NOVEL TARGETS OF FASD PATHOGENESIS IN THE DEVELOPING HIPPOCAMPUS

A Thesis

by

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ABSTRACT

Prenatal alcohol exposure can contribute to fetal alcohol spectrum disorders (FASD), characterized by a myriad of developmental impairments affecting behavior and cognition. Studies show many of these functional impairments are associated with the hippocampus, a structure exhibiting exquisite vulnerability to developmental alcohol exposure and critically implicated in learning and memory, however mechanisms underlying alcohol-induced hippocampal deficits remain poorly understood. Utilizing a high-throughput RNA-Seq approach to address the neurobiological and molecular basis of prenatal alcohol-induced hippocampal functional deficits, we hypothesized that chronic binge prenatal alcohol exposure alters gene expression and global molecular pathways in the fetal hippocampus. Timed-pregnant Sprague Dawley rats were randomly assigned to a pair-fed control (PF) or binge-alcohol treatment (ALC) group on gestational day (GD) 4. ALC dams acclimatized from GD 5-10 with a daily treatment of 4.5 g/kg alcohol and subsequently received 6 g/kg on GDs 11-20. PF dams received a once-daily maltose dextrin gavage on GDs 5-20, isocalorically matching ALC counterparts. On GD 21, bilateral hippocampi were dissected, flash frozen, and stored at -80°C. Total RNA was then isolated from homogenized tissues. Samples were normalized to ~4nM and pooled equally. Sequencing was performed by Illumina NextSeq 500 on a 75 cycle, single end sequencing run. RNA-Seq identified 13,388 genes, of these, 76 genes showed a significant difference ($P < 0.05$, log2 fold change >

2) in expression between the PF and ALC groups. 49 genes showed sex-dependent dysregulation, of which, 23 were significantly altered in ALC-exposed females, 26 were altered in ALC-exposed males, and 2 were altered in both ALC-exposed males and females compared with PF offspring. We conclude that chronic binge alcohol exposure during pregnancy dysregulates fetal hippocampal gene expression in a sex-specific manner. Identification of subtle, transcriptome-level dysregulation in hippocampal molecular pathways offers potential mechanistic insights underlying FASD pathogenesis.

DEDICATION

To my family

“Real living, is living for others.” -Bruce Lee

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NOMENCLATURE

ADHD	Attention Deficit Hyperactivity Disorder
ARBD	Alcohol-Related Birth Defect(s)
ARND	Alcohol-Related Neurodevelopmental Disorder
FAS	Fetal Alcohol Syndrome
FASD	Fetal Alcohol Spectrum Disorder
CNS	Central Nervous System
^{1}H-MRS	Proton Magnetic Resonance Spectroscopy
PAE	Prenatal Alcohol Exposure
pFAS	Partial Fetal Alcohol Syndrome
NMDA	N-Methyl-D-Aspartate
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
MRI	Magnetic Resonance Imaging
PND	Postnatal Day(s)
qPCR	Quantitative Polymerase Chain Reaction
RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
VIP	Vasoactive Intestinal Peptide

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CHAPTER I

INTRODUCTION

Fetal Alcohol Syndrome

Alcohol's teratogenic effects have become widely publicized in today's society, and partly because of federal public health initiatives, scientific reports on alcohol's harmful effects during pregnancy are readily accessible to the general public. However, prior to 1973, there was limited scientific evidence describing the teratogenic effects of alcohol, and gestational alcohol exposure was not linked to congenital birth defects until the late 1960's. In 1968, Dr. Paul Lemione, a French pediatrician, was the first to document the negative effects of prenatal alcohol exposure (PAE) that could manifest as craniofacial dysmorphia and growth restriction in children (O'Malley, 2007, Lemione et al., 1968). Five years later, Jones and Smith combined the patterns of craniofacial dysmorphia and growth restriction with new evidence of abnormal brain development following an autopsy of an affected newborn, coining the term Fetal Alcohol Syndrome (FAS) (Jones and Smith, 1973). The first diagnostic criteria for FAS in the United States was developed in 1989, comprising of the following (Sokol and Clarren, 1989):

1. Pre-/postnatal growth restriction
2. Dysfunction of the central nervous system (CNS):
    a. Neurological
    b. Development

    c. Intelligence

3. Craniofacial dysmorphia
    a. Microcephaly
    b. Short palpebral fissures
    c. Thin upper lip
    d. Elongated, flattened maxilla zone
    e. Poorly-defined philtrum

As PAE became more widely researched, and as awareness increased, there was a realization that not all children exposed to alcohol *in utero* expressed all of the hallmark phenotypes of FAS. Thus, new terms were created to encompass varying combinations of effects: Partial FAS (pFAS), Alcohol-related Neurodevelopmental Disorder (ARND), and Alcohol-related Birth Defect(s) (ARBD). In 2003, the diagnostic criteria describing pFAS, ARND, and ARBD were later consolidated and catalogued with FAS as unique sub-categories of Fetal Alcohol Spectrum Disorders (FASD) (Sokol et al., 2003a). Description criteria for all four subcategories along with FASD continue to be refined as understanding is gained through advances in research. A brief summary of the latest guidelines in the Unites States are as follows (Hoyme et al., 2016):

1. FAS (with or without knowledge of PAE)
    a. Pre-/postnatal growth restriction
    b. Stunted brain growth, morphology, or dysregulated neurophysiology
    c. Neurobehavioral impairment
    d. Characteristic facial dysmorphia

2. pFAS
    a. With knowledge of PAE
        i. Characteristic facial dysmorphia
        ii. Neurobehavioral impairment
    b. Without knowledge of PAE
        i. Pre-/postnatal growth restriction or stunted brain growth, morphology, or dysregulated neurophysiology
        ii. Neurobehavioral impairment
        iii. Characteristic facial dysmorphia
3. ARND
    a. Knowledge of PAE
    b. Neurobehavioral impairment
4. ARBD
    a. Knowledge of PAE
    b. Anatomic malformation(s) known to be caused by PAE

Fetal Alcohol Spectrum Disorders

Fetal Alcohol Spectrum Disorders (FASD) is an umbrella term that comprises all the possible pathological outcomes resulting from PAE, including and in addition to, FAS, pFAS, ARND, and ARBD (Riley et al., 2011b, Sokol et al., 2003a). As more studies discerning the teratogenic effects of alcohol were conducted, the extensive effects of gestational alcohol exposure were found to depend on an array of contributing

factors, such as exposure dose, timing, exposure pattern, maternal nutrition, maternal and fetal genetic susceptibility, and parental history of substance abuse (Smith et al., 2014a, May and Gossage, 2011a, May et al., 2013b, Maier and West, 2001b). A recent study found that individuals affected by FASD are at an increased risk for susceptibility to over 400 comorbid conditions, as a consequence of *in utero* alcohol exposure, than the rest of the general population (Popova et al., 2016b). Among these comorbid conditions, functional abnormalities of the peripheral nervous system (PNS), chronic/acute serous otitis media, visual impairment, congenital malformations of the spine, and hypertelorism had a higher prevalence among individuals with FASD (Popova et al., 2016b).

Simultaneously with efforts to ascertain the effects of PAE, the scientific community has made concerted efforts to raise community awareness of the harmful consequences of alcohol exposure during pregnancy. Despite these efforts and statements from the United States Surgeon Generals recommending that pregnant women or women who may become pregnant abstain from alcohol, 1 in 10 pregnant women reported alcohol consumption within the past 30 days (Health and Services, 1981, Health and Services, 2005, Tan et al., 2015b). Studies have estimated approximately 30-40% of pregnancies in the United States are affected by alcohol consumption (Ethen et al., 2009b). Accurately assessing FASD prevalence is difficult, due in part to the subtler effects of *in utero* alcohol exposure, missed diagnoses, misdiagnoses, and incomplete or inaccurate maternal self-reported histories as a result of the societal stigma accompanying alcohol use in pregnancy. Previous studies indicated

FASD prevalence in the United States was about 2-5% (May et al., 2009, May et al., 2014), however, the most recent estimates may be as high as 3-9% (May et al., 2018b).

The congenital defects resulting from PAE can be costly, as affected individuals often require lifelong, multifaceted services to meet their needs. The annual financial cost per individual with FASD has been estimated to be approximately $24,300 for adults and $22,800 for children, with an estimated lifetime cost of $1.4 million per individual and an annual societal cost exceeding $4 billion (Greenmyer et al., 2018, Lupton et al., 2004). These costs do not include any approved pharmacologic therapy, as there currently are none. At present, the only approved existing treatments focus on the secondary effects of PAE, mainly symptoms such as cognitive deficits, attention deficit hyperactivity disorder (ADHD), depression, anxiety, and language delays (Spohr and Steinhausen, 2008b, Wilhoit et al., 2017). Targets of PAE and its pharmacokinetics are complex in nature, and thus to date, the molecular mechanisms underlying FASD pathogenesis remain insufficiently understood (Burd, 2016b). Despite the limited understanding, researchers from around the globe have continued to investigate the teratogenic effects of alcohol and uncover the etiological mechanisms underlying developmental alcohol-mediated damage. With successful completion of each FASD investigation, novel facets of the disease are being discovered, and each scientific contribution advances our understanding of PAE's teratogenic mechanisms of action on distinct organ systems, most notably of which is the fetal brain.

Prenatal Alcohol Exposure and the Brain

The developing brain is one of the most, well-studied targets of gestational alcohol exposure. Out of all of the developing organs, studies collectively designate the fetal brain as the target most susceptible to the teratogenic effects of alcohol exposure (Caputo et al., 2016a). As a result, alcohol-induced damage to the developing brain has been extensively documented. The following synopsis provides a small glimpse of what has been ascertained thus far with respect to alcohol-mediated pathogenesis of deficits in the developing brain in both human and animal model studies.

Clinical and Human Studies

Human autopsy studies of children heavily exposed to alcohol *in utero* have revealed reduced brain volumes and abnormal brain development (Jones and Smith, 1973). Magnetic resonance imaging (MRI) studies of individuals affected by PAE have revealed similar abnormalities, with additional findings. With regards to brain structure, two consistent observations were: 1) reduced whole-brain volume and 2) reduced grey and white matter volumes across the cerebral cortex (Archibald et al., 2001, Nardelli et al., 2011, Donald et al., 2015). Alterations to the corpus callosum have been consistently described across neurodevelopmentally-centered FASD studies, with reports of alcohol-induced alterations to both the shape and area of this structure to varying extents (Yang et al., 2012, Riikonen et al., 1999, Riley et al., 1995). Analogously, hippocampi of children exposed to alcohol *in utero* exhibit reductions in volume (Nardelli et al., 2011, Willoughby et al., 2008b, Archibald et al., 2001). Functional MRI studies have been

pivotal in revealing subtle changes in brain activity in multiple domains, the most widely investigated being working memory (Norman et al., 2013, Spadoni et al., 2009, Malisza et al., 2005, Diwadkar et al., 2013). Activated brain areas were noted to be dissimilar between unexposed control and PAE groups, and alterations to blood flow regulation were also noted (Spadoni et al., 2009, Malisza et al., 2005). Additional MRI-based PAE studies have detected white matter microstructure abnormalities of the corpus callosum, which indicate a disruption of inter-hemispheric functional connectivity (Wozniak et al., 2009, Wozniak et al., 2011). Children exposed to alcohol *in utero* also exhibited increased characteristic path lengths and lower global efficiency, further substantiating the vulnerability of the developing brain's neural circuitry to PAE (Wozniak et al., 2013). Although the clinical relevance of the observations from Wozniak and colleagues were not completely understood, alcohol's effects on the cerebral network were inferred to have negative functional implications on conscious/subconscious operations. More recently, alcohol's effects on the brain's neurochemistry across different regions have been made apparent by use of proton magnetic resonance spectroscopy (^{1}H-MRS), which detected alterations in abundances of *N*-acetyl aspartate, glutamine, glutamate, and choline-containing and creatine-containing neurometabolites (Astley et al., 2009, Cortese et al., 2006, Fagerlund et al., 2006, du Plessis et al., 2014). The observations from different research groups gave emphasis to the gestational period as a critical developmental window for investigating neurometabolic changes due to their role in brain development (Bluml et al., 2013, Donald et al., 2015).

Non-Human Primates

In non-human primate studies, the striatal dopamine system has been shown to be another vulnerable target of alcohol. PAE beginning in early gestation and persisting throughout the remainder of pregnancy induced a decreased ratio of dopamine (D2) receptors to dopamine levels in the striatum of young adult moneys (Schneider et al., 2005). Alcohol exposure limited to late gestation alone resulted in an increased ratio of D2 receptors to dopamine levels. A follow up study identified an effect of sex, where dopamine (D1) receptor binding was increased in males exposed to alcohol *in utero* (Converse et al., 2014). This disruption of receptor/dopamine ratios was believed to be a major contributing factor towards the alterations of cognitive function and behavioral response seen in subjects of PAE (Schneider et al., 2005, Arnsten, 1997, Berridge and Robinson, 1998, Redgrave et al., 1999). In baboons, an increase in fetal cerebral blood flow was documented following gestational alcohol exposure (Kochunov et al., 2010). However, other studies utilizing a primate model to measure hemodynamics in fetal anterior and middle cerebral arteries reported alcohol-induced decreases in peak systolic velocity (Seleverstov et al., 2017, Tobiasz et al., 2018). Moreover, acceleration and time-velocity integral were decreased in the alcohol-exposed group, suggesting a suppressive effect on the function of fetal brain vasculature.

Ovine

Sheep is an another clinically relevant model for understanding alcohol's pathogenesis on the developing brain, based on their ability to account for maternal/fetal

interactions during all three trimester-equivalents of brain development, and because maternal weight and fetal weight correlate with those of humans, and the ovine gestational length is relatively long (approximately 147 days), and therefore more comparable to humans than those of smaller animal models. Ovine model studies have revealed alcohol exposure *in utero* modulates fetal cerebral blood flow activity pre- and postnatally in response to environmental insults such as hypoxia (Mayock et al., 2007, Gleason et al., 1997). Furthermore, alcohol exposure during the first trimester alone decreased levels of vasoactive intestinal peptide (VIP), a potent vasodilator, in neurons of the fetal brain (Anderson et al., 2008). This alcohol-induced deficiency of the VIP vasodilator in cerebral neurons has been linked with having a substantial role in the chronic attenuation of fetal cerebral blood flow in response to hypoxic conditions. Another ovine model study investigating the persistent effects of alcohol exposure on the fetal brain reported an increase in the reactivity of intracerebral arterioles to VIP during adulthood of the alcohol-exposed offspring (Ngai et al., 2008). Alcohol exposure limited to the third trimester equivalent of brain development specifically targeted and increased blood flow in the cerebellum, and was accompanied by a loss of Purkinje cell neurons within this brain region (Parnell et al., 2007). Increases in arterial stiffness was another alcohol-induced cerebral vascular adaptation that has been reported in this model as a response to PAE (Parkington et al., 2014). The elastic modulus in these vessels increased and was linked with arterial wall compositional changes. In cortical white matter of the fetal brain, gestational alcohol exposure increased the size of microglia-macrophage-rich

regions and this change was associated with increased cortical white matter vulnerability (Watari et al., 2006).

Rodent

Rodent models are widely utilized in the FASD field, as their brain and behavioral patterns are extensively characterized. Studies on rat brain morphology following PAE noted reductions in total brain volume, in alignment with similar reports of brain volume reductions in children with PAE (Zimmerberg and Reuter, 1989). Additionally, motor cortex neuron generation was delayed and defects in neuronal migration were observed (Miller, 1986). In rat pup whole brains, alcohol exposure induced degradation of radial glial fibers and accelerated the astrocyte transformation process from radial glia (Miller and Robertson, 1993). In the cerebellum, Bergmann glia development, which is critical process for supporting proper granule cell migration, was impeded by gestational alcohol exposure (Shetty and Phillips, 1992). Purkinje and granule cells within the developing rat cerebellum were also severely affected by alcohol exposure during development, with exquisite vulnerability observed during the third trimester-equivalent of brain development (West et al., 1990). Of the two types of aforementioned neurons, Purkinje cells were the most susceptible to the teratogenic effects of alcohol, resulting in greater cell death and significant cell loss in comparison with the cerebellar granule cell population. Interestingly, the distinct lobes comprising the cerebellum have exhibited varying windows of vulnerability during development to alcohol exposure (West et al., 1990). Lobes I, II, IX, and X were among the most

vulnerable during postnatal days (GD) 4-9, with West and colleagues suggesting it may due to the timing of the insult taking place during a particular window of neuronal maturation (Sawant et al., 2013, West et al., 1990). With regards to brain vasculature, PAE impaired cerebral arteriole response to eNOS and nNOS-dependent agonists and was suspected to result in dysregulated oxygenation and nutrition (Bukiya and Dopico, 2018, Cananzi and Mayhan, 2019).

Prenatal Alcohol Exposure and Behavior

Behavioral deficits comprise a cardinal feature of FASD, and human and animal studies on alcohol exposure *in utero* have observed a myriad of alcohol-related behavioral deficits across various domains (Mattson et al., 2019). Cognitive abilities are the most commonly reported alcohol-induced behavioral impairment, with vitiated social behavior unmistakably becoming an increasingly prevalent sequelae of developmental alcohol exposure (Steinhausen, 1996). The effects of PAE on behavior are lifelong and diverse manifestations have been observed from infancy through adulthood in human clinical studies and in animal models (O'Connor et al., 1992, O'Connor, 1996, Goldschmidt et al., 1996, Streissguth et al., 1991). Below is a brief review describing the variety of behavioral deficits that have been reported in offspring following alcohol exposure during development.

Early infancy

In one of the first clinical studies which assessed the effects of alcohol on behavior, infants exposed to PAE expressed alterations in their ability to form maternal attachment, attachment being a component of a social behavior system between mother and infant (Bowlby, 1969). Impaired responses were also observed in a subsequent study, whereupon after separation from their mothers, infants previously exposed to alcohol *in utero* displayed heightened responses of conflicted and disoriented behavior (O'Connor et al., 1992). Infants exposed to alcohol prenatally were likewise noted to express increased levels of irritability, which has been shown to negatively affect attachment behavior (Coles and Platzman, 1993, Meares et al., 1982). Alcohol-induced impairment of feeding pattern have been observed, with exposed newborns having altered nursing behaviors (Sundelin-Wahlsten et al., 2017, Ouellette et al., 1977, Martin et al., 1979).

Animal models of PAE assessing neonatal behaviors report comparable early life behavioral aberrations. In a PAE study that utilized macaques, neonates exposed to alcohol *in utero* expressed aberrant hyperactive behaviors, and concurrently exhibited symptomatic tremors (Elton and Wilson, 1977). A similar study assessed cognitive function in macaque infants prenatally exposed to alcohol. These neonates were observed to have reduced visual orientation ability and attention capacity (Schneider et al., 1997). Rat neonates exposed to alcohol *in utero* had impaired suckling behavior when compared with controls. At birth, these alcohol-exposed pups took significantly longer time to adequately latch to the dam’s nipple (Chen et al., 1982). Another alcohol-

induced behavioral alteration previously reported in rat neonates is timing delay of vocalization in response to a physical separation from their respective dams (Barron et al., 2000).

Periadolescence

Human studies concentrating on the periadolescent period have yielded further insights into alcohol-mediated behavioral deficits. Studies have reported children prenatally exposed to alcohol were more inclined to develop academic difficulties in school, with a higher probability of having lower standardized test scores in fundamental subjects, such as arithmetic and reading (Glass et al., 2017, Howell et al., 2006, Goldschmidt et al., 1996). Children affected by alcohol *in utero* have been observed to have difficulties in interacting with others in social situations and display patent deficits in interpersonal skills (Thomas et al., 1998, Rasmussen et al., 2011, Whaley et al., 2001). Moreover, children known to be prenatally exposed to alcohol *in utero* were rated by their teachers to be more aggressive and destructive compared to their classmates (Brown et al., 1991). Impulsivity and distractibility have been widely reported in young adolescent children with FAS, with about 60% having problems with the law (Furtado and Roriz, 2016, Streissguth et al., 1996, Rasmussen and Wyper, 2007).

In periadolescent rats prenatally exposed to alcohol, alterations in affiliative behaviors have been observed, such as the play and/or parental subdomains (Boschen et al., 2014). In one study, alcohol-exposed males did not display as much playful behavior as their control male counterparts (Meyer and Riley, 1986). Moreover, females within

the alcohol treatment group demonstrated increases in play-related behaviors compared to those females in the control group. Conventionally, males display a higher frequency of playful behavior than do females (Kelly et al., 2000). With regards to induced parental behavior, juvenile male and female rats exposed to alcohol during early development had an increased latency of pup retrieval compared to the control male and female groups after being presented with rat pups (Barron and Riley, 1985, Wilson et al., 1996).

Late adolescence and adulthood

FASD behavioral deficits have been shown to persist beyond childhood. A study of adults and adolescents near adulthood exposed to alcohol *in utero* reported multiple deficits in social behavior (Streissguth et al., 1991). Affected individuals failed to adequately take into consideration the potential consequences of their actions. The inability to recognize and properly react to social cues and reciprocate friendship were noted characteristics of young adults who were exposed to alcohol *in utero* (Rangmar et al., 2015, Streissguth et al., 1991). Social withdrawal, high anxiety, and delinquent behaviors were also observed in exposed individuals. Reports on adults with histories of *in utero* alcohol exposure noted findings similar to those of children during periadolescence with respect to trouble with the law (Streissguth et al., 1991).

Animal models have corroborated that the behavioral-related effects of developmental alcohol exposure persist into adulthood. In adult rats prenatally exposed alcohol, an exacerbation of aggressive behavior has been observed, with exposed animals expressing an increase in the frequency of attacking, biting, and/or displaying

more aggressive postures in contrast to controls (Royalty, 1990, Hamilton et al., 2014). Another rodent model study observed impairment of social recognition behaviors in male and female rats exposed during the third trimester-equivalent (Kelly and Tran, 1997). Two studies demonstrated feminization of adult male rat sexual behavior as a consequence of PAE (Dahlgren et al., 1991, Hard et al., 1984). PAE in female rats has been shown to delay behavioral estrus and the timing of vaginal opening, indicating a disruption by alcohol in pubertal maturation processes (Creighton-Taylor and Rudeen, 1991, McGivern et al., 1992, Lan et al., 2009). The long-term effects of PAE were likewise manifested in adult female rats by their display of deficits in maternal instinct behaviors (Barron and Riley, 1985, Hard et al., 1985). Aberrant behaviors of these prenatally exposed dams included building nests of comparatively reduced quality and noticeable pup negligence, with extra preference given to eating, drinking, and grooming themselves.

Utilization of Animal Models for FASD Etiology

When alcohol exposure was first linked with congenital birth defects in the late 1960s and early 1970s, many individuals in the scientific community were skeptical of this association (Cudd, 2005). The predominant thought was that alcohol had been consumed for millennia, and with congenital defects only recently linked to alcohol, the shared belief was that these defects were likely caused by an alternative environmental insult. Contrary to this initial skepticism, the use of animal models in gestational alcohol studies proved to be vital in providing irrefutable evidence that substantiated alcohol's

teratogenic nature (Uban et al., 2011). Further investigative use of animal models revealed an extensive array of malignant consequences due to PAE and helped to identify specific sites targeted by alcohol during fetal development. As human studies were and remain limited due to ethical concerns, the importance of these findings gleaned from animal models were critical for discerning the teratogenic effects seen in individuals exposed to alcohol *in utero*. Presently, animal models are used to identity and further investigate mechanisms of alcohol-mediated damage in various target organs of PAE; development of protective and/or ameliorating strategies are also being strategically investigated by means of animal studies.

Experiments that utilize animal models allow for controlled experimental conditions and offer manifold advantages (Cudd, 2005). Human PAE studies are limited by a plethora of confounding variables, such as exposure timing, dose, and pattern, which can inconsistently influence the outcomes observed in each individual affected by alcohol *in utero*. Furthermore, the unreliability of maternal self-reports and co-environmental insults that may accompany alcohol use during pregnancy make it difficult to discern which outcomes observed are definitively alcohol-derived. Additional confounding variables that complicate human studies include divergence in plane of nutrition and genetics. Another advantage of animal models are their capacity to investigate additional dependent variables. However, it is critical to understand the differences of each species as they compare to humans. Investigators must take into account the dissimilarities, plan a successful design accordingly, and implement their

study designs. This will ensure efficacy in using animal models to accurately assess the teratogenic effects of PAE.

Accounting for species-specific differences is of paramount importance when investigating alcohol's effects on the developing brain. This in part is due to each species' varying windows of accelerated brain growth and development during gestation (Dobbing and Sands, 1979). Structures developing during the brain's growth spurt exhibit greater vulnerability to alcohol's teratogenic effects compared to other time points in gestation (Goodlett et al., 1990). Consequently, consideration of the following factors is essential when selecting a species model for evaluating alcohol's harmful effects: developmental timing of the structures of interest (i.e. hippocampus, cerebellum, olfactory bulbs, etc.), the window of development during which to administer alcohol, and period of development during which to collect dependent measurements (Cudd, 2005). A common example of species differences is the 'brain growth spurt' timing divergence between humans and rats. In humans, this period of accelerated development occurs during the third trimester of gestation and reaches peak velocity at parturition, whereas in rats, the maximum brain growth velocity takes place postnatally, on postnatal day(s) (PND) 1-10 (Dobbing and Sands, 1979). Therefore, the use of rat models to investigate the negative effects of PAE on brain structures during this exquisitely sensitive developmental window occurs postnatally during the 'third trimester-equivalent' of brain development, to correspond appropriately with the third trimester in humans. In other animal models, such as non-human primates and guinea pigs, accelerated brain development takes place earlier in gestation compared to humans,

highlighting the importance of careful planning in order to suitably investigate the effects of PAE on the brain and properly extrapolate findings from animal models and infer these findings to humans (Dobbing and Sands, 1979). The following are brief abstracts of the advantages of various animal models.

Rodent models

The rat has been a widely used animal model for the study of FASD. A popular example is the use of rat models to explore the effects of PAE on the cerebellum during its most profound period of growth (PND 1-10) that corresponds with the third trimester of human pregnancy (West et al., 1990). The sheer volume of knowledge known on the animal's physiology, anatomy, reproduction, and teratology makes it an optimal model for strategically designing studies that focus their investigation on a particular region of interest. This vast knowledge base is a considerable advantage of utilizing this model in an alcohol study. Moreover, rats are a well-suited animal model for FASD behavioral studies because of their simplified and extensively documented behaviors compared to other models and the existing established measures of these behaviors for both rats and humans (Kelly et al., 2009a). Eye-blink conditioning learning measures are frequently used to assess associative learning, whereas the Morris water maze can effectually evaluate spacial learning and memory (Stanton and Goodlett, 1998, Vorhees and Williams, 2006). Furthermore, dam interactions with their offspring litters can model some human maternal behaviors (Wilson and Cudd, 2011). Metabolic studies in rats can also be used to identify biomarkers that form as metabolites resulting from

developmental alcohol exposure (Laposata et al., 2000). Economically, rats are relatively inexpensive to house and properly maintain, with less labor required for their upkeep compared to the larger mammalian animal models.

The mouse is another useful model for the study of FASD. Similar to the rat, the mouse model has multiple advantages that should be taken into consideration when designing FASD experiments. The extensive literature regarding the many domains studied, including physiology and reproductive teratology, allow for investigations that provide further insights into the effects of alcohol *in utero*. With regards to cost, mice are less costly to acquire and house than most other animals, i.e. the sheep model, and have a shorter gestational period, making data acquisition timely and efficient. The genetic manipulation potential in mice is another added benefit, as there are multiple strains available for use, depending on the experimental needs and focus of a particular project. Mice are among the limited number of animal models that display the characteristic facial dysmorphology seen in fetal alcohol syndrome (FAS), making it possible to study this alcohol-induced phenotype (Sulik et al., 1981). Similar to the rat, mice are economically and environmentally friendly to house and maintain.

The guinea pig offers different advantages when used as a model for the study of FASD. The gestational period of the guinea pig is notably longer compared to the rat and mouse, presenting the advantage of being able to study more precisely different time periods in fetal development. One notable aspect is the occurrence of the brain's growth spurt, which happens *in utero* in contrast to rats and mice, allowing for third trimester-equivalent brain studies to take into account maternal and fetal interactions at the

placental interface. Biomarkers of prenatal alcohol exposure can also be reliably investigated, as the guinea pig model has successfully demonstrated the use of fatty acid ethyl ester detection for this purpose (Caprara et al., 2005). Growth restriction in the brain and total body weight coinciding with hyperactive offspring and deficits in the cerebral cortex, hippocampus, and cerebellum all demonstrate that the guinea pig is a useful model for alcohol studies, as this is frequently observed in clinical studies of children exposed to alcohol *in utero* (Cudd, 2005). Similar to rats and mice, guinea pigs are also very economical.

Porcine model

The pig is a larger mammalian animal model that has also successfully been used to study FASD. Similar to that which occurs in humans, the peak brain growth spurt in pigs occur at parturition. Another incentive for using the porcine model is that the pig brain is gyrencephalic, with structure and function resemblant to human brains, making this an ideal candidate model for neuroimaging studies. Complete atlases that detail the brain activation regions are another advantage when studying the effects of alcohol on the developing brain (Saikali et al., 2010, Watanabe et al., 2001). One noteworthy benefit of utilizing this species is its unique trait of having a predilection for alcohol, unlike any other animal model. The pig model has been successfully used to replicate the characteristic traits of FAS such as growth restriction, microcephaly, and cleft palate (Dexter et al., 1976). While the number of behavioral studies conducted using this model is relatively small, pigs have been efficaciously used to investigate alcohol-mediated

effects on offspring behavior (Backus, 2013). Studies assessing the effects of alcohol on maternal and fetal cell structure in various tissue targets have also used the pig model (Romert and Matthiessen, 1984).

Ovine model

The sheep model has also been widely used to study FASD. Due to the extensive amount of work done employing this model to characterize fetal physiology for over 50 years, established, long-term, instrumentation techniques can be implemented in this model to investigate the myriad effects of gestational alcohol exposure. The distinctive advantage of long-term instrumentation in fetal sheep allows for a wide range in depth studies to assess the following: brain metabolism, alcohol disposition in maternal and fetal compartment, fetal brain activity, fetal cerebral blood flow, fetal brain neurotransmitter activity, fetal hypothalamus pituitary adrenal axis function, and maternal and fetal thyroid hormone responses following prenatal alcohol exposure (Richardson et al., 1985, Brien et al., 1985, Patrick et al., 1985, Gleason and Hotchkiss, 1992, Reynolds et al., 1995, Cudd et al., 2001b, Cudd et al., 2002). Levels of carbon dioxide, blood pH, and lactate can also be detected in real time in developing fetuses in response to alcohol (Cudd et al., 2001a, Bocking et al., 1993). The substantially longer gestation period in sheep (147 days) also benefits studies that aim to address temporal vulnerabilities and investigate mechanisms of damage across different organ systems, and this gestational period is more comparable to humans than those of many other animal models. Nutrient availability following alcohol exposure, such as maternal and

fetal plasma amino acid levels, can be investigated in sheep, allowing insight into how alcohol exposure during gestation affects the maternal compartment and how this in turn may have an effect on micro and macro nutrient bioavailability in the developing fetus (Ramadoss et al., 2008).

Non-Human Primate Models

The non-human primate model offers distinct advantages for assessing alcohol-mediated teratogenic outcomes due to the animals' high intelligence, analogous human-like behavior, and high degree of overlapping homologous genes in comparison to humans. Non-human primate models are ideal for conducting cognitive function studies, as they are exceedingly effective for investigating various domains of memory and learning (Harlow, 1959, Gunderson et al., 1989). Attention deficits, hyperactivity, and temperamental responses following alcohol exposure can also be studied and are well-documented (Elton and Wilson, 1977, Clarren et al., 1992, Schneider and Suomi, 1992). Since non-human primates have comparable gestation periods and undergo rapid brain growth *in utero,* FASD studies exploiting this model can be directed at mechanisms altered by alcohol exposure during this particular window of development. Furthermore, the generation interval of non-human primates is truncated, facilitating longitudinal studies of the effects from PAE (Schneider et al., 2002).

Genomic Technology

Alcohol exposure has been observed to have an effect on the expression of various genes in the developing brain by means of transcriptome analysis. The technology used to assess alterations in gene expression between treatment and control groups has been critical for understanding the exquisite vulnerability of the developing brain and consequently for the advancement of the FASD field. Below is a brief summation of some of the most widely-used genomic methods.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

RT-qPCR is among the earliest methods used to assess gene expressions in a sample. One advantage in utilizing RT-qPCR is its sensitivity, allowing the quantification of target genes that are low in number (Fey et al., 2004). Furthermore, it is widely regarded as the most accurate of all methods that quantify gene expression levels (Gilliland et al., 1990, Wang and Brown, 1999). RT-qPCR is relatively inexpensive compared to other methodologies, such as microarray and the NanoString nCounter system. One disadvantage is its inherent limited range of transcript detection. Moreover, RT-qPCR utilizes hybridization, which presents variability that must be accounted for in the samples ran. Lastly, the use of amplification is another disadvantage, as it introduces bias (Hurd and Nelson, 2009).

Microarray

Microarray is a high-throughput technique used in studies to observe alterations in gene expression. It has the advantage of being able to measure a plethora of gene expression levels simultaneously, making it an unequivocally more powerful tool than RT-qPCR (Narrandes and Xu, 2018). Another advantage of microarray is its high reproducibility and well-defined analysis guidelines. Furthermore, there is the convenience of having ready-to-use commercial 'chips,' which can be customized according to the particular bent of a study, reducing the amount of time and labor required to run samples. However, there are some disadvantages to using microarray, which are important for consideration when using this technology. The variability that can be present in sources, such as dye labeling and hybridization, is one downside (Jaluria et al., 2007). Furthermore, cross-hybridization between similar sequences complicates the analysis of related genes. The use of amplification is another disadvantage as it introduces bias into samples (Hurd and Nelson, 2009). Microarrays also have difficulty detecting low transcript levels and splice variants. Lastly, their reliance on an established genome sequence makes microarray unfit to detect novel transcripts in target tissue.

NanoString nCounter System

NanoString nCounter System (NanoString) is a method that relies on the direct imaging of tripartite structures that consist of color-coded probe pairs that are hybridized with specific target mRNAs. NanoString has multiple advantages when assessing gene

dysregulation, such as the elimination of amplification and cloning. Furthermore, it has a large transcript detection range, which is notably larger than microarrays. Another advantage is its accuracy in detecting differentially expressed genes, which was demonstrated to be more accurate than microarray (Geiss et al., 2008). Lastly, NanoString's digital readout reduces the amount of background signal, making analysis less ambiguous. There are some disadvantages to consider when utilizing NanoString technology (Chatterjee et al., 2015). First, detection is limited to pre-existing screening panels, which also hinders the discovery of novel RNA molecules. Second, this method may become prohibitively expensive, especially when running a relatively large number of samples.

RNA sequencing (RNA-seq)

The development of high-throughput sequencing resulted in novel way to map and quantify transcriptomes. Commonly known as 'RNA-seq,' it utilizes deep-sequencing technology that sequences a genomic region multiple times and offers many advantages over earlier generation transcriptomic approaches (Wang et al., 2009b). First, in contrast to hybridization-based methods, RNA-seq can detect novel transcripts without needing an existing genomic sequence for reference. When compared to microarrays, RNA-seq has low, if any, background levels to take into account upon analysis. Furthermore, this technology has a considerably more expansive transcript detection range than microarrays or Nanostring. RNA-seq is robust in its capacity to accurately quantify gene expression levels, which has been recurrently validated by

quantitative PCR (qPCR) (Nagalakshmi et al., 2008). Lastly, only a small amount of RNA sample is required to undergo a sequencing run. As with other genomic technologies, there are some disadvantages worth considering when using RNA-seq. First, unlike smaller RNA molecules, such as microRNA, larger RNA molecules must be fragmented in order to be sequenced, which creates bias in the results. Secondly, library construction may be laborious and time-consuming.

Genomic technology in FASD brain-related studies

The technologies available for detecting alterations in the transcriptome have been used in various target tissues with great success. In alcohol studies, the brain has been investigated by using various approaches. From older, targeted methods to newer high-throughput methods, the results have helped to increase our understanding of the mechanisms of alcohol-mediated neuropathogenesis by way of pinpointing differentially expressed genes. The following summaries provide a brief review of the work done in alcohol-induced transcriptomic alterations in the brain using two different methods: targeted and high-throughput.

Gene expression outcomes from targeted approaches

Utilizing targeted approaches, PAE has been observed to have an effect on the expression of certain genes in either whole or specific regions of the developing brain, with long-term alterations also being noted. Hard and colleagues noted six alcohol-induced downregulated genes in their study of fetal mice whole brains: Tissue Inhibitor

of Metalloproteinase 4 (*Timp4),* Bone morphogenic protein 15 (*Bmp15),* Ring finger protein 25 (*Rnf25),* RAC-alpha serine/threonine-protein kinase *(Akt1),* Tubby-related protein 4 *(Tulp4),* and (Dexamethasone-induced Ras-related protein 1) *Dexras1* (Hard et al., 2005). *Timp4* is known to regulate the degradation of the extracellular matrix and may also mediate hormone signaling and tissue remodeling, but its specific role in brain development is not currently known (Leco et al., 1997). *Bmp15* encodes for a paracrine signaling molecule involved in regulating cellular differentiation and apoptosis, but currently has no known significance in the developing fetal brain. *Rnf25* facilitates post-translational modification most notably via E2-dependent ubiquination, however modulation of NF-κB-mediated transcription activity has been observed (Lorick et al., 1999). *Akt1* is well documented in its role mediating signal transduction, that affects cell growth and survival, particularly in the context of neurons (Lawlor and Alessi, 2001). *Tulp4* brain function is not known, but may be involved in mediating ubiquination and subsequent proteasomal degradation. *Dexras1* is widely expressed in many tissue types, and has been linked with several functions. Notably, it is induced in the brain in response to various types of stress (i.e. glucocorticoid or osmotic), and expression is suspected to modulate cellular proliferation and suppression of aberrant cell growth; its function has also been implicated in playing a role in nitric oxide signaling (Fang et al., 2000). In pooled fetal rat whole brains, Cellular retinol-binding protein-1 (*Crbp)* and Retinoic acid receptor (*Rar)* expression was affected by alcohol exposure (Grummer and Zachman, 1995). *Crbp* is involved in transportation and delivery of vitamin A by means of its encoded protein; in non-neural tissue, it has a role in maintenance of cellular

differentiative states (Folli et al., 2001). *Rar* has been critically implicated in differentiation and development and cellular matrix homeostasis (Collins, 2002). In one study, alcohol exposure during gestation dysregulated the expression of Solute carrier family 6 member 4 (*Slc6a4)* in male and female fetal mice hippocampi and hypothalami (Ngai et al., 2015). *Slc6a4* encodes for serotonin transporters that regulate serotonergic signaling, and dysregulation of this gene has been linked with anxiety and obsessive compulsive disorders (Lesch, 2011). In pooled fetal rat forebrains, Neuroblastoma apoptosis-related RNA-binding protein 3 (*Napor3)* expression was downregulated in the ethanol treatment group (Naha et al., 2009). *Napor3* is reported to affect the regulation of RNA processing events within the cell nucleus and cytoplasm (Choi et al., 1998, Zhang et al., 2002, Ladd and Cooper, 2004). It is also involved in central nervous system development (Oppenheim, 1991).

In the developing whole brain of rat pups, expression of Glial fibrillary acidic protein (*Gfap)* was altered by alcohol exposure (Valles et al., 1997). *Gfap* encodes for a protein that provides structural stability for astrocytes and is involved in synaptic plasticity, neurite outgrowth, and neuronal migration. (Weinstein et al., 1991, Chen and Liem, 1994, Middeldorp and Hol, 2011). In the developing neocortex of neonatal mouse pups, the expression of three genes were altered: Retinoid Z receptor beta (*Rzrβ)*, Cadherin 8 (*Cdh8),* and Inhibitor of DNA binding 2 (*Id2)*. These genes are involved in the regulation of circadian rhythm, synaptic adhesion, and inhibition of transcription factor function, respectively (Greiner et al., 1996, Suzuki et al., 1991, Ward et al., 2010). One study found gene alterations in various brain regions of rat pups and adolescents

exposed to alcohol *in utero* (Wille-Bille et al., 2018). In the rat pups, Prodynorphin (*Pdyn)* expression was altered in the brain's ventral tegmental area and nucleus accumbens. In the adolescent rats, *Pdyn* expression was decreased in the nucleus accumbens. kappa opioid receptor (*Kor)* was altered in the prefrontal cortex of neonates and in the ventral tegmental area in adolescents. *Pdyn* encodes a precursor protein that is eventually processed to form endogenous opioids (Cox, 1982). *Kor* encodes an opioid receptor for endogenous ligands (Yuferov et al., 2004). A study in the developing male rat hippocampus following third trimester-equivalent alcohol exposure observed an increase in the expression of Brain-derived neurotrophic factor (*Bdnf)* (Boschen et al., 2015). *Bdnf* is a member of the canonical neural growth factors, which promote neuron survival and development (Binder and Scharfman, 2004). Li and colleagues observed altered Gamma-aminobutyric acid beta receptor 1 (*Gabbr1)* expression in rat pup forebrains following PAE (Li et al., 2005). *Gabbr1* is implicated in calcium concentration modulation in cells and various modes of synaptic inhibition (Kaupmann et al., 1998, Obrietan and van den Pol, 1998). One study observed an altered expression of Complexin-1 (*Cplx1)* and Complexin-2 (*Cplx2)* in various developing brain regions of rat pups. The hippocampus had a reduced *Cplx2* expression. The anterior cingulate cortex, prefrontal cortex, frontoparietal cortex, caudate nucleus and putamen all had a reduced *Cplx2* expression. *Cplx1* and *Cplx2* encode proteins that play a role in the release of transmitters from neurons via exocytosis of synaptic vesicles (Brose, 2008).

In juvenile rat cerebella exposed to alcohol *in utero*, a reduced expression of Wingless-type MMTV integration site family, member 5A (*Wnt5a)*, Frizzled class

receptor 6 *(Fzd6)*, DIX domain containing 1 *(Dixdc1)*, and Axis inhibition protein 2 *(Axin2)* was observed (Tong et al., 2013). *Wnt5a* encodes for signaling proteins that are critical in the regulation of development (Zhou et al., 2017). *Fzd6* encodes for a protein subunit in a membrane receptor that is involved in cell migration (Zou et al., 2017). *Dixdc1* encodes for proteins that positively regulate the Wnt signaling pathway (Shiomi et al., 2003). *Axin2* is involved in the negative regulation of the Wnt signaling pathway (Mai et al., 1999).

A study of adult male mice exposed to alcohol *in utero* were reported to have an increased expression of Solute carrier 17 family A6 (*Slc17a6)* in the hippocampus (Zhang et al., 2015). Similarly, the increased expression *Slc17a6i* was also noted in a latter study (Zhang and Chong, 2016). *Slc17a6* mediates uptake of glutamate in presynaptic neurons (Schweizer et al., 2014). In another study, gene expression of Dual Specificity Phosphatase 6 *(Dusp6)* and Adaptor related protein complex 1 subunit sigma 2 (*Ap1s2)* were upregulated in the prefrontal cortex of adult female rats exposed to alcohol *in utero*, with Regulator Of G Protein Signaling 13 (*Rgs13)* observed to be upregulated in the hippocampus (Lussier et al., 2015a). *Dusp6* has been associated with bipolar disorder and promotes apoptosis (Kim et al., 2012). *Ap1s2* is linked with intellectual impairment and autism spectrum disorder and plays a role in neurodevelopment (Borck et al., 2008). *Rgs3* is involved in glucose output regulation (Raab et al., 2005). Gangisetty and colleagues observed dysregulation of the following genes in adult female rat pituitary glands that were prenatally exposed to alcohol: Dopamine receptor D2 (*D2r)*, DNA (cytosine-5)-methyltransferase 1 *(Dnmt1);* DNA

(cytosine-5-)-methyltransferase 3 beta *(Dnmt3b);* Methyl CpG binding protein 2 *(Mecp2);* Histone deacetylase 2 *(Hdac2);* Histone deacetylase 2 *(Hdac4); Euchromatic histone lysine methyltransferase 2 (G9*a); and SET domain containing lysine methyltransferase 7 *(Set7)* (Gangisetty et al., 2015). *D2r* encodes for the dopamine receptor D2 (Usiello et al., 2000). *Dnmt1, Dnmt3b, Mecp2, Hdac2, Hdac4, G9a,* and *Set7* are a group of genes that essentially regulate post-translational modification via DNA methylation and histone modification (Esteve et al., 2009, Bertos et al., 2001, Hervouet et al., 2018). In the medial frontal cortex of adult mice prenatally exposed to alcohol, Peptidylprolyl isomerase A (*CypA)* was observed to have decreased expression levels in the medial frontal cortex (Caldwell et al., 2008). Within the same study, *18s rRNA,* had increased expression levels in the hippocampal formation. *Bdnf* was dysregulated in the medial frontal cortex and the hippocampal formation. *CypA* and *18s rRNA* are both housekeeping genes. Specifically, *CypA* encodes for proteins that catalyze protein folding (Hoffmann and Schiene-Fischer, 2014). The *18s rRNA* gene encodes for rRNA that make up the small subunit (40s) of eukaryotic ribosomes within the cell (Hayashi, 2014, Henras et al., 2015). As stated previously, *Bdnf* encodes for brain-derived neurotrophic factor, which is a central promoter of neuron survival (Binder and Scharfman, 2004). Adult rats exposed to alcohol *in utero* had six altered gene expressions in three brain regions compared to control animals (Zink et al., 2011). These regions were the hippocampus Vesicular glutamate transporter (*Vglut1),* Excitatory amino acid transporter 1 (*Eaat1),* N-methyl-D-aspartate receptor 2 alpha *(Nr2a),* N-methyl-D-aspartate receptor 2 beta *(Nr2b),* and N-methyl-D-aspartate receptor 2 delta

(Nr2d), anterior cingulate cortex (*Vglut1*), and prefrontal cortex (*Vglut1*, *Eaat1*, and *Nr2c*). *Vglut1* is associated with synaptic vesicle membranes and glutamate transport (Shen et al., 2009). *Eaat1* encodes for an amino acid transporter that plays a role in glutamate removal from the synaptic cleft (Arriza et al., 1994). *Nr2a*, *Nr2b*, *Nr2c*, and *Nr2d* encode for protein subunits in the N-methyl-D-aspartate (NMDA) receptor that contributes to long-term potentiation (Traynelis et al., 2010).

A study on chicken embryo brains following alcohol exposure observed dysregulated gene expressions of Insulin-like growth factor 1 (*Igf1)* and Glucose transporter 1 (*Glut1)* (Tan et al., 2013). *Igf1* mediates neuronal energy utilization and development in the brain (Cheng et al., 2000). *Glut1* plays a major role in glucose transportation and uptake in the brain, which is an essential process for neuronal function (Yu et al., 2008b, Fladeby et al., 2003).

Gene expression outcomes from high-throughput methods

Utilizing high-throughput methods, PAE has been observed to have an effect on the expression of multiple genes in either whole or specific regions of the brain. One study identified over 250 differentially expressed genes in pooled fetal mouse hippocampi (Mandal et al., 2015). Genes of interest altered by PAE were analyzed and observed to be involved in nervous system development [NOVA alternative splicing regulator 1 (*Nova1)*, Netrin-G1 *(Ntng1)*, Galanin *(Gal)*, Neurogenin-2 *(Neurog2)*, Neurogenic differentiation factor 2 *(Neurod2)*, and FEZ Family zinc finger 2 *(Fezf2*)] and calcium signaling pathways [Calcium/Calmodulin Dependent Protein Kinase II

Delta (*Camk2d)*, Epidermal growth factor receptor 3 *(Erbb3)*, Neurotensin receptor type 1 *(Ntsr1)*, and Inositol 1,4,5-trisphosphate receptor type 1 (*Itpr1*)]. The long-term effects of PAE on gene expression were investigated in adult male mouse whole brains, with 163 genes observed to be differentially expressed (Kleiber et al., 2012). Genes implicated in learning, memory, and intellectual disability [Acetylcholinesterase (*Ache)*, B-cell lymphoma 2 *(Bcl2)*, Cullin-4B *(Cul4b)*, *Dyskerin (Dkc1)*, and Somatostatin receptor type *3 (Sstr3*)] that were relevant to FASD had an altered expression. Furthermore, some differentially expressed genes in this study were associated with human psychopathology [Nuclear receptor binding SET domain protein 1 (*Nsdh1)*, Wilms tumor protein 1 *(Wt1)*, and Pituitary homeobox 3 *(Pitx3*)]. Another study assessed the effects of PAE on fetal and adult male mouse whole brains (Mantha et al., 2014). They observed a total of 116 genes that were altered. In fetal whole brains, altered genes of interest [B Cell Receptor Associated Protein 31 *(Bcap31)*, Hippocalcin *(Hpca)*, Thymine-DNA glycosylase *(Tdg)*, MicroRNA 99a *(mir-99)*, Zinc Finger CCCH-Type Containing 18 *(Zc3h18)*, cAMP responsive element modulator *(Crem)*, MicroRNA *379 (mir-379)*, Leukocyte Associated Immunoglobulin Like Receptor 1 *(Lair1)*, Junction Mediating And Regulatory Protein *(Jmy)*, C-C Motif Chemokine Ligand 3 *(Ccl3)*, Neuromedin-B receptor *(Nmbr)*, and RING1 and YY1 binding protein (*Rybp*)] were implicated in cell death and free radical scavenging pathways. In adult whole brains, altered genes of interest were implicated in neurological disease: Early growth response 3 (*Egr3)*; Polypeptide n-acetylgalactosaminyltransferase 7 (*Galnt7)*; Histone H2B type 1-N (*Hist1h2bn)*; Homer protein homolog 1 (*Homer1)*; X-Box

binding protein 1 (*Xbp1)*; DnaJ heat shock protein family member B11 *(Dnajb11)*; Heat shock protein family A member 5 *(Hspa5)*; Integrin subunit alpha 4 (*Itga4)*; Protein tyrosine phosphatase non-receptor type 22 (*Ptpn22)*; Dynein light chain tctex-type 1 *(Dynlt1)*; Heat shock protein 90, alpha (cytosolic), class A member 1 *(Hsp90aal)*; Tu translation elongation factor, mitochondrial *(Tufm)*; and Prolyl 4-hydroxylase subunit alpha 1 (*P4ha1*).

Gaps in knowledge

To date, despite the genomic studies performed on the developing brain exposed to alcohol *in utero*, much is still unknown regarding the alcohol-induced changes in gene expression and the resulting downstream effects, especially in animal models. Initially, *in vitro* studies investigated the transcriptome of different brain neuron populations exposed to alcohol, revealing alterations in select genes. Although this work has been critical for expanding our understanding of the negative effects of alcohol, cell culture studies do not take into account many physiological factors in a mammalian organism that may yield an altered or completely different outcome. It is therefore crucial to continue using animal models in innovative ways to build upon what has been previously established. This will allow for our understanding of alcohol's teratogenic mechanisms to further develop and result in clinical applications that can aid affected individuals during different stages of life, especially during gestation and early development.

An in-depth search of the literature reveals that a great majority of genomic studies on the alcohol-exposed developing brain utilized older methods (i.e. non-high throughput) such as RT-qPCR. Although RT-qPCR technology is incredibly accurate and has the sensitivity to detect and quantify the target genes that are low in number, it can only be used in targeted mechanistic investigations. Studies utilizing this method are limited by its ability to investigate only a relatively small number of genes at a time. Newer technology that has emerged within the last two decades provides a greater dynamic range while retaining the accuracy of detecting differentially expressed genes. Furthermore, these technologies have continued to develop at a rapid pace, becoming even more robust in terms of the technological innovations during the last decade. Despite these incredible advances, however, there are a limited number of FASD animal model studies that have utilized the available state-of-the-art, high-throughput sequencing technologies, especially to investigate gene expression in the developing brain. Some studies, which have utilized methods such as microarray, have revealed up to hundreds of differentially expressed genes in developing brain alcohol studies. Yet, by current standards, this method is rapidly being regarded as outdated and is now used less frequently, due to its inherent flaws. For example, microarray has a relatively low dynamic range and is known for having an inherent bias due to its hybridization methods. Such flaws are a major drawback that may affect the observed outcome and must be taken into consideration when drawing conclusions.

A large number of genomic studies have assessed gene expression in mature offspring exposed to alcohol *in utero*, a stage in life when additional outcomes such as

learning and behavior can be effectively assessed. These studies performed on the gene expression patterns in the mature brain has provided some insights on the long-term mechanistic consequences of PAE that may be linked to the learning and behavioral deficits seen in adults living with FASD. Gestational alcohol studies have also investigated the differential genome expression in brains of neonatal and adolescent animal models. Data gleaned from these studies have revealed outcomes that may be associated with the learning and behavioral challenges observed in humans during these age-equivalent periods that persist into adulthood. However, these approaches do not inform us of the gene expression alterations that are present in the fetal brain. Consequences of PAE observed during the third trimester-equivalent and in adult animal models are important to investigate, but a re-emphasized focus must be made on the first two trimester equivalents. The effect of PAE on the transcriptome of the developing fetal brain in animal models has been investigated to certain extent, but not to the degree of neonatal and adult brain studies. By understanding how alcohol affects the mechanisms in the brain when the uterus and the placenta are intact, more strategies can be devised to target and ameliorate the damage(s) caused during this period of gestation that often persists into adulthood.

In addition to the limited number of fetal brain FASD studies, less is known regarding the effect of PAE on the transcriptome of the various fetal brain regions. Because each brain region is important in regulating different physiological functions, it is important to further study how the gene expressions in each area are affected by gestational alcohol exposure. It is widely known that each region of the developing brain

is differentially affected by alcohol, but some regions are in particular are more sensitive. The hippocampus, which is associated with learning, memory, and behavior, has been demonstrated to have an exquisite vulnerability to alcohol during development in various animal models. This vulnerability has led FASD investigators to study the genes expressed in this region following PAE. Some of the work done utilized targeted approaches, which resulted in observations of small groups of altered genes at a time. These targeted hippocampal studies were eventually followed up by using early high-throughput methods like microarray, which was able to reveal more than 100 altered genes in this region in response to developmental alcohol exposure. These discoveries provided some insights that increased our understanding of the hippocampal transcriptome alterations that could be initiated by PAE. However, there is an insufficiency of hippocampal FASD studies that use modern high-throughput state-of-the-art technologies. The use of such technology would greatly expand upon what is known, as these methods offer substantial insights into alcohol's effects on the transcriptome.

Consolidation of the knowledge gaps described above reveals the need to further advance our understanding of alcohol-induced dysregulation of the hippocampal transcriptome during early development so that targeted intervention strategies may be effectively applied as soon as possible. By utilizing next generation (next gen) RNA-Seq as a novel means for investigating the multi-mechanistic actions of alcohol on the holistic gene expression of the hippocampus, a more thorough range of differentially expressed genes can be detected. Since alcohol has been shown to affect various aspects

of fetal hippocampal development, it is imperative to further discern how gestational alcohol exposure alters this structure at the level of the transcriptome so that we may understand mechanisms underlying neuropathogenesis and formulate appropriately targeted intervention strategies.

Previously, our FASD model has shown dysregulation of amino acid homeostasis and protein expression in the fetal hippocampus (Lunde-Young et al., 2018, Davis-Anderson et al., 2018b). The purpose of this thesis was to discern the alterations of fetal hippocampal gene expression and their associated pathways following maternal alcohol exposure. We hypothesized that chronic binge gestational alcohol exposure altered fetal hippocampal gene expression and, consequently, their related global canonical pathways.

CHAPTER II

HIPPOCAMPAL TRANSCIRPTOME REVEALS NOVEL TARGETS OF FASD PATHOGENESIS*

Introduction

Fetal Alcohol Spectrum Disorders (FASD) collectively describes an array of physical abnormalities, central nervous system disruptions, and cognitive and behavioral deficits induced by prenatal alcohol exposure (Sokol et al., 2003b, Riley et al., 2011a). In the United States, more than 30% of pregnancies are estimated to be affected by prenatal alcohol exposure (Ethen et al., 2009a), and 1 in 10 pregnant women report alcohol consumption in the past 30 days (Tan et al., 2015a). A recent study estimates that FASD prevalence in the U.S. populations may range from 3% up to 9% (May et al., 2018a). A myriad of factors influence phenotypic severity within FASD, including timing, dose, and duration of exposure, as well as maternal nutrition, genetic susceptibility of both the mother and fetus, and parental history of substance use disorder (Maier and West, 2001a, Smith et al., 2014b, May and Gossage, 2011b, May et al., 2013a). These variables, coupled with the fact that *in utero* alcohol exposure impairs nearly every developing organ system, attribute to the wide-ranging variation in

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presentation and severity of FASD phenotypes among affected individuals (Hofer and Burd, 2009, Popova et al., 2016a, Caputo et al., 2016b). Birth defects resulting from prenatal alcohol exposure are persistent and lifelong, with profound socioeconomic consequences (Thanh et al., 2011); currently no approved pharmacologic therapy exists (Spohr and Steinhausen, 2008a). Targets of prenatal alcohol exposure and its pharmacokinetics are complex in nature, and thus to date, the molecular mechanisms underlying FASD pathogenesis remain insufficiently understood (Burd, 2016a).

The fetal brain is one of the most well studied targets of gestational alcohol exposure. Human and animal model studies have implicated the developing hippocampus, a structure associated with learning and memory function, as exquisitely vulnerable to alcohol-induced developmental damage (Lewis et al., 2015, Dudek et al., 2014). In animal models, gestational alcohol-induced alterations to hippocampal synaptic plasticity have been extensively studied (Fontaine et al., 2016, Bhattacharya et al., 2015), as well as alcohol-induced alterations to hippocampal synaptic activity (Kajimoto et al., 2016) and regional and cellular morphology (Perez et al., 1991, Berman and Hannigan, 2000, Ramos et al., 2002). In humans, prenatal alcohol exposure produces asymmetrical reduction in hippocampal volume, impaired spatial recall, delayed reproduction of a spatial figure, impaired place learning, delayed recognition, and verbal learning tasks relative to controls (Willoughby et al., 2008a, Autti-Rämö et al., 2002, Hamilton et al., 2003).

A limited number of FASD animal model studies have reported alterations in the hippocampal transcriptome using DNA microarray analysis (Chater-Diehl et al., 2016,

Mandal et al., 2015, Lussier et al., 2015b). One study reported that developmental alcohol dysregulates several genes implicated in in nervous system development (*Nova1, Ntng1, Neurog2, and Fexfs*) (Mandal et al., 2015), and another reports that alcohol alters hippocampal gene expression, DNA methylation, and histone methylation in free radical scavenging networks in offspring 70 days after birth (Chater-Diehl et al., 2016). FASD studies have also shown altered hippocampal DNA methylation and gene expression on postnatal day (PND) 28 corresponding with asymmetrical hippocampal volume on PND 60 in offspring exposed to alcohol during early neurulation (GD 0.5-8) (Marjonen et al., 2015), and that alcohol exposure on GD 8-21 dysregulates several candidate genes (*Gabrb3, Ube3a, Mecp2, and SLC25a12*) that overlap with autism spectrum disorders and concurrently produces adverse hippocampal learning outcomes in adult offspring (Tunc-Ozcan et al., 2013, Tunc-Ozcan et al., 2018).

These studies largely utilize microarrays to assess gene expression in mature offspring, a time when hippocampal-based learning outcomes can be effectively assessed. Our study is unique as it is the first to utilize high-throughput next-generation (next-gen) RNA deep-sequencing (RNA-seq) to examine a more thorough, dynamic range of transcriptome-wide effects of chronic prenatal binge alcohol exposure on the developing hippocampus. Alcohol-induced dysregulation to the hippocampal transcriptome during pregnancy could substantially impair hippocampal development, and associated adverse consequences may impair juvenile learning outcomes that could persist into adulthood. It is essential to understand fully alcohol-induced hippocampal transcriptome dysregulation early in life so that targeted intervention strategies may be

effectively applied as soon as possible. However, microarray analysis is limited in its ability to detect differentially expressed genes due to factors such as high background levels caused by cross-hybridization and signal saturation, and also lacks sensitivity for genes with very high or low expression levels (Wang et al., 2009a). By utilizing next gen RNA-Seq as a strategic means for investigating multi-mechanistic actions of alcohol on holistic gene expression of target organ structures, a much larger dynamic range of differentially expressed hippocampal genes can be detected (Wang et al., 2009a). Since alcohol has been shown to affect various aspects of fetal hippocampal development, (Mantha et al., 2013, Boschen and Klintsova, 2017, Gil-Mohapel et al., 2011), it is imperative to further discern how gestational alcohol exposure alters this structure at the level of the transcriptome so that we may understand mechanisms underlying neuropathogenesis and develop appropriately targeted intervention strategies.

Our model of FASD has previously shown distinct dysregulation of amino acid homeostasis and the protein signature in the fetal hippocampus (Davis-Anderson et al., 2018a). The purpose of the study herein was to discern the alterations of fetal hippocampal gene expression and their associated pathways in response to maternal alcohol exposure. We hypothesized that chronic gestational alcohol exposure alters fetal hippocampal gene expression and their related global canonical pathways.

Materials and Methods

Animals

All experimental procedures were in accordance with National Institutes of Health guidelines (NIH Publication No. 85–23, revised 1996) with approval by the Animal Care and Use Committee at Texas A&M University. Timed pregnant Sprague–Dawley rats were purchased from Charles River (Wilmington, MA), and were housed in a temperature-controlled room (23°C) with a 12:12-hour light–dark cycle. Rats were assigned to a pair-fed control (PF) group (n = 6 dams) or an alcohol (ALC) treatment group (n = 6 dams) on GD 4. The ALC-treated animals acclimatized via a once daily orogastric gavage of a 4.5 g/kg (22.5% wt/v, peak BAC, 216 mg/dl) alcohol dose from GD 5-10, and progressed to a 6 g/kg dose (28.5% wt/v, peak BAC, 289 mg/dl) (Davis-Anderson et al., 2018a) from GD 11-20. The PF animals were isocalorically matched to the ALCs by daily dosing with a gavage of maltose dextrin to account for calories derived from alcohol. The exposure regimen utilized in this study is based on both reported binge alcohol consumption patterns in pregnant women and binge exposure patterns implemented across FASD animal models (Caetano, Ramisetty-Mikler, Floyd, & McGrath, 2006; Church & Gerkin, 1988; Cudd, Chen, & West, 2002; May et al., 2013; Ryan, Williams, & Thomas, 2008; Thomas, Idrus, Monk, & Dominguez, 2010). All rats were weighed prior to the start of the study, and each treatment animal was yoked with a control animal of similar weight throughout the duration of the study. Feed intake in both groups was measured daily and the amount of diet consumed by the ALC

animals was matched to the diet administered to PF animals. There was no significant maternal weight difference between treatment groups. Animals were sacrificed on GD 21, one day after the last alcohol exposure.

Fetal Hippocampal Isolation

Fetal brain tissue was collected from an equal number of male and female offspring within each treatment group. Brains were extracted under a dissection microscope via craniotomy and were serially washed in cold phosphate buffered saline (PBS), meninges were removed, and bilateral hippocampi were micro-dissected in ice-cold HEPES buffer. Individual samples were then flash frozen and stored at -80 ˚C until analyses. One pair of male or female hippocampi from each dam was utilized for analysis.

Sample preparation

Each tissue sample was homogenized in TRIzol® Reagent and total RNA was isolated according to manufacturer's protocol (Invitrogen; Carlsbad, CA). Prior to analysis, RNA quality was assessed using an Agilent TapeStation RNA assay. Whole-genome RNA transcripts were quantified via Qubit Fluorometric assay and subsequently, all samples were normalized to an equivalent starting concentration. Sequencing libraries were prepared using the TruSeq Stranded mRNA Library Prep kit (Illumina; San Diego, CA). Each sample was uniquely indexed (barcoded) to allow for

pooling of all samples in a single sequencing run. Library size and quality were then assessed with an Agilent TapeStation D1000 DNA assay. Samples were normalized to ~4nM and pooled equally. Sequencing was performed on an Illumina NextSeq 500 running with a 75 cycle, single end sequencing run.

Bioinformatics

Raw RNA-sequence data was analyzed to identify significant differences in gene expression between the PF and ALC treatment groups, sex-dependent expression differences between these treatment groups, and the global biological pathways associated with disruption of these hippocampal genes. A total of approximately 142 million reads were evaluated and trimmed of all adapter sequences and low quality bases using Trimmomatic read trimmer (Bolger et al., 2014). Using Trimmomatic and the corresponding adapter sequences file for Illumina, reads were scanned with a sliding window of 5, cutting when the average quality per base drops below 20, then trimming reads at the beginning and end if base quality dropped below 20, and finally dropping reads if the read length was less than 50. This resulted in 131 million filtered reads (approximately 92%), out of which a total of 128 million filtered reads (approximately 97%) mapped to the Rattus norvegicus (rn5) genome assembly. Read mapping for our samples was performed using HISAT genomic analysis software platform version 2.0.5 (Kim et al., 2015). Transcript-wise counts were generated using the featureCounts tool from the SUBREAD high-performance read alignment package (Liao et al., 2013). Differential gene expression tests were then performed using DESeq2 software

following the guidelines recommended by Love and colleagues (Love et al., 2014). A heat map and volcano plots were generated from this processed data using the R programming language. The resulting gene expression values for genes that met statistical significance criteria were uploaded to INGENUITY® Pathways (QIAGEN, Venlo, Netherlands; Application Build 261899, Content Version 18030641) for biological pathway analysis. A core analysis was used to identify top canonical pathways effected by the alcohol treatment. Filters utilized for this analysis include species, confidence, mutation, and molecule type.

Statistical Analyses

Raw read counts for each gene in each hippocampal sample were utilized as input into DESeq2, which modeled the read counts as following a negative binomial distribution, with a mean representing the read concentration per gene. This mean was scaled by a normalization factor (median-of-ratios) to account for differences in sequencing depth between samples. During independent filtering, DESeq2 used the average expression strength of each gene, across all samples as its filter criteria, and omitted all genes with mean normalized counts below a filtering threshold from multiple testing adjustments. The gene-set that satisfied $-2 > \log2(\text{fold change}) > 2$ and $P < 0.05$ was deemed differentially expressed. Median-of-ratios for each gene was determined as a raw count of the gene divided by the row-wise geometric mean to yield a ratio and a median of ratios for all genes in each sample, thus producing a normalization factor for the sample. After normalized counts were calculated for each gene in each sample, a

generalized linear model (GLM) with a logarithmic link was fit in order to test for treatment effects (alcohol vs control) and conditional effects (sex), which returned the coefficients indicating overall expression strength of a gene and log2(fold change) between the treatment groups. After GLMs were fit for each gene, DESeq2 utilized a Wald test for significance (to test the null hypothesis that the logarithmic fold change between the treatment and control group is exactly zero for a given gene's expression), and the resulting Wald test P values of a subset of genes that pass independent filtering were adjusted for multiple testing using the Benjamini-Hochberge procedure. During independent filtering, DESeq2 used the average expression strength of each gene, across all samples, as its filter criteria, and omitted all genes with mean normalized counts below a filtering threshold from multiple testing adjustments. By default, DESeq2 chose a threshold that maximized the number of genes found at a user-specified target false discovery rate (FDR; 0.05). Gene sets that satisfied log2(fold change) $\geq$ 2.0 and an FDR adjusted P-value < 0.05 were considered differentially expressed.

Results

High-throughput RNA deep sequencing analysis identified 13,388 hippocampal genes, of which, 76 showed significant dysregulation following chronic binge gestational alcohol exposure ($P < 0.05$; log2(fold change) $\geq$ 2.0). Of these dysregulated genes, 37 exhibited downregulation and 39 expressed upregulation. A heat map illustrates these alterations (Fig 1); expression values based on Pearson correlation values determined the hierarchical clustering structure. Within this group of dysregulated

genes, a subset of 49 genes showed sex-dependent expression differences ($P < 0.05$; log2(fold change) ≥ 2.0), with 23 genes in alcohol-exposed females and 26 genes in alcohol-exposed males showing expression differences when compared to respective PF offspring. Only 2 genes, ATP synthase F1 subunit (*Atp5f1*) and Smad nuclear interacting protein 1 (*Snip1*) exhibited significant dysregulation in both alcohol-exposed females and males. Interestingly, *Atp5f1* expression increased in ALC females but decreased in ALC males. *Snip1* expression decreased in ALC female and male offspring.

Among the 26 hippocampal genes exhibiting expression changes in ALC females, 9 displayed upregulation and 16 displayed downregulation (Fig 2). Two downregulated genes, aldehyde dehydrogenase 18 family, member A1 (*Aldh18a1*, ↓) and microRNA 410 (*Mir410*, ↓), have gene-chemical interactions with choline, an essential nutrient and methyl donor which has been shown to be dysregulated following developmental alcohol exposure and is critically implicated in hippocampal-based learning tasks (Ryan et al., 2008, Monk et al., 2012). Two other downregulated genes, myosin light chain 2 (*Myl2*, ↓) and phosphorylase kinase catalytic subunit gamma 2 (*Phkg2*, ↓), have gene-chemical interactions with ethanol. Proprotein convertase subtilisin/kexin type 1 inhibitor (*Pcsk1*, ↑) has previously been identified as involved in brain development, and is also implicated in neuroendocrine signaling. Other genes of interest include neuraminidase 2 (*Neu2*, ↓), for which response to ethanol is a biological process and solute carrier family 6 member 13 (*Slc6a13*, ↓) which is involved in neurotransmitter transport and binding.

Among female offspring, Bioinformatic INGENUITY® Pathway Analysis (IPA®; Fig 3) identified dysregulation of 24 global biological pathways involving differential expression of hippocampal genes following chronic binge gestational alcohol exposure. IPA® determined the top canonical pathways dysregulated in ALC female hippocampi were Proline Biosynthesis I (P = 0.0056), Regulation of Actin-based Motility by Rho (P = 0.0058), PAK Signaling (P = 0.0079), RhoA Signaling (P = 0.011), and Citruline Biosynthesis (P = 0.012).

Of the 28 male hippocampal genes exhibiting major changes described above, 12 genes showed upregulation and 16 exhibited downregulation (Fig 4). Seven of these dysregulated genes have a known gene-chemical interaction with choline: Aldehyde Dehydrogenase 1 Family Member A3 (*Aldh1a3*, ↓), glutathione S-transferase, mu 5 (*Gstm5* ↑), programmed cell death 5 (*Pdcd5*, ↑), RUN and FYVE domain containing 2 (*Rufy2*, ↓), saccharopine dehydrogenase (putative) (*Sccpdh*, ↑), sperm associated antigen 4 (*Spag4*, ↑), SCO-spondin (*Sspo*, ↑), zinc finger protein 14 (*Zfp14*, ↓). *Gstm5* (↑) and mitogen activated protein kinase kinase 3 (*Map2k3*, ↓) have known gene-chemical interactions with ethanol. Other genes of interest include *Sspo*, involved in cell differentiation and nervous system development; reactive oxygen species modulator 1 (*Romo1*, ↑), involved in the response to reactive oxygen species, and Mitochondrial Fission Factor (*Mff*, ↓), disease annotations for which include developmental disabilities and mitochondrial encephalomyopathy.

Among male offspring, IPA® (Fig 5) identified dysregulation of 32 global biological pathways involving differential expression of hippocampal genes following

chronic binge gestational alcohol exposure. IPA® determined the top canonical pathways dysregulated in ALC male hippocampi were Xenobiotic Metabolism Signaling (P = 0.0003), Anandamide Degradation (P = 0.0012), Alanine Biosynthesis III (P = 0.0012), CD27 Signaling in Lymphocytes (P = 0.0019), and Molybdenum Cofactor Biosynthesis (P =0.0049).

Discussion

To our knowledge, this is the first investigation of the fetal hippocampal transcriptome utilizing next-gen high-throughput RNA-seq following chronic binge gestational alcohol exposure. Three salient findings can be gleaned from this study: 1) a chronic binge paradigm of gestational alcohol exposure dysregulates hippocampal gene expression, 2) this gene dysregulation manifests differently between male and female hippocampi, and 3) gene disruption following our exposure paradigm implicates key global pathways essential for healthy fetal development. Collectively, high-throughput RNA deep-sequencing identified 76 hippocampal genes with a significant expression difference between the pair-fed control and alcohol-treated groups, and within this group, a subset of 49 of these genes exhibited sex-dependent dysregulation.

Among female hippocampi, IPA® determined alcohol dysregulated 24 global canonical pathways following our chronic binge exposure, and includes the following pathways of interest: proline biosynthesis I, citrulline biosynthesis, and the superpathway of citrulline metabolism. *Aldh18a1* encodes for the catalytic enzyme

delta-1-pyrroline-5-carboxylate synthetase (P5CS), which is critical for de novo proline synthesis. Emerging data implicate proline's critical role as a neuroprotectant (Sareddy et al., 2015, Andrade et al., 2018) through opposition to intracellular accumulation of reactive oxygen species (Delwing et al., 2007, Krishnan et al., 2008), which has been extensively documented as a response to alcohol exposure in the developing brain. Taken in conjunction with proline's established roles as an antagonist to abiotic stressors (Dall'Asta et al., 1999, Ignatova and Gierasch, 2006, Wondrak et al., 2005) and an apoptotic regulator (Rivera and Maxwell, 2005, Liu et al., 2006b), alcohol-induced dysregulation of proline biosynthesis I may contribute to alcohol's pathogenesis in the developing hippocampus. Interestingly, *Aldh18a1* is also critically implicated in citrulline biosynthesis and the superpathway of citrulline metabolism. Citrulline biosynthesis occurs downstream from the amino acid precursors glutamate, proline, and arginine, and as arginine is converted to citrulline, nitric oxide (NO) is produced. Interestingly, NO is essential for healthy physiological nervous system regulation and has been shown to have critical roles in synaptic plasticity, learning, and memory (Susswein et al., 2004, Feil and Kleppisch, 2008). It is possible that alcohol-induced dysregulation of citrulline-related biochemical pathways observed in the female hippocampus is reflective of dysregulation of nitric oxide synthase (NOS) activity in this region. *Aldh18a1* downregulation among females may lead to accumulation of its substrate, glutamate, implicating a role for amino acid homeostasis in female hippocampal FASD pathogenesis. Although *Aldh18a1* dysregulation has been linked

with learning disabilities and neurodevelopmental deficits, hippocampal dysregulation in the context of FASD remains unknown.

Other genes of interest dysregulated by alcohol among female hippocampi include *Mir410, Myl2, Phkg2, Pcsk1, and Slc6a13.* Two downregulated genes, *Myl2* and *Phkg2*, have gene-chemical interactions with ethanol, but to our knowledge have not been previously linked with FASD. *Phkg2*, a regulator of neural and hormonal regulation of glycogen breakdown, has been shown to be downregulated in whole brain analysis following prenatal alcohol exposure, but has not been localized to the hippocampus in FASD (Laufer, 2016). *Pcsk1*, a highly expressed gene in the hippocampus that encodes for a serine protease responsible for processing neuropeptides and prohormones, has previously been identified as involved in brain development and is implicated in neuroendocrine signaling (Demoures et al., 2018). In Alzheimer's patients with severe neurodegeneration, the hippocampus is the most vulnerable region of *Pcsk1* dysregulation (Hokama et al., 2013). *Neu2*, for which response to ethanol is a biological process, has shown dysregulation in human embryonic stem cells exposed to alcohol (Khalid et al., 2014) and has been shown to be dysregulated in humans with alcohol dependence (Lingjun et al., 2015). *Slc6a13* (solute carrier family 6 member 13), which is involved in neurotransmitter transport and binding, has also been linked by multiple reports with alcohol use disorders (Hagerty et al., 2016, McClintick et al., 2015, McClintick et al., 2016), but to our knowledge, its relationship to FASD has not been explored.

Among male hippocampi, IPA® determined alcohol dysregulated 31 global pathways following our chronic binge exposure. Pathways of interest include xenobiotic metabolism signaling, anandamide degradation, alanine biosynthesis III, and molybdenum cofactor biosynthesis. Xenobiotic metabolism signaling describes a cellular stress response to xenobiotic exposure and a concomitant metabolism response to detoxify drugs and other organic compounds (Omiecinski et al., 2010). Genes differentially expressed that are associated with this pathway include *Aldh1a3, Gstm3, Map3k7, Map2k3*. Interestingly, *Gstm3* and *Map2k3* have known gene-chemical interactions with ethanol. *Gstm3* is a major detoxification enzyme shown to play a role in the breakdown of xenobiotics including a wide array of drugs and genetic variation is reported to influence susceptibility to toxins (Mei et al., 2008, Dasari et al., 2018). Recent microarray analysis reported dysregulation of glutathione pathways in the synaptoneurosome transcriptome of the mouse amygdala following a chronic alcohol exposure (Most et al., 2015). Anandamide is an endogenous neurotransmitter and *Faah,* a key gene within this pathway chiefly responsible for enzymatic breakdown of anandamide, was dysregulated by alcohol. Anandamide dysregulation is associated with hippocampal-based memory in rats and has been previously speculated to underlie FASD behavioral pathology (Basavarajappa, 2015, Mallet and Beninger, 1996). Molybdenum cofactor biosynthesis and alanine biosynthesis III are directly related, and cysteine desulfurase (*Nfs1)* is implicated in each. Xanthine oxidoreductases are a class of molybdenum cofactor enzymes implicated in cellular responses to senescence and apoptosis (Garattini et al., 2003), and the conversion of cysteine to alanine (alanine

biosynthesis III) through sulfuration of xanthine oxidoreductase renders this class of enzymes catalytically active (Schwarz, 2005). In humans, dysregulation of this process is associated with progressive neurological damage (Johnson, 2001). Collectively, these pathways and their associated genes previously implicated in critical neurodevelopmental processes may play a role in FASD hippocampal pathogenesis observed in male offspring.

Other genes of interest dysregulated among males include: *Sspo*, involved in cell differentiation and nervous system development and has previously been identified as differentially expressed in autism spectrum disorders, bipolar disorder, and schizophrenia (Kember et al., 2015; Krumm et al., 2015; Takata, Ionita-Laza, Gogos, Xu, & Karayiorgou, 2016); *Romo1*, involved in the response to reactive oxygen species and TNF-induced apoptosis (Bae, Oh, Rhee, & Do Yoo, 2011; Lee et al., 2010; Redza-Dutordoir & Averill-Bates, 2016); and *Mff*, which is essential for embryonic development and synapse formation disease and annotations for which include developmental disabilities and mitochondrial encephalomyopathy (Ishihara et al., 2009). Seven dysregulated genes (*Aldh1a3*, *Gstm5*, *Pdcd5*, *Rufy2*, *Sccpdh*, *Spag4*, and *Sspo*) have a known gene-chemical interaction with choline. Prenatal alcohol-induced dysregulation of choline bioavailability is associated with impaired hippocampal development, learning, and memory (Niculescu, Craciunescu, & Zeisel, 2006), and we conjecture that these choline-interacting genes play an underlying role in this established alcohol-induced neuropathology. Though these genes have been previously implicated in

alcohol-related neurological dysfunction, their roles in FASD hippocampal deficits remain to be explored.

Sex-based differences identified in the brain, and specifically in the hippocampus, have been shown to differentially affect susceptibility to disease, neurological function, and behaviors (Ngun et al., 2011). Collaborative reports investigating FASD models have implicated abundant alcohol-induced sex-specific hippocampal effects. Hippocampal neuroimmune response measured in offspring on PND 5 and 8 demonstrated a sex-dependent response to a developmental alcohol challenge (Ruggiero et al., 2018). Adolescent hippocampal functional assessment revealed N-methyl-D-aspartate long-term potentiation reduced by 40% in adolescent males prenatally exposed to alcohol compared to adolescent females, which interestingly exhibited increased hippocampal glutamine synthetase expression (Sickmann et al., 2014). Prenatal alcohol exposure has also been shown to have sex-specific hippocampal effects lasting into adulthood, as Uban and colleagues demonstrated that PND 60 females exhibit a reduced proportion of newly produced neurons and glia in the dentate gyrus compared with males (Uban et al., 2010). These studies indicate alcohol has the potential to affect this brain region differentially based on sex, however, a knowledge gap persists regarding differences in hippocampal gene expression profiles between male and female rats alone and even more so in the context of FASD (Schneider et al., 2011, Tunc-Ozcan et al., 2013, Van den Hove et al., 2013). An understanding of these differential outcomes at the transcriptome level is fundamental for developing novel

therapeutic strategies that account for these sex-based differences for maximum effectiveness.

Perspectives and significance

Thus far, the effects of chronic binge gestational alcohol exposure on hippocampal transcriptome-wide gene expression have remained largely limited to microarray analyses. The majority of hippocampal microarray analyses in animal models of FASD has been performed on: 1) adolescent or adult animals exposed to alcohol during development (Chater-Diehl et al., 2016, Lussier et al., 2015b, Marjonen et al., 2015), or 2) on animals whose exposure paradigm did not mimic a chronic binge exposure paradigm throughout pregnancy (Mandal et al., 2015). To our knowledge, no microarray has analyzed rat hippocampal gene expression using a chronic binge model of gestational alcohol exposure. By utilizing next-gen high-throughput RNA-seq, our goal was to elucidate novel molecular targets underlying FASD hippocampal deficits to better understand FASD pathogenesis. In summary, our results indicate a chronic binge paradigm of gestational alcohol exposure differentially alters hippocampal gene expression, and that this alcohol-induced gene expression exhibits sex-specific variation in the developing hippocampus, as do their associated global canonical pathways. Detection of subtle gene expression changes within specific brain regions such as the hippocampus through advances in next-generation sequencing may yield critical new understanding of vulnerable genes and genetic networks underlying FASD neuropathogenesis. Insights acquired from this advanced genomic technology offer

novel findings essential for pinpointing targets of developmental alcohol exposure necessary for the development of urgently needed, targeted therapeutic intervention strategies.

Data availability statement: The data that support the findings will be available in U.S. National Library of Medicine, NCBI Sequence Read Archive (SRA) following an embargo that ends on February 14, 2020 (Ramadoss, 2019).

CHAPTER III

SUMMARY AND CONCLUSIONS

FASD and the Hippocampus

Much of the behavioral, learning, and memory deficits observed in FASD are linked to alcohol's effect on the developing hippocampus. Outcomes observed in children and adults with known alcohol exposure *in utero* are consistent with animal models of FASD. These deficits are largely attributed to unknown etiological mechanisms that alter the developing hippocampus at cellular and gross structural levels.

Alcohol's mechanisms of action, which are complex, are known to have an exquisite detrimental effect on the developing hippocampus. Morphological alterations in the mitochondria are evident and the distribution of rough endoplasmic reticulum within pyramidal cells is also affected (Smith and Davies, 1990, Tanaka et al., 1983). Pyramidal and granule cell numbers of the developing hippocampal formation are reduced as a consequence of alcohol exposure (Perez et al., 1991). Furthermore, alcohol exposure has been shown to reduce the area of certain regions of the hippocampus proper, such as CA1 (Greene et al., 1992). Abnormal branching is observed in the CA3 region of the hippocampus proper, where mossy fiber invasion and hyper-development are evident (West et al., 1981, West and Hodges-Savola, 1983). Alcohol exposure stunts dendritic arborization in the CA1 region and decreases the density of dendritic spines across the pyramidal cells of the developing hippocampus proper (Davies and Smith,

1981, Perez et al., 1991). Abnormal hippocampal electrophysiology is also observed, such as alterations in theta brain wave activity (Cortese et al., 1997).

The result of neuroanatomical and electrophysiological alterations of the hippocampus are linked to deficits in reverse learning, acquisition, and spacial learning. Animal models have observed findings consistent with individuals with FASD. PAE in mice resulted in deficits in reverse learning (Wainwright et al., 1990). Alcohol exposure pre- and postnatally impaired acquisition in the treatment group (Zimmerberg et al., 1991, Thomas et al., 1996, Thomas et al., 1997). Goodlett and colleagues tested spatial learning and memory in juvenile rats exposed during the third trimester-equivalent, with deficits observed in both male and females (Goodlett et al., 1987).

In brief, the alcohol-induced damage to the developing hippocampus is long-lasting and affects multiple domains that are observed in the FASD phenotype. Understanding why the hippocampus is such a delicate target compared to other brain regions warrants future studies.

Sex differences in FASD

The effects of alcohol exposure during development can be influenced by sex, however, the factors underlying these sex differences have yet to be thoroughly understood. For instance, animal models of FASD have observed male rats to have a greater impairment of spatial memory (Blanchard et al., 1987). In contrast, a different study using one episode of PAE observed females having a greater deficit in retention learning (Minetti et al., 1996). Similar to Minetti and colleagues, females in the alcohol

treatment group had greater learning deficits in a binge-like alcohol model (Kelly et al., 1988). One study observed deficits in social recognition that were significantly different in male rats exposed to alcohol *in utero* (Kelly et al., 2009b). In the same study, female rats only exhibited deficits when the tasks were of greater difficulty. PAE in macaque monkeys resulted in male offspring having an increased binding to dopamine (D_1R) receptors (Converse et al., 2014). Hippocampal cell survival was reduced in alcohol-exposed male rat offspring, with no significant effects observed in females (Sliwowska et al., 2010, Uban et al., 2010). Long-term potentiation (LTP) was reduced in the dentate gyrus of alcohol exposed male offspring (Titterness and Christie, 2012). Tonic seizure prevalence was increased in male offspring prenatally exposed to alcohol, but not females (Cho et al., 2017). Corticotropic-releasing hormone (CRH) was increased in male rats exposed to alcohol *in utero* (Gabriel et al., 2005). Glucocorticoid receptor mRNA levels were increased following PAE in male offspring (Redei et al., 1993). With respect to functional connectivity, a greater number of alcohol-induced alterations were observed in males compared to females (Rodriguez et al., 2016). PAE reduced the number of neurons from the medial septum in female offspring (Moore et al., 1997).

The differences between male and female physiology and how alcohol affects each sex is not fully understood. The wide array of outcomes due to these differences add another layer of complexity that will require additional investigation in FASD studies.

Temporal/Regional Effects of Alcohol

The magnitude of alcohol's effect on the developing brain depends on many factors, two of which are the most important: timing and dosing. Regions of neurogenesis and development juxtaposed with specific windows of vulnerability compound alcohol's already complex effects, especially in the hippocampus, cortex, and cerebellum (Riley and McGee, 2005). Rodent models of gestational alcohol exposure, such the chronic binge alcohol model used in this study, replicate human brain development during a period when the uterus and the placenta are intact. Transient alcohol exposure, such as one day of treatment, alters amino acid availability within the maternal plasma and the shapes of various brain regions (Padmanabhan et al., 2002, Fish et al., 2016). Chronic exposure to alcohol during gestation results in disruption of cell migration and synapse formation (Cuzon et al., 2008). Amino acid availability is also affected and fetal brain regions are altered (Lunde-Young et al., 2018). These anatomical changes are detrimental to brain function as alcohol impairs memory, spatial navigation, and nociceptive response (Sutherland et al., 1997, Zimmerberg et al., 1991, Fish et al., 2016, Blanchard et al., 1987, Reyes et al., 1989). In rodent models, the first week of the postnatal period is approximate to the third trimester in humans. During this period of development, the brain undergoes a growth spurt and its sensitivity to alcohol is reflected by the reduction of brain volume and loss of cortical, hippocampal, and cerebellar neurons due to exposure (Coleman et al., 2012, Maier et al., 1999, Bonthius and West, 1990, Livy et al., 2003). Alcohol exposure during this period also impairs adult-onset dentate gyrus neurogenesis and is responsible for triggering neuronal

apoptosis (Klintsova et al., 2007, Ikonomidou et al., 2000). These long-term disruptions in the brain's circuitry are associated with deficits in behavior that persist into adulthood (Sadrian et al., 2013). Furthermore, alcohol exposure in the postnatal model is linked to spatial memory deficits, and learning and memory impairment (Savage et al., 2010, Wozniak et al., 2004). Despite the body of literature cataloguing the consequences of alcohol exposure in the developing brain, many questions remain. In particular, the underlying mechanisms of alcohol-induced neuropathogenesis in highly vulnerable brain regions.

Alcohol-Affected Global Pathways

Citrulline biosynthesis and the superpathway of citrulline metabolism are two canonical pathways shown to be dysregulated in the hippocampi of males in response to our chronic gestational alcohol exposure paradigm. Dysregulation of citrulline biosynthesis by means of inhibition in a rat model has previously been linked with severe growth restriction, a hallmark feature of FASD that was also observed in our study (Fig 6) (Hoogenraad et al., 1985). A noteworthy component of the citrulline biosynthesis pathway is the production of nitric oxide (NO), a potent vasodilator, by the conversion of L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS). In addition to playing important roles in endothelial-derived vasodilation, and in embryonic cardiac and vascular development, nitric oxide is a key regulator in blood flow to the developing brain and is an important signaling molecule in neurotransmission (Michell et al., 2004, Duncan and Heales, 2005, Stojanovic et al., 2003, Stojanovic et al., 2004,

Yamamoto et al., 2015, Yassin et al., 2014, Džoljić et al., 2015). Dysregulation of citrulline biosynthesis aligns with findings from previous FASD studies showing alcohol exposure during gestation dysregulates eNOS expression and vascular function, and we conjecture this dysregulation is linked with dysregulation of citrulline biosynthesis, as observed in this study (Ramadoss and Magness, 2012, Naik et al., 2016, Naik et al., 2018, Subramanian et al., 2014, Ramadoss et al., 2011). Dysregulation of NO has also been linked with neuroprotective and neurotoxic effects, and thus dysregulation of citrulline biosynthesis during development could lead to marked neuropathological outcomes in the fetal brain (Boje, 2004, Akyol et al., 2004, Colasanti and Suzuki, 2000).

Xenobiotic metabolism signaling is an essential pathway comprised of a network of receptors and enzymes whose main purpose is to serve as a coordinated defense system by means of drug metabolism and clearance in response to endogenous and exogenous cellular stimuli. However, more recent studies have implicated xenobiotic metabolism signaling in cellular processes beyond its primary conventional function, including tissue injury and repair, energy homeostasis, inflammation, cell proliferation, and immune response (Mackowiak et al., 2018). Dysregulation of this pathway has been previously linked with Parkinson's disease, Alzheimer's disease, and neurodegenerative disorders, but this pathway has not been previously linked to the brain in the context of FASD (McFadden, 1996). As alcohol is a xenobiotic, dysregulation of the ability to metabolize xenobiotic substrates may translate into an impaired defense response to these at the cellular level. Dysregulation of functional roles such as cellular proliferation may be particularly harmful to hippocampal development.

The anandamide degradation pathway was also shown to be dysregulated in the fetal hippocampus in response to chronic binge gestational alcohol exposure. Anandamide is a fatty acid neurotransmitter that binds with CB1 cannabinoid receptors in the central nervous system and has been previously implicated to affect working memory, feeding behavior patterns, and anxiety, often with beneficial results. However, one study of particular interest showed that an overabundance of anandamide resulted in hippocampal cellular toxicity (Cernak et al., 2004). In a separate study, Subbanna and colleagues noted increased levels of anandamide in the hippocampus following PAE, noting neurodegeneration and impaired synaptic plasticity in treatment animals (Subbanna et al., 2013). These findings indicate the anandamide degradation pathway may play a crucial role in negative effects seen in the developing hippocampus.

Review of Altered Genes

The following sections provide a brief overview of the literature that describes the general physiological processes of the genes observed to be altered in fetal male and female hippocampi following alcohol exposure. In addition, associated diseases of these genes are briefly mentioned.

Fetal Male Specific Alterations in our study

Aldh1a3 is involved in the oxidation of retinal to retinoic acid (Zhao et al., 1996). In pregnancy, retinoic acid from *Aldh1a3* encoded enzymes contributes to the development of various organs such as skin, brain, lungs, kidneys, seminal vesicles, eyes

and skeletal muscle (Niederreither et al., 2002, Dupe et al., 2003, Everts et al., 2004). More recently, *Aldh1a3* has been implicated in the differentiation or maintenance of placental cells (Outhwaite et al., 2015).

Bmp15 is involved various processes such as the positive regulation of transcription and regulation of cell apoptosis (Gaudet et al., 2011). *Bmp15* also plays a role in granulosa cell proliferation and regulation of steroid hormones (Juengel and McNatty, 2005, Chang et al., 2013). Mutation or deletion of *Bmp15* has been associated with subfertility (Dixit et al., 2006). There is also evidence of *Bmp15* being involved in germ cell development during the process of oogenesis (Lochab and Extavour, 2017).

Ebna1bp2 encodes a dynamic scaffolding protein that is required for 27S pre-rRNA processing (Hirano et al., 2009). Downregulation of this gene is seen during pregnancy, specifically in the endometrium upon implantation of the embryo (Kashiwagi et al., 2007).

Echs1 is involved in fatty acid beta oxidation (Yamada et al., 2015). *Echs1* deficiency in children results in delayed milestones, dystonia, and basal ganglia abnormalities in the brain (Yamada et al., 2015).

Faah plays a role in negatively regulating blood vessel diameter (Baranowska-Kuczko et al., 2016). It is also involved in fatty acid metabolic processes such as the degradation of bioactive fatty acid amides (Giang and Cravatt, 1997). Initially inactive, expression is first detected in the blastocyst stage of embryonic development (Paria et al., 1999). Active *Faah* is suggested to serve as a method to regulate local uterine levels

of endogenous cannabinoids, which are known to interfere with implantation and be detrimental for embryonic development (Paria et al., 1999).

Gstm5 encodes proteins that are involved in the metabolic processing of glutathione (Patskovsky et al., 1999). During development, *Gstm5* expression in fetuses is high prenatally and gradually decreases postpartum in neonates (Cui et al., 2010). Cui and colleagues have postulated that there is a special function during gestation to explain this pattern of expression. Interestingly, *Gstm5* is upregulated in the myometrium during pregnancy (Rehman et al., 2003). In the placenta, *Gstm5* is assumed to be upregulated as a protective mechanism response against high levels of reactive oxygen species during pregnancy (Alexander et al., 2018).

Haus1 is involved in the cell division cycle by contributing to mitotic spindle assemble (Lawo et al., 2009). Encoded *Haus1* proteins are involved in the regulation of cellular migration, polarization, and neuronal development (Cunha-Ferreira et al., 2018).

Hiat1 encodes for proteins that play a role in transmembrane transport (Sreedharan et al., 2011). *Hiat1* has recently been implicated in spermatogenesis (Doran et al., 2016). *Hiat1* expression was also been shown to be affected by nutrient availability, mainly the lack of amino acids (Lekholm et al., 2017).

Hist1h2ail is hypothesized to be involved in the regulation of gene silencing according to various genome databases. To our knowledge, there are no scientific studies on this gene's role.

Hnrnpab is suspected to be involved in editing apolipoprotein B mRNA (Lau et al., 1997). Adequate expression is essential for the maintenance of neuronal connectivity (Berson et al., 2012).

Loc680489 is predicted by genome databases to be involved in membrane structure and play a role in bicellular tight junctions. To our knowledge, there is no further investigation on this gene.

Map2k3 (*Mkk3*) is involved in the positive regulation of endothelial cell migration of blood vessels and protein kinase activity (Pin et al., 2012). *Map2k3* also stimulates the signal cascade that leads to the induction of TNFα in microglia (Yoshino et al., 2011). *Map2k3* is required for the modification of actin cytoskeletons in endothelial cells (Wang et al., 2005). *Map2k3* is responsible for the stimulation of wound closure (Bulat et al., 2009).

Map3k7 plays a role in the positive regulation of macroautophagy and production of T-cell cytokines (Sun et al., 2004). During pregnancy, *Map3k7* in the embryo is sensitive to maternal diabetes and is associated with neural tube defects (Salbaum and Kappen, 2010). Intake of low or high amounts of protein in the diet has been observed to dysregulate *Map3k7* expression prenatally (Oster et al., 2011, Oster et al., 2012b).

Mff is involved in the regulation of mitochondrial and peroxisomal organization and fission (Gandre-Babbe and van der Bliek, 2008). *Mff* malfunction has been associated with encephalopathy that may be present as neurological regression, intellectual disability, and/or microcephaly, among other clinical features (Nasca et al., 2018).

Miip is involved in the negative regulation of cell migration and suspected to play a role in the inhibition of cancer progression (Song et al., 2003). Overexpression of *Miip* has an inhibitory effect on cell growth and delays mitosis (Ji et al., 2010, Wang et al., 2011).

Nfs1 encoded proteins catalyze the removal of elemental sulfur to make alanine from cysteine (Li et al., 2006). Infantile mitochondrial complex II/III deficiency has been linked to *Nfs1*, as depletions of its transcript and protein levels have been observed in affected children (Farhan et al., 2014).

Pdcd5 encoded proteins are involved in the negative regulation of cellular proliferation (Chen et al., 2006). Additionally, *Pdcd5* plays a role in the positive regulation of apoptosis (Liu et al., 1999). Osteoarthritis and rheumatoid arthritis pathogenesis have been linked to *Pdcd5* dysregulation (Cheng et al., 2004, Li et al., 2014). *Pdcd5* therapy has been demonstrated to promote apoptosis in cancer cells with low transcript levels (Wang et al., 2016). *Pdcd5* has been demonstrated to be important for placenta development and embryonic viability (Li et al., 2017).

Polr2l encodes a protein subunit of a polymerase that is involved in synthesizing mRNA (Forget et al., 2013). *Polr2l* has been observed to have a sensitivity to high protein intake during gestation (Oster et al., 2012a). *Polr2l* participates in Huntington's disease pathway.

Romo1 activity positively regulates cell proliferation and the metabolic process of reactive oxygen species (Na et al., 2008, Chung et al., 2006). *Romo1*-encoded proteins have antibacterial activity via bacterial membrane destruction, indicating an antibiotic

potential (Sha et al., 2012). It is considered to be crucial for the proliferation and invasion of cancer cells, with high expression related to recurrence and poor prognosis in patients (Wu, 2006). Complications during pregnancy has been observed to alter *Romo1* expression (Baptista et al., 2019).

Rufy2 is involved in the regulation of endocytosis and suspected to play a role in neurodegenerative diseases such as Alzheimer's (Yang et al., 2002, Kitagishi and Matsuda, 2013).

Sccpdh encoded enzymes oxidize saccharopine in the metabolic pathway of lysine. These enzymatic proteins protect mitochondrial dynamics and functions from the pathological accumulation of saccharopine (Leandro and Houten, 2019).

Serpina11 is involved in the negative regulation of endopeptidase activity (Gaudet et al., 2011).

Slc10a6 is involved in sodium-dependent sulfated bile acid and steroid hormone transportation (Gaudet et al., 2011, Claro da Silva et al., 2013). Inflammation has been shown to elicit an upregulation of *Slc10a6* expression (Kosters et al., 2016). It is postulated that *Slc10a6*-encoded proteins in the placenta are involved in DHEA transport and progesterone synthesis (Geyer et al., 2007). More recently, *Slc10a6* hormonal transportation activity has been discovered in male germ cells and throughout the different stages of sperm development (Fietz et al., 2013).

Spag4 plays an important role in cytokinesis and is upregulated to protect against formation of tetraploid cells (Shoji et al., 2013). *Spag4* is involved in the process of sperm cell maturation and maintenance of the nuclear envelope (Shao et al., 1999,

Gaudet et al., 2011). Expression of *Spag4* correlates with cancer prognosis (Shoji et al., 2013).

Sspo-encoded proteins play a role in neuronal development, presumably in axon growth and/or guidance (Meiniel, 2001, Gobron et al., 1999). During embryonic development, *Sspo*-encoded proteins promote neuronal differentiation in the diencephalon and mesencephalon by binding to their respective neuroepithelial cells (Vera et al., 2013).

Zfp14 has been associated with amyotrophic lateral sclerosis (Morello et al., 2018). In the aged brain, *Zfp14* was found to be among the top differentially expressed genes (Pardo et al., 2013).

Female Specific Alterations in our study

Aldh18a1 encodes for enzymes involved in the processes of proline, ornithine, and arginine biosynthesis (Scriver et al., 1983, Phang, 1995, Hu et al., 2008). Abnormal *Aldh18a1* activity can present with clinical features such as facial dysmorphism (Fischer et al., 2014). Cutis laxa has been associated with *Aldh18a1* deficits, with patients commonly presenting with features such as microecphaly, retinopathy, and developmental delay (Pardo et al., 2013, Wolthuis et al., 2014). In addition, intrauterine growth restriction and white matter abnormalities are two unique features observed in a clinical setting (Bicknell et al., 2008).

Cwh43 plays a critical role in cellular nutrient metabolism and lipid homeostasis, with a possible involvement in cytokinesis and overall cell proliferation (Nakazawa et

al., 2018, Nakazawa et al., 2019). During the endometrium's receptive period necessary for blastocyst implantation, *Cwh43* is upregulated (Moreno-Moya et al., 2014). *Cwh43* methylation is associated with maternal plasma folate levels (Joubert et al., 2016).

Cysltr2 is involved in the immune response mechanism (Takasaki et al., 2000). In certain tissues, *Cysltr2* is postulated to produce vasodilation responses through a pathway involving nitric oxide (Secrest et al., 1988, Allen et al., 1992, Evans, 2002). In the lung, *Cysltr2*-encoded proteins are involved in asthma via a pulmonary vasoconstriction and bronchoconstriction response (Haeggstrom and Funk, 2011). High *Cysltr2* expression is associated with a good prognosis in cancer patients (Magnusson et al., 2010). During the late luteal phase, *Cysltr2* expression is upregulated in the endometrium (Korzekwa et al., 2016).

Dmrt2 is one of many genes involved in transcription regulation (Herrera et al., 2010). During gestation, *Dmrt2*-encoded proteins play a role in somite differentiation and patterns the axial skeleton during embryonic development (Seo et al., 2006). In addition, *Dmrt2* is involved in a developmental cascade that is responsible for the onset of myogenesis (Sato et al., 2010).

Efemp1 negatively regulates chondrocyte differentiation by inhibiting the formation of cartilage nodules, the production of proteoglycans and, modulating matrix gene expression (Wakabayashi et al., 2010). *Efemp1* encoded proteins make up part of the extracellular matrix in the aorta (Didangelos et al., 2010). *Efemp1* participates in cancer development and is considered to be a better diagnostic marker for prostate cancer than prostate-specific antigen (PSA) (Shen et al., 2017). *Efemp1* is also involved

in the regulation of body weight (Weedon et al., 2008). *Efemp1* is associated with uterine leiomyomas, and benign tumors that present with multiple symptoms such as recurrent pregnancy loss. (Pritts et al., 2009, Marsh et al., 2016)

Faim is involved in the positive regulation of neurogenesis and neurite outgrowth (Sole et al., 2004). Furthermore, *Faim* protects neurons by antagonizing the stimulus for cell death and is posited to be involved as a modulator during embryonic neuron development (Segura et al., 2007). *Faim* also plays a role in the negative regulation of lymphocyte and hepatocyte apoptosis (Gaudet et al., 2011, Huo et al., 2009). In the incidence of early pregnancy loss, *Faim* expression in placental villi has been observed to be decreased (Liu et al., 2006a).

Fat2 is involved in cellular adhesion and epithelial cell migration (Matsui et al., 2007). *Fat2* is associated with spinocerebellar ataxia (Nibbeling et al., 2017).

Fscn2 is involved in cell polarity establishment, migration and actin filament assembly (Gaudet et al., 2011). *Fscn2* plays a role in photoreceptor disk morphogenesis and visual perception (Tubb et al., 2000).

Ism1 is critical for the generation of hematopoietic stem and progenitor cells (Berrun et al., 2018). Ism1-encoded proteins are involved in lymphocyte function and may play a role in immune response (Valle-Rios et al., 2014). *Ism1* activity inhibits angiogenesis and is a tumor suppressor (Yuan et al., 2012). *Ism1* is expressed in various embryonic structures and suspected to be crucial for proper development of tissues and organs, such as the brain (Osorio et al., 2014). Furthermore, *Ism1* plays a role in clefting and craniofacial development (Lansdon et al., 2018).

MGC94199 is crucial for the development of photoreceptor structure formation and alignment (Sharif et al., 2018). *MGC94199* mutations have been linked to Bardet Biedl syndrome, characterized by phenotypes such as photoreceptor degeneration and cognitive impairment (Heon et al., 2016, Kerr et al., 2016).

Milr1 is involved in the suppression of mast cells activation and suspected to play a role in negatively regulating hypersensitivity responses to allergens (Nagai et al., 2013).

Mir410 negatively regulate angiogenesis by suppressing *Vegfr2* (Su et al., 2017). *Mir410* functions as a glioma tumor suppressor (Chen et al., 2012). Downregulation of *Mir410* is involved in proliferation and invasion of oral cancer (Shiah et al., 2014).

Myl1-encoded proteins comprise a structural constituent of fast-twitch skeletal muscle and are involved in muscular contraction via filament sliding (Barton and Buckingham, 1985).

Myl2 is involved in the negative regulation of cell growth (Maina et al., 2005). *Myl2* also plays a role in cardiac tissue morphogenesis (Poetter et al., 1996). During early development, *Myl2* is involved in the formation and function of the heart (Sheikh et al., 2015).

Neu2 plays a role in myoblast and neuronal differentiation (Sato and Miyagi, 1996, Fanzani et al., 2004). *Neu2* is suspected to be involved in muscle hypertrophy (Fanzani et al., 2012). In cancer cells, *Neu2* expression induces apoptosis (Tringali et al., 2007). *Neu2* is involved in controlling membrane structural properties (Rondelli et al., 2017).

Pcsk1n inhibits enzyme activity that regulates neuroendocrine precursor cleavage (Fricker et al., 2000). *Pcsk1n* is suspected to be involved in development due to its expression being present in the forming embryonic brain, with pituitary expression evident later in gestation (Morgan et al., 2005).

Phkg2 is involved in generating precursor metabolites and energy (Maichele et al., 1996). Furthermore, *Phkg2* plays a role in positive regulation of glycogen breakdown (Brushia and Walsh, 1999). *Phkg2* is associated with the hepatic phenotype observed within glycogen storage disease type IX (Albash et al., 2014).

Pnpla1 is involved in biosynthesizing a key lipid component of the skin's permeability barrier (Hirabayashi et al., 2017). Furthermore, expression of this gene is required for the differentiation of epidermal keratinocytes (Hirabayashi et al., 2019). *Pnpla1* also plays a role in the catabolism of triglycerides (Gaudet et al., 2011). *Pnpla1* is linked with autosomal recessive congenital ichthyosis (Traupe et al., 2014, Grall et al., 2012).

Rps9-encoded proteins are required for cell proliferation (Lindstrom and Zhang, 2008). Inhibition of *Rps9* is suspected to reinitiate differentiation or apoptosis in proliferating cancer cells (Lindstrom and Nister, 2010).

Slc13a4 encoded proteins regulate sterol uptake (Ness et al., 2001). *Slc13a4* negatively regulates filamentation (Foster et al., 2013). *Slc13a4* is suspected to be involved in sulfate transport across the placenta to be utilized by the fetus (Girard et al., 1999). Fetal development and viability is dependent on placental *Slc13a4* (Rakoczy et al., 2015).

Slc6a13 contributes to oxidative stress resistance by uptake of hypotaurine across the plasma membrane (Nishimura et al., 2018). *Slc6a13*-encoded proteins serve as major taurine transporters in the liver (Zhou et al., 2012).

Slco4c1-encoded proteins transport digoxin, thyroid hormones, ouabain, and cAMP (Mikkaichi et al., 2004). Moreover, *Slco4c1* is involved in the elimination of uremic toxins, decreases renal inflammation, and attenuates hypertension (Toyohara et al., 2009). Modulation of *Slco4c1* expression has been proposed as a therapeutic approach in preventing chronic renal disease (Suzuki et al., 2011).

Wdr63 activity enhances osteogenic differentiation in apical papilla stem cells (Diao et al., 2015). *Wdr63* is suspected to play a role in encephalocele formation during development (Hofmeister et al., 2018). *Wdr63* expression in the lung may contribute to ciliogenesis (Lonergan et al., 2006). During pregnancy, *Wdr63* expression in the maternal endometrium is altered (Zhou et al., 2008).

Shared Gene Fetal Male and Female Alterations in our study

Atp5f1 is involved in the lipid metabolism, ATP metabolism, and electron transport chain pathways (Gaudet et al., 2011). Inadequate intake of maternal dietary riboflavin during development downregulates *Atp5f1* expression, which impairs oxidative phosphorylation and plays a role in the total deficit of ATP that is lethal for embryos (Tang et al., 2019). Gestational exposure to smoking has also been shown to affect *Atp5f1* expression in placenta (Huuskonen et al., 2016).

Snip1 is involved in microRNA (miRNA) biosynthesis (Gaudet et al., 2011, Yu et al., 2008a). In addition to miRNA biosynthesis, *Snip1*-encoded proteins regulate cell proliferation and embryonic fibroblast transformation (Fujii et al., 2006, Roche et al., 2004). Over-expression of *Snip1* has been observed to result in gene silencing (Kim et al., 2000). *Snip1* expression is important for embryonic viability during development (Yu et al., 2008a). *Snip1*-encoded proteins play a crucial role in brain development; absence of these proteins result in structural brain defects and cell death (Fernandez et al., 2018).

Future Direction And Perspectives

The biological activities of each gene, as mentioned in the literature, make the results gleaned from our study of the fetal hippocampus following PAE, quite intriguing. New questions are raised with regards to certain physiological roles during development and they cannot be answered due to the glaring gaps in the literature. Moreover, technological advances in the field of genetics have revealed novel insights regarding interactions between PAE and gene expression that pose new questions. Future studies are recommended to investigate the novel findings of our work.

One physiological area of emphasis that needs to be expanded upon is gene expression and their roles during development. A majority of the studies performed on gene function are described within the context of mature organisms. For example, some of the previously described genes have been observed to have activity in the maternal endometrium during early pregnancy. Logically, functions such as cell proliferation,

differentiation, or migration make sense within the context of the developing embryo/fetus. However, there is limited, if any, evidence to validate these suspicions. Therefore, it is of great importance to expand our understanding on embryonic/fetal gene expression and their roles in various tissues by using robust and replicable methods. An increased physiological comprehension will aid in deducing alcohol's effects on the genomic activity of the embryo/fetus, especially in the developing brain.

In addition to the gaps of knowledge in gene function during development, literature on targeted gene expression and their roles in certain organs, such as the brain, is limited. At the time of this writing, approximately 80% of the genes detected in our study have not been described in the brain, much less the developing hippocampus. Thus future investigation in this area is recommended. Some of the gene functions from our study can logically be applied within the context of brain growth and development (e.g. cell polarity, neuronal differentiation, and cell migration). However, to our knowledge, no evidence exists that validates and describes the gene functions and processes in the brain. Without a thorough physiological understanding of these genes, it becomes difficult to ascertain what mechanisms in the developing brain could be affected by alcohol exposure *in utero*.

Lastly, we advocate for investigating the associated pathways of the altered genes in the hippocampus. In doing so, novel insights for understanding how these physiological mechanisms of the hippocampus are affected by alcohol will be gleaned. We suspect that alterations of these pathways play a critical role in the FASD phenotype.

The future direction in FASD is a massive undertaking that will require a collaborative effort. In doing so, not only will our physiological understanding during development be greatly improved, but novel ideas may be synthesized to further the cause of understanding the mechanistic etiologies of alcohol during development. Moreover, advances in FASD research may carry over into the clinical setting and allow the incorporation of appropriate strategies for all current subcategories of FASD (Fig 7).

REFERENCES

AKYOL, O., ZOROGLU, S. S., ARMUTCU, F., SAHIN, S. & GUREL, A. 2004. Nitric oxide as a physiopathological factor in neuropsychiatric disorders. *In vivo,* 18**,** 377-390.

ALBASH, B., IMTIAZ, F., AL-ZAIDAN, H., AL-MANEA, H., BANEMAI, M., ALLAM, R., AL-SUHEEL, A. & AL-OWAIN, M. 2014. Novel PHKG2 mutation causing GSD IX with prominent liver disease: report of three cases and review of literature. *Eur J Pediatr,* 173**,** 647-53.

ALEXANDER, J., TEAGUE, A. M., CHEN, J., ASTON, C. E., LEUNG, Y. K., CHERNAUSEK, S., SIMMONS, R. A. & PINNEY, S. E. 2018. Offspring sex impacts DNA methylation and gene expression in placentae from women with diabetes during pregnancy. *PLoS One,* 13**,** e0190698.

ALLEN, S. P., CHESTER, A. H., PIPER, P. J., SAMPSON, A. P., AKL, E. S. & YACOUB, M. H. 1992. Effects of leukotrienes C4 and D4 on human isolated saphenous veins. *Br J Clin Pharmacol,* 34**,** 409-14.

ANDERSON, D. J., MONDARES, R. L., BORN, D. E. & GLEASON, C. A. 2008. The effect of binge fetal alcohol exposure on the number of vasoactive intestinal peptide-producing neurons in fetal sheep brain. *Dev Neurosci,* 30**,** 276-84.

ANDRADE, V. S., ROJAS, D. B., DE ANDRADE, R. B., KIM, T. D. H., VIZUETE, A. F., ZANATTA, Â., WAJNER, M., GONÇALVES, C.-A. S. & WANNMACHER, C. M. D. 2018. A possible anti-inflammatory effect of proline in the brain cortex and cerebellum of rats. *Molecular neurobiology,* 55**,** 4068-4077.

ARCHIBALD, S. L., FENNEMA-NOTESTINE, C., GAMST, A., RILEY, E. P., MATTSON, S. N. & JERNIGAN, T. L. 2001. Brain dysmorphology in individuals with severe prenatal alcohol exposure. *Dev Med Child Neurol,* 43**,** 148-54.

ARNSTEN, A. F. 1997. Catecholamine regulation of the prefrontal cortex. *J Psychopharmacol,* 11**,** 151-62.

ARRIZA, J. L., FAIRMAN, W. A., WADICHE, J. I., MURDOCH, G. H., KAVANAUGH, M. P. & AMARA, S. G. 1994. Functional comparisons of three glutamate transporter subtypes cloned from human motor cortex. *J Neurosci,* 14**,** 5559-69.

ASTLEY, S. J., RICHARDS, T., AYLWARD, E. H., OLSON, H. C., KERNS, K., BROOKS, A., COGGINS, T. E., DAVIES, J., DORN, S., GENDLER, B., JIRIKOWIC, T., KRAEGEL, P. & MARAVILLA, K. 2009. Magnetic resonance spectroscopy outcomes from a comprehensive magnetic resonance study of children with fetal alcohol spectrum disorders. *Magn Reson Imaging,* 27**,** 760-78.

AUTTI-RÄMÖ, I., AUTTI, T., KORKMAN, M., KETTUNEN, S., SALONEN, O. & VALANNE, L. 2002. MRI findings in children with school problems who had been exposed prenatally to alcohol. *Developmental medicine and child neurology,* 44**,** 98-106.

BACKUS, B. L. 2013. *A swine model for Fetal Alcohol Spectrum Disorder-The effect of maternal free-choice ethanol consumption on the behavior of female offspring.*

BAPTISTA, L. C., FIGUEIRA, C. O., SOUZA, B. B., FERTRIN, K. Y., ANTOLINI, A., COSTA, F. F., DE MELO, M. B. & COSTA, M. L. 2019. Highlight Article: Different morphological and gene expression profile in placentas of the same sickle cell anemia patient in pregnancies of opposite outcomes. *Exp Biol Med (Maywood),* 244**,** 395-403.

BARANOWSKA-KUCZKO, M., KOZLOWSKA, H., KLOZA, M., KARPINSKA, O., TOCZEK, M., HARASIM, E., KASACKA, I. & MALINOWSKA, B. 2016. Protective role of cannabinoid CB1 receptors and vascular effects of chronic administration of FAAH inhibitor URB597 in DOCA-salt hypertensive rats. *Life Sci,* 151**,** 288-299.

BARRON, S. & RILEY, E. P. 1985. Pup-induced maternal behavior in adult and juvenile rats exposed to alcohol prenatally. *Alcohol Clin Exp Res,* 9**,** 360-5.

BARRON, S., SEGAR, T. M., YAHR, J. S., BASEHEART, B. J. & WILLFORD, J. A. 2000. The effects of neonatal ethanol and/or cocaine exposure on isolation-induced ultrasonic vocalizations. *Pharmacol Biochem Behav,* 67**,** 1-9.

BARTON, P. J. & BUCKINGHAM, M. E. 1985. The myosin alkali light chain proteins and their genes. *Biochem J,* 231**,** 249-61.

BASAVARAJAPPA, B. 2015. Fetal alcohol spectrum disorder: potential role of endocannabinoids signaling. *Brain sciences,* 5**,** 456-493.

BERMAN, R. F. & HANNIGAN, J. H. 2000. Effects of prenatal alcohol exposure on the hippocampus: spatial behavior, electrophysiology, and neuroanatomy. *Hippocampus,* 10**,** 94-110.

BERRIDGE, K. C. & ROBINSON, T. E. 1998. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev,* 28**,** 309-69.

BERRUN, A., HARRIS, E. & STACHURA, D. L. 2018. Isthmin 1 (ism1) is required for normal hematopoiesis in developing zebrafish. *PLoS One,* 13**,** e0196872.

BERSON, A., BARBASH, S., SHALTIEL, G., GOLL, Y., HANIN, G., GREENBERG, D. S., KETZEF, M., BECKER, A. J., FRIEDMAN, A. & SOREQ, H. 2012. Cholinergic-associated loss of hnRNP-A/B in Alzheimer's disease impairs cortical splicing and cognitive function in mice. *EMBO Mol Med,* 4**,** 730-42.

BERTOS, N. R., WANG, A. H. & YANG, X. J. 2001. Class II histone deacetylases: structure, function, and regulation. *Biochem Cell Biol,* 79**,** 243-52.

BHATTACHARYA, D., DUNAWAY, E., BHATTACHARYA, S., BLOEMER, J., BUABEID, M., ESCOBAR, M., SUPPIRAMANIAM, V. & DHANASEKARAN, M. 2015. Impaired ILK function is associated with deficits in hippocampal based memory and synaptic plasticity in a FASD rat model. *PloS one,* 10**,** e0135700.

BICKNELL, L. S., PITT, J., AFTIMOS, S., RAMADAS, R., MAW, M. A. & ROBERTSON, S. P. 2008. A missense mutation in ALDH18A1, encoding

Delta1-pyrroline-5-carboxylate synthase (P5CS), causes an autosomal recessive neurocutaneous syndrome. *Eur J Hum Genet,* 16**,** 1176-86.

BINDER, D. K. & SCHARFMAN, H. E. 2004. Brain-derived neurotrophic factor. *Growth Factors,* 22**,** 123-31.

BLANCHARD, B. A., RILEY, E. P. & HANNIGAN, J. H. 1987. Deficits on a spatial navigation task following prenatal exposure to ethanol. *Neurotoxicol Teratol,* 9**,** 253-8.

BLUML, S., WISNOWSKI, J. L., NELSON, M. D., JR., PAQUETTE, L., GILLES, F. H., KINNEY, H. C. & PANIGRAHY, A. 2013. Metabolic maturation of the human brain from birth through adolescence: insights from in vivo magnetic resonance spectroscopy. *Cereb Cortex,* 23**,** 2944-55.

BOCKING, A. D., SINERVO, K. R., SMITH, G. N., CARMICHAEL, L., CHALLIS, J. R., OLSON, D. M. & BRIEN, J. F. 1993. Increased uteroplacental production of prostaglandin E2 during ethanol infusion. *Am J Physiol,* 265**,** R640-5.

BOJE, K. 2004. Nitric oxide neurotoxicity in neurodegenerative diseases. *Front Biosci,* 9**,** 763-776.

BOLGER, A. M., LOHSE, M. & USADEL, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics,* 30**,** 2114-2120.

BONTHIUS, D. J. & WEST, J. R. 1990. Alcohol-induced neuronal loss in developing rats: increased brain damage with binge exposure. *Alcohol Clin Exp Res,* 14**,** 107-18.

BORCK, G., MOLLA-HERMAN, A., BODDAERT, N., ENCHA-RAZAVI, F., PHILIPPE, A., ROBEL, L., DESGUERRE, I., BRUNELLE, F., BENMERAH, A., MUNNICH, A. & COLLEAUX, L. 2008. Clinical, cellular, and neuropathological consequences of AP1S2 mutations: further delineation of a recognizable X-linked mental retardation syndrome. *Hum Mutat,* 29**,** 966-74.

BOSCHEN, K. E., CRISS, K. J., PALAMARCHOUK, V., ROTH, T. L. & KLINTSOVA, A. Y. 2015. Effects of developmental alcohol exposure vs.

intubation stress on BDNF and TrkB expression in the hippocampus and frontal cortex of neonatal rats. *Int J Dev Neurosci,* 43**,** 16-24.

BOSCHEN, K. E., HAMILTON, G. F., DELORME, J. E. & KLINTSOVA, A. Y. 2014. Activity and social behavior in a complex environment in rats neonatally exposed to alcohol. *Alcohol,* 48**,** 533-41.

BOSCHEN, K. E. & KLINTSOVA, A. Y. 2017. Disruptions to hippocampal adult neurogenesis in rodent models of fetal alcohol spectrum disorders. *Neurogenesis,* 4**,** e1324259.

BOWLBY, J. 1969. 1982. Attachment and loss: vol. 1. Attachment. *New York Basic Books*.

BRIEN, J. F., CLARKE, D. W., RICHARDSON, B. & PATRICK, J. 1985. Disposition of ethanol in maternal blood, fetal blood, and amniotic fluid of third-trimester pregnant ewes. *Am J Obstet Gynecol,* 152**,** 583-90.

BROSE, N. 2008. For better or for worse: complexins regulate SNARE function and vesicle fusion. *Traffic,* 9**,** 1403-13.

BROWN, R. T., COLES, C. D., SMITH, I. E., PLATZMAN, K. A., SILVERSTEIN, J., ERICKSON, S. & FALEK, A. 1991. Effects of prenatal alcohol exposure at school age. II. Attention and behavior. *Neurotoxicol Teratol,* 13**,** 369-76.

BRUSHIA, R. J. & WALSH, D. A. 1999. Phosphorylase kinase: the complexity of its regulation is reflected in the complexity of its structure. *Front Biosci,* 4**,** D618-41.

BUKIYA, A. N. & DOPICO, A. M. 2018. Fetal Cerebral Circulation as Target of Maternal Alcohol Consumption. *Alcohol Clin Exp Res,* 42**,** 1006-1018.

BULAT, N., WAEBER, G. & WIDMANN, C. 2009. LDLs stimulate p38 MAPKs and wound healing through SR-BI independently of Ras and PI3 kinase. *J Lipid Res,* 50**,** 81-9.

BURD, L. 2016a. Fetal alcohol spectrum disorder: complexity from comorbidity. *The Lancet,* 387**,** 926-927.

BURD, L. 2016b. Fetal alcohol spectrum disorder: complexity from comorbidity. *Lancet,* 387**,** 926-927.

CALDWELL, K. K., SHEEMA, S., PAZ, R. D., SAMUDIO-RUIZ, S. L., LAUGHLIN, M. H., SPENCE, N. E., ROEHLK, M. J., ALCON, S. N. & ALLAN, A. M. 2008. Fetal alcohol spectrum disorder-associated depression: evidence for reductions in the levels of brain-derived neurotrophic factor in a mouse model. *Pharmacol Biochem Behav,* 90**,** 614-24.

CANANZI, S. G. & MAYHAN, W. G. 2019. In utero exposure to alcohol alters reactivity of cerebral arterioles. *J Cereb Blood Flow Metab,* 39**,** 332-341.

CAPRARA, D. L., BRIEN, J. F., IQBAL, U., REYNOLDS, J. N., KLEIN, J. & KOREN, G. 2005. A Guinea pig model for the identification of in utero alcohol exposure using fatty acid ethyl esters in neonatal hair. *Pediatr Res,* 58**,** 1158-63.

CAPUTO, C., WOOD, E. & JABBOUR, L. 2016a. Impact of fetal alcohol exposure on body systems: A systematic review. *Birth Defects Res C Embryo Today,* 108**,** 174-80.

CAPUTO, C., WOOD, E. & JABBOUR, L. 2016b. Impact of fetal alcohol exposure on body systems: A systematic review. *Birth Defects Research Part C: Embryo Today: Reviews,* 108**,** 174-180.

CERNAK, I., VINK, R., NATALE, J., STOICA, B., PAUL IV, M. L., MOVSESYAN, V., AHMED, F., KNOBLACH, S. M., FRICKE, S. T. & FADEN, A. I. 2004. The “dark side” of endocannabinoids: a neurotoxic role for anandamide. *Journal of Cerebral Blood Flow & Metabolism,* 24**,** 564-578.

CHANG, H. M., CHENG, J. C., KLAUSEN, C. & LEUNG, P. C. 2013. BMP15 suppresses progesterone production by down-regulating StAR via ALK3 in human granulosa cells. *Mol Endocrinol,* 27**,** 2093-104.

CHATER-DIEHL, E. J., LAUFER, B. I., CASTELLANI, C. A., ALBERRY, B. L. & SINGH, S. M. 2016. Alteration of gene expression, DNA methylation, and histone methylation in free radical scavenging networks in adult mouse hippocampus following fetal alcohol exposure. *PLoS One,* 11**,** e0154836.

CHATTERJEE, A., LEICHTER, A. L., FAN, V., TSAI, P., PURCELL, R. V., SULLIVAN, M. J. & ECCLES, M. R. 2015. A cross comparison of technologies for the detection of microRNAs in clinical FFPE samples of hepatoblastoma patients. *Sci Rep,* 5**,** 10438.

CHEN, J. S., DRISCOLL, C. D. & RILEY, E. P. 1982. Ontogeny of suckling behavior in rats prenatally exposed to alcohol. *Teratology,* 26**,** 145-53.

CHEN, L., ZHANG, J., FENG, Y., LI, R., SUN, X., DU, W., PIAO, X., WANG, H., YANG, D., SUN, Y., LI, X., JIANG, T., KANG, C., LI, Y. & JIANG, C. 2012. MiR-410 regulates MET to influence the proliferation and invasion of glioma. *Int J Biochem Cell Biol,* 44**,** 1711-7.

CHEN, L. N., WANG, Y., MA, D. L. & CHEN, Y. Y. 2006. Short interfering RNA against the PDCD5 attenuates cell apoptosis and caspase-3 activity induced by Bax overexpression. *Apoptosis,* 11**,** 101-11.

CHEN, W. J. & LIEM, R. K. 1994. Reexpression of glial fibrillary acidic protein rescues the ability of astrocytoma cells to form processes in response to neurons. *J Cell Biol,* 127**,** 813-23.

CHENG, A. X., LOU, S. Q., ZHOU, H. W., WANG, Y. & MA, D. L. 2004. Expression of PDCD5, a novel apoptosis related protein, in human osteoarthritic cartilage. *Acta Pharmacol Sin,* 25**,** 685-90.

CHENG, C. M., REINHARDT, R. R., LEE, W. H., JONCAS, G., PATEL, S. C. & BONDY, C. A. 2000. Insulin-like growth factor 1 regulates developing brain glucose metabolism. *Proc Natl Acad Sci U S A,* 97**,** 10236-41.

CHO, S. J., LOVINGER, D. M. & N'GOUEMO, P. 2017. Prenatal alcohol exposure enhances the susceptibility to NMDA-induced generalized tonic-clonic seizures in developing rats. *CNS Neurosci Ther,* 23**,** 808-817.

CHOI, D. K., ITO, T., MITSUI, Y. & SAKAKI, Y. 1998. Fluorescent differential display analysis of gene expression in apoptotic neuroblastoma cells. *Gene,* 223**,** 21-31.

CHUNG, Y. M., KIM, J. S. & YOO, Y. D. 2006. A novel protein, Romo1, induces ROS production in the mitochondria. *Biochem Biophys Res Commun,* 347**,** 649-55.

CLARO DA SILVA, T., POLLI, J. E. & SWAAN, P. W. 2013. The solute carrier family 10 (SLC10): beyond bile acid transport. *Mol Aspects Med,* 34**,** 252-69.

CLARREN, S. K., ASTLEY, S. J., GUNDERSON, V. M. & SPELLMAN, D. 1992. Cognitive and behavioral deficits in nonhuman primates associated with very early embryonic binge exposures to ethanol. *J Pediatr,* 121**,** 789-96.

COLASANTI, M. & SUZUKI, H. 2000. The dual personality of NO. *Trends in pharmacological sciences,* 21**,** 249-252.

COLEMAN, L. G., JR., OGUZ, I., LEE, J., STYNER, M. & CREWS, F. T. 2012. Postnatal day 7 ethanol treatment causes persistent reductions in adult mouse brain volume and cortical neurons with sex specific effects on neurogenesis. *Alcohol,* 46**,** 603-12.

COLES, C. D. & PLATZMAN, K. A. 1993. Behavioral development in children prenatally exposed to drugs and alcohol. *Int J Addict,* 28**,** 1393-433.

COLLINS, S. J. 2002. The role of retinoids and retinoic acid receptors in normal hematopoiesis. *Leukemia,* 16**,** 1896-905.

CONVERSE, A. K., MOORE, C. F., HOLDEN, J. E., AHLERS, E. O., MOIRANO, J. M., LARSON, J. A., RESCH, L. M., DEJESUS, O. T., BARNHART, T. E., NICKLES, R. J., MURALI, D., CHRISTIAN, B. T. & SCHNEIDER, M. L. 2014. Moderate-level prenatal alcohol exposure induces sex differences in dopamine d1 receptor binding in adult rhesus monkeys. *Alcohol Clin Exp Res,* 38**,** 2934-43.

CORTESE, B. M., KRAHL, S. E., BERMAN, R. F. & HANNIGAN, J. H. 1997. Effects of prenatal ethanol exposure on hippocampal theta activity in the rat. *Alcohol,* 14**,** 231-5.

CORTESE, B. M., MOORE, G. J., BAILEY, B. A., JACOBSON, S. W., DELANEY-BLACK, V. & HANNIGAN, J. H. 2006. Magnetic resonance and spectroscopic imaging in prenatal alcohol-exposed children: preliminary findings in the caudate nucleus. *Neurotoxicol Teratol,* 28**,** 597-606.

COX, B. M. 1982. Endogenous opioid peptides: a guide to structures and terminology. *Life Sci,* 31**,** 1645-58.

CREIGHTON-TAYLOR, J. A. & RUDEEN, P. K. 1991. Prenatal ethanol exposure and opiatergic influence on puberty in the female rat. *Alcohol,* 8**,** 187-91.

CUDD, T. A. 2005. Animal model systems for the study of alcohol teratology. *Exp Biol Med (Maywood),* 230**,** 389-93.

CUDD, T. A., CHEN, W. J., PARNELL, S. E. & WEST, J. R. 2001a. Third trimester binge ethanol exposure results in fetal hypercapnea and acidemia but not hypoxemia in pregnant sheep. *Alcohol Clin Exp Res,* 25**,** 269-76.

CUDD, T. A., CHEN, W. J. & WEST, J. R. 2001b. Fetal and maternal sheep hypothalamus pituitary adrenal axis responses to chronic binge ethanol exposure during the third trimester equivalent. *Alcohol Clin Exp Res,* 25**,** 1065-71.

CUDD, T. A., CHEN, W. J. & WEST, J. R. 2002. Fetal and maternal thyroid hormone responses to ethanol exposure during the third trimester equivalent of gestation in sheep. *Alcohol Clin Exp Res,* 26**,** 53-8.

CUI, J. Y., CHOUDHURI, S., KNIGHT, T. R. & KLAASSEN, C. D. 2010. Genetic and epigenetic regulation and expression signatures of glutathione S-transferases in developing mouse liver. *Toxicol Sci,* 116**,** 32-43.

CUNHA-FERREIRA, I., CHAZEAU, A., BUIJS, R. R., STUCCHI, R., WILL, L., PAN, X., ADOLFS, Y., VAN DER MEER, C., WOLTHUIS, J. C., KAHN, O. I., SCHATZLE, P., ALTELAAR, M., PASTERKAMP, R. J., KAPITEIN, L. C. &

HOOGENRAAD, C. C. 2018. The HAUS Complex Is a Key Regulator of Non-centrosomal Microtubule Organization during Neuronal Development. *Cell Rep,* 24**,** 791-800.

CUZON, V. C., YEH, P. W., YANAGAWA, Y., OBATA, K. & YEH, H. H. 2008. Ethanol consumption during early pregnancy alters the disposition of tangentially migrating GABAergic interneurons in the fetal cortex. *J Neurosci,* 28**,** 1854-64.

DAHLGREN, I. L., MATUSZCZYK, J. V. & HARD, E. 1991. Sexual orientation in male rats prenatally exposed to ethanol. *Neurotoxicol Teratol,* 13**,** 267-9.

DALL'ASTA, V., BUSSOLATI, O., SALA, R., PAROLARI, A., ALAMANNI, F., BIGLIOLI, P. & GAZZOLA, G. C. 1999. Amino acids are compatible osmolytes for volume recovery after hypertonic shrinkage in vascular endothelial cells. *American Journal of Physiology-Cell Physiology,* 276**,** C865-C872.

DASARI, S., GONUGUNTLA, S., GANJAYI, M. S., BUKKE, S., SREENIVASULU, B. & MERIGA, B. 2018. Genetic polymorphism of glutathione S-transferases: Relevance to neurological disorders. *Pathophysiology*.

DAVIES, D. L. & SMITH, D. E. 1981. A Golgi study of mouse hippocampal CA1 pyramidal neurons following perinatal ethanol exposure. *Neurosci Lett,* 26**,** 49-54.

DAVIS-ANDERSON, K. L., WESSELING, H., SIEBERT, L. M., LUNDE-YOUNG, E. R., NAIK, V. D., STEEN, H. & RAMADOSS, J. 2018a. Fetal regional brain protein signature in FASD rat model. *Reproductive Toxicology,* 76**,** 84-92.

DAVIS-ANDERSON, K. L., WESSELING, H., SIEBERT, L. M., LUNDE-YOUNG, E. R., NAIK, V. D., STEEN, H. & RAMADOSS, J. 2018b. Fetal regional brain protein signature in FASD rat model. *Reprod Toxicol,* 76**,** 84-92.

DELWING, D., DELWING, D., CHIARANI, F., KUREK, A. G. & WYSE, A. T. 2007. Proline reduces brain cytochrome c oxidase: prevention by antioxidants. *International journal of developmental neuroscience,* 25**,** 17-22.

DEMOURES, B., SIEGFRIED, G. & KHATIB, A.-M. 2018. PCSK1 (proprotein convertase subtilisin/kexin type 1). *Atlas of Genetics and Cytogenetics in Oncology and Haematology*.

DEXTER, J. D., TUMBLESON, M. E., HUTCHESON, D. P. & MIDDLETON, C. C. 1976. Sinclair(S-1) miniature swine as a model for the study of human alcoholism. *Ann N Y Acad Sci,* 273**,** 188-93.

DIAO, S., YANG, D. M., DONG, R., WANG, L. P., WANG, J. S., DU, J., WANG, S. L. & FAN, Z. 2015. Enriched trimethylation of lysine 4 of histone H3 of WDR63 enhanced osteogenic differentiation potentials of stem cells from apical papilla. *J Endod,* 41**,** 205-11.

DIDANGELOS, A., YIN, X., MANDAL, K., BAUMERT, M., JAHANGIRI, M. & MAYR, M. 2010. Proteomics characterization of extracellular space components in the human aorta. *Mol Cell Proteomics,* 9**,** 2048-62.

DIWADKAR, V. A., MEINTJES, E. M., GORADIA, D., DODGE, N. C., WARTON, C., MOLTENO, C. D., JACOBSON, S. W. & JACOBSON, J. L. 2013. Differences in cortico-striatal-cerebellar activation during working memory in syndromal and nonsyndromal children with prenatal alcohol exposure. *Hum Brain Mapp,* 34**,** 1931-45.

DIXIT, H., RAO, L. K., PADMALATHA, V. V., KANAKAVALLI, M., DEENADAYAL, M., GUPTA, N., CHAKRABARTY, B. & SINGH, L. 2006. Missense mutations in the BMP15 gene are associated with ovarian failure. *Hum Genet,* 119**,** 408-15.

DOBBING, J. & SANDS, J. 1979. Comparative aspects of the brain growth spurt. *Early Hum Dev,* 3**,** 79-83.

DONALD, K. A., EASTMAN, E., HOWELLS, F. M., ADNAMS, C., RILEY, E. P., WOODS, R. P., NARR, K. L. & STEIN, D. J. 2015. Neuroimaging effects of prenatal alcohol exposure on the developing human brain: a magnetic resonance imaging review. *Acta Neuropsychiatr,* 27**,** 251-69.

DORAN, J., WALTERS, C., KYLE, V., WOODING, P., HAMMETT-BURKE, R. & COLLEDGE, W. H. 2016. Mfsd14a (Hiat1) gene disruption causes globozoospermia and infertility in male mice. *Reproduction,* 152**,** 91-9.

DU PLESSIS, L., JACOBSON, J. L., JACOBSON, S. W., HESS, A. T., VAN DER KOUWE, A., AVISON, M. J., MOLTENO, C. D., STANTON, M. E., STANLEY, J. A., PETERSON, B. S. & MEINTJES, E. M. 2014. An in vivo 1H magnetic resonance spectroscopy study of the deep cerebellar nuclei in children with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res,* 38**,** 1330-8.

DUDEK, J., SKOCIC, J., SHEARD, E. & ROVET, J. 2014. Hippocampal abnormalities in youth with alcohol-related neurodevelopmental disorder. *Journal of the International Neuropsychological Society,* 20**,** 181-191.

DUNCAN, A. J. & HEALES, S. J. 2005. Nitric oxide and neurological disorders. *Molecular aspects of medicine,* 26**,** 67-96.

DUPE, V., MATT, N., GARNIER, J. M., CHAMBON, P., MARK, M. & GHYSELINCK, N. B. 2003. A newborn lethal defect due to inactivation of retinaldehyde dehydrogenase type 3 is prevented by maternal retinoic acid treatment. *Proc Natl Acad Sci U S A,* 100**,** 14036-41.

DŽOLJIĆ, E., GRABATINIĆ, I. & KOSTIĆ, V. 2015. Why is nitric oxide important for our brain? *Functional neurology,* 30**,** 159.

ELTON, R. H. & WILSON, M. E. 1977. Changes in ethanol consumption by pregnant pigtailed macaques. *J Stud Alcohol,* 38**,** 2181-3.

ESTEVE, P. O., CHIN, H. G., BENNER, J., FEEHERY, G. R., SAMARANAYAKE, M., HORWITZ, G. A., JACOBSEN, S. E. & PRADHAN, S. 2009. Regulation of DNMT1 stability through SET7-mediated lysine methylation in mammalian cells. *Proc Natl Acad Sci U S A,* 106**,** 5076-81.

ETHEN, M. K., RAMADHANI, T. A., SCHEUERLE, A. E., CANFIELD, M. A., WYSZYNSKI, D. F., DRUSCHEL, C. M. & ROMITTI, P. A. 2009a. Alcohol consumption by women before and during pregnancy. *Maternal and child health journal,* 13**,** 274-285.

ETHEN, M. K., RAMADHANI, T. A., SCHEUERLE, A. E., CANFIELD, M. A., WYSZYNSKI, D. F., DRUSCHEL, C. M., ROMITTI, P. A. & NATIONAL BIRTH DEFECTS PREVENTION, S. 2009b. Alcohol consumption by women before and during pregnancy. *Matern Child Health J,* 13**,** 274-85.

EVANS, J. F. 2002. Cysteinyl leukotriene receptors. *Prostaglandins Other Lipid Mediat,* 68-69**,** 587-97.

EVERTS, H. B., KING, L. E., JR., SUNDBERG, J. P. & ONG, D. E. 2004. Hair cycle-specific immunolocalization of retinoic acid synthesizing enzymes Aldh1a2 and Aldh1a3 indicate complex regulation. *J Invest Dermatol,* 123**,** 258-63.

FAGERLUND, A., HEIKKINEN, S., AUTTI-RAMO, I., KORKMAN, M., TIMONEN, M., KUUSI, T., RILEY, E. P. & LUNDBOM, N. 2006. Brain metabolic alterations in adolescents and young adults with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res,* 30**,** 2097-104.

FANG, M., JAFFREY, S. R., SAWA, A., YE, K., LUO, X. & SNYDER, S. H. 2000. Dexras1: a G protein specifically coupled to neuronal nitric oxide synthase via CAPON. *Neuron,* 28**,** 183-93.

FANZANI, A., COLOMBO, F., GIULIANI, R., PRETI, A. & MARCHESINI, S. 2004. Cytosolic sialidase Neu2 upregulation during PC12 cells differentiation. *FEBS Lett,* 566**,** 178-82.

FANZANI, A., ZANOLA, A., FAGGI, F., PAPINI, N., VENERANDO, B., TETTAMANTI, G., SAMPAOLESI, M. & MONTI, E. 2012. Implications for the mammalian sialidases in the physiopathology of skeletal muscle. *Skelet Muscle,* 2**,** 23.

FARHAN, S. M., WANG, J., ROBINSON, J. F., LAHIRY, P., SIU, V. M., PRASAD, C., KRONICK, J. B., RAMSAY, D. A., RUPAR, C. A. & HEGELE, R. A. 2014. Exome sequencing identifies NFS1 deficiency in a novel Fe-S cluster disease, infantile mitochondrial complex II/III deficiency. *Mol Genet Genomic Med,* 2**,** 73-80.

FEIL, R. & KLEPPISCH, T. 2008. NO/cGMP-dependent modulation of synaptic transmission. *Pharmacology of neurotransmitter release.* Springer.

FERNANDEZ, J. P., MORENO-MATEOS, M. A., GOHR, A., MIAO, L., CHAN, S. H., IRIMIA, M. & GIRALDEZ, A. J. 2018. RES complex is associated with intron definition and required for zebrafish early embryogenesis. *PLoS Genet,* 14**,** e1007473.

FEY, A., EICHLER, S., FLAVIER, S., CHRISTEN, R., HOFLE, M. G. & GUZMAN, C. A. 2004. Establishment of a real-time PCR-based approach for accurate quantification of bacterial RNA targets in water, using Salmonella as a model organism. *Appl Environ Microbiol,* 70**,** 3618-23.

FIETZ, D., BAKHAUS, K., WAPELHORST, B., GROSSER, G., GUNTHER, S., ALBER, J., DORING, B., KLIESCH, S., WEIDNER, W., GALUSKA, C. E., HARTMANN, M. F., WUDY, S. A., BERGMANN, M. & GEYER, J. 2013. Membrane transporters for sulfated steroids in the human testis--cellular localization, expression pattern and functional analysis. *PLoS One,* 8**,** e62638.

FISCHER, B., CALLEWAERT, B., SCHROTER, P., COUCKE, P. J., SCHLACK, C., OTT, C. E., MORRONI, M., HOMANN, W., MUNDLOS, S., MORAVA, E., FICCADENTI, A. & KORNAK, U. 2014. Severe congenital cutis laxa with cardiovascular manifestations due to homozygous deletions in ALDH18A1. *Mol Genet Metab,* 112**,** 310-6.

FISH, E. W., HOLLOWAY, H. T., RUMPLE, A., BAKER, L. K., WIECZOREK, L. A., MOY, S. S., PANIAGUA, B. & PARNELL, S. E. 2016. Acute alcohol exposure during neurulation: Behavioral and brain structural consequences in adolescent C57BL/6J mice. *Behav Brain Res,* 311**,** 70-80.

FLADEBY, C., SKAR, R. & SERCK-HANSSEN, G. 2003. Distinct regulation of glucose transport and GLUT1/GLUT3 transporters by glucose deprivation and IGF-I in chromaffin cells. *Biochim Biophys Acta,* 1593**,** 201-8.

FOLLI, C., CALDERONE, V., OTTONELLO, S., BOLCHI, A., ZANOTTI, G., STOPPINI, M. & BERNI, R. 2001. Identification, retinoid binding, and x-ray analysis of a human retinol-binding protein. *Proc Natl Acad Sci U S A,* 98**,** 3710-5.

FONTAINE, C. J., PATTEN, A. R., SICKMANN, H. M., HELFER, J. L. & CHRISTIE, B. R. 2016. Effects of pre-natal alcohol exposure on hippocampal synaptic

plasticity: sex, age and methodological considerations. *Neuroscience & Biobehavioral Reviews,* 64**,** 12-34.

FORGET, D., LACOMBE, A. A., CLOUTIER, P., LAVALLEE-ADAM, M., BLANCHETTE, M. & COULOMBE, B. 2013. Nuclear import of RNA polymerase II is coupled with nucleocytoplasmic shuttling of the RNA polymerase II-associated protein 2. *Nucleic Acids Res,* 41**,** 6881-91.

FOSTER, H. A., CUI, M., NAVEENATHAYALAN, A., UNDEN, H., SCHWANBECK, R. & HOFKEN, T. 2013. The zinc cluster protein Sut1 contributes to filamentation in Saccharomyces cerevisiae. *Eukaryot Cell,* 12**,** 244-53.

FRICKER, L. D., MCKINZIE, A. A., SUN, J., CURRAN, E., QIAN, Y., YAN, L., PATTERSON, S. D., COURCHESNE, P. L., RICHARDS, B., LEVIN, N., MZHAVIA, N., DEVI, L. A. & DOUGLASS, J. 2000. Identification and characterization of proSAAS, a granin-like neuroendocrine peptide precursor that inhibits prohormone processing. *J Neurosci,* 20**,** 639-48.

FUJII, M., LYAKH, L. A., BRACKEN, C. P., FUKUOKA, J., HAYAKAWA, M., TSUKIYAMA, T., SOLL, S. J., HARRIS, M., ROCHA, S., ROCHE, K. C., TOMINAGA, S. I., JEN, J., PERKINS, N. D., LECHLEIDER, R. J. & ROBERTS, A. B. 2006. SNIP1 is a candidate modifier of the transcriptional activity of c-Myc on E box-dependent target genes. *Mol Cell,* 24**,** 771-783.

FURTADO, E. F. & RORIZ, S. T. 2016. Inattention and impulsivity associated with prenatal alcohol exposure in a prospective cohort study with 11-years-old Brazilian children. *Eur Child Adolesc Psychiatry,* 25**,** 1327-1335.

GABRIEL, K. I., GLAVAS, M. M., ELLIS, L. & WEINBERG, J. 2005. Postnatal handling does not normalize hypothalamic corticotropin-releasing factor mRNA levels in animals prenatally exposed to ethanol. *Brain Res Dev Brain Res,* 157**,** 74-82.

GANDRE-BABBE, S. & VAN DER BLIEK, A. M. 2008. The novel tail-anchored membrane protein Mff controls mitochondrial and peroxisomal fission in mammalian cells. *Mol Biol Cell,* 19**,** 2402-12.

GANGISETTY, O., WYNNE, O., JABBAR, S., NASELLO, C. & SARKAR, D. K. 2015. Fetal Alcohol Exposure Reduces Dopamine Receptor D2 and Increases Pituitary Weight and Prolactin Production via Epigenetic Mechanisms. *PLoS One,* 10**,** e0140699.

GARATTINI, E., MENDEL, R., ROMÃO, M. J., WRIGHT, R. & TERAO, M. 2003. Mammalian molybdo-flavoenzymes, an expanding family of proteins: structure, genetics, regulation, function and pathophysiology. *Biochemical Journal,* 372**,** 15-32.

GAUDET, P., LIVSTONE, M. S., LEWIS, S. E. & THOMAS, P. D. 2011. Phylogenetic-based propagation of functional annotations within the Gene Ontology consortium. *Brief Bioinform,* 12**,** 449-62.

GEISS, G. K., BUMGARNER, R. E., BIRDITT, B., DAHL, T., DOWIDAR, N., DUNAWAY, D. L., FELL, H. P., FERREE, S., GEORGE, R. D., GROGAN, T., JAMES, J. J., MAYSURIA, M., MITTON, J. D., OLIVERI, P., OSBORN, J. L., PENG, T., RATCLIFFE, A. L., WEBSTER, P. J., DAVIDSON, E. H., HOOD, L. & DIMITROV, K. 2008. Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat Biotechnol,* 26**,** 317-25.

GEYER, J., DORING, B., MEERKAMP, K., UGELE, B., BAKHIYA, N., FERNANDES, C. F., GODOY, J. R., GLATT, H. & PETZINGER, E. 2007. Cloning and functional characterization of human sodium-dependent organic anion transporter (SLC10A6). *J Biol Chem,* 282**,** 19728-41.

GIANG, D. K. & CRAVATT, B. F. 1997. Molecular characterization of human and mouse fatty acid amide hydrolases. *Proc Natl Acad Sci U S A,* 94**,** 2238-42.

GIL-MOHAPEL, J., BOEHME, F., PATTEN, A., COX, A., KAINER, L., GILES, E., BROCARDO, P. S. & CHRISTIE, B. R. 2011. Altered adult hippocampal neuronal maturation in a rat model of fetal alcohol syndrome. *Brain research,* 1384**,** 29-41.

GILLILAND, G., PERRIN, S., BLANCHARD, K. & BUNN, H. F. 1990. Analysis of cytokine mRNA and DNA: detection and quantitation by competitive polymerase chain reaction. *Proc Natl Acad Sci U S A,* 87**,** 2725-9.

GIRARD, J. P., BAEKKEVOLD, E. S., FELIU, J., BRANDTZAEG, P. & AMALRIC, F. 1999. Molecular cloning and functional analysis of SUT-1, a sulfate transporter from human high endothelial venules. *Proc Natl Acad Sci U S A,* 96**,** 12772-7.

GLASS, L., MOORE, E. M., AKSHOOMOFF, N., JONES, K. L., RILEY, E. P. & MATTSON, S. N. 2017. Academic Difficulties in Children with Prenatal Alcohol Exposure: Presence, Profile, and Neural Correlates. *Alcohol Clin Exp Res,* 41**,** 1024-1034.

GLEASON, C. A. & HOTCHKISS, K. J. 1992. Cerebral responses to acute maternal alcohol intoxication in immature fetal sheep. *Pediatr Res,* 31**,** 645-8.

GLEASON, C. A., IIDA, H., HOTCHKISS, K. J., NORTHINGTON, F. J. & TRAYSTMAN, R. J. 1997. Newborn cerebrovascular responses after first trimester moderate maternal ethanol exposure in sheep. *Pediatr Res,* 42**,** 39-45.

GOBRON, S., CREVEAUX, I., MEINIEL, R., DIDIER, R., DASTUGUE, B. & MEINIEL, A. 1999. SCO-spondin is evolutionarily conserved in the central nervous system of the chordate phylum. *Neuroscience,* 88**,** 655-64.

GOLDSCHMIDT, L., RICHARDSON, G. A., STOFFER, D. S., GEVA, D. & DAY, N. L. 1996. Prenatal alcohol exposure and academic achievement at age six: a nonlinear fit. *Alcohol Clin Exp Res,* 20**,** 763-70.

GOODLETT, C. R., KELLY, S. J. & WEST, J. R. 1987. Early postnatal alcohol exposure that produces high blood alcohol levels impairs development of spatial navigation learning. *Psychobiology,* 15**,** 64-74.

GOODLETT, C. R., MARCUSSEN, B. L. & WEST, J. R. 1990. A single day of alcohol exposure during the brain growth spurt induces brain weight restriction and cerebellar Purkinje cell loss. *Alcohol,* 7**,** 107-14.

GRALL, A., GUAGUERE, E., PLANCHAIS, S., GROND, S., BOURRAT, E., HAUSSER, I., HITTE, C., LE GALLO, M., DERBOIS, C., KIM, G. J., LAGOUTTE, L., DEGORCE-RUBIALES, F., RADNER, F. P., THOMAS, A., KURY, S., BENSIGNOR, E., FONTAINE, J., PIN, D., ZIMMERMANN, R., ZECHNER, R., LATHROP, M., GALIBERT, F., ANDRE, C. & FISCHER, J.

2012. PNPLA1 mutations cause autosomal recessive congenital ichthyosis in golden retriever dogs and humans. *Nat Genet,* 44**,** 140-7.

GREENE, P. L., DIAZ-GRANADOS, J. L. & AMSEL, A. 1992. Blood ethanol concentration from early postnatal exposure: effects on memory-based learning and hippocampal neuroanatomy in infant and adult rats. *Behav Neurosci,* 106**,** 51-61.

GREENMYER, J. R., KLUG, M. G., KAMBEITZ, C., POPOVA, S. & BURD, L. 2018. A Multicountry Updated Assessment of the Economic Impact of Fetal Alcohol Spectrum Disorder: Costs for Children and Adults. *J Addict Med,* 12**,** 466-473.

GREINER, E. F., KIRFEL, J., GRESCHIK, H., DORFLINGER, U., BECKER, P., MERCEP, A. & SCHULE, R. 1996. Functional analysis of retinoid Z receptor beta, a brain-specific nuclear orphan receptor. *Proc Natl Acad Sci U S A,* 93**,** 10105-10.

GRUMMER, M. A. & ZACHMAN, R. D. 1995. Prenatal ethanol consumption alters the expression of cellular retinol binding protein and retinoic acid receptor mRNA in fetal rat embryo and brain. *Alcohol Clin Exp Res,* 19**,** 1376-81.

GUNDERSON, V. M., GRANT-WEBSTER, K. S. & SACKETT, G. P. 1989. Deficits in visual recognition in low birth weight infant pigtailed monkeys (Macaca nemestrina). *Child Dev,* 60**,** 119-27.

HAEGGSTROM, J. Z. & FUNK, C. D. 2011. Lipoxygenase and leukotriene pathways: biochemistry, biology, and roles in disease. *Chem Rev,* 111**,** 5866-98.

HAGERTY, S. L., BIDWELL, L. C., HARLAAR, N. & HUTCHISON, K. E. 2016. An exploratory association study of alcohol use disorder and DNA methylation. *Alcoholism: Clinical and Experimental Research,* 40**,** 1633-1640.

HAMILTON, D. A., BARTO, D., RODRIGUEZ, C. I., MAGCALAS, C. M., FINK, B. C., RICE, J. P., BIRD, C. W., DAVIES, S. & SAVAGE, D. D. 2014. Effects of moderate prenatal ethanol exposure and age on social behavior, spatial response perseveration errors and motor behavior. *Behav Brain Res,* 269**,** 44-54.

HAMILTON, D. A., KODITUWAKKU, P., SUTHERLAND, R. J. & SAVAGE, D. D. 2003. Children with Fetal Alcohol Syndrome are impaired at place learning but not cued-navigation in a virtual Morris water task. *Behavioural brain research,* 143**,** 85-94.

HARD, E., DAHLGREN, I. L., ENGEL, J., LARSSON, K., LILJEQUIST, S., LINDH, A. S. & MUSI, B. 1984. Development of sexual behavior in prenatally ethanol-exposed rats. *Drug Alcohol Depend,* 14**,** 51-61.

HARD, E., MUSI, B., DAHLGREN, I. L., ENGEL, J., LARSSON, K., LILJEQUIST, S. & LINDH, A. S. 1985. Impaired maternal behaviour and altered central serotonergic activity in the adult offspring of chronically ethanol treated dams. *Acta Pharmacol Toxicol (Copenh),* 56**,** 347-53.

HARD, M. L., ABDOLELL, M., ROBINSON, B. H. & KOREN, G. 2005. Gene-expression analysis after alcohol exposure in the developing mouse. *J Lab Clin Med,* 145**,** 47-54.

HARLOW, H. F. 1959. The development of learning in the rhesus monkey. *American Scientist*.

HAYASHI, R. 2014. Chapter 52 - Gene Expression and the Impact of Antioxidant Supplements in the Cataractous Lens. *In:* PREEDY, V. R. (ed.) *Handbook of Nutrition, Diet and the Eye.* San Diego: Academic Press.

HEALTH, U. D. O. & SERVICES, H. 1981. Surgeon General's advisory on alcohol and pregnancy. *FDA Drug Bulletin,* 11**,** 9-10.

HEALTH, U. D. O. & SERVICES, H. 2005. US Surgeon General releases advisory on alcohol use in pregnancy. *Washington, DC: DHHS*.

HENRAS, A. K., PLISSON-CHASTANG, C., O'DONOHUE, M. F., CHAKRABORTY, A. & GLEIZES, P. E. 2015. An overview of pre-ribosomal RNA processing in eukaryotes. *Wiley Interdiscip Rev RNA,* 6**,** 225-42.

HEON, E., KIM, G., QIN, S., GARRISON, J. E., TAVARES, E., VINCENT, A., NUANGCHAMNONG, N., SCOTT, C. A., SLUSARSKI, D. C. & SHEFFIELD,

V. C. 2016. Mutations in C8ORF37 cause Bardet Biedl syndrome (BBS21). *Hum Mol Genet,* 25**,** 2283-2294.

HERRERA, M. B., FONSATO, V., GATTI, S., DEREGIBUS, M. C., SORDI, A., CANTARELLA, D., CALOGERO, R., BUSSOLATI, B., TETTA, C. & CAMUSSI, G. 2010. Human liver stem cell-derived microvesicles accelerate hepatic regeneration in hepatectomized rats. *J Cell Mol Med,* 14**,** 1605-18.

HERVOUET, E., PEIXOTO, P., DELAGE-MOURROUX, R., BOYER-GUITTAUT, M. & CARTRON, P. F. 2018. Specific or not specific recruitment of DNMTs for DNA methylation, an epigenetic dilemma. *Clin Epigenetics,* 10**,** 17.

HIRABAYASHI, T., ANJO, T., KANEKO, A., SENOO, Y., SHIBATA, A., TAKAMA, H., YOKOYAMA, K., NISHITO, Y., ONO, T., TAYA, C., MURAMATSU, K., FUKAMI, K., MUNOZ-GARCIA, A., BRASH, A. R., IKEDA, K., ARITA, M., AKIYAMA, M. & MURAKAMI, M. 2017. PNPLA1 has a crucial role in skin barrier function by directing acylceramide biosynthesis. *Nat Commun,* 8**,** 14609.

HIRABAYASHI, T., MURAKAMI, M. & KIHARA, A. 2019. The role of PNPLA1 in omega-O-acylceramide synthesis and skin barrier function. *Biochim Biophys Acta Mol Cell Biol Lipids,* 1864**,** 869-879.

HIRANO, Y., ISHII, K., KUMETA, M., FURUKAWA, K., TAKEYASU, K. & HORIGOME, T. 2009. Proteomic and targeted analytical identification of BXDC1 and EBNA1BP2 as dynamic scaffold proteins in the nucleolus. *Genes Cells,* 14**,** 155-66.

HOFER, R. & BURD, L. 2009. Review of published studies of kidney, liver, and gastrointestinal birth defects in fetal alcohol spectrum disorders. *Birth Defects Research Part A: Clinical and Molecular Teratology,* 85**,** 179-183.

HOFFMANN, H. & SCHIENE-FISCHER, C. 2014. Functional aspects of extracellular cyclophilins. *Biol Chem,* 395**,** 721-35.

HOFMEISTER, W., PETTERSSON, M., KURTOGLU, D., ARMENIO, M., EISFELDT, J., PAPADOGIANNAKIS, N., GUSTAVSSON, P. & LINDSTRAND, A. 2018. Targeted copy number screening highlights an intragenic deletion of WDR63 as the likely cause of human occipital

encephalocele and abnormal CNS development in zebrafish. *Hum Mutat,* 39**,** 495-505.

HOKAMA, M., OKA, S., LEON, J., NINOMIYA, T., HONDA, H., SASAKI, K., IWAKI, T., OHARA, T., SASAKI, T. & LAFERLA, F. M. 2013. Altered expression of diabetes-related genes in Alzheimer's disease brains: the Hisayama study. *Cerebral cortex,* 24**,** 2476-2488.

HOOGENRAAD, N., TOTINO, N., ELMER, H., WRAIGHT, C., ALEWOOD, P. & JOHNS, R. B. 1985. Inhibition of intestinal citrulline synthesis causes severe growth retardation in rats. *American Journal of Physiology-Gastrointestinal and Liver Physiology,* 249**,** G792-G799.

HOWELL, K. K., LYNCH, M. E., PLATZMAN, K. A., SMITH, G. H. & COLES, C. D. 2006. Prenatal alcohol exposure and ability, academic achievement, and school functioning in adolescence: a longitudinal follow-up. *J Pediatr Psychol,* 31**,** 116-26.

HOYME, H. E., KALBERG, W. O., ELLIOTT, A. J., BLANKENSHIP, J., BUCKLEY, D., MARAIS, A. S., MANNING, M. A., ROBINSON, L. K., ADAM, M. P., ABDUL-RAHMAN, O., JEWETT, T., COLES, C. D., CHAMBERS, C., JONES, K. L., ADNAMS, C. M., SHAH, P. E., RILEY, E. P., CHARNESS, M. E., WARREN, K. R. & MAY, P. A. 2016. Updated Clinical Guidelines for Diagnosing Fetal Alcohol Spectrum Disorders. *Pediatrics,* 138.

HU, C. A., KHALIL, S., ZHAORIGETU, S., LIU, Z., TYLER, M., WAN, G. & VALLE, D. 2008. Human Delta1-pyrroline-5-carboxylate synthase: function and regulation. *Amino Acids,* 35**,** 665-72.

HUO, J., XU, S., GUO, K., ZENG, Q. & LAM, K. P. 2009. Genetic deletion of faim reveals its role in modulating c-FLIP expression during CD95-mediated apoptosis of lymphocytes and hepatocytes. *Cell Death Differ,* 16**,** 1062-70.

HURD, P. J. & NELSON, C. J. 2009. Advantages of next-generation sequencing versus the microarray in epigenetic research. *Briefings in Functional Genomics,* 8**,** 174-183.

HUUSKONEN, P., AMEZAGA, M. R., BELLINGHAM, M., JONES, L. H., STORVIK, M., HAKKINEN, M., KESKI-NISULA, L., HEINONEN, S., O'SHAUGHNESSY, P. J., FOWLER, P. A. & PASANEN, M. 2016. The human placental proteome is affected by maternal smoking. *Reprod Toxicol,* 63**,** 22-31.

IGNATOVA, Z. & GIERASCH, L. M. 2006. Inhibition of protein aggregation in vitro and in vivo by a natural osmoprotectant. *Proceedings of the National Academy of Sciences,* 103**,** 13357-13361.

IKONOMIDOU, C., BITTIGAU, P., ISHIMARU, M. J., WOZNIAK, D. F., KOCH, C., GENZ, K., PRICE, M. T., STEFOVSKA, V., HORSTER, F., TENKOVA, T., DIKRANIAN, K. & OLNEY, J. W. 2000. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science,* 287**,** 1056-60.

JALURIA, P., KONSTANTOPOULOS, K., BETENBAUGH, M. & SHILOACH, J. 2007. A perspective on microarrays: current applications, pitfalls, and potential uses. *Microb Cell Fact,* 6**,** 4.

JI, P., SMITH, S. M., WANG, Y., JIANG, R., SONG, S. W., LI, B., SAWAYA, R., BRUNER, J. M., KUANG, J., YU, H., FULLER, G. N. & ZHANG, W. 2010. Inhibition of gliomagenesis and attenuation of mitotic transition by MIIP. *Oncogene,* 29**,** 3501-8.

JOHNSON, J. 2001. Molybdenum cofactor deficiency and isolated sulfite oxidase deficiency. *The metabolic and molecular bases of inherited disease***,** 3163-3177.

JONES, K. L. & SMITH, D. W. 1973. Recognition of the fetal alcohol syndrome in early infancy. *Lancet,* 302**,** 999-1001.

JOUBERT, B. R., DEN DEKKER, H. T., FELIX, J. F., BOHLIN, J., LIGTHART, S., BECKETT, E., TIEMEIER, H., VAN MEURS, J. B., UITTERLINDEN, A. G., HOFMAN, A., HABERG, S. E., REESE, S. E., PETERS, M. J., ANDREASSEN, B. K., STEEGERS, E. A., NILSEN, R. M., VOLLSET, S. E., MIDTTUN, O., UELAND, P. M., FRANCO, O. H., DEHGHAN, A., DE JONGSTE, J. C., WU, M. C., WANG, T., PEDDADA, S. D., JADDOE, V. W., NYSTAD, W., DUIJTS, L. & LONDON, S. J. 2016. Maternal plasma folate impacts differential DNA methylation in an epigenome-wide meta-analysis of newborns. *Nat Commun,* 7**,** 10577.

JUENGEL, J. L. & MCNATTY, K. P. 2005. The role of proteins of the transforming growth factor-beta superfamily in the intraovarian regulation of follicular development. *Hum Reprod Update,* 11**,** 143-60.

KAJIMOTO, K., VALENZUELA, C. F., ALLAN, A. M., GE, S., GU, Y. & CUNNINGHAM, L. A. 2016. Prenatal alcohol exposure alters synaptic activity of adult hippocampal dentate granule cells under conditions of enriched environment. *Hippocampus,* 26**,** 1078-1087.

KASHIWAGI, A., DIGIROLAMO, C. M., KANDA, Y., NIIKURA, Y., ESMON, C. T., HANSEN, T. R., SHIODA, T. & PRU, J. K. 2007. The postimplantation embryo differentially regulates endometrial gene expression and decidualization. *Endocrinology,* 148**,** 4173-84.

KAUPMANN, K., SCHULER, V., MOSBACHER, J., BISCHOFF, S., BITTIGER, H., HEID, J., FROESTL, W., LEONHARD, S., PFAFF, T., KARSCHIN, A. & BETTLER, B. 1998. Human gamma-aminobutyric acid type B receptors are differentially expressed and regulate inwardly rectifying K+ channels. *Proc Natl Acad Sci U S A,* 95**,** 14991-6.

KELLY, S. J., DAY, N. & STREISSGUTH, A. P. 2000. Effects of prenatal alcohol exposure on social behavior in humans and other species. *Neurotoxicol Teratol,* 22**,** 143-9.

KELLY, S. J., GOODLETT, C. R. & HANNIGAN, J. H. 2009a. Animal models of fetal alcohol spectrum disorders: impact of the social environment. *Dev Disabil Res Rev,* 15**,** 200-8.

KELLY, S. J., GOODLETT, C. R., HULSETHER, S. A. & WEST, J. R. 1988. Impaired spatial navigation in adult female but not adult male rats exposed to alcohol during the brain growth spurt. *Behav Brain Res,* 27**,** 247-57.

KELLY, S. J., LEGGETT, D. C. & CRONISE, K. 2009b. Sexually dimorphic effects of alcohol exposure during development on the processing of social cues. *Alcohol Alcohol,* 44**,** 555-60.

KELLY, S. J. & TRAN, T. D. 1997. Alcohol exposure during development alters social recognition and social communication in rats. *Neurotoxicol Teratol,* 19**,** 383-9.

KERR, E. N., BHAN, A. & HEON, E. 2016. Exploration of the cognitive, adaptive and behavioral functioning of patients affected with Bardet-Biedl syndrome. *Clin Genet,* 89**,** 426-433.

KHALID, O., KIM, J. J., KIM, H.-S., HOANG, M., TU, T. G., ELIE, O., LEE, C., VU, C., HORVATH, S. & SPIGELMAN, I. 2014. Gene expression signatures affected by alcohol-induced DNA methylomic deregulation in human embryonic stem cells. *Stem cell research,* 12**,** 791-806.

KIM, D., LANGMEAD, B. & SALZBERG, S. L. 2015. HISAT: a fast spliced aligner with low memory requirements. *Nature methods,* 12**,** 357.

KIM, R. H., WANG, D., TSANG, M., MARTIN, J., HUFF, C., DE CAESTECKER, M. P., PARKS, W. T., MENG, X., LECHLEIDER, R. J., WANG, T. & ROBERTS, A. B. 2000. A novel smad nuclear interacting protein, SNIP1, suppresses p300-dependent TGF-beta signal transduction. *Genes Dev,* 14**,** 1605-16.

KIM, S. H., SHIN, S. Y., LEE, K. Y., JOO, E. J., SONG, J. Y., AHN, Y. M., LEE, Y. H. & KIM, Y. S. 2012. The genetic association of DUSP6 with bipolar disorder and its effect on ERK activity. *Prog Neuropsychopharmacol Biol Psychiatry,* 37**,** 41-9.

KITAGISHI, Y. & MATSUDA, S. 2013. RUFY, Rab and Rap Family Proteins Involved in a Regulation of Cell Polarity and Membrane Trafficking. *Int J Mol Sci,* 14**,** 6487-98.

KLEIBER, M. L., LAUFER, B. I., WRIGHT, E., DIEHL, E. J. & SINGH, S. M. 2012. Long-term alterations to the brain transcriptome in a maternal voluntary consumption model of fetal alcohol spectrum disorders. *Brain Res,* 1458**,** 18-33.

KLINTSOVA, A. Y., HELFER, J. L., CALIZO, L. H., DONG, W. K., GOODLETT, C. R. & GREENOUGH, W. T. 2007. Persistent impairment of hippocampal neurogenesis in young adult rats following early postnatal alcohol exposure. *Alcohol Clin Exp Res,* 31**,** 2073-82.

KOCHUNOV, P., CASTRO, C., DAVIS, D. M., DUDLEY, D., WEY, H. Y., PURDY, D., FOX, P. T., SIMERLY, C. & SCHATTEN, G. 2010. Fetal brain during a

binge drinking episode: a dynamic susceptibility contrast MRI fetal brain perfusion study. *Neuroreport,* 21**,** 716-21.

KORZEKWA, A. J., MILEWSKI, R., ŁUPICKA, M. & SKARZYNSKI, D. J. 2016. Leukotriene production profiles and actions in the bovine endometrium during the oestrous cycle. *Reproduction, Fertility and Development,* 28**,** 682-689.

KOSTERS, A., ABEBE, D. F., FELIX, J. C., DAWSON, P. A. & KARPEN, S. J. 2016. Inflammation-associated upregulation of the sulfated steroid transporter Slc10a6 in mouse liver and macrophage cell lines. *Hepatol Res,* 46**,** 794-803.

KRISHNAN, N., DICKMAN, M. B. & BECKER, D. F. 2008. Proline modulates the intracellular redox environment and protects mammalian cells against oxidative stress. *Free Radical Biology and Medicine,* 44**,** 671-681.

LADD, A. N. & COOPER, T. A. 2004. Multiple domains control the subcellular localization and activity of ETR-3, a regulator of nuclear and cytoplasmic RNA processing events. *J Cell Sci,* 117**,** 3519-29.

LAN, N., YAMASHITA, F., HALPERT, A. G., SLIWOWSKA, J. H., VIAU, V. & WEINBERG, J. 2009. Effects of prenatal ethanol exposure on hypothalamic-pituitary-adrenal function across the estrous cycle. *Alcohol Clin Exp Res,* 33**,** 1075-88.

LANSDON, L. A., DARBRO, B. W., PETRIN, A. L., HULSTRAND, A. M., STANDLEY, J. M., BROUILLETTE, R. B., LONG, A., MANSILLA, M. A., CORNELL, R. A., MURRAY, J. C., HOUSTON, D. W. & MANAK, J. R. 2018. Identification of Isthmin 1 as a Novel Clefting and Craniofacial Patterning Gene in Humans. *Genetics,* 208**,** 283-296.

LAPOSATA, M., KABAKIBI, A., WALDEN, M. P., CLUETTE-BROWN, J. E., NANJI, A. A., REFAAI, M. A., WERNER, J. & NANJI, A. A. 2000. Differences in the fatty acid composition of fatty acid ethyl esters in organs and their secretions. *Alcohol Clin Exp Res,* 24**,** 1488-91.

LAU, P. P., ZHU, H. J., NAKAMUTA, M. & CHAN, L. 1997. Cloning of an Apobec-1-binding protein that also interacts with apolipoprotein B mRNA and evidence for its involvement in RNA editing. *J Biol Chem,* 272**,** 1452-5.

LAUFER, B. I. 2016. A Long-Term Neuroepigenomic Profile of Prenatal Alcohol Exposure.

LAWLOR, M. A. & ALESSI, D. R. 2001. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? *J Cell Sci,* 114**,** 2903-10.

LAWO, S., BASHKUROV, M., MULLIN, M., FERRERIA, M. G., KITTLER, R., HABERMANN, B., TAGLIAFERRO, A., POSER, I., HUTCHINS, J. R., HEGEMANN, B., PINCHEV, D., BUCHHOLZ, F., PETERS, J. M., HYMAN, A. A., GINGRAS, A. C. & PELLETIER, L. 2009. HAUS, the 8-subunit human Augmin complex, regulates centrosome and spindle integrity. *Curr Biol,* 19**,** 816-26.

LEANDRO, J. & HOUTEN, S. M. 2019. Saccharopine, a lysine degradation intermediate, is a mitochondrial toxin. *J Cell Biol,* 218**,** 391-392.

LECO, K. J., APTE, S. S., TANIGUCHI, G. T., HAWKES, S. P., KHOKHA, R., SCHULTZ, G. A. & EDWARDS, D. R. 1997. Murine tissue inhibitor of metalloproteinases-4 (Timp-4): cDNA isolation and expression in adult mouse tissues. *FEBS Lett,* 401**,** 213-7.

LEKHOLM, E., PERLAND, E., ERIKSSON, M. M., HELLSTEN, S. V., LINDBERG, F. A., ROSTAMI, J. & FREDRIKSSON, R. 2017. Putative Membrane-Bound Transporters MFSD14A and MFSD14B Are Neuronal and Affected by Nutrient Availability. *Front Mol Neurosci,* 10**,** 11.

LEMIONE, P., HARASSEAU, H., BORTERYU, J. & MENUET, J. 1968. Les enfants de parents alcooliques: anomalies observées à propos de 127 cas [The children of alcoholic parents: anomalies observed in 127 cases]. *Quest Medicale,* 21.

LESCH, K. P. 2011. When the serotonin transporter gene meets adversity: the contribution of animal models to understanding epigenetic mechanisms in affective disorders and resilience. *Curr Top Behav Neurosci,* 7**,** 251-80.

LEWIS, C. E., THOMAS, K. G., DODGE, N. C., MOLTENO, C. D., MEINTJES, E. M., JACOBSON, J. L. & JACOBSON, S. W. 2015. Verbal learning and memory impairment in children with fetal alcohol spectrum disorders. *Alcoholism: Clinical and Experimental Research,* 39**,** 724-732.

LI, G., XU, C., LIN, X., QU, L., XIA, D., HONGDU, B., XIA, Y., WANG, X., LOU, Y., HE, Q., MA, D. & CHEN, Y. 2017. Deletion of Pdcd5 in mice led to the deficiency of placenta development and embryonic lethality. *Cell Death Dis,* 8**,** e2811.

LI, K., TONG, W. H., HUGHES, R. M. & ROUAULT, T. A. 2006. Roles of the mammalian cytosolic cysteine desulfurase, ISCS, and scaffold protein, ISCU, in iron-sulfur cluster assembly. *J Biol Chem,* 281**,** 12344-51.

LI, K., ZHOU, Z., WANG, Y. O., LIU, J., ZHAO, H. B., YANG, J. & WANG, S. Q. 2014. Pretreatment of mice with oligonucleotide prop5 protects them from influenza virus infections. *Viruses,* 6**,** 573-81.

LI, S. P., KIM, J. H., PARK, M. S., BAHK, J. Y., CHUNG, B. C. & KIM, M. O. 2005. Ethanol modulates the expression of GABA(B) receptor mRNAs in the prenatal rat brain in an age and area dependent manner. *Neuroscience,* 134**,** 857-66.

LIAO, Y., SMYTH, G. K. & SHI, W. 2013. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics,* 30**,** 923-930.

LINDSTROM, M. S. & NISTER, M. 2010. Silencing of ribosomal protein S9 elicits a multitude of cellular responses inhibiting the growth of cancer cells subsequent to p53 activation. *PLoS One,* 5**,** e9578.

LINDSTROM, M. S. & ZHANG, Y. 2008. Ribosomal protein S9 is a novel B23/NPM-binding protein required for normal cell proliferation. *J Biol Chem,* 283**,** 15568-76.

LINGJUN, Z., ZHANG, C. K., SAYWARD, F. G., CHEUNG, K.-H., KESHENG, W., KRYSTAL, J. H., HONGYU, Z. & XINGGUANG, L. 2015. Gene-based and pathway-based genome-wide association study of alcohol dependence. *Shanghai archives of psychiatry,* 27**,** 111.

LIU, A. X., JIN, F., ZHANG, W. W., ZHOU, T. H., ZHOU, C. Y., YAO, W. M., QIAN, Y. L. & HUANG, H. F. 2006a. Proteomic analysis on the alteration of protein expression in the placental villous tissue of early pregnancy loss. *Biol Reprod,* 75**,** 414-20.

LIU, H., WANG, Y., ZHANG, Y., SONG, Q., DI, C., CHEN, G., TANG, J. & MA, D. 1999. TFAR19, a novel apoptosis-related gene cloned from human leukemia cell line TF-1, could enhance apoptosis of some tumor cells induced by growth factor withdrawal. *Biochem Biophys Res Commun,* 254**,** 203-10.

LIU, Y., BORCHERT, G., SURAZYNSKI, A., HU, C. & PHANG, J. 2006b. Proline oxidase activates both intrinsic and extrinsic pathways for apoptosis: the role of ROS/superoxides, NFAT and MEK/ERK signaling. *Oncogene,* 25**,** 5640.

LIVY, D. J., MILLER, E. K., MAIER, S. E. & WEST, J. R. 2003. Fetal alcohol exposure and temporal vulnerability: effects of binge-like alcohol exposure on the developing rat hippocampus. *Neurotoxicol Teratol,* 25**,** 447-58.

LOCHAB, A. K. & EXTAVOUR, C. G. 2017. Bone Morphogenetic Protein (BMP) signaling in animal reproductive system development and function. *Dev Biol,* 427**,** 258-269.

LONERGAN, K. M., CHARI, R., DELEEUW, R. J., SHADEO, A., CHI, B., TSAO, M. S., JONES, S., MARRA, M., LING, V., NG, R., MACAULAY, C., LAM, S. & LAM, W. L. 2006. Identification of novel lung genes in bronchial epithelium by serial analysis of gene expression. *Am J Respir Cell Mol Biol,* 35**,** 651-61.

LORICK, K. L., JENSEN, J. P., FANG, S., ONG, A. M., HATAKEYAMA, S. & WEISSMAN, A. M. 1999. RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc Natl Acad Sci U S A,* 96**,** 11364-9.

LOVE, M. I., HUBER, W. & ANDERS, S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology,* 15**,** 550.

LUNDE-YOUNG, R., DAVIS-ANDERSON, K., NAIK, V., NEMEC, M., WU, G. & RAMADOSS, J. 2018. Regional dysregulation of taurine and related amino acids in the fetal rat brain following gestational alcohol exposure. *Alcohol,* 66**,** 27-33.

LUPTON, C., BURD, L. & HARWOOD, R. 2004. Cost of fetal alcohol spectrum disorders. *Am J Med Genet C Semin Med Genet,* 127C**,** 42-50.

LUSSIER, A. A., STEPIEN, K. A., NEUMANN, S. M., PAVLIDIS, P., KOBOR, M. S. & WEINBERG, J. 2015a. Prenatal alcohol exposure alters steady-state and activated gene expression in the adult rat brain. *Alcohol Clin Exp Res,* 39**,** 251-61.

LUSSIER, A. A., STEPIEN, K. A., WEINBERG, J. & KOBOR, M. S. 2015b. Prenatal alcohol exposure alters gene expression in the rat brain: Experimental design and bioinformatic analysis of microarray data. *Data in brief,* 4**,** 239-252.

MACKOWIAK, B., HODGE, J., STERN, S. & WANG, H. 2018. The Roles of Xenobiotic Receptors: Beyond Chemical Disposition. *Drug Metabolism and Disposition,* 46**,** 1361-1371.

MAGNUSSON, C., MEZHYBOVSKA, M., LORINC, E., FERNEBRO, E., NILBERT, M. & SJOLANDER, A. 2010. Low expression of CysLT1R and high expression of CysLT2R mediate good prognosis in colorectal cancer. *Eur J Cancer,* 46**,** 826-35.

MAI, M., QIAN, C., YOKOMIZO, A., SMITH, D. I. & LIU, W. 1999. Cloning of the human homolog of conductin (AXIN2), a gene mapping to chromosome 17q23–q24. *Genomics,* 55**,** 341-344.

MAICHELE, A. J., BURWINKEL, B., MAIRE, I., SOVIK, O. & KILIMANN, M. W. 1996. Mutations in the testis/liver isoform of the phosphorylase kinase gamma subunit (PHKG2) cause autosomal liver glycogenosis in the gsd rat and in humans. *Nat Genet,* 14**,** 337-40.

MAIER, S. E., MILLER, J. A. & WEST, J. R. 1999. Prenatal binge-like alcohol exposure in the rat results in region-specific deficits in brain growth. *Neurotoxicol Teratol,* 21**,** 285-91.

MAIER, S. E. & WEST, J. R. 2001a. Drinking patterns and alcohol-related birth defects. *Alcohol Research and Health,* 25**,** 168-174.

MAIER, S. E. & WEST, J. R. 2001b. Drinking patterns and alcohol-related birth defects. *Alcohol Res Health,* 25**,** 168-74.

MAINA, E. N., MORRIS, M. R., ZATYKA, M., RAVAL, R. R., BANKS, R. E., RICHARDS, F. M., JOHNSON, C. M. & MAHER, E. R. 2005. Identification of novel VHL target genes and relationship to hypoxic response pathways. *Oncogene,* 24**,** 4549-58.

MALISZA, K. L., ALLMAN, A. A., SHILOFF, D., JAKOBSON, L., LONGSTAFFE, S. & CHUDLEY, A. E. 2005. Evaluation of spatial working memory function in children and adults with fetal alcohol spectrum disorders: a functional magnetic resonance imaging study. *Pediatr Res,* 58**,** 1150-7.

MALLET, P. & BENINGER, R. 1996. The endogenous cannabinoid receptor agonist. *Behavioural pharmacology,* 7**,** 276-284.

MANDAL, C., PARK, K. S., JUNG, K. H. & CHAI, Y. G. 2015. Ethanol-related alterations in gene expression patterns in the developing murine hippocampus. *Acta biochimica et biophysica Sinica,* 47**,** 581-587.

MANTHA, K., KLEIBER, M. & SINGH, S. 2013. Neurodevelopmental timing of ethanol exposure may contribute to observed heterogeneity of behavioral deficits in a mouse model of fetal alcohol spectrum disorder (FASD). *Journal of Behavioral and Brain Science,* 3**,** 85.

MANTHA, K., LAUFER, B. I. & SINGH, S. M. 2014. Molecular changes during neurodevelopment following second-trimester binge ethanol exposure in a mouse model of fetal alcohol spectrum disorder: from immediate effects to long-term adaptation. *Dev Neurosci,* 36**,** 29-43.

MARJONEN, H., SIERRA, A., NYMAN, A., ROGOJIN, V., GRÖHN, O., LINDEN, A.-M., HAUTANIEMI, S. & KAMINEN-AHOLA, N. 2015. Early maternal alcohol consumption alters hippocampal DNA methylation, gene expression and volume in a mouse model. *PLoS One,* 10**,** e0124931.

MARSH, E. E., CHIBBER, S., WU, J., SIEGERSMA, K., KIM, J. & BULUN, S. 2016. Epidermal growth factor-containing fibulin-like extracellular matrix protein 1 expression and regulation in uterine leiomyoma. *Fertil Steril,* 105**,** 1070-5.

MARTIN, D., MARTIN, J., STREISSGUTH, A. & LUND, C. 1979. Sucking frequency and amplitude in newborns as a function of maternal drinking and smoking. *Currents in alcoholism,* 5**,** 359-366.

MATSUI, S., UTANI, A., TAKAHASHI, K., MUKOYAMA, Y., MIYACHI, Y. & MATSUYOSHI, N. 2007. Human Fat2 is localized at immature adherens junctions in epidermal keratinocytes. *Journal of dermatological science,* 48**,** 233-236.

MATTSON, S. N., BERNES, G. A. & DOYLE, L. R. 2019. Fetal Alcohol Spectrum Disorders: A Review of the Neurobehavioral Deficits Associated With Prenatal Alcohol Exposure. *Alcohol Clin Exp Res,* 43**,** 1046-1062.

MAY, P. A., BAETE, A., RUSSO, J., ELLIOTT, A. J., BLANKENSHIP, J., KALBERG, W. O., BUCKLEY, D., BROOKS, M., HASKEN, J., ABDUL-RAHMAN, O., ADAM, M. P., ROBINSON, L. K., MANNING, M. & HOYME, H. E. 2014. Prevalence and characteristics of fetal alcohol spectrum disorders. *Pediatrics,* 134**,** 855-66.

MAY, P. A., BLANKENSHIP, J., MARAIS, A.-S., GOSSAGE, J. P., KALBERG, W. O., JOUBERT, B., CLOETE, M., BARNARD, R., DE VRIES, M. & HASKEN, J. 2013a. Maternal alcohol consumption producing fetal alcohol spectrum disorders (FASD): quantity, frequency, and timing of drinking. *Drug and alcohol dependence,* 133**,** 502-512.

MAY, P. A., BLANKENSHIP, J., MARAIS, A. S., GOSSAGE, J. P., KALBERG, W. O., JOUBERT, B., CLOETE, M., BARNARD, R., DE VRIES, M., HASKEN, J., ROBINSON, L. K., ADNAMS, C. M., BUCKLEY, D., MANNING, M., PARRY, C. D., HOYME, H. E., TABACHNICK, B. & SEEDAT, S. 2013b. Maternal alcohol consumption producing fetal alcohol spectrum disorders (FASD): quantity, frequency, and timing of drinking. *Drug Alcohol Depend,* 133**,** 502-12.

MAY, P. A., CHAMBERS, C. D., KALBERG, W. O., ZELLNER, J., FELDMAN, H., BUCKLEY, D., KOPALD, D., HASKEN, J. M., XU, R. & HONERKAMP-SMITH, G. 2018a. Prevalence of fetal alcohol spectrum disorders in 4 US communities. *Jama,* 319**,** 474-482.

MAY, P. A., CHAMBERS, C. D., KALBERG, W. O., ZELLNER, J., FELDMAN, H., BUCKLEY, D., KOPALD, D., HASKEN, J. M., XU, R., HONERKAMP-SMITH, G., TARAS, H., MANNING, M. A., ROBINSON, L. K., ADAM, M. P., ABDUL-RAHMAN, O., VAUX, K., JEWETT, T., ELLIOTT, A. J., KABLE, J. A., AKSHOOMOFF, N., FALK, D., ARROYO, J. A., HERELD, D., RILEY, E. P., CHARNESS, M. E., COLES, C. D., WARREN, K. R., JONES, K. L. & HOYME, H. E. 2018b. Prevalence of Fetal Alcohol Spectrum Disorders in 4 US Communities. *JAMA,* 319**,** 474-482.

MAY, P. A. & GOSSAGE, J. P. 2011a. Maternal risk factors for fetal alcohol spectrum disorders: not as simple as it might seem. *Alcohol Res Health,* 34**,** 15-26.

MAY, P. A. & GOSSAGE, J. P. 2011b. Maternal risk factors for fetal alcohol spectrum disorders: not as simple as it might seem. *Alcohol Research & Health,* 34**,** 15.

MAY, P. A., GOSSAGE, J. P., KALBERG, W. O., ROBINSON, L. K., BUCKLEY, D., MANNING, M. & HOYME, H. E. 2009. Prevalence and epidemiologic characteristics of FASD from various research methods with an emphasis on recent in-school studies. *Dev Disabil Res Rev,* 15**,** 176-92.

MAYOCK, D. E., NESS, D., MONDARES, R. L. & GLEASON, C. A. 2007. Binge alcohol exposure in the second trimester attenuates fetal cerebral blood flow response to hypoxia. *J Appl Physiol (1985),* 102**,** 972-7.

MCCLINTICK, J. N., MCBRIDE, W. J., BELL, R. L., DING, Z.-M., LIU, Y., XUEI, X. & EDENBERG, H. J. 2015. Gene expression changes in serotonin, GABA-A receptors, neuropeptides and ion channels in the dorsal raphe nucleus of adolescent alcohol-preferring (P) rats following binge-like alcohol drinking. *Pharmacology Biochemistry and Behavior,* 129**,** 87-96.

MCCLINTICK, J. N., MCBRIDE, W. J., BELL, R. L., DING, Z. M., LIU, Y., XUEI, X. & EDENBERG, H. J. 2016. Gene Expression Changes in Glutamate and GABA-A Receptors, Neuropeptides, Ion Channels, and Cholesterol Synthesis in the Periaqueductal Gray Following Binge-Like Alcohol Drinking by Adolescent Alcohol-Preferring (P) Rats. *Alcoholism: Clinical and Experimental Research,* 40**,** 955-968.

MCFADDEN, S. A. 1996. Phenotypic variation in xenobiotic metabolism and adverse environmental response: focus on sulfur-dependent detoxification pathways. *Toxicology,* 111**,** 43-65.

MCGIVERN, R. F., RAUM, W. J., HANDA, R. J. & SOKOL, R. Z. 1992. Comparison of two weeks versus one week of prenatal ethanol exposure in the rat on gonadal organ weights, sperm count, and onset of puberty. *Neurotoxicol Teratol,* 14**,** 351-8.

MEARES, R., PENMAN, R., MILGROM-FRIEDMAN, J. & BAKER, K. 1982. Some origins of the 'difficult' child: the Brazelton scale and the mother's view of her new-born's character. *Br J Med Psychol,* 55**,** 77-86.

MEI, N., GUO, L., TSENG, J., DIAL, S. L., LIAO, W. & MANJANATHA, M. G. 2008. Gene expression changes associated with xenobiotic metabolism pathways in mice exposed to acrylamide. *Environmental and molecular mutagenesis,* 49**,** 741-745.

MEINIEL, A. 2001. SCO-spondin, a glycoprotein of the subcommissural organ/Reissner's fiber complex: evidence of a potent activity on neuronal development in primary cell cultures. *Microsc Res Tech,* 52**,** 484-95.

MEYER, L. S. & RILEY, E. P. 1986. Social play in juvenile rats prenatally exposed to alcohol. *Teratology,* 34**,** 1-7.

MICHELL, A., LEWIS, S., FOLTYNIE, T. & BARKER, R. 2004. Biomarkers and Parkinson's disease. *Brain,* 127**,** 1693-1705.

MIDDELDORP, J. & HOL, E. M. 2011. GFAP in health and disease. *Prog Neurobiol,* 93**,** 421-43.

MIKKAICHI, T., SUZUKI, T., ONOGAWA, T., TANEMOTO, M., MIZUTAMARI, H., OKADA, M., CHAKI, T., MASUDA, S., TOKUI, T., ETO, N., ABE, M., SATOH, F., UNNO, M., HISHINUMA, T., INUI, K., ITO, S., GOTO, J. & ABE, T. 2004. Isolation and characterization of a digoxin transporter and its rat homologue expressed in the kidney. *Proc Natl Acad Sci U S A,* 101**,** 3569-74.

MILLER, M. W. 1986. Effects of alcohol on the generation and migration of cerebral cortical neurons. *Science,* 233**,** 1308-11.

MILLER, M. W. & ROBERTSON, S. 1993. Prenatal exposure to ethanol alters the postnatal development and transformation of radial glia to astrocytes in the cortex. *J Comp Neurol,* 337**,** 253-66.

MINETTI, A., AROLFO, M. P., VIRGOLINI, M. B., BRIONI, J. D. & FULGINITI, S. 1996. Spatial learning in rats exposed to acute ethanol intoxication on gestational day 8. *Pharmacol Biochem Behav,* 53**,** 361-7.

MONK, B. R., LESLIE, F. M. & THOMAS, J. D. 2012. The effects of perinatal choline supplementation on hippocampal cholinergic development in rats exposed to alcohol during the brain growth spurt. *Hippocampus,* 22**,** 1750-1757.

MOORE, D. B., RUYGROK, A. C., WALKER, D. W. & HEATON, M. B. 1997. Effects of prenatal ethanol exposure on parvalbumin-expressing GABAergic neurons in the adult rat medial septum. *Alcohol Clin Exp Res,* 21**,** 849-56.

MORELLO, G., GUARNACCIA, M., SPAMPINATO, A. G., LA COGNATA, V., D'AGATA, V. & CAVALLARO, S. 2018. Copy Number Variations in Amyotrophic Lateral Sclerosis: Piecing the Mosaic Tiles Together through a Systems Biology Approach. *Mol Neurobiol,* 55**,** 1299-1322.

MORENO-MOYA, J. M., VILELLA, F., MARTINEZ, S., PELLICER, A. & SIMON, C. 2014. The transcriptomic and proteomic effects of ectopic overexpression of miR-30d in human endometrial epithelial cells. *Mol Hum Reprod,* 20**,** 550-66.

MORGAN, D. J., MZHAVIA, N., PENG, B., PAN, H., DEVI, L. A. & PINTAR, J. E. 2005. Embryonic gene expression and pro-protein processing of proSAAS during rodent development. *J Neurochem,* 93**,** 1454-62.

MOST, D., FERGUSON, L., BLEDNOV, Y., MAYFIELD, R. & HARRIS, R. 2015. The synaptoneurosome transcriptome: a model for profiling the emolecular effects of alcohol. *The pharmacogenomics journal,* 15**,** 177.

NA, A. R., CHUNG, Y. M., LEE, S. B., PARK, S. H., LEE, M. S. & YOO, Y. D. 2008. A critical role for Romo1-derived ROS in cell proliferation. *Biochem Biophys Res Commun,* 369**,** 672-8.

NAGAI, K., TAHARA-HANAOKA, S., MORISHIMA, Y., TOKUNAGA, T., IMOTO, Y., NOGUCHI, E., KANEMARU, K., IMAI, M., SHIBAYAMA, S., HIZAWA, N., FUJIEDA, S., YAMAGATA, K. & SHIBUYA, A. 2013. Expression and function of Allergin-1 on human primary mast cells. *PLoS One,* 8**,** e76160.

NAGALAKSHMI, U., WANG, Z., WAERN, K., SHOU, C., RAHA, D., GERSTEIN, M. & SNYDER, M. 2008. The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science,* 320**,** 1344-9.

NAHA, N., LEE, H. Y., NASER, M. I., PARK, T. J., KIM, S. H. & KIM, M. O. 2009. Ethanol inhibited apoptosis-related RNA binding protein, Napor-3 gene expression in the prenatal rat brain. *Med Sci Monit,* 15**,** BR6-12.

NAIK, V. D., DAVIS-ANDERSON, K., SUBRAMANIAN, K., LUNDE-YOUNG, R., NEMEC, M. J. & RAMADOSS, J. 2018. Mechanisms Underlying Chronic Binge Alcohol Exposure-Induced Uterine Artery Dysfunction in Pregnant Rat. *Alcoholism: Clinical and Experimental Research,* 42**,** 682-690.

NAIK, V. D., LUNDE-YOUNG, E. R., DAVIS-ANDERSON, K. L., ORZABAL, M., IVANOV, I. & RAMADOSS, J. 2016. Chronic binge alcohol consumption during pregnancy alters rat maternal uterine artery pressure response. *Alcohol,* 56**,** 59-64.

NAKAZAWA, N., TERUYA, T., SAJIKI, K., KUMADA, K., VILLAR-BRIONES, A., ARAKAWA, O., TAKADA, J., SAITOH, S. & YANAGIDA, M. 2018. The putative ceramide-conjugation protein Cwh43 regulates G0 quiescence, nutrient metabolism and lipid homeostasis in fission yeast. *J Cell Sci,* 131.

NAKAZAWA, N., XU, X., ARAKAWA, O. & YANAGIDA, M. 2019. Coordinated Roles of the Putative Ceramide-Conjugation Protein, Cwh43, and a Mn(2+)-Transporting, P-Type ATPase, Pmr1, in Fission Yeast. *G3 (Bethesda),* 9**,** 2667-2676.

NARDELLI, A., LEBEL, C., RASMUSSEN, C., ANDREW, G. & BEAULIEU, C. 2011. Extensive deep gray matter volume reductions in children and adolescents with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res,* 35**,** 1404-17.

NARRANDES, S. & XU, W. 2018. Gene Expression Detection Assay for Cancer Clinical Use. *J Cancer,* 9**,** 2249-2265.

NASCA, A., NARDECCHIA, F., COMMONE, A., SEMERARO, M., LEGATI, A., GARAVAGLIA, B., GHEZZI, D. & LEUZZI, V. 2018. Clinical and Biochemical Features in a Patient With Mitochondrial Fission Factor Gene Alteration. *Front Genet,* 9**,** 625.

NESS, F., BOUROT, S., REGNACQ, M., SPAGNOLI, R., BERGES, T. & KARST, F. 2001. SUT1 is a putative Zn[II]2Cys6-transcription factor whose upregulation enhances both sterol uptake and synthesis in aerobically growing Saccharomyces cerevisiae cells. *Eur J Biochem,* 268**,** 1585-95.

NGAI, A. C., MONDARES, R. L., MAYOCK, D. E. & GLEASON, C. A. 2008. Fetal alcohol exposure alters cerebrovascular reactivity to vasoactive intestinal peptide in adult sheep. *Neonatology,* 93**,** 45-51.

NGAI, Y. F., SULISTYONINGRUM, D. C., O'NEILL, R., INNIS, S. M., WEINBERG, J. & DEVLIN, A. M. 2015. Prenatal alcohol exposure alters methyl metabolism and programs serotonin transporter and glucocorticoid receptor expression in brain. *Am J Physiol Regul Integr Comp Physiol,* 309**,** R613-22.

NGUN, T. C., GHAHRAMANI, N., SÁNCHEZ, F. J., BOCKLANDT, S. & VILAIN, E. 2011. The genetics of sex differences in brain and behavior. *Frontiers in neuroendocrinology,* 32**,** 227-246.

NIBBELING, E. A. R., DUARRI, A., VERSCHUUREN-BEMELMANS, C. C., FOKKENS, M. R., KARJALAINEN, J. M., SMEETS, C., DE BOER-BERGSMA, J. J., VAN DER VRIES, G., DOOIJES, D., BAMPI, G. B., VAN DIEMEN, C., BRUNT, E., IPPEL, E., KREMER, B., VLAK, M., ADIR, N., WIJMENGA, C., VAN DE WARRENBURG, B. P. C., FRANKE, L., SINKE, R. J. & VERBEEK, D. S. 2017. Exome sequencing and network analysis identifies shared mechanisms underlying spinocerebellar ataxia. *Brain,* 140**,** 2860-2878.

NIEDERREITHER, K., FRAULOB, V., GARNIER, J. M., CHAMBON, P. & DOLLE, P. 2002. Differential expression of retinoic acid-synthesizing (RALDH) enzymes during fetal development and organ differentiation in the mouse. *Mech Dev,* 110**,** 165-71.

NISHIMURA, T., HIGUCHI, K., YOSHIDA, Y., SUGITA-FUJISAWA, Y., KOJIMA, K., SUGIMOTO, M., SANTO, M., TOMI, M. & NAKASHIMA, E. 2018. Hypotaurine Is a Substrate of GABA Transporter Family Members GAT2/Slc6a13 and TAUT/Slc6a6. *Biol Pharm Bull,* 41**,** 1523-1529.

NORMAN, A. L., O'BRIEN, J. W., SPADONI, A. D., TAPERT, S. F., JONES, K. L., RILEY, E. P. & MATTSON, S. N. 2013. A functional magnetic resonance imaging study of spatial working memory in children with prenatal alcohol exposure: contribution of familial history of alcohol use disorders. *Alcohol Clin Exp Res,* 37**,** 132-40.

O'CONNOR, M. J., SIGMAN, M. & KASARI, C. 1992. Attachment behavior of infants exposed prenatally to alcohol: Mediating effects of infant affect and mother-infant interaction. *Development and Psychopathology,* 4**,** 243-256.

O'MALLEY, K. D. 2007. *ADHD and fetal alcohol spectrum disorders (FASD)*, Nova Publishers.

O'CONNOR, M. J. 1996. The implications of attachment theory for the socioemotional development of children exposed to alcohol prenatally. *Alcohol, pregnancy, and the developing child. Cambridge, UK: Press Syndicate of the University of Cambridge***,** 183-206.

OBRIETAN, K. & VAN DEN POL, A. N. 1998. GABAB receptor-mediated inhibition of GABAA receptor calcium elevations in developing hypothalamic neurons. *J Neurophysiol,* 79**,** 1360-70.

OMIECINSKI, C. J., VANDEN HEUVEL, J. P., PERDEW, G. H. & PETERS, J. M. 2010. Xenobiotic metabolism, disposition, and regulation by receptors: from biochemical phenomenon to predictors of major toxicities. *Toxicological Sciences,* 120**,** S49-S75.

OPPENHEIM, R. W. 1991. Cell death during development of the nervous system. *Annu Rev Neurosci,* 14**,** 453-501.

OSORIO, L., WU, X. & ZHOU, Z. 2014. Distinct spatiotemporal expression of ISM1 during mouse and chick development. *Cell Cycle,* 13**,** 1571-82.

OSTER, M., MURANI, E., METGES, C. C., PONSUKSILI, S. & WIMMERS, K. 2011. A high protein diet during pregnancy affects hepatic gene expression of energy sensing pathways along ontogenesis in a porcine model. *PLoS One,* 6**,** e21691.

OSTER, M., MURANI, E., METGES, C. C., PONSUKSILI, S. & WIMMERS, K. 2012a. A gestational high protein diet affects the abundance of muscle transcripts related to cell cycle regulation throughout development in porcine progeny. *PLoS One,* 7**,** e34519.

OSTER, M., MURANI, E., METGES, C. C., PONSUKSILI, S. & WIMMERS, K. 2012b. A low protein diet during pregnancy provokes a lasting shift of hepatic expression of genes related to cell cycle throughout ontogenesis in a porcine model. *BMC Genomics,* 13**,** 93.

OUELLETTE, E. M., ROSETT, H. L., ROSMAN, N. P. & WEINER, L. 1977. Adverse effects on offspring of maternal alcohol abuse during pregnancy. *N Engl J Med,* 297**,** 528-30.

OUTHWAITE, J. E., NATALE, B. V., NATALE, D. R. & SIMMONS, D. G. 2015. Expression of aldehyde dehydrogenase family 1, member A3 in glycogen trophoblast cells of the murine placenta. *Placenta,* 36**,** 304-11.

PADMANABHAN, R., IBRAHIM, A. & BENER, A. 2002. Effect of maternal methionine pre-treatment on alcohol-induced exencephaly and axial skeletal dysmorphogenesis in mouse fetuses. *Drug Alcohol Depend,* 65**,** 263-81.

PARDO, L. M., RIZZU, P., FRANCESCATTO, M., VITEZIC, M., LEDAY, G. G., SANCHEZ, J. S., KHAMIS, A., TAKAHASHI, H., VAN DE BERG, W. D., MEDVEDEVA, Y. A., VAN DE WIEL, M. A., DAUB, C. O., CARNINCI, P. & HEUTINK, P. 2013. Regional differences in gene expression and promoter usage in aged human brains. *Neurobiol Aging,* 34**,** 1825-36.

PARIA, B. C., ZHAO, X., WANG, J., DAS, S. K. & DEY, S. K. 1999. Fatty-acid amide hydrolase is expressed in the mouse uterus and embryo during the periimplantation period. *Biol Reprod,* 60**,** 1151-7.

PARKINGTON, H. C., KENNA, K. R., SOZO, F., COLEMAN, H. A., BOCKING, A., BRIEN, J. F., HARDING, R., WALKER, D. W., MORLEY, R. & TARE, M. 2014. Maternal alcohol consumption in pregnancy enhances arterial stiffness and alters vasodilator function that varies between vascular beds in fetal sheep. *J Physiol,* 592**,** 2591-603.

PARNELL, S. E., RAMADOSS, J., DELP, M. D., RAMSEY, M. W., CHEN, W. J., WEST, J. R. & CUDD, T. A. 2007. Chronic ethanol increases fetal cerebral blood flow specific to the ethanol-sensitive cerebellum under normoxaemic, hypercapnic and acidaemic conditions: ovine model. *Exp Physiol,* 92**,** 933-43.

PATRICK, J., RICHARDSON, B., HASEN, G., CLARKE, D., WLODEK, M., BOUSQUET, J. & BRIEN, J. 1985. Effects of maternal ethanol infusion on fetal cardiovascular and brain activity in lambs. *Am J Obstet Gynecol,* 151**,** 859-67.

PATSKOVSKY, Y. V., PATSKOVSKA, L. N. & LISTOWSKY, I. 1999. An asparagine-phenylalanine substitution accounts for catalytic differences between hGSTM3-3 and other human class mu glutathione S-transferases. *Biochemistry,* 38**,** 16187-94.

PEREZ, H. D., VILLANUEVA, J. E. & SALAS, J. M. 1991. Behavioral and Hippocampal Morphological Changes Induced by Ethanol Administered to Pregnant Rats. *Annals of the New York Academy of Sciences,* 625**,** 300-304.

PHANG, J. M. 1995. Disorders of proline and hydroxyproline metabolism. *The metabolic basis of inherited disease*.

PIN, A. L., HOULE, F., GUILLONNEAU, M., PAQUET, E. R., SIMARD, M. J. & HUOT, J. 2012. miR-20a represses endothelial cell migration by targeting MKK3 and inhibiting p38 MAP kinase activation in response to VEGF. *Angiogenesis,* 15**,** 593-608.

POETTER, K., JIANG, H., HASSANZADEH, S., MASTER, S. R., CHANG, A., DALAKAS, M. C., RAYMENT, I., SELLERS, J. R., FANANAPAZIR, L. &

EPSTEIN, N. D. 1996. Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. *Nat Genet,* 13**,** 63-9.

POPOVA, S., LANGE, S., SHIELD, K., MIHIC, A., CHUDLEY, A. E., MUKHERJEE, R. A., BEKMURADOV, D. & REHM, J. 2016a. Comorbidity of fetal alcohol spectrum disorder: a systematic review and meta-analysis. *The Lancet,* 387**,** 978-987.

POPOVA, S., LANGE, S., SHIELD, K., MIHIC, A., CHUDLEY, A. E., MUKHERJEE, R. A. S., BEKMURADOV, D. & REHM, J. 2016b. Comorbidity of fetal alcohol spectrum disorder: a systematic review and meta-analysis. *Lancet,* 387**,** 978-987.

PRITTS, E. A., PARKER, W. H. & OLIVE, D. L. 2009. Fibroids and infertility: an updated systematic review of the evidence. *Fertil Steril,* 91**,** 1215-23.

RAAB, R. M., BULLEN, J., KELLEHER, J., MANTZOROS, C. & STEPHANOPOULOS, G. 2005. Regulation of mouse hepatic genes in response to diet induced obesity, insulin resistance and fasting induced weight reduction. *Nutr Metab (Lond),* 2**,** 15.

RAKOCZY, J., ZHANG, Z., BOWLING, F. G., DAWSON, P. A. & SIMMONS, D. G. 2015. Loss of the sulfate transporter Slc13a4 in placenta causes severe fetal abnormalities and death in mice. *Cell Res,* 25**,** 1273-6.

RAMADOSS, J. 2019. Fetal hippocampus FASD RNA Sequencing.

RAMADOSS, J., JOBE, S. O. & MAGNESS, R. R. 2011. Alcohol and Maternal Uterine Vascular Adaptations During Pregnancy—Part I: Effects of Chronic In Vitro Binge-Like Alcohol on Uterine Endothelial Nitric Oxide System and Function. *Alcoholism: Clinical and Experimental Research,* 35**,** 1686-1693.

RAMADOSS, J. & MAGNESS, R. R. 2012. Vascular effects of maternal alcohol consumption. *American Journal of Physiology-Heart and Circulatory Physiology,* 303**,** H414-H421.

RAMADOSS, J., WU, G. & CUDD, T. A. 2008. Chronic binge ethanol-mediated acidemia reduces availability of glutamine and related amino acids in maternal plasma of pregnant sheep. *Alcohol,* 42**,** 657-66.

RAMOS, A. J., EVRARD, S. G., TAGLIAFERRO, P., TRICÁRICO, M. V. & BRUSCO, A. 2002. Effects of chronic maternal ethanol exposure on hippocampal and striatal morphology in offspring. *Annals of the New York Academy of Sciences,* 965**,** 343-353.

RANGMAR, J., SANDBERG, A. D., ARONSON, M. & FAHLKE, C. 2015. Cognitive and executive functions, social cognition and sense of coherence in adults with fetal alcohol syndrome. *Nord J Psychiatry,* 69**,** 472-8.

RASMUSSEN, C., BECKER, M., MCLENNAN, J., URICHUK, L. & ANDREW, G. 2011. An evaluation of social skills in children with and without prenatal alcohol exposure. *Child Care Health Dev,* 37**,** 711-8.

RASMUSSEN, C. & WYPER, K. 2007. Decision making, executive functioning and risky behaviors in adolescents with prenatal alcohol exposure. *International Journal on Disability and Human Development,* 6**,** 405-416.

REDEI, E., HALASZ, I., LI, L. F., PRYSTOWSKY, M. B. & AIRD, F. 1993. Maternal adrenalectomy alters the immune and endocrine functions of fetal alcohol-exposed male offspring. *Endocrinology,* 133**,** 452-60.

REDGRAVE, P., PRESCOTT, T. J. & GURNEY, K. 1999. Is the short-latency dopamine response too short to signal reward error? *Trends Neurosci,* 22**,** 146-51.

REHMAN, K. S., YIN, S., MAYHEW, B. A., WORD, R. A. & RAINEY, W. E. 2003. Human myometrial adaptation to pregnancy: cDNA microarray gene expression profiling of myometrium from non-pregnant and pregnant women. *Mol Hum Reprod,* 9**,** 681-700.

REYES, E., WOLFE, J. & SAVAGE, D. D. 1989. The effects of prenatal alcohol exposure on radial arm maze performance in adult rats. *Physiol Behav,* 46**,** 45-8.

REYNOLDS, J. D., PENNING, D. H., DEXTER, F., ATKINS, B., HRDY, J., PODUSKA, D., CHESTNUT, D. H. & BRIEN, J. F. 1995. Dose-dependent effects of acute in vivo ethanol exposure on extracellular glutamate concentration in the cerebral cortex of the near-term fetal sheep. *Alcohol Clin Exp Res,* 19**,** 1447-53.

RICHARDSON, B. S., PATRICK, J. E., BOUSQUET, J., HOMAN, J. & BRIEN, J. F. 1985. Cerebral metabolism in fetal lamb after maternal infusion of ethanol. *Am J Physiol,* 249**,** R505-9.

RIIKONEN, R., SALONEN, I., PARTANEN, K. & VERHO, S. 1999. Brain perfusion SPECT and MRI in foetal alcohol syndrome. *Dev Med Child Neurol,* 41**,** 652-9.

RILEY, E. P., INFANTE, M. A. & WARREN, K. R. 2011a. Fetal alcohol spectrum disorders: an overview. *Neuropsychology review,* 21**,** 73.

RILEY, E. P., INFANTE, M. A. & WARREN, K. R. 2011b. Fetal alcohol spectrum disorders: an overview. *Neuropsychol Rev,* 21**,** 73-80.

RILEY, E. P., MATTSON, S. N., SOWELL, E. R., JERNIGAN, T. L., SOBEL, D. F. & JONES, K. L. 1995. Abnormalities of the corpus callosum in children prenatally exposed to alcohol. *Alcohol Clin Exp Res,* 19**,** 1198-202.

RILEY, E. P. & MCGEE, C. L. 2005. Fetal alcohol spectrum disorders: an overview with emphasis on changes in brain and behavior. *Exp Biol Med (Maywood),* 230**,** 357-65.

RIVERA, A. & MAXWELL, S. A. 2005. The p53-induced gene-6 (proline oxidase) mediates apoptosis through a calcineurin-dependent pathway. *Journal of Biological Chemistry*.

ROCHE, K. C., WIECHENS, N., OWEN-HUGHES, T. & PERKINS, N. D. 2004. The FHA domain protein SNIP1 is a regulator of the cell cycle and cyclin D1 expression. *Oncogene,* 23**,** 8185-95.

RODRIGUEZ, C. I., DAVIES, S., CALHOUN, V., SAVAGE, D. D. & HAMILTON, D. A. 2016. Moderate Prenatal Alcohol Exposure Alters Functional Connectivity in the Adult Rat Brain. *Alcohol Clin Exp Res,* 40**,** 2134-2146.

ROMERT, P. & MATTHIESSEN, M. E. 1984. Fine structure of hepatocytes from mini-pigs and mini-pig fetuses exposed to alcohol (ethanol) in vivo. *Acta Anat (Basel),* 120**,** 190-5.

RONDELLI, V., BROCCA, P., FRAGNETO, G., DAILLANT, J., TRINGALI, C., CANTU, L. & DEL FAVERO, E. 2017. Membrane restructuring following in situ sialidase digestion of gangliosides: Complex model bilayers by synchrotron radiation reflectivity. *Biochim Biophys Acta Biomembr,* 1859**,** 845-851.

ROYALTY, J. 1990. Effects of prenatal ethanol exposure on juvenile play-fighting and postpubertal aggression in rats. *Psychol Rep,* 66**,** 551-60.

RYAN, S. H., WILLIAMS, J. K. & THOMAS, J. D. 2008. Choline supplementation attenuates learning deficits associated with neonatal alcohol exposure in the rat: effects of varying the timing of choline administration. *Brain research,* 1237**,** 91-100.

SADRIAN, B., WILSON, D. A. & SAITO, M. 2013. Long-lasting neural circuit dysfunction following developmental ethanol exposure. *Brain Sci,* 3**,** 704-27.

SAIKALI, S., MEURICE, P., SAULEAU, P., ELIAT, P. A., BELLAUD, P., RANDUINEAU, G., VERIN, M. & MALBERT, C. H. 2010. A three-dimensional digital segmented and deformable brain atlas of the domestic pig. *J Neurosci Methods,* 192**,** 102-9.

SALBAUM, J. M. & KAPPEN, C. 2010. Neural tube defect genes and maternal diabetes during pregnancy. *Birth Defects Res A Clin Mol Teratol,* 88**,** 601-11.

SAREDDY, G. R., ZHANG, Q., WANG, R., SCOTT, E., ZOU, Y., O'CONNOR, J. C., CHEN, Y., DONG, Y., VADLAMUDI, R. K. & BRANN, D. 2015. Proline-, glutamic acid-, and leucine-rich protein 1 mediates estrogen rapid signaling and neuroprotection in the brain. *Proceedings of the National Academy of Sciences,* 112**,** E6673-E6682.

SATO, K. & MIYAGI, T. 1996. Involvement of an endogenous sialidase in skeletal muscle cell differentiation. *Biochem Biophys Res Commun,* 221**,** 826-30.

SATO, T., ROCANCOURT, D., MARQUES, L., THORSTEINSDOTTIR, S. & BUCKINGHAM, M. 2010. A Pax3/Dmrt2/Myf5 regulatory cascade functions at the onset of myogenesis. *PLoS Genet,* 6**,** e1000897.

SAVAGE, D. D., ROSENBERG, M. J., WOLFF, C. R., AKERS, K. G., EL-EMAWY, A., STAPLES, M. C., VARASCHIN, R. K., WRIGHT, C. A., SEIDEL, J. L., CALDWELL, K. K. & HAMILTON, D. A. 2010. Effects of a novel cognition-enhancing agent on fetal ethanol-induced learning deficits. *Alcohol Clin Exp Res,* 34**,** 1793-802.

SAWANT, O. B., LUNDE, E. R., WASHBURN, S. E., CHEN, W. J., GOODLETT, C. R. & CUDD, T. A. 2013. Different patterns of regional Purkinje cell loss in the cerebellar vermis as a function of the timing of prenatal ethanol exposure in an ovine model. *Neurotoxicol Teratol,* 35**,** 7-13.

SCHNEIDER, J., ANDERSON, D., SONNENAHALLI, H. & VADIGEPALLI, R. 2011. Sex-based differences in gene expression in hippocampus following postnatal lead exposure. *Toxicology and applied pharmacology,* 256**,** 179-190.

SCHNEIDER, M. L., MOORE, C. F., BARNHART, T. E., LARSON, J. A., DEJESUS, O. T., MUKHERJEE, J., NICKLES, R. J., CONVERSE, A. K., ROBERTS, A. D. & KRAEMER, G. W. 2005. Moderate-level prenatal alcohol exposure alters striatal dopamine system function in rhesus monkeys. *Alcohol Clin Exp Res,* 29**,** 1685-97.

SCHNEIDER, M. L., MOORE, C. F., KRAEMER, G. W., ROBERTS, A. D. & DEJESUS, O. T. 2002. The impact of prenatal stress, fetal alcohol exposure, or both on development: perspectives from a primate model. *Psychoneuroendocrinology,* 27**,** 285-98.

SCHNEIDER, M. L., ROUGHTON, E. C. & LUBACH, G. R. 1997. Moderate Alcohol Consumption and Psychological Stress during Pregnancy Induce Attention and Neuromotor Impairments in Primate Infants. *Child Dev,* 68**,** 747-759.

SCHNEIDER, M. L. & SUOMI, S. J. 1992. Neurobehavioral assessment in rhesus monkey neonates (Macaca mulatta): developmental changes, behavioral stability, and early experience. *Infant behavior and development,* 15**,** 155-177.

SCHWARZ, G. 2005. Molybdenum cofactor biosynthesis and deficiency. *Cellular and Molecular Life Sciences CMLS,* 62**,** 2792-2810.

SCHWEIZER, N., PUPE, S., ARVIDSSON, E., NORDENANKAR, K., SMITH-ANTTILA, C. J., MAHMOUDI, S., ANDREN, A., DUMAS, S., RAJAGOPALAN, A., LEVESQUE, D., LEAO, R. N. & WALLEN-MACKENZIE, A. 2014. Limiting glutamate transmission in a Vglut2-expressing subpopulation of the subthalamic nucleus is sufficient to cause hyperlocomotion. *Proc Natl Acad Sci U S A,* 111**,** 7837-42.

SCRIVER, C. R., SMITH, R. & PHANG, J. 1983. Disorders of proline and hydroxyproline metabolism. *Metabolic basis of inherited disease/[edited by] John B. Stanbury...[et al.]*.

SECREST, R. J., OHLSTEIN, E. H. & CHAPNICK, B. M. 1988. Relationship between LTD4-induced endothelium-dependent vasomotor relaxation and cGMP. *J Pharmacol Exp Ther,* 245**,** 47-52.

SEGURA, M. F., SOLE, C., PASCUAL, M., MOUBARAK, R. S., PEREZ-GARCIA, M. J., GOZZELINO, R., IGLESIAS, V., BADIOLA, N., BAYASCAS, J. R., LLECHA, N., RODRIGUEZ-ALVAREZ, J., SORIANO, E., YUSTE, V. J. & COMELLA, J. X. 2007. The long form of Fas apoptotic inhibitory molecule is expressed specifically in neurons and protects them against death receptor-triggered apoptosis. *J Neurosci,* 27**,** 11228-41.

SELEVERSTOV, O., TOBIASZ, A., JACKSON, J. S., SULLIVAN, R., MA, D., SULLIVAN, J. P., DAVISON, S., AKKHAWATTANANGKUL, Y., TATE, D. L., COSTELLO, T., BARNETT, S., LI, W., MARI, G., DOPICO, A. M. & BUKIYA, A. N. 2017. Maternal alcohol exposure during mid-pregnancy dilates fetal cerebral arteries via endocannabinoid receptors. *Alcohol,* 61**,** 51-61.

SEO, K. W., WANG, Y., KOKUBO, H., KETTLEWELL, J. R., ZARKOWER, D. A. & JOHNSON, R. L. 2006. Targeted disruption of the DM domain containing transcription factor Dmrt2 reveals an essential role in somite patterning. *Dev Biol,* 290**,** 200-10.

SHA, J., ZHAO, G., CHEN, X., GUAN, W., HE, Y. & WANG, Z. 2012. Antibacterial potential of hGlyrichin encoded by a human gene. *J Pept Sci,* 18**,** 97-104.

SHAO, X., TARNASKY, H. A., LEE, J. P., OKO, R. & VAN DER HOORN, F. A. 1999. Spag4, a novel sperm protein, binds outer dense-fiber protein Odf1 and localizes to microtubules of manchette and axoneme. *Dev Biol,* 211**,** 109-23.

SHARIF, A. S., YU, D., LOERTSCHER, S., AUSTIN, R., NGUYEN, K., MATHUR, P. D., CLARK, A. M., ZOU, J., LOBANOVA, E. S., ARSHAVSKY, V. Y. & YANG, J. 2018. C8ORF37 Is Required for Photoreceptor Outer Segment Disc Morphogenesis by Maintaining Outer Segment Membrane Protein Homeostasis. *J Neurosci,* 38**,** 3160-3176.

SHEIKH, F., LYON, R. C. & CHEN, J. 2015. Functions of myosin light chain-2 (MYL2) in cardiac muscle and disease. *Gene,* 569**,** 14-20.

SHEN, H., ZHANG, L., ZHOU, J., CHEN, Z., YANG, G., LIAO, Y. & ZHU, M. 2017. Epidermal Growth Factor-Containing Fibulin-Like Extracellular Matrix Protein 1 (EFEMP1) Acts as a Potential Diagnostic Biomarker for Prostate Cancer. *Med Sci Monit,* 23**,** 216-222.

SHEN, Y. C., LIAO, D. L., CHEN, J. Y., WANG, Y. C., LAI, I. C., LIOU, Y. J., CHEN, Y. J., LUU, S. U. & CHEN, C. H. 2009. Resequencing and association study of vesicular glutamate transporter 1 gene (VGLUT1) with schizophrenia. *Schizophr Res,* 115**,** 254-60.

SHETTY, A. K. & PHILLIPS, D. E. 1992. Effects of prenatal ethanol exposure on the development of Bergmann glia and astrocytes in the rat cerebellum: an immunohistochemical study. *J Comp Neurol,* 321**,** 19-32.

SHIAH, S. G., HSIAO, J. R., CHANG, W. M., CHEN, Y. W., JIN, Y. T., WONG, T. Y., HUANG, J. S., TSAI, S. T., HSU, Y. M., CHOU, S. T., YEN, Y. C., JIANG, S. S., SHIEH, Y. S., CHANG, I. S., HSIAO, M. & CHANG, J. Y. 2014. Downregulated miR329 and miR410 promote the proliferation and invasion of oral squamous cell carcinoma by targeting Wnt-7b. *Cancer Res,* 74**,** 7560-72.

SHIOMI, K., UCHIDA, H., KEINO-MASU, K. & MASU, M. 2003. Ccd1, a novel protein with a DIX domain, is a positive regulator in the Wnt signaling during zebrafish neural patterning. *Curr Biol,* 13**,** 73-7.

SHOJI, K., MURAYAMA, T., MIMURA, I., WADA, T., KUME, H., GOTO, A., OHSE, T., TANAKA, T., INAGI, R., VAN DER HOORN, F. A., MANABE, I., HOMMA, Y., FUKAYAMA, M., SAKURAI, T., HASEGAWA, T., ABURATANI, H., KODAMA, T. & NANGAKU, M. 2013. Sperm-associated antigen 4, a novel hypoxia-inducible factor 1 target, regulates cytokinesis, and its expression correlates with the prognosis of renal cell carcinoma. *Am J Pathol,* 182**,** 2191-203.

SLIWOWSKA, J. H., BARKER, J. M., BARHA, C. K., LAN, N., WEINBERG, J. & GALEA, L. A. 2010. Stress-induced suppression of hippocampal neurogenesis in adult male rats is altered by prenatal ethanol exposure. *Stress,* 13**,** 301-13.

SMITH, D. E. & DAVIES, D. L. 1990. Effect of perinatal administration of ethanol on the CA1 pyramidal cell of the hippocampus and Purkinje cell of the cerebellum: an ultrastructural survey. *J Neurocytol,* 19**,** 708-17.

SMITH, S. M., GARIC, A., BERRES, M. E. & FLENTKE, G. R. 2014a. Genomic factors that shape craniofacial outcome and neural crest vulnerability in FASD. *Front Genet,* 5**,** 224.

SMITH, S. M., GARIC, A., BERRES, M. E. & FLENTKE, G. R. 2014b. Genomic factors that shape craniofacial outcome and neural crest vulnerability in FASD. *Frontiers in genetics,* 5**,** 224.

SOKOL, R. J. & CLARREN, S. K. 1989. Guidelines for use of terminology describing the impact of prenatal alcohol on the offspring. *Alcohol Clin Exp Res,* 13**,** 597-8.

SOKOL, R. J., DELANEY-BLACK, V. & NORDSTROM, B. 2003a. Fetal alcohol spectrum disorder. *JAMA,* 290**,** 2996-9.

SOKOL, R. J., DELANEY-BLACK, V. & NORDSTROM, B. 2003b. Fetal alcohol spectrum disorder. *Jama,* 290**,** 2996-2999.

SOLE, C., DOLCET, X., SEGURA, M. F., GUTIERREZ, H., DIAZ-MECO, M. T., GOZZELINO, R., SANCHIS, D., BAYASCAS, J. R., GALLEGO, C., MOSCAT, J., DAVIES, A. M. & COMELLA, J. X. 2004. The death receptor antagonist FAIM promotes neurite outgrowth by a mechanism that depends on ERK and NF-kapp B signaling. *J Cell Biol,* 167**,** 479-92.

SONG, S. W., FULLER, G. N., KHAN, A., KONG, S., SHEN, W., TAYLOR, E., RAMDAS, L., LANG, F. F. & ZHANG, W. 2003. IIp45, an insulin-like growth factor binding protein 2 (IGFBP-2) binding protein, antagonizes IGFBP-2 stimulation of glioma cell invasion. *Proc Natl Acad Sci U S A,* 100**,** 13970-5.

SPADONI, A. D., BAZINET, A. D., FRYER, S. L., TAPERT, S. F., MATTSON, S. N. & RILEY, E. P. 2009. BOLD response during spatial working memory in youth with heavy prenatal alcohol exposure. *Alcohol Clin Exp Res,* 33**,** 2067-76.

SPOHR, H.-L. & STEINHAUSEN, H.-C. 2008a. Fetal alcohol spectrum disorders and their persisting sequelae in adult life. *Deutsches Ärzteblatt International,* 105**,** 693.

SPOHR, H. L. & STEINHAUSEN, H. C. 2008b. Fetal alcohol spectrum disorders and their persisting sequelae in adult life. *Dtsch Arztebl Int,* 105**,** 693-8.

SREEDHARAN, S., STEPHANSSON, O., SCHIOTH, H. B. & FREDRIKSSON, R. 2011. Long evolutionary conservation and considerable tissue specificity of several atypical solute carrier transporters. *Gene,* 478**,** 11-8.

STANTON, M. E. & GOODLETT, C. R. 1998. Neonatal ethanol exposure impairs eyeblink conditioning in weanling rats. *Alcohol Clin Exp Res,* 22**,** 270-5.

STEINHAUSEN, H.-C. 1996. Psychopathology and cognitive functioning in children with fetal alcohol syndrome. *Alcohol, pregnancy and the developing child***,** 227-246.

STOJANOVIC, R., TODOROVIC, Z., NESIC, Z., VUCKOVIC, S., CEROVAC-COSIC, N. & PROSTRAN, M. 2004. NG-nitro-L-arginine methyl ester-induced potentiaton of the effect of aminophylline on rat diaphragm: the role of extracellular calcium. *Journal of pharmacological sciences,* 96**,** 493-498.

STOJANOVIC, R., TODOROVIC, Z., VUCKOVIC, S., NESIC, Z. & PROSTRAN, M. 2003. NG-nitro-L-arginine methyl ester potentiates the effect of aminophylline on the isolated rat hemidiaphragm. *Journal of pharmacological sciences,* 92**,** 157-162.

STREISSGUTH, A. P., AASE, J. M., CLARREN, S. K., RANDELS, S. P., LADUE, R. A. & SMITH, D. F. 1991. Fetal alcohol syndrome in adolescents and adults. *JAMA,* 265**,** 1961-7.

STREISSGUTH, A. P., BARR, H. M., KOGAN, J. & BOOKSTEIN, F. L. 1996. Understanding the occurrence of secondary disabilities in clients with fetal alcohol syndrome (FAS) and fetal alcohol effects (FAE). *Final report to the Centers for Disease Control and Prevention (CDC)***,** 96-06.

SU, S. H., WU, C. H., CHIU, Y. L., CHANG, S. J., LO, H. H., LIAO, K. H., TSAI, C. F., TSAI, T. N., LIN, C. H., CHENG, S. M., CHENG, C. C. & WANG, H. W. 2017. Dysregulation of Vascular Endothelial Growth Factor Receptor-2 by Multiple miRNAs in Endothelial Colony-Forming Cells of Coronary Artery Disease. *J Vasc Res,* 54**,** 22-32.

SUBBANNA, S., SHIVAKUMAR, M., PSYCHOYOS, D., XIE, S. & BASAVARAJAPPA, B. S. 2013. Anandamide-CB1 receptor signaling contributes to postnatal ethanol-induced neonatal neurodegeneration, adult synaptic, and memory deficits. *J Neurosci,* 33**,** 6350-66.

SUBRAMANIAN, K., NAIK, V. D., SATHISHKUMAR, K., YALLAMPALLI, C., SAADE, G. R., HANKINS, G. D. & RAMADOSS, J. 2014. Chronic binge alcohol exposure during pregnancy impairs rat maternal uterine vascular function. *Alcoholism: Clinical and Experimental Research,* 38**,** 1832-1838.

SULIK, K. K., JOHNSTON, M. C. & WEBB, M. A. 1981. Fetal alcohol syndrome: embryogenesis in a mouse model. *Science,* 214**,** 936-8.

SUN, L., DENG, L., EA, C. K., XIA, Z. P. & CHEN, Z. J. 2004. The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol Cell,* 14**,** 289-301.

SUNDELIN-WAHLSTEN, V., HALLBERG, G. & HELANDER, A. 2017. Higher alcohol consumption in early pregnancy or low-to-moderate drinking during pregnancy may affect children's behaviour and development at one year and six months. *Acta Paediatr,* 106**,** 446-453.

SUSSWEIN, A. J., KATZOFF, A., MILLER, N. & HURWITZ, I. 2004. Nitric oxide and memory. *The Neuroscientist,* 10**,** 153-162.

SUTHERLAND, R. J., MCDONALD, R. J. & SAVAGE, D. D. 1997. Prenatal exposure to moderate levels of ethanol can have long-lasting effects on hippocampal synaptic plasticity in adult offspring. *Hippocampus,* 7**,** 232-8.

SUZUKI, S., SANO, K. & TANIHARA, H. 1991. Diversity of the cadherin family: evidence for eight new cadherins in nervous tissue. *Cell Regul,* 2**,** 261-70.

SUZUKI, T., TOYOHARA, T., AKIYAMA, Y., TAKEUCHI, Y., MISHIMA, E., SUZUKI, C., ITO, S., SOGA, T. & ABE, T. 2011. Transcriptional regulation of organic anion transporting polypeptide SLCO4C1 as a new therapeutic modality to prevent chronic kidney disease. *J Pharm Sci,* 100**,** 3696-707.

TAKASAKI, J., KAMOHARA, M., MATSUMOTO, M., SAITO, T., SUGIMOTO, T., OHISHI, T., ISHII, H., OTA, T., NISHIKAWA, T., KAWAI, Y., MASUHO, Y., ISOGAI, T., SUZUKI, Y., SUGANO, S. & FURUICHI, K. 2000. The molecular characterization and tissue distribution of the human cysteinyl leukotriene CysLT(2) receptor. *Biochem Biophys Res Commun,* 274**,** 316-22.

TAN, C. H., DENNY, C. H., CHEAL, N. E., SNIEZEK, J. E. & KANNY, D. 2015a. Alcohol Use and Binge Drinking Among Women of Childbearing Age - United States, 2011-2013. *Mmwr-Morbidity and Mortality Weekly Report,* 64**,** 1042-1046.

TAN, C. H., DENNY, C. H., CHEAL, N. E., SNIEZEK, J. E. & KANNY, D. 2015b. Alcohol use and binge drinking among women of childbearing age - United States, 2011-2013. *MMWR Morb Mortal Wkly Rep,* 64**,** 1042-6.

TAN, R. R., LI, Y. F., ZHANG, X. T., HUANG, Y. H., WU, Y. P., OUYANG, S. H., TSOI, B., YI, R. N., YANG, X., KURIHARA, H. & HE, R. R. 2013. Glucose

metabolism disorder is a risk factor in ethanol exposure induced malformation in embryonic brain. *Food Chem Toxicol,* 60**,** 238-45.

TANAKA, H., INOMATA, K. & ARIMA, M. 1983. Zinc supplementation in ethanol-treated pregnant rats increases the metabolic activity in the fetal hippocampus. *Brain Dev,* 5**,** 549-54.

TANG, J., HU, J., XUE, M., GUO, Z., XIE, M., ZHANG, B., ZHOU, Z., HUANG, W. & HOU, S. 2019. Maternal diet deficient in riboflavin induces embryonic death associated with alterations in the hepatic proteome of duck embryos. *Nutr Metab (Lond),* 16**,** 19.

THANH, N. X., JONSSON, E., DENNETT, L. & JACOBS, P. 2011. Costs of FASD. *Fetal Alcohol Spectrum Disorder,* 4**,** 1.

THOMAS, J. D., WASSERMAN, E. A., WEST, J. R. & GOODLETT, C. R. 1996. Behavioral deficits induced by bingelike exposure to alcohol in neonatal rats: importance of developmental timing and number of episodes. *Dev Psychobiol,* 29**,** 433-52.

THOMAS, J. D., WEINERT, S. P., SHARIF, S. & RILEY, E. P. 1997. MK-801 administration during ethanol withdrawal in neonatal rat pups attenuates ethanol-induced behavioral deficits. *Alcohol Clin Exp Res,* 21**,** 1218-25.

THOMAS, S. E., KELLY, S. J., MATTSON, S. N. & RILEY, E. P. 1998. Comparison of social abilities of children with fetal alcohol syndrome to those of children with similar IQ scores and normal controls. *Alcohol Clin Exp Res,* 22**,** 528-33.

TITTERNESS, A. K. & CHRISTIE, B. R. 2012. Prenatal ethanol exposure enhances NMDAR-dependent long-term potentiation in the adolescent female dentate gyrus. *Hippocampus,* 22**,** 69-81.

TOBIASZ, A. M., DUNCAN, J. R., BURSAC, Z., SULLIVAN, R. D., TATE, D. L., DOPICO, A. M., BUKIYA, A. N. & MARI, G. 2018. The Effect of Prenatal Alcohol Exposure on Fetal Growth and Cardiovascular Parameters in a Baboon Model of Pregnancy. *Reprod Sci,* 25**,** 1116-1123.

TONG, M., ZIPLOW, J., CHEN, W. C., NGUYEN, Q. G., KIM, C. & DE LA MONTE, S. M. 2013. Motor Function Deficits Following Chronic Prenatal Ethanol Exposure are Linked to Impairments in Insulin/IGF, Notch and Wnt Signaling in the Cerebellum. *J Diabetes Metab,* 4**,** 238.

TOYOHARA, T., SUZUKI, T., MORIMOTO, R., AKIYAMA, Y., SOUMA, T., SHIWAKU, H. O., TAKEUCHI, Y., MISHIMA, E., ABE, M., TANEMOTO, M., MASUDA, S., KAWANO, H., MAEMURA, K., NAKAYAMA, M., SATO, H., MIKKAICHI, T., YAMAGUCHI, H., FUKUI, S., FUKUMOTO, Y., SHIMOKAWA, H., INUI, K., TERASAKI, T., GOTO, J., ITO, S., HISHINUMA, T., RUBERA, I., TAUC, M., FUJII-KURIYAMA, Y., YABUUCHI, H., MORIYAMA, Y., SOGA, T. & ABE, T. 2009. SLCO4C1 transporter eliminates uremic toxins and attenuates hypertension and renal inflammation. *J Am Soc Nephrol,* 20**,** 2546-55.

TRAUPE, H., FISCHER, J. & OJI, V. 2014. Nonsyndromic types of ichthyoses - an update. *J Dtsch Dermatol Ges,* 12**,** 109-21.

TRAYNELIS, S. F., WOLLMUTH, L. P., MCBAIN, C. J., MENNITI, F. S., VANCE, K. M., OGDEN, K. K., HANSEN, K. B., YUAN, H., MYERS, S. J. & DINGLEDINE, R. 2010. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev,* 62**,** 405-96.

TRINGALI, C., LUPO, B., ANASTASIA, L., PAPINI, N., MONTI, E., BRESCIANI, R., TETTAMANTI, G. & VENERANDO, B. 2007. Expression of sialidase Neu2 in leukemic K562 cells induces apoptosis by impairing Bcr-Abl/Src kinases signaling. *J Biol Chem,* 282**,** 14364-72.

TUBB, B. E., BARDIEN-KRUGER, S., KASHORK, C. D., SHAFFER, L. G., RAMAGLI, L. S., XU, J., SICILIANO, M. J. & BRYAN, J. 2000. Characterization of human retinal fascin gene (FSCN2) at 17q25: close physical linkage of fascin and cytoplasmic actin genes. *Genomics,* 65**,** 146-56.

TUNC-OZCAN, E., ULLMANN, T. M., SHUKLA, P. K. & REDEI, E. E. 2013. Low-dose thyroxine attenuates autism-associated adverse effects of fetal alcohol in male offspring's social behavior and hippocampal gene expression. *Alcoholism: Clinical and Experimental Research,* 37**,** 1986-1995.

UBAN, K. A., BODNAR, T., BUTTS, K., SLIWOWSKA, J. H., COMEAU, W. & WEINBERG, J. 2011. Direct and indirect mechanisms of alcohol teratogenesis: Implications for understanding alterations in brain and behavior in FASD. *Fetal Alcohol Spectrum Disorder***,** 71-108.

UBAN, K. A., SLIWOWSKA, J. H., LIEBLICH, S., ELLIS, L. A., YU, W. K., WEINBERG, J. & GALEA, L. A. 2010. Prenatal alcohol exposure reduces the proportion of newly produced neurons and glia in the dentate gyrus of the hippocampus in female rats. *Horm Behav,* 58**,** 835-43.

USIELLO, A., BAIK, J. H., ROUGE-PONT, F., PICETTI, R., DIERICH, A., LEMEUR, M., PIAZZA, P. V. & BORRELLI, E. 2000. Distinct functions of the two isoforms of dopamine D2 receptors. *Nature,* 408**,** 199-203.

VALLE-RIOS, R., MARAVILLAS-MONTERO, J. L., BURKHARDT, A. M., MARTINEZ, C., BUHREN, B. A., HOMEY, B., GERBER, P. A., ROBINSON, O., HEVEZI, P. & ZLOTNIK, A. 2014. Isthmin 1 is a secreted protein expressed in skin, mucosal tissues, and NK, NKT, and th17 cells. *J Interferon Cytokine Res,* 34**,** 795-801.

VALLES, S., PITARCH, J., RENAU-PIQUERAS, J. & GUERRI, C. 1997. Ethanol exposure affects glial fibrillary acidic protein gene expression and transcription during rat brain development. *J Neurochem,* 69**,** 2484-93.

VAN DEN HOVE, D., KENIS, G., BRASS, A., OPSTELTEN, R., RUTTEN, B., BRUSCHETTINI, M., BLANCO, C., LESCH, K., STEINBUSCH, H. & PRICKAERTS, J. 2013. Vulnerability versus resilience to prenatal stress in male and female rats; implications from gene expression profiles in the hippocampus and frontal cortex. *European Neuropsychopharmacology,* 23**,** 1226-1246.

VERA, A., STANIC, K., MONTECINOS, H., TORREJON, M., MARCELLINI, S. & CAPRILE, T. 2013. SCO-spondin from embryonic cerebrospinal fluid is required for neurogenesis during early brain development. *Front Cell Neurosci,* 7**,** 80.

VORHEES, C. V. & WILLIAMS, M. T. 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc,* 1**,** 848-58.

WAINWRIGHT, P. E., HUANG, Y. S., SIMMONS, V., MILLS, D. E., WARD, R. P., WARD, G. R., WINFIELD, D. & MCCUTCHEON, D. 1990. Effects of prenatal ethanol and long-chain n-3 fatty acid supplementation on development in mice. 2. Fatty acid composition of brain membrane phospholipids. *Alcohol Clin Exp Res,* 14**,** 413-20.

WAKABAYASHI, T., MATSUMINE, A., NAKAZORA, S., HASEGAWA, M., IINO, T., OTA, H., SONODA, H., SUDO, A. & UCHIDA, A. 2010. Fibulin-3 negatively regulates chondrocyte differentiation. *Biochem Biophys Res Commun,* 391**,** 1116-21.

WANG, Q., YERUKHIMOVICH, M., GAARDE, W. A., POPOFF, I. J. & DOERSCHUK, C. M. 2005. MKK3 and -6-dependent activation of p38alpha MAP kinase is required for cytoskeletal changes in pulmonary microvascular endothelial cells induced by ICAM-1 ligation. *Am J Physiol Lung Cell Mol Physiol,* 288**,** L359-69.

WANG, T. & BROWN, M. J. 1999. mRNA quantification by real time TaqMan polymerase chain reaction: validation and comparison with RNase protection. *Analytical biochemistry,* 269**,** 198-201.

WANG, W., SONG, X. W. & ZHAO, C. H. 2016. Roles of programmed cell death protein 5 in inflammation and cancer (Review). *Int J Oncol,* 49**,** 1801-1806.

WANG, Y., WEN, J. & ZHANG, W. 2011. MIIP, a cytoskeleton regulator that blocks cell migration and invasion, delays mitosis, and suppresses tumorogenesis. *Curr Protein Pept Sci,* 12**,** 68-73.

WANG, Z., GERSTEIN, M. & SNYDER, M. 2009a. RNA-Seq: a revolutionary tool for transcriptomics. *Nature reviews genetics,* 10**,** 57.

WANG, Z., GERSTEIN, M. & SNYDER, M. 2009b. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet,* 10**,** 57-63.

WARD, S. M., FERNANDO, S. J., HOU, T. Y. & DUFFIELD, G. E. 2010. The transcriptional repressor ID2 can interact with the canonical clock components CLOCK and BMAL1 and mediate inhibitory effects on mPer1 expression. *J Biol Chem,* 285**,** 38987-9000.

WATANABE, H., ANDERSEN, F., SIMONSEN, C. Z., EVANS, S. M., GJEDDE, A., CUMMING, P. & DANE, X. S. G. 2001. MR-based statistical atlas of the Gottingen minipig brain. *Neuroimage,* 14**,** 1089-96.

WATARI, H., BORN, D. E. & GLEASON, C. A. 2006. Effects of first trimester binge alcohol exposure on developing white matter in fetal sheep. *Pediatr Res,* 59**,** 560-4.

WEEDON, M. N., LANGO, H., LINDGREN, C. M., WALLACE, C., EVANS, D. M., MANGINO, M., FREATHY, R. M., PERRY, J. R., STEVENS, S., HALL, A. S., SAMANI, N. J., SHIELDS, B., PROKOPENKO, I., FARRALL, M., DOMINICZAK, A., DIABETES GENETICS, I., WELLCOME TRUST CASE CONTROL, C., JOHNSON, T., BERGMANN, S., BECKMANN, J. S., VOLLENWEIDER, P., WATERWORTH, D. M., MOOSER, V., PALMER, C. N., MORRIS, A. D., OUWEHAND, W. H., CAMBRIDGE, G. E. M. C., ZHAO, J. H., LI, S., LOOS, R. J., BARROSO, I., DELOUKAS, P., SANDHU, M. S., WHEELER, E., SORANZO, N., INOUYE, M., WAREHAM, N. J., CAULFIELD, M., MUNROE, P. B., HATTERSLEY, A. T., MCCARTHY, M. I. & FRAYLING, T. M. 2008. Genome-wide association analysis identifies 20 loci that influence adult height. *Nat Genet,* 40**,** 575-83.

WEINSTEIN, D. E., SHELANSKI, M. L. & LIEM, R. K. 1991. Suppression by antisense mRNA demonstrates a requirement for the glial fibrillary acidic protein in the formation of stable astrocytic processes in response to neurons. *J Cell Biol,* 112**,** 1205-13.

WEST, J. R., GOODLETT, C. R., BONTHIUS, D. J., HAMRE, K. M. & MARCUSSEN, B. L. 1990. Cell population depletion associated with fetal alcohol brain damage: mechanisms of BAC-dependent cell loss. *Alcohol Clin Exp Res,* 14**,** 813-8.

WEST, J. R. & HODGES-SAVOLA, C. A. 1983. Permanent hippocampal mossy fiber hyperdevelopment following prenatal ethanol exposure. *Neurobehav Toxicol Teratol,* 5**,** 139-50.

WEST, J. R., HODGES, C. A. & BLACK, A. C., JR. 1981. Prenatal exposure to ethanol alters the organization of hippocampal mossy fibers in rats. *Science,* 211**,** 957-9.

WHALEY, S. E., O'CONNOR, MJ & GUNDERSON, B. 2001. Comparison of the adaptive functioning of children prenatally exposed to alcohol to a nonexposed clinical sample. *Alcohol Clin Exp Res,* 25**,** 1018-24.

WILHOIT, L. F., SCOTT, D. A. & SIMECKA, B. A. 2017. Fetal Alcohol Spectrum Disorders: Characteristics, Complications, and Treatment. *Community Ment Health J,* 53**,** 711-718.

WILLE-BILLE, A., MIRANDA-MORALES, R. S., PUCCI, M., BELLIA, F., D'ADDARIO, C. & PAUTASSI, R. M. 2018. Prenatal ethanol induces an anxiety phenotype and alters expression of dynorphin & nociceptin/orphanin FQ genes. *Prog Neuropsychopharmacol Biol Psychiatry,* 85**,** 77-88.

WILLOUGHBY, K. A., SHEARD, E. D., NASH, K. & ROVET, J. 2008a. Effects of prenatal alcohol exposure on hippocampal volume, verbal learning, and verbal and spatial recall in late childhood. *Journal of the International Neuropsychological Society,* 14**,** 1022-1033.

WILLOUGHBY, K. A., SHEARD, E. D., NASH, K. & ROVET, J. 2008b. Effects of prenatal alcohol exposure on hippocampal volume, verbal learning, and verbal and spatial recall in late childhood. *J Int Neuropsychol Soc,* 14**,** 1022-33.

WILSON, J. H., KELLY, S. J. & WILSON, M. A. 1996. Early postnatal alcohol exposure in rats: maternal behavior and estradiol levels. *Physiol Behav,* 59**,** 287-93.

WILSON, S. E. & CUDD, T. A. 2011. Focus on: the use of animal models for the study of fetal alcohol spectrum disorders. *Alcohol Res Health,* 34**,** 92-8.

WOLTHUIS, D. F., VAN ASBECK, E., MOHAMED, M., GARDEITCHIK, T., LIM-MELIA, E. R., WEVERS, R. A. & MORAVA, E. 2014. Cutis laxa, fat pads and retinopathy due to ALDH18A1 mutation and review of the literature. *Eur J Paediatr Neurol,* 18**,** 511-5.

WONDRAK, G. T., JACOBSON, M. K. & JACOBSON, E. L. 2005. Identification of quenchers of photoexcited states as novel agents for skin photoprotection. *Journal of Pharmacology and Experimental Therapeutics,* 312**,** 482-491.

WOZNIAK, D. F., HARTMAN, R. E., BOYLE, M. P., VOGT, S. K., BROOKS, A. R., TENKOVA, T., YOUNG, C., OLNEY, J. W. & MUGLIA, L. J. 2004. Apoptotic neurodegeneration induced by ethanol in neonatal mice is associated with profound learning/memory deficits in juveniles followed by progressive functional recovery in adults. *Neurobiol Dis,* 17**,** 403-14.

WOZNIAK, J. R., MUELLER, B. A., BELL, C. J., MUETZEL, R. L., HOECKER, H. L., BOYS, C. J. & LIM, K. O. 2013. Global functional connectivity abnormalities in children with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res,* 37**,** 748-56.

WOZNIAK, J. R., MUELLER, B. A., MUETZEL, R. L., BELL, C. J., HOECKER, H. L., NELSON, M. L., CHANG, P. N. & LIM, K. O. 2011. Inter-hemispheric functional connectivity disruption in children with prenatal alcohol exposure. *Alcohol Clin Exp Res,* 35**,** 849-61.

WOZNIAK, J. R., MUETZEL, R. L., MUELLER, B. A., MCGEE, C. L., FREERKS, M. A., WARD, E. E., NELSON, M. L., CHANG, P. N. & LIM, K. O. 2009. Microstructural corpus callosum anomalies in children with prenatal alcohol exposure: an extension of previous diffusion tensor imaging findings. *Alcohol Clin Exp Res,* 33**,** 1825-35.

WU, W. S. 2006. The signaling mechanism of ROS in tumor progression. *Cancer Metastasis Rev,* 25**,** 695-705.

YAMADA, K., AIBA, K., KITAURA, Y., KONDO, Y., NOMURA, N., NAKAMURA, Y., FUKUSHI, D., MURAYAMA, K., SHIMOMURA, Y., PITT, J., YAMAGUCHI, S., YOKOCHI, K. & WAKAMATSU, N. 2015. Clinical, biochemical and metabolic characterisation of a mild form of human short-chain enoyl-CoA hydratase deficiency: significance of increased N-acetyl-S-(2-carboxypropyl)cysteine excretion. *J Med Genet,* 52**,** 691-8.

YAMAMOTO, K., TAKEI, H., KOYANAGI, Y., KOSHIKAWA, N. & KOBAYASHI, M. 2015. Presynaptic cell type-dependent regulation of GABAergic synaptic transmission by nitric oxide in rat insular cortex. *Neuroscience,* 284**,** 65-77.

YANG, J., KIM, O., WU, J. & QIU, Y. 2002. Interaction between tyrosine kinase Etk and a RUN domain- and FYVE domain-containing protein RUFY1. A possible role of ETK in regulation of vesicle trafficking. *J Biol Chem,* 277**,** 30219-26.

YANG, Y., PHILLIPS, O. R., KAN, E., SULIK, K. K., MATTSON, S. N., RILEY, E. P., JONES, K. L., ADNAMS, C. M., MAY, P. A., O'CONNOR, M. J., NARR, K. L. & SOWELL, E. R. 2012. Callosal thickness reductions relate to facial dysmorphology in fetal alcohol spectrum disorders. *Alcohol Clin Exp Res,* 36**,** 798-806.

YASSIN, L., RADTKE-SCHULLER, S., ASRAF, H., GROTHE, B., HERSHFINKEL, M., FORSYTHE, I. D. & KOPP-SCHEINPFLUG, C. 2014. Nitric oxide signaling modulates synaptic inhibition in the superior paraolivary nucleus (SPN) via cGMP-dependent suppression of KCC2. *Frontiers in neural circuits,* 8**,** 65.

YOSHINO, Y., YAMAMOTO, S., KOHSAKA, S., OSHIRO, S. & NAKAJIMA, K. 2011. Superoxide anion contributes to the induction of tumor necrosis factor alpha (TNFalpha) through activation of the MKK3/6-p38 MAPK cascade in rat microglia. *Brain Res,* 1422**,** 1-12.

YU, B., BI, L., ZHENG, B., JI, L., CHEVALIER, D., AGARWAL, M., RAMACHANDRAN, V., LI, W., LAGRANGE, T., WALKER, J. C. & CHEN, X. 2008a. The FHA domain proteins DAWDLE in Arabidopsis and SNIP1 in humans act in small RNA biogenesis. *Proc Natl Acad Sci U S A,* 105**,** 10073-8.

YU, S., ZHAO, T., GUO, M., FANG, H., MA, J., DING, A., WANG, F., CHAN, P. & FAN, M. 2008b. Hypoxic preconditioning up-regulates glucose transport activity and glucose transporter (GLUT1 and GLUT3) gene expression after acute anoxic exposure in the cultured rat hippocampal neurons and astrocytes. *Brain Res,* 1211**,** 22-9.

YUAN, B., XIAN, R., MA, J., CHEN, Y., LIN, C. & SONG, Y. 2012. Isthmin inhibits glioma growth through antiangiogenesis in vivo. *J Neurooncol,* 109**,** 245-52.

YUFEROV, V., FUSSELL, D., LAFORGE, K. S., NIELSEN, D. A., GORDON, D., HO, A., LEAL, S. M., OTT, J. & KREEK, M. J. 2004. Redefinition of the human kappa opioid receptor gene (OPRK1) structure and association of haplotypes with opiate addiction. *Pharmacogenetics,* 14**,** 793-804.

ZHANG, C. R. & CHONG, S. 2016. Gene expression in the mouse brain following early pregnancy exposure to ethanol. *Genom Data,* 10**,** 107-108.

ZHANG, C. R., HO, M. F., VEGA, M. C., BURNE, T. H. & CHONG, S. 2015. Prenatal ethanol exposure alters adult hippocampal VGLUT2 expression with concomitant changes in promoter DNA methylation, H3K4 trimethylation and miR-467b-5p levels. *Epigenetics Chromatin,* 8**,** 40.

ZHANG, W., LIU, H., HAN, K. & GRABOWSKI, P. J. 2002. Region-specific alternative splicing in the nervous system: implications for regulation by the RNA-binding protein NAPOR. *RNA,* 8**,** 671-85.

ZHAO, D., MCCAFFERY, P., IVINS, K. J., NEVE, R. L., HOGAN, P., CHIN, W. W. & DRAGER, U. C. 1996. Molecular identification of a major retinoic-acid-synthesizing enzyme, a retinaldehyde-specific dehydrogenase. *Eur J Biochem,* 240**,** 15-22.

ZHOU, L., LI, R., WANG, R., HUANG, H. X. & ZHONG, K. 2008. Local injury to the endometrium in controlled ovarian hyperstimulation cycles improves implantation rates. *Fertil Steril,* 89**,** 1166-76.

ZHOU, Y., HOLMSETH, S., GUO, C., HASSEL, B., HOFNER, G., HUITFELDT, H. S., WANNER, K. T. & DANBOLT, N. C. 2012. Deletion of the gamma-aminobutyric acid transporter 2 (GAT2 and SLC6A13) gene in mice leads to changes in liver and brain taurine contents. *J Biol Chem,* 287**,** 35733-46.

ZHOU, Y., KIPPS, T. J. & ZHANG, S. 2017. Wnt5a Signaling in Normal and Cancer Stem Cells. *Stem Cells Int,* 2017**,** 5295286.

ZIMMERBERG, B. & REUTER, J. M. 1989. Sexually dimorphic behavioral and brain asymmetries in neonatal rats: effects of prenatal alcohol exposure. *Brain Res Dev Brain Res,* 46**,** 281-90.

ZIMMERBERG, B., SUKEL, H. L. & STEKLER, J. D. 1991. Spatial learning of adult rats with fetal alcohol exposure: deficits are sex-dependent. *Behav Brain Res,* 42**,** 49-56.

ZINK, M., FERBERT, T., FRANK, S. T., SEUFERT, P., GEBICKE-HAERTER, P. J. & SPANAGEL, R. 2011. Perinatal exposure to alcohol disturbs spatial learning and glutamate transmission-related gene expression in the adult hippocampus. *Eur J Neurosci,* 34**,** 457-68.

ZOU, L., WANG, X., JIANG, L., WANG, S., XIONG, X., YANG, H., GAO, W., GONG, M., HU, C. A. & YIN, Y. 2017. Molecular cloning, characterization and expression analysis of Frizzled 6 in the small intestine of pigs (Sus scrofa). *PLoS One*, 12**,** e0179421.

APPENDIX A

FIGURES*

* Reprinted with permission from "Hippocampal Transcriptome Reveals Novel Targets of FASD Pathogenesis" by Raine Lunde-Young[1], Josue Ramirez[1], Vishal Naik, Marcus Orzabal, JeHoon Lee, Kranti Konganti, Andrew Hillhouse, David Threadgill, and Jayanth Ramadoss, 2019. *Brain and Behavior*, Volume 9, e01334, Copyright 2019 by The Authors.

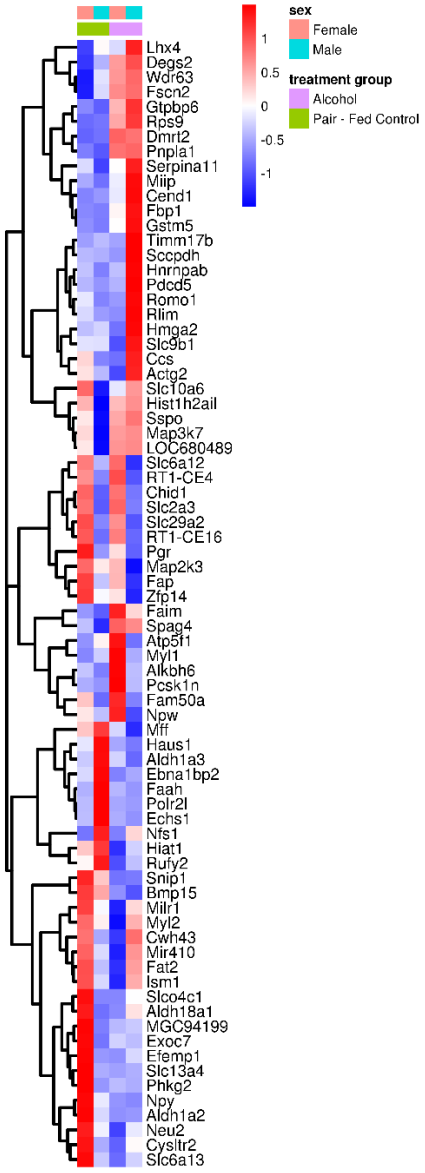

Figure 1: **Heat map of RNA-Seq transcriptome analysis of significantly altered hippocampal genes following our chronic binge prenatal alcohol paradigm. Heat map representation of 76 differentially expressed genes in the fetal hippocampus between Pair-Fed Control and Alcohol treatment groups, with 37 genes exhibiting downregulation and 39 genes exhibiting upregulation. Map was constructed from the normalized and log-transformed expression values and subtracted from the row means for each treatment group ($P < 0.05$, and log2(fold change) > 2.0).**

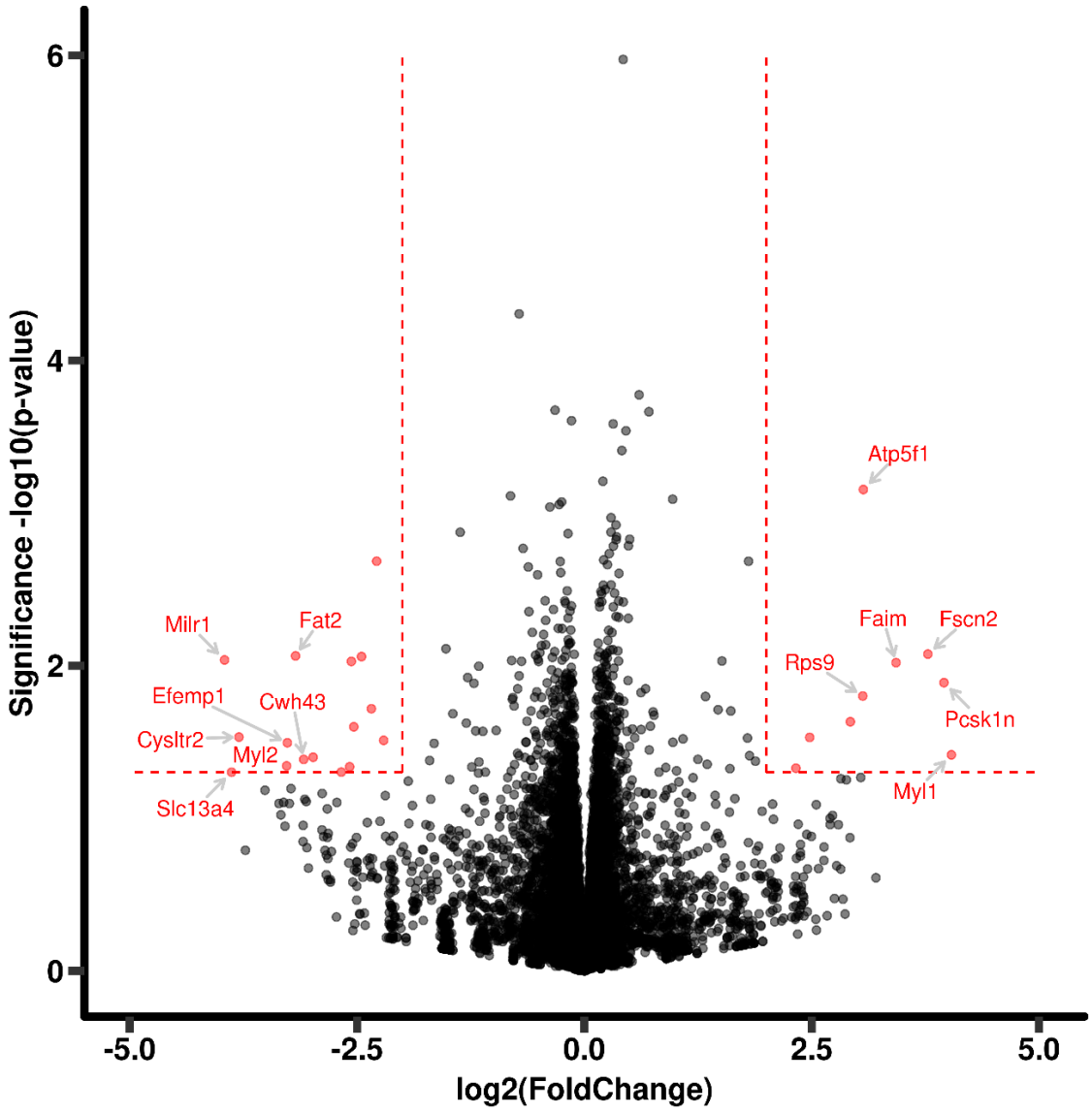

Figure 2: **Volcano plot representation of female hippocampal gene expression between the Pair-Fed Control and Alcohol groups. In alcohol-exposed females, 25 hippocampal genes exhibited sex-specific alcohol-induced dysregulation, of which 9 were upregulated and 16 were downregulated. Dotted lines denote selection criteria for significance (P < 0.05, and log2(fold change) > 2) and separate differentially expressed genes and similarly expressed genes.**

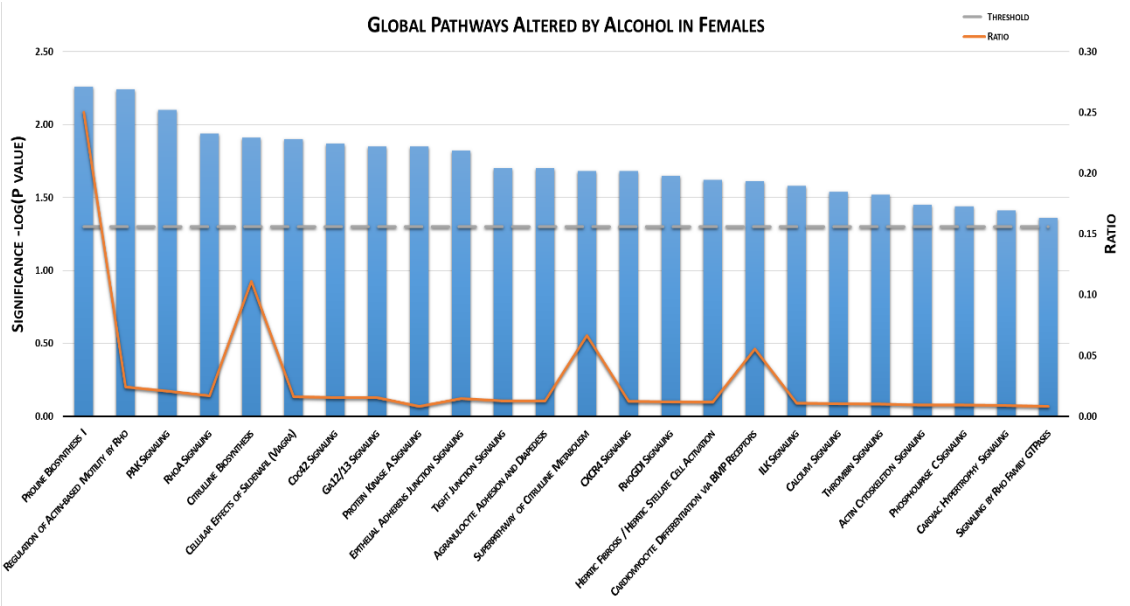

Figure 3: INGENUITY® Pathway Analysis of female hippocampal differentially expressed genes. In alcohol-exposed females, 24 global pathways were altered compared to the Pair-Fed Control group ($P < 0.05$). Ratio represents number of molecules affected to total number of molecules in each pathway.

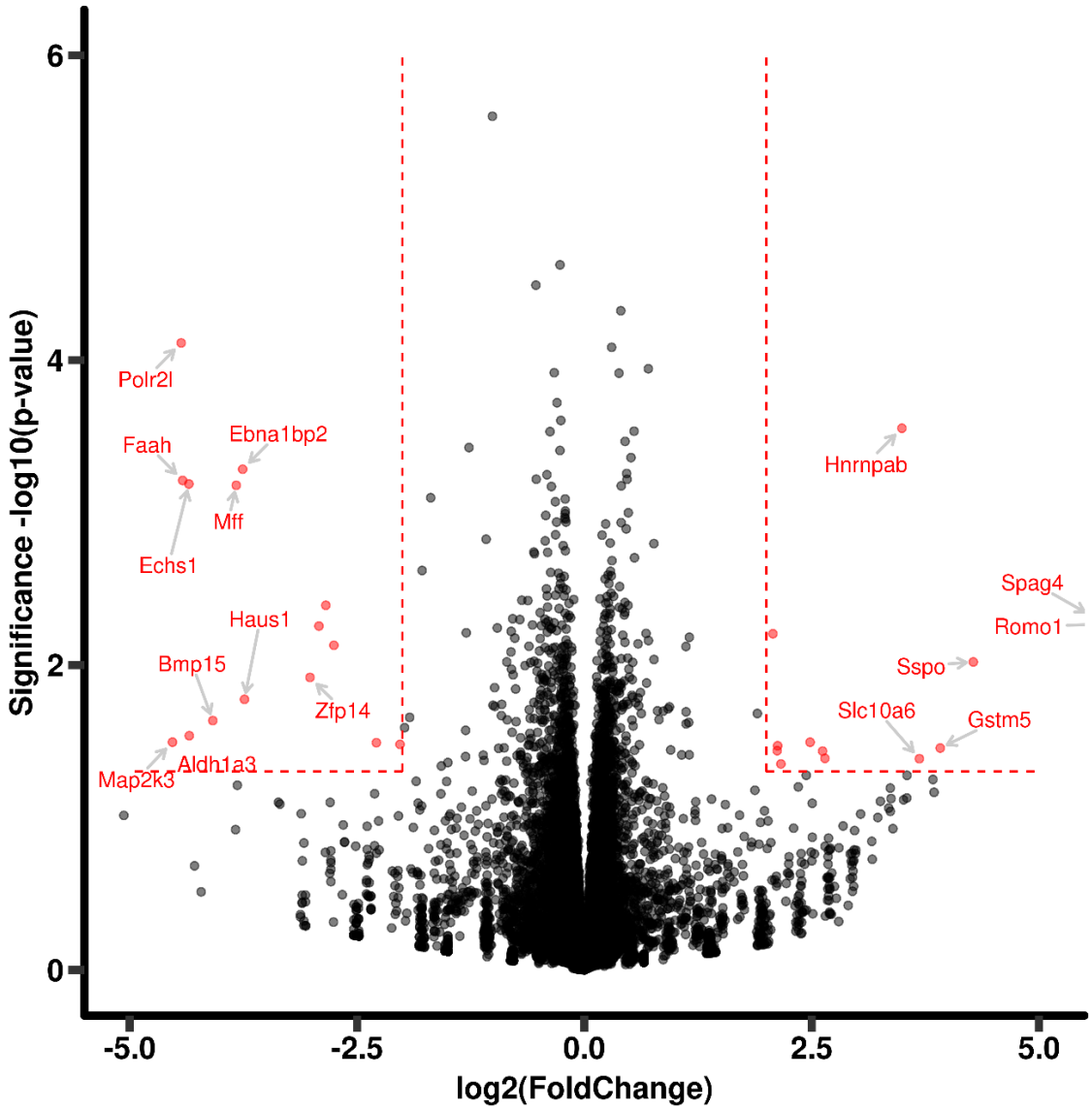

Figure 4: Volcano plot representation of male hippocampal gene expression between the Pair-fed Control and Alcohol groups. In alcohol-exposed males, 28 hippocampal genes exhibited sex-specific alcohol-induced dysregulation, of which 12 genes were upregulated and 16 genes were downregulated. Dotted lines denote selection criteria for significance (P < 0.05, and log2(fold change) > 2) and separate differentially expressed genes and similarly expressed genes.

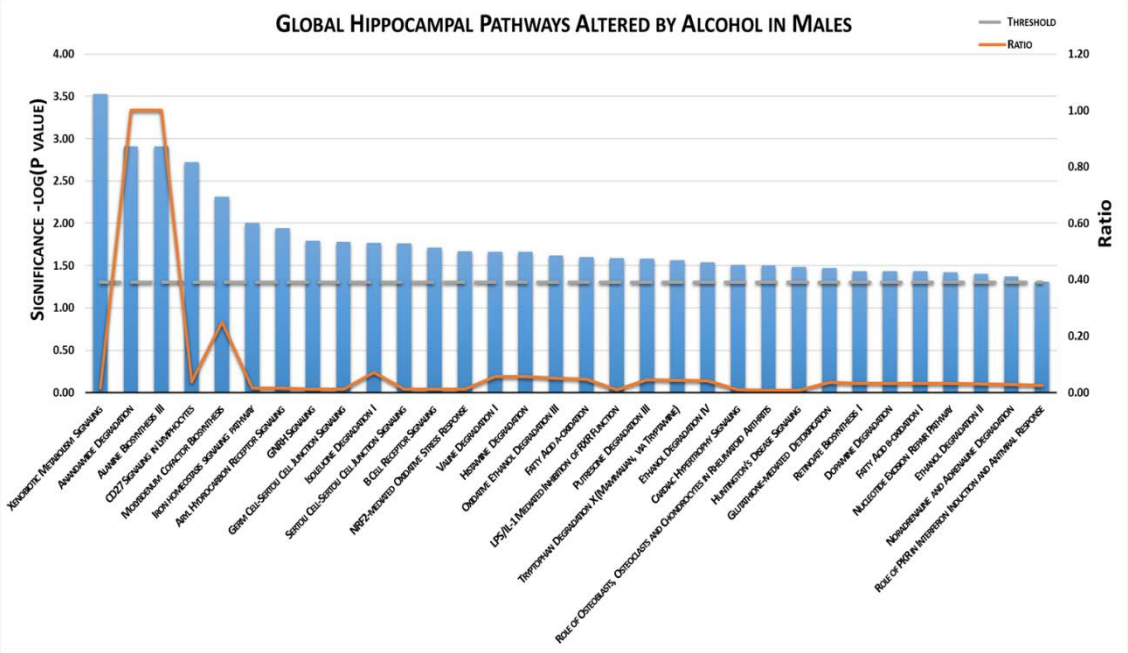

Figure 5: INGENUITY® Pathway Analysis of male hippocampal differentially expressed genes. In alcohol-exposed males, 32 global pathways were altered compared to the Pair-Fed Control group ($P < 0.05$). Ratio represents number of molecules affected to total number of molecules in each pathway.

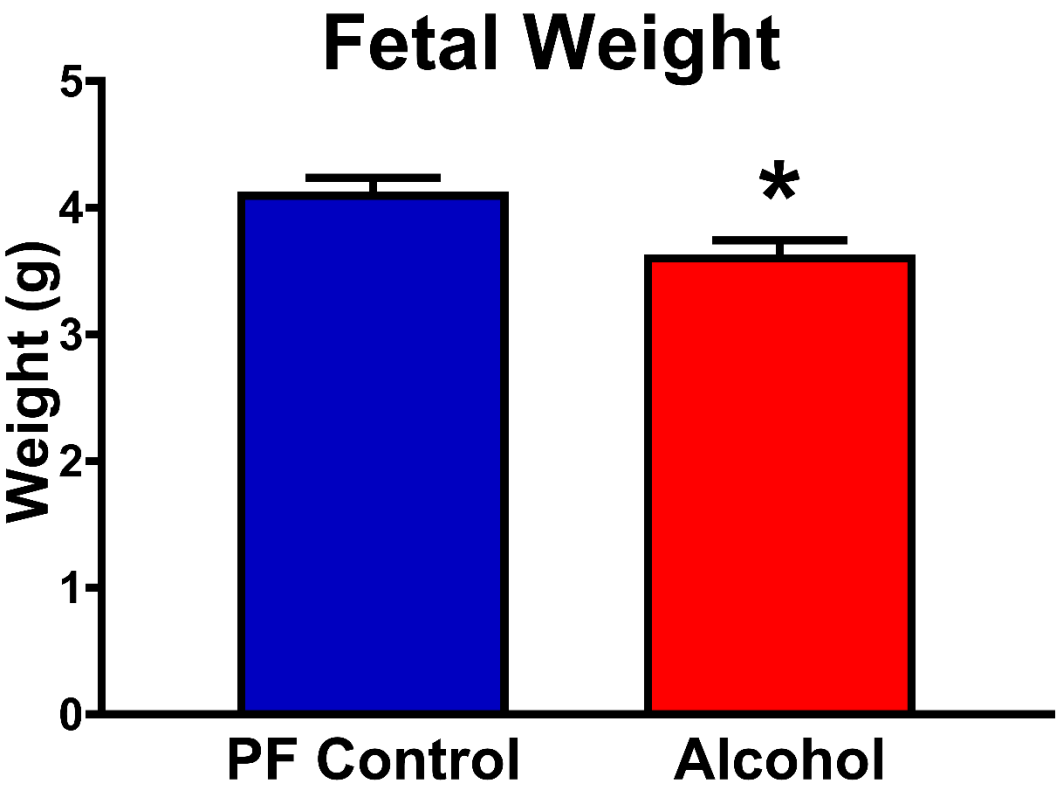

Figure 6: Effect of chronic binge alcohol exposure on fetal weight. Measurements were obtained on gestation day (GD) 21. Following prenatal alcohol exposure, mean fetal body weight was significantly decreased compared with that in the Pair-Fed Control group. Values are mean ± SEM (P<0.05).

A. Fetal Alcohol Syndrome

With or without knowledge of PAE:

I. Pre-/postnatal growth restriction

II. Stunted brain growth, morphology, or dysregulated neurophysiology

III. Neurobehavioral impairment

IV. Characteristic facial dysmorphia

B. Partial Fetal Alcohol Syndrome

I. With knowledge of PAE

- **Characteristic facial dysmorphia**
- **Neurobehavioral impairment**

II. Without knowledge of PAE

- **Pre-/postnatal growth restriction or stunted brain growth, morphology, or dysregulated neurophysiology**
- **Neurobehavioral impairment**
- **Characteristic facial dysmorphia**

C. Alcohol-related Neurodevelopmental Disorder

I. Knowledge of PAE

II. Neurobehavioral impairment

D. Alcohol-related Birth Defects(s)

I. Knowledge of PAE

II. Anatomic malformation(s) known to be caused by PAE

Figure 7: Clinical criteria of Fetal Alcohol Syndrome (FAS), partial FAS, Alcohol-related Neurodevelopmental Disorder, (ARND) and Alcohol-related Birth Defects (ARBD) as described by Hoyme et al, 2016.