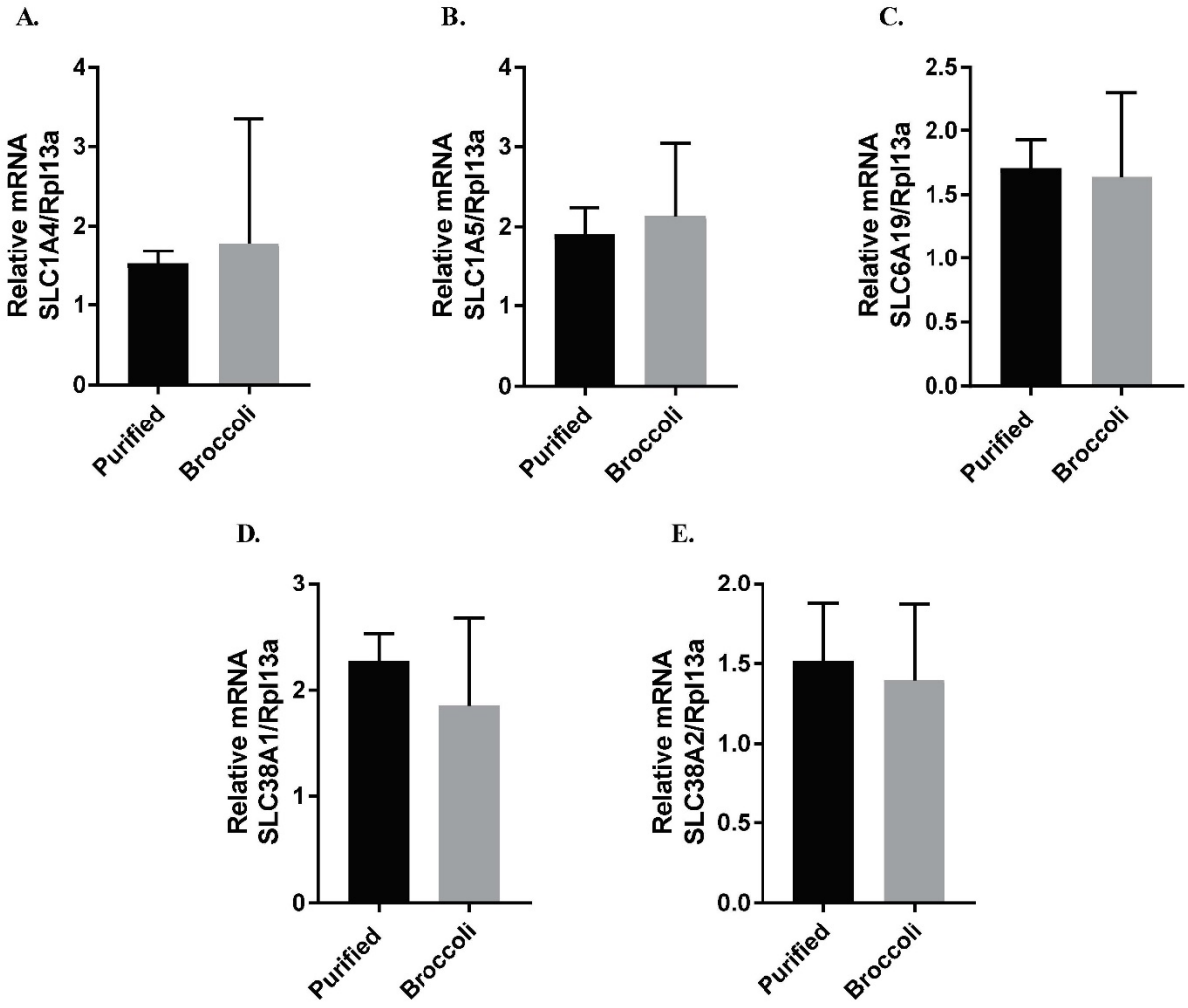

Figure 11: Neutral Amino Acid Transporter Expression in the Mouse Jejunum

Wildtype *Ahr*$^{+/+}$ mice were fed a purified chow diet (4) or one supplemented with broccoli (6) for two weeks. Both diets were isocalorically matched. RNA was isolated from the jejunum and analyzed by qPCR normalized to *Rpl13a* for *SLC1A4* (A) *SLC1A5* (B) *SLC6A19* (C), *SLC38A1* (D), and *SLC38A2* (E). Statistical significance is indicated by an asterisk ($P < 0.05$ (*), $P < 0.01$ (**), and $P< 0.001$ (***)).

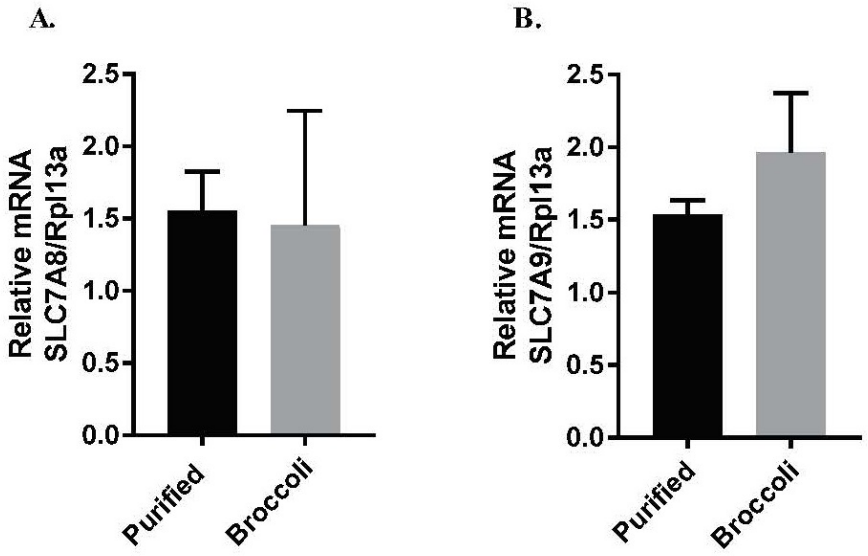

Figure 12: Cationic Amino Acid Transporter Expression in the Mouse Jejunum

Wildtype *Ahr*$^{+/+}$ mice were fed a purified chow diet (4) or one supplemented with broccoli (6) for two weeks. Both diets were isocalorically matched. RNA was isolated from the jejunum and analyzed by qPCR normalized to *Rpl13a* for *SLC7A8* (A) and *SLC7A9* (B). Statistical significance is indicated by an asterisk ($P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***)).

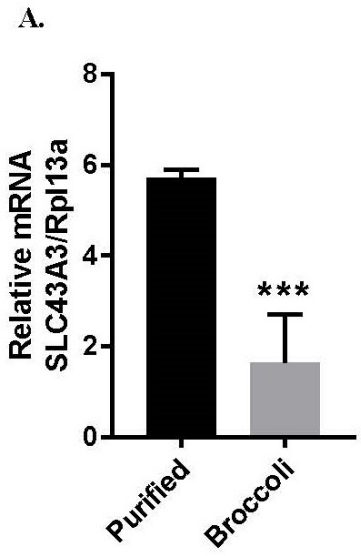

Figure 13: Broccoli Enriched Diet Decreases SLC43A3 Expression

Wildtype *Ahr*$^{+/+}$ mice were fed a purified chow diet (4) or one supplemented with broccoli (6) for two weeks. Both diets were isocalorically matched. RNA was isolated from the jejunum and analyzed by qPCR normalized to *Rpl13a* for *SLC43A3 (A)*. Statistical significance is indicated by an asterisk ($P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***)).

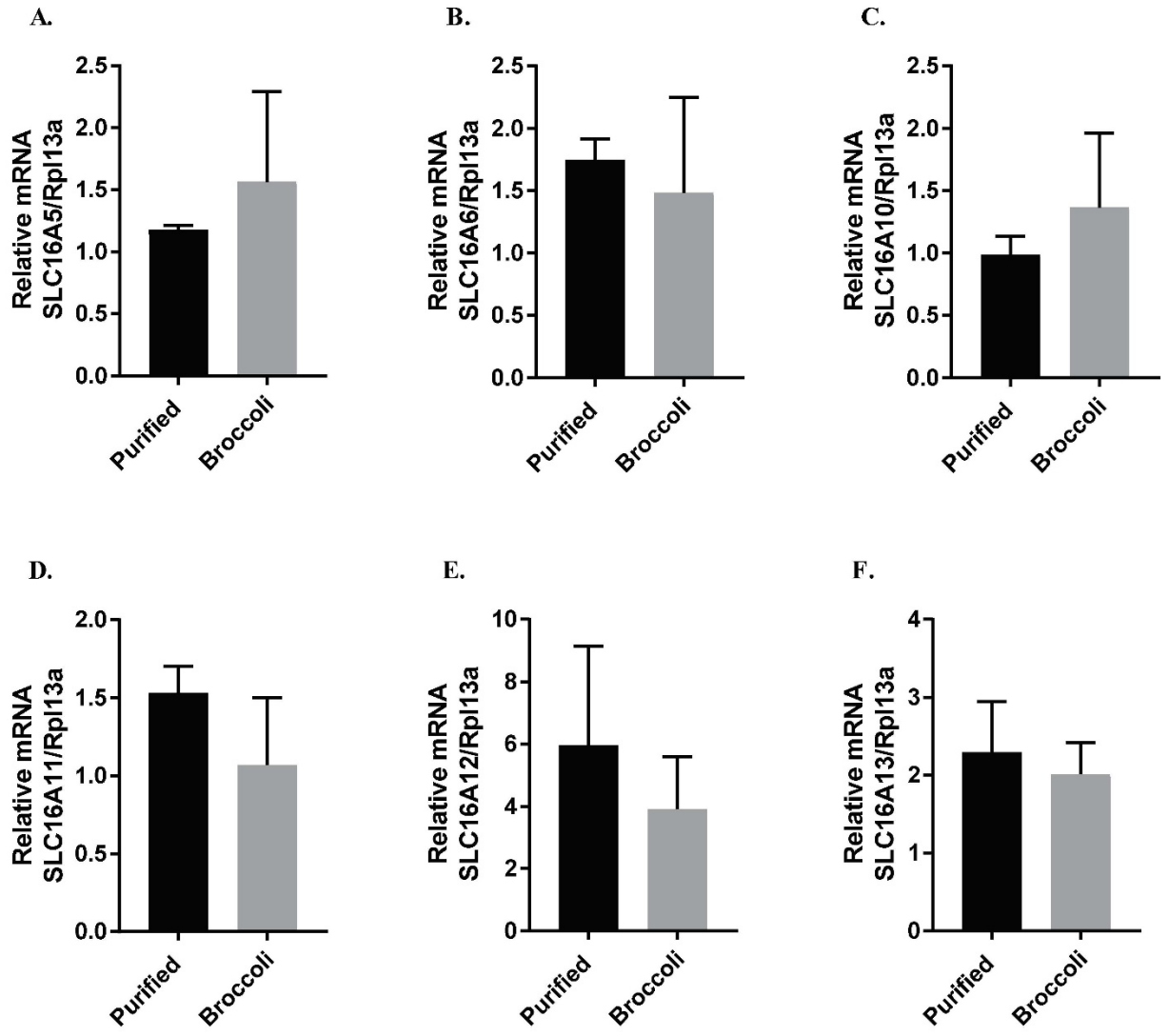

Figure 14: Monocarboxylate Transporter Expression in the Mouse Jejunum

Wildtype *Ahr*$^{+/+}$ mice were fed a purified chow diet (4) or one supplemented with broccoli (6) for two weeks. Both diets were isocalorically matched. RNA was isolated from the jejunum and analyzed by qPCR normalized to *Rpl13a* for *SLC16A5* (A) and *SLC16A6* (B) *SLC16A10* (C), *SLC16A11* (D), *SLC16A12* (E), *SLC16A13* (F). Statistical significance is indicated by an asterisk ($P < 0.05$ (*), $P < 0.01$ (**), and $P< 0.001$ (***)).

DISCUSSION

The purpose of this study was to characterize the expression of amino acid transporters within the gastrointestinal tract, specifically the jejunum where most amino acid uptake occurs[59]. Amino acids serve an important physiologic role as precursors of protein synthesis[28]. As such, regulation of intracellular pools of amino acids is vital to maintaining a proper supply of proteins in cells. When the cells are starved of nutrients, amino acid synthesis and transport is reduced to conserve constituents, while the opposite is true when the cells are dividing[28]. It was hypothesized that AHR transcriptionally upregulates amino acids transporters. In particular, transporters responsible for the uptake of tryptophan were of interest, since intracellular metabolism by enzymes such as IDO/TDO could produce additional amino acid derivatives such as kynurenine, kynurenic, xanthurenic and cinnabarinic acids that function as AHR ligands[26]. Moreover, these metabolites could further stimulate AHR activity in a positive feedback loop. While AHR has exhibited tissue and ligand specific effects[1,4], recent evidence supports an anti-inflammatory and beneficial immunological response in the gut[13]. The presence of AHR is required for the development of healthy immune populations and mediates anti-inflammatory responses through interaction with prostaglandin endoperoxide synthase 2 (PTGS2) and cytokine production[60]. However, the data failed to support our hypothesis and it appears that AHR regulation of amino acid transporters is limited outside of LAT1.

In order to characterize expression of these amino acid transporters in regards to AHR, they were first screened by qPCR analysis in OSC-19 cells. It was previously established that *LAT1* was upregulated in certain breast[37] and colorectal cancers[36] and that DREs were present in the

promoter of *LAT1*[37]. Furthermore, amino acid restriction has been shown to promote angiogenesis by upregulating the vascular endothelial growth factor (VEGF), which can lead to metabolic reprogramming of cellular processes (e.g. amino acid transport) and to enhance cancer cell survival and proliferation[54]. When OSC-19 cells were treated with TCDD, *LAT1* expression increased which was consistent with previous studies[34-37] and experiments conducted in the Perdew laboratory. Upregulation of *LAT1* was also significantly greater when cells were treated with both TCDD and amino acid deficiency. When other transporters were examined, only *SLC16A14* was upregulated significantly in response to TCDD, but only within the cells under amino acid deficiency. The functions of SLC16A14 have yet to be characterized, but due to the homology with SLC16A10 it may be involved in the transport of tryptophan and other aromatic amino acids[55]. As more information is discovered regarding the functions of SLC16A14, it may be interesting to further examine any potential interactions of this transporter with AHR mediated processes. A link between AHR and SLC16A14 would add to our understanding of the carcinogenic mechanism behind AHR activation and may lead to identification of a therapeutic target.

AHR is already known to be involved in inflammatory signaling and immune regulation in the gastrointestinal tract[13], but the influence on amino acid transporters has not been explored in detail. Despite the significant increased expression of *CYP1A1*, we did not see a significant increase in the expression of *LAT1*. However, AHR is known to have ligand and cell specific effects which could explain why no changes were observed or perhaps the endogenous ligands were not potent enough to observe an effect. It may be beneficial to analyze the expression of these transporters in mice exposed to xenobiotics or other endogenous AHR ligands to determine if this may be the case. The only other significant change was a decrease in *SLC43A3*, a purine

nucleobase transporter, expression[44]. The function of the transporter has been only recently elucidated as a purine selective nucleobase transporter that may participate in nucleotide salvage pathways[44]. This data would suggest that a broccoli supplemented diet and potentially AHR induction would inhibit these anabolic pathways. Inhibition of nucleotide biosynthesis could be potentially beneficial depending on tissue location and any preexisting disease pathologies. These pathways work in concert with de novo synthesis of nucleotides to provide DNA repair mechanisms with components needed to fix damaged or mutated DNA. Disruption of nucleotide salvage mechanisms has been linked to multiple conditions, including neurodegenerative diseases[61]. On the other hand, nucleotide biosynthesis and salvage is often upregulated in tumors in order to support cell growth and proliferation, and downregulation of those pathways could offer a protective effect[62]. Future studies could determine whether or not the reduced expression was AHR dependent by treating mice with AHR antagonists or by using *Ahr*$^{-/-}$ null mice.

In conclusion, AHR activation had no effect on the majority of amino acid transporters. *LAT1* expression was significantly increased in OSC-19 cells which is consistent with previous studies, but these findings did not translate to mouse jejunum samples. However, there was some evidence of altered *SLC16A14* expression in OSC-19 cells, and *SLC43A3* expression in mice jejunum samples. Since this experiment only looked at mRNA transcript levels and did not evaluate any post transcriptional, translational, or post translational modifications, observed activity of these transporters may differ. Future experiments could examine these effects and see if this observed gene expression ultimately influences organism physiology.

REFERENCES

1. Beischlag, Timothy V. et al. “The Aryl Hydrocarbon Receptor Complex and the Control of Gene Expression.” *Critical Reviews in Eukaryotic Gene Expression* 18.3 (2008): 207–250.

2. Denison, M S, J M Fisher, and J P Whitlock. “Inducible, Receptor-Dependent Protein-DNA Interactions at a Dioxin-Responsive Transcriptional Enhancer.” *Proceedings of the National Academy of Sciences of the United States of America* 85.8 (1988): 2528–2532.

3. Bohonowych, Jessica E., and Michael S. Denison. “Persistent Binding of Ligands to the Aryl Hydrocarbon Receptor.” *Toxicological Sciences* 98.1 (2007): 99–109.

4. Murray, Iain A., et al. “Aryl hydrocarbon receptor ligands in cancer: friend and foe.” *Nature Reviews Cancer*, vol. 14, no. 12, 14 Dec. 2014, pp. 801– 814.

5. Forrester, Alison R. et al. “Induction of a Chloracne Phenotype in an Epidermal Equivalent Model by 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) Is Dependent on Aryl Hydrocarbon Receptor Activation and Is Not Reproduced by Aryl Hydrocarbon Receptor Knock down.” *Journal of Dermatological Science* 73.1 (2014): 10–22.

6. Gelhaus, Stacy L. et al. “Regulation of Benzo[a]pyrene-Mediated DNA- and Glutathione-Adduct Formation by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin in Human Lung Cells.” *Chemical Research in Toxicology* 24.1 (2011): 89–98.

7. Shiizaki, Kazuhiro, Masanobu Kawanishi, and Takashi Yagi. “Modulation of benzo[a]pyrene–DNA Adduct Formation by CYP1 Inducer and Inhibitor.” *Genes and Environment* 39 (2017): 14.

8. Lin, Bernice C., et al. “Deletion of the Aryl Hydrocarbon Receptor-Associated Protein 9 Leads to Cardiac Malformation and Embryonic Lethality.” *Journal of Biological Chemistry*, vol. 282, no. 49, Dec. 2007, pp. 35924–32.

9. Abbott, Barbara D., et al. “Adverse Reproductive Outcomes in the Transgenic Ah Receptor-Deficient Mouse.” *Toxicology and Applied Pharmacology*, vol. 155, no. 1, Feb. 1999, pp. 62–70.

10. Sekine, Hiroki et al. “Hypersensitivity of Aryl Hydrocarbon Receptor-Deficient Mice to Lipopolysaccharide-Induced Septic Shock” *Molecular and Cellular Biology* 29.24 (2009): 6391–6400.

11. Nguyen, Linh P., and Christopher A. Bradfield. “The Search for Endogenous Activators of the Aryl Hydrocarbon Receptor.” *Chemical Research in Toxicology* 21.1 (2008): 102–116.

12. Swedenborg, E., et al. “Endocrine Disruptive Chemicals: Mechanisms of Action and Involvement in Metabolic Disorders.” *Journal of Molecular Endocrinology*, vol. 43, no. 1, July 2009, pp. 1–10.

13. Murray, Iain A., and Gary H. Perdew. “Ligand Activation of the Ah Receptor Contributes to Gastrointestinal Homeostasis.” *Current Opinion in Toxicology* 2 (2017): 15–23.

14. Haas, Katharina, et al. “Aryl Hydrocarbon Receptor in Keratinocytes Is Essential for Murine Skin Barrier Integrity.” *Journal of Investigative Dermatology*, vol. 136, no. 11, Nov. 2016, pp. 2260–69.

15. Fribourgh, Jennifer L., and Carrie L. Partch. “Assembly and Function of bHLH–PAS Complexes.” *Proceedings of the National Academy of Sciences of the United States of America* 114.21 (2017): 5330–5332.

16. Seok, Seung-Hyeon et al. “Structural Hierarchy Controlling Dimerization and Target DNA Recognition in the AHR Transcriptional Complex.” *Proceedings of the National Academy of Sciences of the United States of America* 114.21 (2017): 5431–5436.

17. Wu, Dalei et al. “Structure and Dimerization Properties of the Aryl Hydrocarbon Receptor PAS-A Domain.” *Molecular and Cellular Biology* 33.21 (2013): 4346–4356

18. Fukunaga, Bert N., et al. “Identification of Functional Domains of the Aryl Hydrocarbon Receptor.” *Journal of Biological Chemistry*, vol. 270, no. 49, Dec. 1995, pp. 29270–78.

19. Antonsson, C et al. “Distinct Roles of the Molecular Chaperone hsp90 in Modulating Dioxin Receptor Function via the Basic Helix-Loop-Helix and PAS Domains.” *Molecular and Cellular Biology* 15.2 (1995): 756–765.

20. Soshilov, Anatoly, and Michael S. Denison. “Ligand Displaces Heat Shock Protein 90 from Overlapping Binding Sites within the Aryl Hydrocarbon Receptor Ligand-Binding Domain.” *The Journal of Biological Chemistry* 286.40 (2011): 35275–35282.

21. Dull, Angie B., et al. “Characterization of the Phosphorylation Status of the Hepatitis B Virus X-Associated Protein 2.” *Archives of Biochemistry and Biophysics*, vol. 406, no. 2, Oct. 2002, pp. 209–21.

22. Meyer, Brian K., and Gary H. Perdew. “Characterization of the AhR−hsp90−XAP2 Core Complex and the Role of the Immunophilin-Related Protein XAP2 in AhR Stabilization †.” *Biochemistry*, vol. 38, no. 28, July 1999, pp. 8907–17.

23. Ma, Qiang. “Induction of CYP1A1. The AhR / DRE Paradigm Transcription, Receptor Regulation, and Expanding Biological Roles.” *Current Drug Metabolism*, vol. 2, no. 2, June 2001, pp. 149–64.

24. Soshilov, Anatoly A., and Michael S. Denison. “Ligand Promiscuity of Aryl Hydrocarbon Receptor Agonists and Antagonists Revealed by Site-Directed Mutagenesis.” *Molecular and Cellular Biology* 34.9 (2014): 1707–1719.

25. Stejskalova, Lucie, et al. “Endogenous and Exogenous Ligands of Aryl Hydrocarbon Receptor: Current State of Art.” *Current Drug Metabolism*, vol. 12, no. 2, Feb. 2011, pp. 198–212.

26. Hubbard, T. D., et al. “Indole and Tryptophan Metabolism: Endogenous and Dietary Routes to Ah Receptor Activation.” *Drug Metabolism and Disposition*, vol. 43, no. 10, Sept. 2015, pp. 1522–35.

27. Bjeldanes, L F et al. “Aromatic Hydrocarbon Responsiveness-Receptor Agonists Generated from Indole-3-Carbinol in Vitro and in Vivo: Comparisons with 2,3,7,8-Tetrachlorodibenzo-P-Dioxin.” *Proceedings of the National Academy of Sciences of the United States of America* 88.21 (1991): 9543–9547.

28. Bröer, Stefan, and Angelika Bröer. “Amino Acid Homeostasis and Signaling in Mammalian Cells and Organisms.” *Biochemical Journal* 474.12 (2017): 1935–1963.

29. Hou, Yongqing, Yulong Yin, and Guoyao Wu. “Dietary Essentiality of ‘nutritionally Non-Essential Amino Acids’ for Animals and Humans.” *Experimental Biology and Medicine* 240.8 (2015): 997–1007.

30. Goberdhan, Deborah C.I., Clive Wilson, and Adrian L. Harris. “Amino Acid Sensing by mTORC1: Intracellular Transporters Mark the Spot.” *Cell Metabolism* 23.4 (2016): 580–589.

31. Ye, Jiangbin et al. “The GCN2-ATF4 Pathway Is Critical for Tumour Cell Survival and Proliferation in Response to Nutrient Deprivation.” *The EMBO Journal* 29.12 (2010): 2082–2096.

32. Koromilas, Antonis E. “Roles of the Translation Initiation Factor EIF2α Serine 51 Phosphorylation in Cancer Formation and Treatment.” *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, vol. 1849, no. 7, July 2015, pp. 871–80.

33. Proud, Christopher G. “Regulation of Eukaryotic Initiation Factor EIF2B.” *Signaling Pathways for Translation*, edited by Robert E. Rhoads, vol. 26, Springer Berlin Heidelberg, 2001, pp. 95–114.

34. Wang, Qian, and Jeff Holst. “L-Type Amino Acid Transport and Cancer: Targeting the mTORC1 Pathway to Inhibit Neoplasia.” *American Journal of Cancer Research* 5.4 (2015): 1281–1294.

35. Yanagida, Osamu, et al. “Human L-Type Amino Acid Transporter 1 (LAT1): Characterization of Function and Expression in Tumor Cell Lines.” *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1514, no. 2 (October 2001): 291–302.

36. Hayase, Suguru et al. “L-Type Amino Acid Transporter 1 Expression Is Upregulated and Associated with Cellular Proliferation in Colorectal Cancer.” *Oncology Letters* 14.6 (2017): 7410–7416.

37. Tomblin, Justin K. et al. “Aryl Hydrocarbon Receptor (AHR) Regulation of L-Type Amino Acid Transporter 1 (LAT-1) Expression in MCF-7 and MDA-MB-231 Breast Cancer Cells.” *Biochemical Pharmacology* 106 (2016): 94–103.

38. Badawy, Abdulla A-B. “Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects.” *International Journal of Tryptophan Research,* 10 (2017)

39. Bilir, Cemil, and Can Sarisozen. “Indoleamine 2,3-Dioxygenase (IDO): Only an Enzyme or a Checkpoint Controller?” *Journal of Oncological Sciences*, vol. 3, no. 2, July 2017, pp. 52–56.

40. Hollingshead, Brett D. et al. “Inflammatory Signaling and Aryl Hydrocarbon Receptor Mediate Synergistic Induction of Interleukin 6 in MCF-7 Cells.” *Cancer research* 68.10 (2008): 3609–3617.

41. Litzenburger, Ulrike M. et al. “Constitutive IDO Expression in Human Cancer Is Sustained by an Autocrine Signaling Loop Involving IL-6, STAT3 and the AHR.” *Oncotarget* 5.4 (2014): 1038–1051.

42. Khayati, Farah, et al. “EMMPRIN/CD147 Is a Novel Coreceptor of VEGFR-2 Mediating Its Activation by VEGF.” *Oncotarget*, vol. 6, no. 12, Apr. 2015.

43. Pras, Elon et al. “Mutations in the SLC3A1 Transporter Gene in Cystinuria.” *American Journal of Human Genetics* 56.6 (1995): 1297–1303.

44. Furukawa, Junji, et al. “Functional Identification of SLC43A3 as an Equilibrative Nucleobase Transporter Involved in Purine Salvage in Mammals.” *Scientific Reports*, vol. 5, Oct. 2015, p. 15057.

45. “Monocarboxylate Transporters as Targets and Mediators in Cancer Therapy Response.” *Histology and Histopathology*, no. 29, Nov. 2014, pp. 1511–1524.

46. Bhutia, Y. D., et al. “Amino Acid Transporters in Cancer and Their Relevance to ‘Glutamine Addiction’: Novel Targets for the Design of a New Class of Anticancer Drugs.” *Cancer Research*, vol. 75, no. 9, May 2015, pp. 1782–88. 14-3745

47. Timosenko, Elina, et al. “Nutritional Stress Induced by Tryptophan-Degrading Enzymes Results in ATF4-Dependent Reprogramming of the Amino Acid Transporter Profile in Tumor Cells.” *Cancer Research*, vol. 76, no. 21, Nov. 2016, pp. 6193–204.

48. Ye, Jiangbin et al. “The GCN2-ATF4 Pathway Is Critical for Tumour Cell Survival and Proliferation in Response to Nutrient Deprivation.” *The EMBO Journal* 29.12 (2010): 2082–2096.

49. Curtis, Kevin M., et al. “EF1alpha and RPL13a Represent Normalization Genes Suitable for RT-QPCR Analysis of Bone Marrow Derived Mesenchymal Stem Cells.” *BMC Molecular Biology*, vol. 11, no. 1, 2010, p. 61.

50. Bian, Zehua, et al. “RPL13A as a Reference Gene for Normalizing MRNA Transcription of Ovarian Cancer Cells with Paclitaxel and 10-Hydroxycamptothecin Treatments.” *Molecular Medicine Reports*, vol. 11, no. 4, Apr. 2015, pp. 3188–94.

51. Johnson, Mark A., et al. “Amino Acid Starvation Has Opposite Effects on Mitochondrial and Cytosolic Protein Synthesis.” *PLoS ONE*, edited by Robert Lightowlers, vol. 9, no. 4, Apr. 2014, p. e93597

52. Fader, Claudio Marcelo, et al. “Autophagy Response: Manipulating the MTOR-Controlled Machinery by Amino Acids and Pathogens.” *Amino Acids*, vol. 47, no. 10, Oct. 2015, pp. 2101–12.

53. Gaccioliy, Francesca, et al. “Amino Acid Starvation Induces the SNAT2 Neutral Amino Acid Transporter by a Mechanism That Involves Eukaryotic Initiation Factor 2α Phosphorylation and Cap-Independent Translation.” *Journal of Biological Chemistry*, vol. 281, no. 26, June 2006, pp. 17929–40.

54. Wang, Yugang et al. “Amino Acid Deprivation Promotes Tumor Angiogenesis through the GCN2/ATF4 Pathway.” *Neoplasia* (New York, N.Y.) 15.8 (2013): 989–997.

55. Roshanbin, Sahar et al. “Histological Characterization of Orphan Transporter MCT14 (SLC16A14) Shows Abundant Expression in Mouse CNS and Kidney.” *BMC Neuroscience* 17 (2016): 43.

56. Svenningsen, Sine Lo, et al. “Transfer RNA Is Highly Unstable during Early Amino Acid Starvation in Escherichia Coli.” *Nucleic Acids Research*, vol. 45, no. 2, Jan. 2017, pp. 793–804.

57. Kim, Sunghoon, et al. “Aminoacyl-TRNA Synthetases and Tumorigenesis: More than Housekeeping.” *Nature Reviews Cancer*, vol. 11, no. 10, Oct. 2011, pp. 708–18.

58. Lee, Yi-Hsuan, et al. “Aryl Hydrocarbon Receptor Mediates Both Proinflammatory and Anti-Inflammatory Effects in Lipopolysaccharide-Activated Microglia: AhR in LPS-Induced Microglial Activation.” *Glia*, vol. 63, no. 7, July 2015, pp. 1138–54.

59. Dave, Mital H., et al. “Expression of Heteromeric Amino Acid Transporters along the Murine Intestine: Intestinal Amino Acid Transporters.” *The Journal of Physiology*, vol. 558, no. 2, July 2004, pp. 597–610.

60. Julliard, Walker, John H. Fechner, and Joshua D. Mezrich. “The Aryl Hydrocarbon Receptor Meets Immunology: Friend or Foe? A Little of Both.” *Frontiers in Immunology* 5 (2014): 458.

61. Fasullo, Michael, and Lauren Endres. “Nucleotide Salvage Deficiencies, DNA Damage and Neurodegeneration.” *International Journal of Molecular Sciences*, vol. 16, no. 12, Apr. 2015, pp. 9431–49.

62. Shuvalov, Oleg, et al. “One-Carbon Metabolism and Nucleotide Biosynthesis as Attractive Targets for Anticancer Therapy.” *Oncotarget*, vol. 8, no. 14, Apr. 2017.

APPENDIX

Quantitative PCR Primers

Human Gene	Forward Primer 5' to 3'	Reverse Primer 3' to 5'
	Control Genes	
RPL13a	CCTGGAGGAGAAGAGGAAAGAGA	GAGGACCTCTGTGTATTTGTCAA
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
	Known Target Genes	
CYP1A1	TACCTCAGCCACCTCCAAGAT	GAGGTCTTGAGGCCCTGAT
	Amino Acid Recycling Genes	
ATF4	CTATACCCAACAGGGCATCC	GTCCCTCCAACAACAGCAAG
GARS	AATGGAAAGTGTTTTGGCCC	TCATTTCCAGTAATGGGAGATTT
GCN2	ACCGTCAGACTCTGGAGGCTTT	GGCTAGATGGTCTGTCGCCAAA
QARS	GCGTCTTGGATATTTCTCCG	GCTTCCAGCTCACACCTTTC
WARS	CGGCAGCTTTGTAGCTCATT	GAGCTCGTAAGGTCCCTCAA
	Membrane Transporter Genes	
EMMPRIN	TCGTCGGGAGTCCACCTTGA	CAGTCGTGCTAGTCCTGGAA
SLC1A4	GGCCTTGGCGTTCATCATCA	GTTGCATACGTACGGAAAGCTG
SLC1A5	GCCATCAACGCCTCCGTGGGA	ACGGGCACCTTCACCCTGGTTC
SLC3A1	TCCACCCAACAACTGGTTAAG	ACCCTTTGTGAGCCAGAACC
SLC3A2	CTCGTGGTTCTCCACTCAGG	CCGCAATCAAGAGCCTGTCT
SLC6A14	GGGTCTCGATTCTCAGTTTGCT	CCCAGTAAATTCCAGCCTGAGT
SLC6A19	TCCATCCACCCGGCCCTGAAG	ACTCGTCCACATACCCTGTCTGGT
SLC7A1	CTCGGGTGCCGTTGCTGCTGT	CAGGTTAGGCTGCTCTGGCTGG
SLC7A2	TGTCAACAAGTCTTCTGGGCT	CAAGCGCCTTCAGGTCAAAC
SLC7A5	CCGTGCCGTCCCTCGTGTTC	GGTTCACCTTGATGGGCCGCT

SLC7A8	TCGGCTCCTGGCTGCCATCT	CCAGCCAGAAGTACTCTCCTTTGC
SLC7A9	CCTGGCCCAAGGAAACACAA	ATGGCCAAAGGCAGGTTTCT
SLC7A11	TGGACGGTGTGTGGGGTCCT	CAGCAGTAGCTGCAGGGCGTA
SLC16A4	GGTTGGATTGGATCCATCATGT	CAGAACCCAAACCGGGTAGA
SLC16A5	GCGGACAAAGTAGAAGCCCA	GCCAGCTCTACTTCACAGCA
SLC16A6	TCATATGTACGTCGCCATCGG	TCAGAGCCATGATTGCTGGT
SLC16A9	GATCCCTGGCAAGTGGAGTT	ACCACATCCAAGACCTACAACA
SLC16A10	TCGGCTCCAAAGACGATG	CAGAGGCTCGATGGAACTTAC
SLC16A11	CCGCTGGTTTTCGGTGTACT	TTAGGAAGCCTGACAGGGGA
SLC16A12	ATTGTGGCTGGCTGTTTCCT	CTCCCAAGTGGAGCACAGAG
SLC16A13	TTTGGGGAGCCCGGTAGGC	GGTCGGAGCGAAGGTCAAA
SLC16A14	AAGAATTCCACCAGAGCCGC	CGGCACCCACAGGTGTTAAT
SLC36A4	ATGTTGGTCAGGAACATGCCAG	TTCAACGCTTGAGGGAAACG
SLC38A1	GCCATTATGGGCAGTGGGAT	ACACCATGCAGCCTGTTTCT
SLC38A2	TGGGCAGTGGAATCCTTGGGC	AAAGACCCTCCTTCATTGGCAGTCT
SLC38A3	ATCGGAGCCATGTCCAGCTA	AGGGGCAGAATGATGGTGAC
SLC43A3	ACTACAGCTATGGCCTGTGC	CAGAAGGAGCGGAGTTCCTG

Table 2: Human quantitative qPCR primers.

Mouse Gene	**Forward Primer (5'-3')**	**Reverse Complement Primer (3'-5')**
Control Gene		
Rpl13a	TTCGGCTGAAGCCTACCAGAAAGT	GCATCTTGGCCTTTTTCCGTT
Known Target Genes		
CYP1A1	CTCTTCCCTGGATGCCTTGAA	GGATGTGGCCCTTCTCAAATG
Membrane Transporter Genes		
EMMPRIN	GCCTGCGCGGCGGCTGGTTT	CACGGTCCAGCCGGGCACCA
SLC1A4	TGCTCTGGCGTTCATCATCA	AGTGAATGCGGCAACCACAA
SLC1A5	TGGCCAGCAAGATTGTGGAGAT	TTTGCGGGTGAAGAGGAAGT

SLC3A1	ATGTCAACGCCATGCACASTGCT	AGGTGTGGTTGGCCTCAGTAAA
SLC3A2	TGCAACCAAGAACTCAGAGC	TCATTTTGGACCTCACTCCC
SLC6A14	CGGCCAGGACAACTTCCCAGT	GCCACTAGGCCACCCCAAGC
SLC7A1	ATGCCATGGCTGAAGATGGACT	AAACACAGGCAGCCACCAAA
SLC7A2	ACTTCTTTGCCGTGTGCCTTGT	TTTCACAAACCCAGCCACCA
SLC7A5	ACAGCTGTGAGGAGCAGCAC	TCTTCGCCACCTACTTGCTC
SLC7A8	TGCAGAGCTTGGCACAATGA	AGGAGGCTTGCAACCTGAGTAA
SLC7A9	AGCGAAACAACACCGCGAAGAA	TTTCCAGCACACCTTTCGGT
SLC7A11	TGCAATCTGCATCTCCATGGC	AAGCAGGAGAGGGCAACAAA
SLC16A4	GGTGGCTACCTGGCACTAAT	ATCCAGCCTGCTATTGGTGGT
SLC16A5	GGCATGGTAGTCAGCACCTT	ACAAGCCCACTACCGTGATG
SLC16A6	CCTCCTTCTCCCAAAGGGTC	GCTGTGATAGCTGGTGCGAA
SLC16A9	GAGCAGTCTTGCCCCCAATA	ACCCACACTCGAACCTGTTG
SLC16A10	GTGCCTTACGTTCACTTGATGA	TGGACATCAGGCCAATGAAGAA
SLC16A11	CGGAGCACTTTGAACGAAGC	AAGCCAAGCGAGGTTAGGAC
SLC16A12	GGCAGTCACCAGATGTATCTCCA	ATGATCCCCGCTTGACAGGA
SLC16A13	CTTCCTGAACTGGTGGGGAC	ATCCCGGAGGTAGCCTGATA
SLC16A14	GATCGTGGGACCTTTCATCG	CACTGCAGGTAGGTAAGCCA
SLC16A19	TTCACATCTGTGTATGCGGCCA	AGTGGCATTGCACCACTGTT
SLC36A4	GGATTTCGTTCCTGCAGACCT	TGTGCATACAGTGGACGGAA
SLC38A1	ACGCGTGCACACCAAAGTAT	AAAGATGGCCGTCAGGAAGT
SLC38A2	TTGCAGGCCACGCTATTTCA	AGCACAGCCAATCGGACAACAA
SLC38A3	TGGCCTGCTTACGTGCATCAAT	AGCGAAGGGCCAGGATT
SLC43A3	GCCCGCCTGATAGCCATATT	CCCAAAGAGGTTCCCAATCTGT

Table 3: Mouse quantitative qPCR primers.

ACADEMIC VITA

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Education

Schreyer Honors College at The Pennsylvania State University

Major: Toxicology

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Thesis Title: Profiling the Expression of Amino Acid Transporters within the Context of the Aryl Hydrocarbon Receptor

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Research Experience

Undergraduate Researcher — 2016-Present
Perdew Lab, State College, PA

Research Intern — 2012 - 2014
Ran Lab, San Antonio Texas

Awards

Lester Earl and Veronica Casida Scholarship — 2017
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Galen Dreibelbis Endowment Scholarship — 2015-2016
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General Chemistry Learning Assistant — 2016-2017

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