EXTERNALIZING DISORDERS: GENETICS OR PRENATAL ALCOHOL EXPOSURE?

by

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To John: We did it!
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<tr>
<td>AA</td>
<td>African American</td>
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<tr>
<td>AD</td>
<td>alcohol dependent</td>
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<td>ADHD</td>
<td>attention deficit hyperactivity disorder</td>
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<td>CD</td>
<td>conduct disorder</td>
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<tr>
<td>CIFASD</td>
<td>Collaborative Initiative on Fetal Alcohol Spectrum Disorders</td>
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<td>COA</td>
<td>children of alcoholics</td>
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<td>COGA</td>
<td>Collaborative Studies on the Genetics of Alcoholism</td>
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<tr>
<td>EA</td>
<td>European American</td>
</tr>
<tr>
<td>ODD</td>
<td>oppositional defiant disorder</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>PAE</td>
<td>prenatal alcohol exposure</td>
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<td>PFC</td>
<td>prefrontal cortex</td>
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<td>PGC</td>
<td>Psychiatric Genetics Consortium</td>
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<tr>
<td>PRS</td>
<td>polygenic risk score(s)</td>
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<td>SNP</td>
<td>single nucleotide polymorphism</td>
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ABSTRACT

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Introduction: Externalizing disorders such as attention deficit hyperactivity disorder (ADHD), conduct disorder (CD), and oppositional defiant disorder (ODD) have a high prevalence rate in both children of alcoholics and in those with prenatal alcohol exposure (PAE). These disorders are also predictors of alcohol dependence (alcdep), heritable, and share an underlying genetic liability with alcdep. Furthermore, a mother who drinks while pregnant is likely to be alcohol dependent (AD), and vice-versa. This study incorporated these factors into one model, including as well as a measure of broad genetic risk for ADHD and alcdep to test for the contributions of these effects simultaneously. An independent sample was used to confirm the results for PAE and broad genetic risk. The hypothesis is that PAE will increase the risk to ADHD but not to CD or ODD.

Methods: Each of these factors was evaluated independently to test if that effect on its own, significantly contributed to each disorder. Another model included several demographic covariates, to determine which of these environmental effects also contributed to the disorder. The final model for each disorder included environmental effects along with the primary effects of interest.

Results: PAE resulted in increased risk for the inattentive (INATT) sub-type of ADHD and conduct disorder (CD) in the discovery sample and for the hyperactive-impulsive (HYPIMP), INATT and CD in the replication sample. PAE and the PAE*maternal alcohol dependence interaction increased the risk for ADHD and INATT. A broad genetic risk for ADHD was associated with all disorders except HYPIMP in the replication sample.
Conclusion: This study further supports the trending evidence of a unique etiology of ADHD in those with PAE, and more specifically, that INATT and HYPIMP are affected according to two different mechanisms of action, independent of a genetic contribution due to either ADHD or alcohol dependence, both of which also were associated with a risk for INATT. The contribution of PAE to INATT and CD were the only consistent results across all definitions of alcohol exposure and in both datasets, indicating that PAE is a veritable risk for INATT and CD.
CHAPTER 1. INTRODUCTION

Prenatal alcohol exposure (PAE) is associated with deficits in executive function, motor function, and cognitive impairment (Mattson, Crocker, & Nguyen, 2011). In addition, individuals with PAE are often diagnosed with comorbid externalizing disorders such as attention deficit hyperactivity disorder (ADHD), conduct disorder (CD), and oppositional defiant disorder (ODD) (Fryer, McGee, Matt, Riley, & Mattson, 2007). These externalizing disorders are similarly prevalent in individuals also exhibiting executive function deficits, including children of alcoholics and those with a family history of alcohol dependence (Kuperman, Schlosser, Lidral, & Reich, 1999; Waldron, Martin, & Heath, 2009), which could be due to the high heritability rates of these disorders (Krueger et al., 2002). Although not all mothers who drink while pregnant are alcohol dependent (AD), the majority of mothers who continue to drink while pregnant are AD (Knopik et al., 2005; May et al., 2014; May et al., 2013; O'Brien & Hill, 2014). This evidence begs the question: is the high comorbidity of these externalizing disorders in individuals with PAE attributable to (1) the direct alcohol exposure on the brain during fetal development, or to heritable traits such as (2) alcohol dependence in the mother, or the father due to assortative mating or (3) the likelihood that these externalizing disorders are observed in the mother and/or father, or finally (4) a multiplicative effect of an AD mother drinking alcohol during the pregnancy, resulting in a gene by environment effect. The objective of this project is to dissect the variability of risk for these disorders that is due to PAE from that due to a genetic liability. Recent results demonstrated that the risk of ADHD is increased in individuals with PAE compared to individuals with maternal alcohol dependence, but the risk of CD and ODD were about the same or less (Wetherill, Foroud, & Goodlett, 2018). Thus the central hypothesis of this work is that PAE contributes a unique risk to ADHD due to its effects on the developing brain and gene pathways. The secondary hypothesis is that there is no such increased risk due to PAE that contributes to CD or ODD.

1.1 Prenatal alcohol exposure

As described above, PAE is associated with a range of deleterious outcomes, with the severity of the effects depending on timing, frequency and amount of alcohol exposure to the fetus (Fryer, McGee, et al., 2007; Guerri, Bazinet, & Riley, 2009; Mattson et al., 2011; Streissguth,
Sampson, & Barr, 1989). The continuum of these outcomes is encompassed under the umbrella term of Fetal Alcohol Spectrum Disorders (FASD). While not a diagnosis, individuals at the extreme end of the continuum, with more severe mental retardation and specific facial dysmorphic features typically meet criteria for Fetal Alcohol Syndrome (Jones & Smith, 1975), with specific criteria described in Hoyme et al (Hoyme et al., 2005). Other individuals encompassed under the FASD term could have a diagnosis of alcohol-related neurodevelopmental disorder, may or may not have facial dysmorphology, and typically have some type of disability. A description of the range of phenotypes, including executive function, attention and behavioral problems, and comparisons of FASD individuals to controls is provided in Mattson et al (Mattson et al., 2011).

Despite the preventability of these negative outcomes, the prevalence of drinking during pregnancy is surprisingly high. The Center for Disease Control (CDC) found the risk of an alcohol-exposed pregnancy in women of reproductive age to be 7.3%, with 3.3 million women at risk during a 1-month period (Green, McKnight-Eily, Tan, Mejia, & Denny, 2016). Other studies reported that 27-46% of women continued to drink after discovery of the pregnancy (McDonald et al., 2014; Muggli et al., 2016). In the United States specific case-ascertainment estimates ranged from 10.9-25.2 per 1,000 children (1.1-2.5%) in a Rocky Mountain city (May et al., 2015) to 24-48 per 1,000 children (2.4%-4.8%) in a Midwestern city (May et al., 2014). A review of ~1600 individuals in a clinical setting implementing five different diagnostic systems of fetal alcohol spectrum disorders found differences in rates depending on the criteria, ranging from 4.7% (using CDC guidelines) to 59.6% (using Hoyme criteria) (Coles et al., 2016; Hoyme et al., 2005). A recent meta-analysis of prevalence rates from around the world found a global prevalence of 22.8 per 1,000, with slightly higher rates in Canada (30.5) and the United States (33.5) (Roozen et al., 2016).

1.2 Prenatal alcohol exposure, the prefrontal cortex, and gene pathways

A review of rodent, fish, and other animal models of PAE clearly shows that several behavioral systems are affected by PAE, including inhibiting natural responses, shifting attention and working memory, and executive function in general (Patten, Fontaine, & Christie, 2014). More specific studies suggest that alcohol exposure to the developing brain damages the neural tube and midline brain development (Sulik, Lauder, & Dehart, 1984; Zhou, Sari, Powrozek, Goodlett, & Li, 2003) and interferes with neurogenesis and cell migration in the forebrain.
(Goodlett, Horn, & Zhou, 2005). PAE affects the medial prefrontal cortex (PFC) in particular, reducing the number of neuronal cells (Mihalick, Crandall, Langlois, Krienke, & Dube, 2001), diminishing dendritic branching as well as density (Hamilton, Whitcher, & Klintsova, 2010; Lawrence, Otero, & Kelly, 2012; Whitcher & Klintsova, 2008), and reducing proteins associated with cognitive function (Barr, Hofmann, Phillips, Weinberg, & Honer, 2005). Furthermore, attention deficits in rodents with PAE were associated with dysregulation of gene pathways in the medial PFC, including the migration of GABAergic interneurons (Skorput, Gupta, Yeh, & Yeh, 2015) and glucocorticoid receptor transport (Allan, Goggin, & Caldwell, 2014; Talpos & Shoaib, 2015). Other pathways implicated more broadly in dysregulation due to PAE include serotonin (Ngai et al., 2015), genes associated with the hypothalamic-pituitary-adrenal axis (Workman, Raineke, Weinberg, & Galea, 2015) and genes related to the corticotropin-releasing hormone (Lan, Hellemans, Ellis, & Weinberg, 2015).

Clinical studies also demonstrate that PAE results in dysregulation of frontal and striatal brain regions (Fryer, Tapert, et al., 2007; Ware et al., 2015) and differential brain activation (Diwadkar et al., 2013). In particular, the medial PFC was identified in a review of neuroimaging studies to be affected by PAE (Moore, Migliorini, Infante, & Riley, 2014). Furthermore, individuals with PAE exhibited decreased activity of the frontal lobes with increased task difficulty (Malisza et al., 2012) and reduced volume of the frontal lobes (Astley et al., 2009; Sowell et al., 2002) compared to controls. Thus prenatal alcohol exposure results in some degree of insult to the developing fetal brain, including the prefrontal cortex, a region of the brain shown to be necessary in executive function tasks including attention to relevant stimuli and response inhibition.

1.3 The prefrontal cortex and executive function

Several studies show that the PFC is involved in planning, working memory, attention, inhibition, and monitoring of actions to achieve a goal, abilities which are considered to be part of executive function (Talpos & Shoaib, 2015; Tekin & Cummings, 2002). A review of the role of frontal lobe activity in tasks involving set-shifting (shifting attention from one goal to a different goal) and response inhibition, found that the frontal lobes are necessary, although not sufficient, for these tasks (Alvarez & Emory, 2006). General results in human subjects have confirmed
findings from animal models that set-shifting is mediated by the PFC (Rogers, Andrews, Grasby, Brooks, & Robbins, 2000).

Inhibitory control, another core domain of executive function, allows selective attention on specific stimuli, as well as subduing impulsive thoughts and activity. Having inhibitory control allows a person to shift attention to appropriate stimuli and ignore inappropriate stimuli, and to exhibit behavioral self-control, including the ability to give up an immediate reward in favor of a greater reward which occurs later in time (Diamond, 2013). Various types of inhibition (motor, behavioral, voluntary, involuntary), the role of inhibition in impulsivity (the inability to inhibit), attending to appropriate cues, and shifting attention and behavior when necessary are all part of executive function (Bari & Robbins, 2013).

1.4 Executive function deficits: externalizing disorders

Impairment in executive functioning is associated with deficits of attention and inhibition which typically are manifested as externalizing disorders. These disorders include attention deficit and hyperactive disorder (ADHD) (Barkley, 2010; Sun & Buys, 2012), conduct disorder (CD) (Johnson, Kemp, Heard, Lennings, & Hickie, 2015), and oppositional defiant disorder (ODD) (Matthys, Vanderschuren, & Schutter, 2013). ADHD is characterized by a continued pattern of hyperactivity and impulsivity or of inattention which negatively impacts functioning or development in at least two settings, with multiple symptoms being diagnosed before age 12 (Diagnostic and Statistical Manual of Mental Disorders, fifth edition; DSM5). Prevalence rates range from 5% throughout the world, but are higher in the USA, ranging from 7%-9%, with males presenting primarily with hyperactivity and impulsivity, and girls typically exhibiting inattention symptoms (Brahmbhatt et al., 2016). Conduct disorder is defined by DSM5 as the persistent violation of rights of others, including aggression to people and animals, destruction of property, deceitfulness, and violation of rules, with no lower age limit. The prevalence rate for CD based on a nationwide study was 5.4% (Kessler et al., 2012). The DSM5 defines ODD as persistent anger or irritability, argumentative or defiant behavior, or vindictiveness with someone other than a sibling, with a negative impact on important areas of functioning. The rate of ODD from the same nationwide study was 8.3% (Kessler et al., 2012). Rates of both CD and ODD are higher in males than females (Cortese, Faraone, Bernardi, Wang, & Blanco, 2016; Kessler et al., 2012). Although similar at first glance, CD is characterized by opposition to authority and is associated
in young adulthood with headstrong behavior and substance use, whereas ODD is characterized by a negative, angry mood and is associated with anxiety and emotional disorders in young adults (Rowe, Costello, Angold, Copeland, & Maughan, 2010).

1.5 Prenatal alcohol exposure: executive function deficits and externalizing disorders

As described above direct exposure of alcohol to the developing brain compromises the function of the PFC, an area of the brain necessary for executive function, which entails a specific set of cognitive skills involving attention, inhibition, and cognitive flexibility such as set-shifting in order to plan for and attain a goal. Not surprisingly, many clinical studies show that individuals with PAE exhibit impairment of executive functioning (Rasmussen, Andrew, Zwaigenbaum, & Tough, 2008), and in particular, compromised response inhibition (Kodituwakku, 2007; Mattson et al., 2011). A meta-analysis examining the association between executive function and FASD yielded the greatest effect size for set-shifting and medium effect size for inhibition (Khoury, Milligan, & Girard, 2015). Furthermore, brain activation was increased in the prefrontal region of individuals with PAE compared to non-exposed individuals when inhibition was required, implying that more cognitive resources were recruited and thus inhibition may be more demanding in individuals with PAE compared to controls (Fryer, Tapert, et al., 2007).

An evaluation of overall behavior problems across several studies demonstrated that individuals with PAE have more externalizing problems (e.g., rule breaking and aggressive behavior), compared to those without PAE (Tsang, Lucas, Carmichael Olson, Pinto, & Elliott, 2016), with developmental timing and amount of alcohol exposure moderating the degree of problems (Irner, 2012; May et al., 2013; Sayal et al., 2014). More specifically, individuals with PAE also have high comorbidities of externalizing disorders (Mattson et al., 2011; Nash, Sheard, Rovet, & Koren, 2008). The most striking comorbidity is with ADHD, which ranges from 49% to 95% (Burd, Klug, Martsolf, & Kerbeshian, 2003; Fryer, McGee, et al., 2007; Mattson et al., 2011). A recent meta-analysis estimated the overall prevalence of “disturbance of activity and attention” to range from 24% - 78% in FASD individuals (Popova et al., 2016). Similarities between children with PAE and children with ADHD were recognized early (Nanson & Hiscock, 1990), and confirmed in a large longitudinal study of babies born to mothers who consumed alcohol while pregnant (Streissguth, Barr, Sampson, & Bookstein, 1994).
Elevated rates of conduct disorder are also observed in individuals with PAE, as evidenced by increased symptoms (Disney, Iacono, McGue, Tully, & Legrand, 2008) and diagnosis (Fryer, McGee, et al., 2007; Popova et al., 2016). The pooled prevalence of CD across several studies evaluating fetal alcohol spectrum disorders was 91% (Popova et al., 2016). Oppositional defiant disorder, also common in individuals with PAE, has moderate comorbidity rates estimated at 38% in one study (Fryer, McGee, et al., 2007). The higher rates of these disruptive behavior disorders remain elevated even after accounting for environmental effects and other covariates (D’Onofrio et al., 2007). Although rates of all three disorders are common in individuals with PAE, results from a large cohort of more than 2,000 individuals revealed that ADHD was the most prevalent (41%), with ODD and CD having a prevalence of less than half that (~17%) (Bhatara, Loudenberg, & Ellis, 2006).

1.6 Maternal and paternal alcohol dependence

A comparison of mothers with and without children with fetal alcohol spectrum disorders (FASD) demonstrated that mothers of children with a FASD drank more drinks on the days they drank, and binged more often, compared to mothers without a FASD (May et al., 2014). These mothers who drink while pregnant are likely to be alcohol dependent. Mothers of children with a FASD reported twice as many alcohol problems as community-sampled mothers, and 5 times as many problems as control mothers who did not consume alcohol while pregnant (Ceccanti et al., 2014). Pregnant mothers who are alcohol dependent are at risk for consuming alcohol during their pregnancy. Almost four times as many alcohol dependent mothers continued to drink into the second and third trimesters compared to non-dependent mothers (Hill, Lowers, Locke-Wellman, & Shen, 2000; O'Brien & Hill, 2014). Due to assortative mating, there is a 3.4 times greater risk that the father is also AD (Tyrfingsson et al., 2010). This has a substantial impact on the fetus, as drinking late into the pregnancy increases the odds of the child having an FASD by 61 (May et al., 2013).

1.7 Alcohol dependence: executive function deficits and externalizing disorders

Inhibition and attention problems are hallmark features of individuals with alcohol dependence and abuse (Potenza & de Wit, 2010), which could be a result of executive function deficits. A review of the reward circuitry in addiction demonstrated that the PFC plays a major
role in inhibiting drug-seeking behavior, partly through cognitive control (Feil et al., 2010). Cognitive control is the ability to shift attention from one thought or goal to a more appropriate goal, commonly measured as set-shifting in PAE studies. Feil and colleagues demonstrated that chronic alcohol consumption is associated with deficiencies in executive functioning tasks, especially impulsivity and cognitive inhibition. They also reported that reduced impulse control was accompanied by a decrease in activity in the PFC (Feil et al., 2010). This conclusion was supported by another review of studies specifically measuring inhibition, which found deficits of medium effect size in inhibition for all tasks involving alcohol dependence, in that larger doses of alcohol consumption were correlated with larger effects on inhibition (Smith, Mattick, Jamadar, & Iredale, 2014).

Not surprising then, that studies show increased rates of ADHD, CD, and ODD in alcohol dependent individuals, due to the impairments in executive function, inhibition, and ability to attend to appropriate goals, (Marmorstein, Iacono, & McGue, 2009), as was described above in individuals with PAE. In fact children of alcoholic mothers are more likely to have these disorders compared to children in families with no history of alcohol dependence (Hill, Locke, Lovers, & Connolly, 1999; Hill & Muka, 1996; Hill, Tessner, & McDermott, 2011). More broadly there is much evidence demonstrating that individuals with these disorders in childhood are at increased risk for alcohol dependence in adulthood (De Alwis, Lynskey, Reiersen, & Agrawal, 2014; Ghosh, Malhotra, & Basu, 2014; Groenman et al., 2013; Hopfer et al., 2013; Kuperman et al., 2001; Merikangas & Avenevoli, 2000), largely due to impairment in inhibitory control (Shirley & Sirocco, 2014).

1.8 Genetic underpinnings of externalizing disorders and alcohol dependence

The commonality of these externalizing disorders with AD individuals and their offspring is likely due to the heritable traits of these disorders, i.e., the traits accompanying these disorders are likely to be transmitted from the parents to the offspring. Heritability is a measure of the proportion of genetic variation in a trait, and ranges from 0 (no heritability) to 100% (the trait is completely heritable). Any remaining proportion of trait variance can be attributed to shared or unique environmental factors between individuals (Visscher, Hill, & Wray, 2008). Heritability estimates are highest for ADHD and range from 70-90%, with a mean estimate of ~76% (Brikell, Kuja-Halkola, & Larsson, 2015; Faraone et al., 2005; Larsson, Chang, D’Onofrio, & Lichtenstein,
Heritability of CD and ODD is slightly lower, ranging from 50-80% for CD (Goldstein, Prescott, & Kendler, 2001; Porsch et al., 2016; Slutske et al., 1997) and 60-61% for ODD (Coolidge, Thede, & Young, 2000; Nadder, Silberg, Eaves, Maes, & Meyer, 1998).

Whether or not externalizing disorders in those with PAE are inherited from one or both parents, each likely to be AD, or are due to an underlying genetic factor shared between these externalizing disorders is unclear. One obvious underlying component common to ADHD, CD, ODD and to alcohol dependence is impairment of behavioral or cognitive inhibition, often studied jointly under the umbrella term of behavioral undercontrol. Behavioral undercontrol was a construct introduced by Sher and colleagues as way to summarize common personality traits of hyperactivity, impulsivity, aggressiveness, and antisociality in AD individuals (Sher, Walitzer, Wood, & Brent, 1991). Clinical studies have shown that behavioral undercontrol is associated with AD (Schuckit & Smith, 2006), and in particular, the traits of impulsivity and interpersonal exploitiveness accounted for 40% of the genetic variability in AD (Slutske et al., 2002). These authors also reported that behavioral undercontrol accounted for 90% of the genetic variation that was common to both AD and CD.

Twin studies have been an excellent resource to study this underlying genetic component. One twin study demonstrated that CD and ADHD in adolescence are both genetically correlated with adult alcohol dependence, indicating a shared genetic component to all three disorders (Edwards & Kendler, 2012). Another twin study found increased rates of ADHD in offspring of AD mothers and fathers, and mothers who drank during pregnancy (Knopik, Heath, Bucholz, Madden, & Waldron, 2009), although a different study did not find evidence of a common genetic liability between paternal alcohol dependence and ADHD in offspring, after correcting for maternal factors (Knopik, Jacob, Haber, Swenson, & Howell, 2009). This supported earlier work showing that parental alcohol dependence or perinatal factors such as maternal alcohol consumption or smoking during pregnancy did not account for a substantial proportion of the heritability estimate of ADHD, indicating other factors are moderating this genetic risk (Knopik et al., 2005). Other studies have confirmed a common genetic liability to all four disorders (Coolidge et al., 2000; Hicks, Foster, Iacono, & McGue, 2013; Knopik et al., 2014) and that this genetic liability is heritable and therefore transmitted from parent to offspring (Bornovalova, Hicks, Iacono, & McGue, 2010; Hicks et al., 2013).
1.9 Summary

All of the above describes the perfect entanglement of PAE, alcohol dependence, and genetics that potentially plays a role in the etiology of externalizing disorders. PAE adversely affects the PFC. Deficits in the PFC are associated with impaired executive function. These impairments are associated with alcohol dependence and externalizing disorders. There is a common genetic liability contributing to these externalizing disorders as well as to alcohol dependence. And finally – women who are AD are likely to drink while pregnant, and vice-versa, those who drink heavily during pregnancy are likely to be AD. This brings us back to the question posed above: is the high comorbidity of these externalizing disorders in individuals with PAE attributable to (1) the direct alcohol exposure on the brain during fetal development, or to heritable traits such as (2) alcohol dependence in the mother, or the father due to assortative mating or (3) the likelihood that these externalizing disorders are observed in the mother and/or father, or finally (4) a multiplicative effect of an AD mother drinking alcohol during the pregnancy, resulting in a gene by environment effect. This study seeks to disentangle the variability of risk of these disorders due to PAE from that due to a genetic liability or to parental alcohol dependence.

It is possible that the three externalizing disorders are not equally affected by PAE and/or parental alcohol dependence. The observation that impulsivity, hyperactivity and inattention are seen in children of alcoholics as well as in those with PAE was identified decades ago (Lippmann, 1980; Weinberg, 1997). Such comparisons for CD and ODD are not found in the literature. Early studies showed different types of attention deficits in those with ADHD without PAE compared to those with PAE (Coles, 2001). Recent studies have started to further delineate these differences, finding for example, that individuals with PAE and ADHD tend to have problems with sustained attention rather than exhibit hyperactivity and impulsivity (Furtado & Roriz, 2016; Infante et al., 2015; Irner, 2012; Mattson et al., 2011). Results from a recent meta-analysis (Wetherill et al., 2018) provided evidence that rates of ADHD were increased in those with PAE compared to individuals with an AD mother or to individuals with any AD parent, but the risk was not elevated for CD or ODD in those with PAE compared to parental alcohol dependence, indicating a unique etiology of ADHD. Therefore the primary hypothesis of this study is that PAE will increase the risk of ADHD above and beyond parental alcohol dependence or a measure of genetic liability. More specifically, PAE is expected to increase the risk for the sub-diagnosis of inattention but not
for hyperactivity/impulsivity. The secondary hypothesis is there will not be an increased risk for CD or ODD due to PAE compared to risk attributable to parental alcohol dependence.
CHAPTER 2. METHODS

2.1 Samples

**COGA**: Data from the Collaborative Studies on the Genetics of Alcoholism (COGA) was utilized as the primary sample. Individuals seeking treatment at inpatient and outpatient alcohol treatment centers were recruited by COGA as probands across seven sites between 1994 – 1998. First-degree and extended family members were then invited to participate in the study. Individuals were administered an extensive interview called the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994) which allows for DSM-IV diagnosis of several disorders, including alcohol dependence, ADHD, CD, and ODD. Families with three or more first-degree relatives with alcohol dependence were identified as high-risk families (about 85% of families), which were targeted for further follow-up. A set of comparison families (about 15%), collected at each site using public records such as license bureaus and public phone records, was ascertained similarly, but were not followed as closely as the high-density AD families.

**CIFASD**: When applicable, data from the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) will be used. Ascertainedment of these participants, age 7-17, has been ongoing since 2003, with methods of recruitment varying by site, including clinic referrals and public advertising. Children were recruited on the basis of PAE, fetal alcohol syndrome (FAS) diagnosis, verifiable non-exposed controls, as well as two contrast groups of children with ADHD, and children with low IQ scores without PAE (Mattson et al., 2010). Although CIFASD includes multiple sites around the world, only subjects recruited across four sites within the United States were used in these analyses, as these were the only participants with available DNA to be used in genome-wide association study (GWAS) analyses. Most of these children were adopted and therefore parental alcohol dependence could not ascertained in this sample; thus this is not seen as a complete replication sample. This dataset will inherently include a more balanced sample of individuals with and without prenatal alcohol exposure, as well as individuals with and without parental alcohol dependence.
2.2 Primary variables

**COGA PAE:** As part of the COGA interview process, the mother or a reliable relative was asked about maternal drinking and smoking during the pregnancy of each offspring. Three questions were asked in the same way across the first two phases of COGA. The first question asked “How frequently did you (the mother) drink anything with alcohol in it during the pregnancy?” Possible responses were every day, couple of times a week, once a week, twice a month, once a month, less than once a month, once or twice, never. The second question asked “How much would you (the mother) usually drink at one time?” Responses included 1 drink, 2 drinks, 3 drinks, 4 drinks, 5+ drinks. The last question was “When you (the mother) were pregnant, what was the most you (the mother) drank at one time?” Responses were the same as above.

Responses to the frequency (first) question were recoded based on a 38-week pregnancy to account for premature births, and a numerical value for the reported number of drinking occasions was recorded as: every day = 266 drinks (38*7), couple of times a week = 76 (38*2), once a week = 38, twice a month = 19 (38/2), once a month = 9.5 (19/2), less than once a month = 5, once or twice = 2, never = 0. The number of drinks per occasion were coded as 0, 1, 2, 3, 4, 5. The total number of reported drinks consumed during pregnancy was coded as the number of drinks per occasion times the number of occasions. If there was no data for the second question, then data from the last question (maximum drinks at one time) was utilized. This number was used as a quantitative estimate of PAE in COGA. In order to maximize the sample, the response from the section of the interview inquiring about alcohol consumption was included. This question asked: “Did you ever drink one whole drink of alcohol?” If the mother responded “no” to this question, and a numerical value of PAE was missing after applying the above computation, then PAE was assigned to be 0. These data were used to define a secondary measure of binge drinking to more closely align with PAE data from CIFASD. Specifically, if the mother (or respondent) reported drinking 4 or more drinks on one occasion, or if the maximum number of drinks consumed on one time as 4 or more, then binge=yes. If these numbers were <4 then binge=no.

**COGA Externalizing Disorders:** Participants under the age of 18 are asked a child-version of the SSAGA and adults are asked SSAGA questions about their offspring <18 years of age. Adults age 18 and older are directly interviewed using the SSAGA. The responses across the three potential interviews were examined to assign DSM-IV diagnosis for CD and ODD, such that if a person ever met DSM-IV criteria for CD or ODD, then they were considered to be affected, and if
they did not meet criteria for any interview, they were considered to be unaffected. Applying DSM-IV criteria to older versions of the SSAGA, which were crafted to assign DSM-3R ADHD diagnosis, was more difficult for ADHD. Therefore the maximum number of criteria met within one interview for hyperactive-impulsive, and separately for inattentive presentations were employed to assign a diagnosis. If the maximum number of hyperactive-impulsive symptoms was $\geq 6$ then HYMPIMP = affected, if the number was $< 6$ then HYPIMP = unaffected. The inattention, INATT, diagnosis was assigned similarly, if the number of inattentive symptoms $\geq 6$ then INATT = affected, if the number was $< 6$ then INATT = unaffected. An estimate of the combined ADHD was defined as ADHD = affected if HYMPIMP = affected and INATT = affected, and ADHD = unaffected if both HYP and INATT were unaffected. Thus individuals with HYMPIMP alone were included in both HYMPIMP and ADHD, and similarly for those with INATT alone, creating non-mutually exclusive groups. Aim 1a will examine if PAE increases the risk for ADHD, CD, and ODD in three separate models. Aim 1b will similarly test if binge drinking increases the risk of the three disorders in COGA data. A pairwise comparison of the odds ratios across the disorders will be computed to examine if PAE or binge is associated with increased risk for one disorder compared to another. Details are described below.

**COGA Parental Alcohol Dependence:** Genetic data was used to confirm or assign biological parents when possible. In the absence of genetic data, the reported family structure was used. SSAGA data was used to determine the number of DSM-IV criteria met for alcohol dependence. If an individual was assessed more than once, the SSAGA interview for which a person endorsed the maximum number of criteria was used. DSM-IV alcohol dependence was assigned as: a person meeting 3 or more criteria were affected, and those not meeting criteria were unaffected. To try to distinguish between AD mothers before or during their pregnancy from mothers who became AD after the pregnancy, a mother with an age of onset for alcohol dependence before or during the year of birth of the child was assigned to be AD (MOM$_{AD}$=yes). If the mother’s age of onset was after the year of birth of the child, or if was not DSM-IV AD (i.e., she met 0, 1, or 2 criteria), she was coded as assigned MOM$_{AD}$=no. The usual DSM-IV criteria was employed for fathers: a father who met 3 or more criteria was coded as DAD$_{AD}$=yes, and a father who met 0, 1, or 2 criteria was coded as DAD$_{AD}$=no. Parental AD will be employed in Aim 5 to test if MOM$_{AD}$, DAD$_{AD}$, or the combination increases the risk of the three disorders in COGA data.
**CIFASD PAE:** Children were recruited as part of CIFASD to have heavy PAE, defined as >4 drinks per occasion at least once per week or >13 drinks per week. Exposure was confirmed by maternal or a relative report when possible, or by reviewing public records. Children with no PAE, or with minimal PAE, >1 drink per week average but never more than 2 drinks on any one occasion, were considered to be controls. Therefore the CIFASD definition of PAE was yes (heavy or binge drinking) or no (none or minimal PAE).

**CIFASD Externalizing Disorders:** Multiple neurobehavioral scales were administered to CIFASD participants to obtain ADHD combined type, HYPIMP, INATT, CD, and ODD diagnoses, including the diagnostic interview schedule for children (DISC), the disruptive behavior disorders (DBD) rating scale, and the child behavior checklist (CBCL). As these scales were administered in different phases of this study, the same scale was not administered to the entire CIFASD sample. Therefore, DSM-IV status for externalizing disorders in these individuals was obtained in preferential order based on the neurobehavioral scale, by first employing the diagnosis from the DISC scale. If this diagnosis was missing then data from the DBD were employed. For the ADHD combined diagnosis, if DISC or DBD diagnoses were missing, individuals with a CBCL T-score ≥ 70 were considered ADHD affected and those with a T-score < 70 were considered ADHD unaffected. Diagnoses for INATT and HYPIMP were assigned similarly, without employing the CBCL scores. Individuals with INATT alone or with HYPIMP alone were not included in the ADHD group, creating three mutually exclusive diagnostic groups. Aim 1c will examine if the binary definition of PAE increases the risk for ADHD, CD, and ODD in three separate models in CIFASD data. A pairwise comparison of the odds ratios across the disorders will be computed to examine if PAE or binge is associated with increased risk for one disorder compared to another. Details are described below.

**CIFASD Parental Alcohol Dependence:** As most individuals were adopted, this information was not collected in the CIFASD data.

### 2.3 Secondary variables

**COGA Demographic Variables:** One SSAGA question asking about smoking during pregnancy, “Did you smoke at all during your pregnancy?” was used to assign maternal smoking during pregnancy (yes vs no). For both parents, if the individual reported daily smoking of cigarettes or if the individual met the Fagerstrom test for nicotine dependence, then smoking = yes,
if the person reported not smoking daily or was not nicotine dependent then smoking = no. Other variables utilized for both parents included the maximum number of drinks consumed in a typical drinking week, education, and income. Further maternal substance use diagnoses were examined but due to small sample size, only marijuana dependence was included in analyses. To account for marijuana use during pregnancy, with affection status coded as above, i.e., if the mother met DSM-IV criteria before or during the year of birth of the child, she was coded as affected, and as unaffected if she did not meet criteria or met criteria after the year of birth of the child. Maternal year of birth was included as a potential environmental covariate. Demographic variables describing the child included race and sex. Race was defined using genome-wide association study genotype data when available, as European American (EA), African American (AA), or other, and described below in more detail. Self-reported race was used for individuals without genotype data. Individuals with no race information were coded as unknown. Sex was also defined using GWAS data when available, and otherwise by self-report. These demographic variables will be employed in Aim 2a and 2b to examine (1) which demographic variables are associated with the externalizing disorder, and (2) if PAE or binge increases the risk of the three disorders after accounting for these environmental effects. The method of selecting the demographic variables to remain in the models for further analyses is described below.

*CIFASD Demographic and Environmental Variables:* Information describing maternal smoking or drinking during pregnancy, parental alcohol dependence or maternal substance use and dependence was not available for the majority of CIFASD participants. As these participants were recruited over three phases of data collection, there was not a set of demographic variables describing the primary caregiver(s) that was consistent across all three phases. Therefore, information describing the participant was implemented, including if there was a head injury (those with a severe injury were excluded), number of people living in the household, a learning disability and a reported history of alcohol abuse in the family. Race and sex of the child were defined using GWAS data from the CIFASD sample or from self-report, as described above. The recruitment group (ADHD vs not) and site (Atlanta, Minneapolis, San Diego, Los Angeles) were also included as covariates. These demographic variables will be employed in Aim 2c to examine if PAE increases the risk of the three disorders after accounting for these environmental effects. The method of selecting the demographic variables to remain in the models for further analyses is described below.
COGA and CIFASD: Differences in qualitative demographic variables were examined using X² tests of association. To simplify associations describing PAE defined in COGA as quantitative, a binary measure of exposed (PAE > 0) vs unexposed (PAE = 0) was utilized. T-tests were employed to test if quantitative measures were different between binary demographic variables. An analysis of variance (ANOVA) was used to test for differences in quantitative measures and race.

2.4 Genome-wide association study (GWAS) data

Overview: GWAS data is necessary to apply polygenic risk scores (PRS). In summary, a GWAS array contains 500,000 to 2.5 million genotyped SNPs. Quality control based on genotyping (each SNP) and based on each sample (each genotyped person) must be applied. Then additional SNP genotypes are imputed, i.e., genotype data for each person on the array is estimated for additional SNPs that were not on the GWAS array, by using information from public databases. For example assume SNP1 and SNP3 were genotyped on the GWAS array, but SNP2, in between SNP1 and SNP2 (on the genome) was not genotyped on the array. Information from public databases about SNP1, SNP2, and SNP3, can be used along with genotype data from SNP1 and SNP3, to impute genotype data for SNP2 for everyone who was on the GWAS array. For each imputed SNP, a probability of the genotype is assigned for each person, and only genotypes with a high probability are kept.

CIFASD: CIFASD samples were genotyped on two different arrays: (1) the MEGA array, which included >1.5 million SNPs, and the (2) Human OMniExpress array, which included >700,000 SNPs. As these individuals are primarily unrelated, there was no family structure to confirm. SNPs with A/T or C/G alleles were removed to avoid strand ambiguities, SNPs were also excluded by array based on high missing rates (>0.05), HWE p<0.0001 and MAF<0.03 and samples were imputed to 1000 Genomes within each array (EUR and AFR, Phase 3, b37, October 2014; build hg19, http://www.internationalgenome.org/) using IMPUTE2. Imputed SNPs with information scores <0.30 were excluded, and SNPs with genotype probabilities ≥ 0.90 were merged with the genotype data. The final GWAS dataset consisted of common SNPs (MAF ≥ 0.01) in HWE (p>0.000001), with low missing rates (<0.20). All variants were mapped to NCBI GRCh37.
2.5 Polygenic risk scores (PRS)

Overview: One SNP can be tested for association with a particular phenotype, and might even attain genome-wide significance ($p \leq 5 \times 10^{-8}$). However, these significant SNPs account for a very small amount of variability in the phenotype of interest. The theory behind PRS is that by using information from several thousand SNPs which show modest association with a trait of interest (i.e., small effect size), the sum of these several hundred(s) to thousands of additive genetic effects will account for a larger proportion of variability in the trait, i.e., a polygenic effect. To implement PRS, a GWAS analysis is run for a particular trait in the discovery sample. The discovery sample is typically very large (several thousand people) and the GWAS is performed under strict replicable analysis using an additive model (approximately equal increased risk is inferred with each copy of the risk allele). The analysis tests for association between each SNP and the trait of interest. The test statistic and standard error for the statistic based on each SNP is stored in a dataset, along with the corresponding p-value for the association and the risk allele of that SNP. Then in the target sample, the number of risk alleles (as identified in the discovery sample) for a particular SNP is weighted by the test statistic from the discovery sample, and this number is computed for SNPs that are in both the discovery and target sample. The sum of all these weights is called the PRS, and one PRS is computed for each person.

Example: Recall that each SNP has only 2 alleles. Using ADHD as an example, consider SNP1, with alleles C and G, and G being the risk allele, and an odds ratio of 3.0 from the discovery sample. That means for each copy of the G (risk) allele, the odds of having ADHD is 3 times more likely than someone with no copies of the G allele (i.e., with a CC genotype). Person 100 in the target sample has a genotype of CG for SNP1. Then Person 100 we compute $OR \times \# \text{risk alleles} = 3 \times 1 = 3$ for SNP1. Person 101 with genotype GG would have a score of $3 \times 2 = 6$. Person 102 with a genotype of CC would have a score of $3 \times 0 = 0$. The PRS is the sum of all these scores for all available SNPs. A typical discovery sample PRS dataset consists of sets of SNPs with increasingly stringent p-value thresholds, called profiles, usually ranging from $p < 0.50$, $p < 0.40$, $p < 0.30$, $p < 0.20$, $p < 0.10$, $p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$, and the PRS from the set of SNPs that best predicts the outcome in the target sample is typically used in analyses.

COGA: The best predictor of the genetic contribution to ADHD in the offspring is parental diagnosis. Therefore maternal and paternal ADHD diagnoses were implemented in models to account for genetic risk of ADHD in the offspring. Similarly, parental diagnoses of HYPIMP and
INATT were implemented for these respective analyses, to account for genetic contributions to these disorders.

**CIFASD:** PRS from GWAS analysis performed by the Psychiatric Genomics Consortium (PGC) for ADHD were implemented in CIFASD as the target sample. SNPs from the PGC with A/T or C/G alleles were removed, to avoid ambiguous strand issues between the discovery PGC sample and the target CIFASD sample. The clump and score procedures as implemented in PLINK (Purcell et al., 2007) was used to sum across the number of risk alleles weighted by multiplying the sign of the beta statistic times the negative log base 10 of the PGC GWAS association p value. Clumping was based on the linkage disequilibrium (LD) pattern in the 1000 Genome Phase 3 sample using a 500kb physical distance and an LD threshold of $r^2 >= 0.25$. PRS were calculated based on increasing p-value thresholds as described above (i.e., $p<0.0001$ to $p<0.50$). PRS based on ADHD were constructed by using the entire PGC sample (EA and AA combined) and then applying the algorithm to all CIFASD individuals with available GWAS data.

PRS with weights estimated for ADHD were incorporated in analyses for ADHD, CD and ODD due to the literature demonstrating an underlying genetic basis for all three of these disorders as part of CIFASD Aim 3c to determine if a genetic risk for ADHD increases the risk for each disorder in the CIFASD data. The best PRS weights corresponding to the best prediction p-value across all three ADHD diagnoses were utilized for the three ADHD diagnoses; the best PRS weights predicting CD and ODD respectively, were employed for these analyses. Aim 4c then incorporated the PRS profile identified as part of Aim 3, along with the measure of PAE and covariates identified as part of Aim 2 for ADHD, HYPIMP, INATT, CD, and ODD.

### 2.6 Methods

**Overview:** Each ADHD diagnosis was evaluated, in addition to the combined ADHD diagnosis: ADHD, HYPIMP, and INATT, along with CD and ODD, resulting in five models (i.e, five diagnoses). This was done for PAE defined as a quantitative phenotype and as a binge phenotype in COGA (i.e., 10 sets of analyses), and for the binary PAE (heavy/binge vs none/minimal) in CIFASD (5 sets of analyses). These 15 sets of analyses were performed for each of Aims 1, 2, 3, 4 (i.e., 60 sets of analyses). Since there is no parental alcohol dependence data for CIFASD parents, only COGA data was analyzed for Aims 5 and 6; therefore 10 sets of analyses for each of those two Aims (i.e., 20 sets of analyses) were performed.
To Examine Differences in Demographic Variables – COGA and CIFASD: Associations between demographic variables were evaluated to identify potential interaction terms that should be included in subsequent analyses. Differences in quantitative variables such as grade, education, income, and maternal age, between traits such as sex, maternal or paternal smoking, smoking during pregnancy, and substance use diagnoses were tested using t-tests. Differences for quantitative variables between race were examined using analysis of variance (ANOVA) models. Association between qualitative variables such as sex, smoking, race, and substance use diagnosis were evaluated using $X^2$ tests. Any pairs of qualitative variables that were associated with each other (i.e., non-independent) (p<0.05) were included as interaction terms in subsequent analyses to analyze the Aims. If a demographic variable was involved in multiple pairwise associations, the 3-way interaction term was included as a potential predictor variable and all relevant 2-way interaction terms were included.

To Analyze Specific Aims – COGA: For all aims, generalized estimating equations were utilized to account for correlated data within COGA families by using family ID as the subject-effect. The SAS procedure GENMOD was used to estimate the marginal effects; i.e., overall effects based on MOMAD (yes vs no), maternal smoking (yes vs no), etc. The correlation matrix for each family was treated as independent. Since all outcome variables were binary (affected vs unaffected), the binomial distribution was used to employ a logistic regression model. Odds ratios were obtained using the LSMEANS option which accounts for all variables and covariates incorporated in the model. This model was utilized for all Aims. This model provides the p-value for each variable based on a z-score of association of that particular variable with the outcome, as well as the type 3 sum of squares (SS), which accounts for all other variables in the model. Both p-values were utilized for Aims 1-5, which sought to identify variables that predict the externalizing disorder. The type 3 SS p-values are primarily reported for Aim 6, which identifies the final model of PAE, parental alcohol dependence, relevant covariates, and PRS which predict the externalizing disorder. In all analyses, only individuals with data for the outcomes (each externalizing disorder diagnosis) and for all independent variables and covariates were included. Individuals with missing data for the outcome or any independent variable were excluded by default in SAS.
Aim 1: PAE was used as an independent variable to predict each disorder, with a separate model for each disorder (separate models for all following aims as well). This was repeated in a separate set of analyses using binge drinking.

Aim 2: PAE and available covariates were used as independent variables to predict each disorder. This was repeated using binge drinking for all disorders except ADHD. PAE was always retained in the model, despite p-value. Other variables with a z-score or type 3 SS p-value \( \leq 0.15 \) were retained. Variables were excluded one at a time. There were several individuals with missing data (N=600-900) for all paternal variables, i.e., the father of that individual did not have data from any SSAGA interview. Therefore, due to the high missing rate of paternal data, paternal variables were prioritized to be excluded based on the highest p-value. This ensured that if no paternal variables predicted the diagnosis, the sample size was increased, thereby increasing power to detect association with maternal and other demographic variables. This strategy was implemented as well in Aims 4, 5, and 6.

Aim 3: Maternal and paternal diagnoses of each externalizing disorder were implemented to account for genetic risk of each disorder in the offspring. For example, maternal HYPIMP, paternal HYPIMP and the combination was examined for their predictive value to HYPIMP in the offspring. This was done for all 5 diagnoses.

Aim 4: PAE, covariates identified in Aim 2, maternal diagnosis of the externalizing disorder, and paternal diagnosis of the externalizing disorder were used as independent variables to predict each disorder in COGA. This was repeated using binge drinking.

Aim 5: MOMAD, DADAD and the interaction term were used as independent variables to predict each disorder. A second model was run using the number of alcohol dependent parents, for each disorder.

Aim 6: PAE, covariates identified in Aim 2, parental externalizing disorder diagnosis, and either MOMAD, DADAD or both, were used as independent variables to predict each disorder. This was repeated using binge drinking. The number of alcohol dependent parents was not utilized due to low available numbers of DADAD. PAE, MOMAD, and PAE* MOMAD were retained in all final models. Otherwise variables only variables with a type 3 SS p<0.10 were retained for the final model. Summary statistics such as odds ratios, p-values, are provided for PAE across all aims and disorders, for parental alcohol dependence for all disorders for Aim 5, and for primary variables remaining in the final model of Aim 6.
To Analyze Specific Aims – CIFASD: Due to the small number of sibling pairs in the CIFASD sample, logistic regression as implemented in SAS was employed. This model provides the p-value for as the type 3 sum of squares (SS), which accounts for all other variables in the model. Summary statistics such as odds ratios, p-values, are provided for PAE across all aims and disorders, and for all primary variables remaining in the final model of Aim 4. The full available sample was utilized for Aims 1-2. Aim 3 explored the predictive value of PRS on each disorder diagnosis and Aim 4 included appropriate covariates and PRS; therefore only individuals with available GWAS date were included in Aims 3-4.

Aim 1: PAE was used as an independent variable to predict each disorder, with a separate model for each disorder (separate models for all following aims as well).

Aim 2: PAE and available covariates were used as independent variables to predict each disorder. Other variables type 3 SS p-value ≤ 0.15 were retained. Variables were excluded one at a time.

Aim 3: The PRS-ADHD were used to predict each disorder for each set of profile SNPs.

Aim 4: PAE, covariates identified in Aim 2 and the appropriate PRS profile for ADHD were used as independent variables to predict each disorder. This is the final Aim for the CIFASD data, due to the unavailability of parental alcohol dependence in this sample.

To compare the odds ratio (OR) for PAE between the externalizing disorders: The natural log of the OR (LOR) for PAE from Aim 1 was computed for each disorder diagnosis. The pairwise LORs was compared by calculating the z-score based on the difference in LOR and the standard error. The LOR between HYPIMP, INATT, CD, and ODD, were compared, 6 tests total. The alpha was adjusted for 6 tests, such that p<0.0083 was considered a significant difference. Since the OR for PAE is an estimate of the increase in risk for each single drink, the LOR was based on 266 drinks, which is 1 drink every day for 38 weeks. This was done for COGA and CIFASD results.
CHAPTER 3. RESULTS

3.1 Demographics

COGA Sample: The average year of birth (YOB) of mothers was 1960 and the average YOB for offspring was 1986. Mothers with a child diagnosed with an ADHD-related disorder were younger on average (average YOB=1961 vs 1960, all p<0.009), as were the diagnosed children (average YOB=1988 vs 1987, all p<0.07). Although mothers of ODD children were also younger (YOB=1961 vs 1960, p=0.004), the children were about the same age (YOB=1987, p=0.18). There was no difference in the age of the mothers (YOB=1960, p=0.20) of offspring with CD, although those with CD were younger (YOB=1985 vs 1987, p<0.0001). As seen in Table 1A, more males were affected across all diagnoses. Of those with an externalizing disorder, the % with MOMAD=Yes was 24-27%, and the % with DADAD=Yes ranged from 54-63%. The highest level of education was 13.0 years (equivalent to technical school or 1 year of college, standard error (se) = 0.040) on average for mothers and 13.1 (0.052) for fathers. Income levels were slightly higher for fathers (about $40,000-$49,999) than for mothers (between $30,000-$49,999). A child with an AD mother was more likely to also have an AD father (p<0.0001). There was no association between sex and prenatal exposure (exposed vs unexposed), maternal smoking during pregnancy, nicotine dependence of the mother or father, race, or marijuana dependency diagnoses (all p>0.11). Individuals who were not EA or AA (i.e., “other”) were marginally less likely to have a mother who smoked during pregnancy (p=0.08). EAs and AAs were more likely to have a father with nicotine dependence (p=0.03). If the child’s mother smoked during pregnancy, or drank during pregnancy, the father was more likely to be nicotine dependent (p<0.0001, p=0.025 respectively). If the mother was AD, she was more likely to smoke during pregnancy and to drink while pregnant (both p<0.0001). There was no association between maternal drinking during pregnancy and the father being AD (p=0.64).

Tests of associations of quantitative measures with binary traits revealed that the level of education and income of both mothers and fathers were lower if the mother was AD (all p<0.0001) or if the father was AD (all p<0.0001). Individuals who were exposed to alcohol had mothers and fathers with (slightly) lower levels of education (mother p=0.079, father p<0.0001) and income (mother p=0.0035, father p<0.0001) compared to those without PAE. Mothers who smoked during
pregnancy were younger (p<0.0001), as were mothers with a partner with nicotine dependence (p=0.013), or alcohol dependence (p=0.0064). On average AA mothers were younger and EA mothers were older (p<0.0001). Mothers with alcohol dependencies were older on average (p=0.0033). Overall, 204 mothers were marijuana dependent (10.2%).

**CIFASD Sample:** Table 1B provides summary statistics for affection status of the disorders. Similar to the COGA sample, more males were diagnosed with the disorders in general. CIFASD. The average age of individuals was about the same for all disorders (p>0.29) except HYPIMP, for which affected individuals were slightly younger (17.8 years) compared to unaffecteds (18.9 years, p=0.017). Males were slightly older than females (p=0.025). Across the sites, participants from the Minneapolis site were youngest (mean age = 14.1) while those from the San Diego site were oldest (18.7, p=0.0002). There were no differences in the highest grade level (p=0.82) or employment status (p=0.85) of caregivers of exposed vs non-exposed individuals with data for these variables. Individuals who were EA were more likely to be recruited in San Diego, whereas AA individuals were more likely to be from the Atlanta site. Individuals of unknown or other race were primarily recruited from the Los Angeles or Minneapolis sites (p<0.0001; Table 1C).

### 3.2 Aim 1: Does PAE (alone) increase the risk for the disorder?

**COGA Sample:** The quantitative measure of PAE, reported number of drinks during the pregnancy, did not increased the risk for ADHD (p=0.70; OR for 1 drink per day=0.90), HYPIMP (p=0.85; OR=1.03) or INATT (p=0.18; OR=1.14). The number of people with the combined ADHD trait with a mother who binge drank was only 6. Therefore results for binge drinking alone are presented here, but no other results for binge drinking and ADHD-combined will be presented. Binge drinking did not increase the risk of any ADHD-related disorder (all p>0.23). However both PAE and binge drinking increased the risk for CD (PAE p=0.015, OR=1.24; binge p=0.042, OR=1.49) and for ODD (PAE p=0.0.0051, OR=1.24; binge OR=1.94, p=0.0015). The LOR computed from drinking one drink per day for 38 weeks was greater for INATT compared to HYPIMP (p=0.0028). The LOR was larger for CD compared to HYPIMP (p=3.25x10-7) but was not significantly greater than INATT (p=0.024) or ODD (p=0.80). Contrary to the hypothesis, the LOR computed from the binge drinking was higher for ODD than for ADHD and CD (both
p<0.0001), and the LOR for CD was higher than ADHD (p<0.0001). All ORs and 95% confidence intervals are provided in Table 2A.

**CIFASD Sample:** PAE, defined as heavy vs none or minimal in this sample, significantly increased the risk for all disorders (all p<0.0001; ADHD OR=10.8, HYPIMP OR=7.4, INATT OR=9.6, CD OR=7.5, ODD OR=4.2). The comparison of the LOR across disorders revealed that the LOR for ADHD was marginally higher than for CD (p=0.053) and significantly higher than for ODD (p<0.0001). The LOR was higher for CD than for ODD (p=0.0013). All ORs and 95% confidence intervals are provided in Table 2B.

### 3.3 Aim 2: Does PAE increase risk after accounting for demographic covariates?

**COGA Sample:** A binary PAE measure was implemented (PAE=yes if number of drinks > 0, and no if number of drinks = 0) and the ORs were estimated. These ORs and 95% CIs for PAE and binge drinking are provided in Table 3A. The p-values reported in the tables are from the quantitative PAE variable.

Demographic covariates which were retained in the model (p<0.15 for either z-score or type 3 SS) as part of this aim differed for the various disorders. Due to the numerous models analyzed (5 diagnoses x 2 measures of PAE), and the fact that some of these demographic variables will not be significant after accounting for parental alcohol dependence and externalizing disorder diagnosis, only detailed results for PAE are reported here. PAE was included in all analyses regardless of p-value. Unless otherwise indicated, only the type 3 SS p-values are reported, to reflect significance after adjusting for other variables in the model.

Neither PAE nor binge drinking was associated with increased risk of ADHD or HYPIMP after adjusting for covariates as evaluated by the type 3 SS p-value (all p>0.43). Although PAE was significantly associated with INATT (p=0.0003), the OR=0.89 with a confidence interval that included 1 (0.56, 1.52). Binge drinking was not associated with INATT (p=0.11). PAE was marginally associated with increased risk of CD (OR=1.32, p=0.052) and ODD (OR=1.91, p=0.067), although confidence intervals for both ORs included 1. Binge drinking was associated with increased risk of CDD (OR=2.36, p=0.046) and with a moderate increased risk of ODD (OR=2.56, p=0.07). All ORs and 95% CIs for PAE and binge are reported in Table 3A.

**CIFASD Sample:** Race was assigned using GWAS data as described above, and by using self-report for those without GWAS data. Individuals were assigned to EA, AA, other, and
unknown categories. Gender and site (of recruitment) were included as covariates, in addition to whether the child was recruited as an ADHD control or not and other demographic variables. As described above, only PAE results will be reported here. PAE increased the risk for all disorders (p<0.0001). All ORs and 95% CIs for PAE are reported in Table 3B.

3.4 Aim 3: Does parental diagnosis/polygenic risk scores increase risk?

**COGA Sample:** Since each offspring inherits 50% of their DNA from each parent, the best predictor of an underlying hereditary component of each externalizing disorder is the presence of the disorder in the parents. This was confirmed by testing if the parental diagnosis for a specific disorder predicted the disorder in the offspring. The strongest association was for ADHD with the odds of ADHD in the offspring increasing by 8.65 for each parent with an ADHD diagnosis (X²(1)=1249.9, p<0.0001). This effect was not as strong for HYPIMP (X²(1)=304.3, p<0.0001) or INATT (X²(1)=361.2, p<0.0001), with the odds increasing by about 2.6 for each parent with the disorder. Parental diagnosis was also predictive of CD (X²(1)=648.7, p<0.0001; OR=6.9 for each parent with a CD diagnosis), and ODD (X²(1)=914.2, p<0.0001; OR=6.7 for each parent with an ODD diagnosis),

**CIFASD Sample:** The PRS-ADHD predicted all ADHD-related diagnoses and ODD for all p-value profiles except p<0.001 (all p<0.08). Only the most strict PRS-ADHD profile, based on SNPs in the PGC sample with p<0.001, predicted CD diagnosis (p=0.065, % variability=2.76). The best profile for ADHD was based on SNPs with p<0.20 (p=0.0099, % variability=2.21). The best profiles for HYPIMP and INATT were based on SNPs with p<0.30 (HYPIMP: p=0.040, % variability=2.21, INATT p=0.0068, % variability=3.04). For ODD, the p<0.05 profile was the most significant for ODD (p=3.96x10⁻⁵, % variability=7.71). In summary: PRS-ADHD increased the risk of all five diagnoses, including CD and most significantly for ODD.

3.5 Aim 4: Does PAE increase risk after accounting for parental diagnosis/PRS?

**COGA Sample:** Due to the fact that PAE was a factor in several interactions, it is not possible to provide an overall OR for PAE as a quantitative measure. Therefore, once the final models were identified for PAE analyses, a binary PAE measure was implemented (PAE=yes if number of drinks > 0, and no if number of drinks = 0) and the ORs were estimated. These ORs
and 95% CIs for PAE and binge drinking are provided in Table 4A; reported p-values are from the quantitative PAE variable.

Parental diagnosis was added to each model with PAE and demographic covariates as described above. All variables were retained. Although PAE increased the risk for ADHD before adjusting for covariates (p=0.016), it did not significantly increase the risk of any of the ADHD-related disorders after adjusting for other effects (all p>0.97). PAE was not associated with increased risk of CD or ODD after accounting for demographic covariates and parental diagnoses (p>0.21).

Similar results were obtained using the binge variable in that binge did not increase the risk of any ADHD-related disorder after accounting for parental diagnoses and other demographic covariates (all p>0.66). Similarly, there was no increased risk of binge drinking with CD or ODD after accounting for demographics and parental diagnoses (both p>0.11). All ORs and 95% CIs for PAE and binge are reported in Table 4A.

**CIFASD Sample:** As this is the last analyses for CIFASD data, any PRS-ADHD or demographic variable that was not significantly associated with the disorder after adjusting for all other effects (type 3 SS p<0.10) was excluded from the final model. PAE and PRS-ADHD were both retained in the final model since they were *a priori* hypothesized to increase the risk for the externalizing disorder. PAE significantly increased increase the risk for HYPIMP, INATT and CD, but only marginally increased the risk for ODD (p=0.13) after adjusting for demographic covariates and a broad genetic risk for ADHD (PRS for all diagnoses p<0.0001); all ORs are provided in Table 4B. In addition, a broad genetic risk as measured by the PRS-ADHD was associated with increased risk in ADHD (p=0.019), INATT (p=0.10), CD (p=0.0031), and ODD (p=0.0026). There was an interaction of the PRS-ADHD and PAE for ADHD (p=0.05) and ODD (p=0.0038), and a modest effect for CD (p=0.087). As seen in Figures 1A, 1C, 1D, this was due to an elevated risk for the disorder in exposed individuals which gradually increased as the genetic risk for ADHD also increased (i.e., PRS scores on the x-axis became larger), whereas in unexposed individuals, the risk for the disorder increased dramatically with high genetic risk values. Thus for high genetic risk (high PRS on the x-axis) there was approximately equal risk of the disorder in exposed and unexposed individuals. Although there was a modest effect of genetic risk on INATT (PRS-ADHD p=0.10), there was no interaction (p=0.99). As seen in Figure 1B, there was almost no increase in risk for INATT with increased genetic risk (PRS-ADHD scores on the x-
There was no increase in risk for HYPIMP due genetic risk for ADHD (PRS-ADHD p=0.91). A family history of alcohol dependence modestly increased the risk for ADHD (OR=1.76, p=0.068) and INATT OR=1.98, p=0.06), although both confidence intervals included 1. There was an increased risk of ADHD (p=0.026), INATT (p=0.01) and ODD (p<0.0001) for EA individuals compared to AA individuals (Figures 2A-2C).

3.6 Aim 5: Does parental alcohol dependence increase the risk of the disorder?

**COGA Sample**: This aim only applies to COGA data. This was tested two ways, as the number of parents with alcohol dependence (0, 1, 2) and whether or not the mother was AD (MOMAD) or the father was AD (DADAD) separately, since MOMAD is a primary variable of interest. The number of AD parents increased the risk of CD and ODD (all p<0.005). Neither were significantly associated with HYPIMP (both p>0.14). MOMAD significantly increased the risk of INATT (p=0.0054) and ODD (p=0.0088). Conversely, DADAD significantly increased the risk for CD (p=0.023) but not MOMAD (p=0.27).

3.7 Aim 6: Do PAE, MOMAD, and the interaction effect PAE*MOMAD increase risk?

For this final aim, all initial models included the respective demographic covariates identified as part of Aim 2, as well as parental externalizing disorder diagnoses, parental alcohol dependence diagnoses, PAE (or binge), MOMAD, PAE*MOMAD. Final models retained PAE, MOMAD, PAE*MOMAD and any demographic covariate and/or interaction with type 3 SS p<0.10 for all models. The same strategy was applied to the analyses using binge drinking. To simplify the OR and 95% confidence interval for the quantitative PAE estimate, once the final models were identified for PAE analyses, a binary PAE measure was implemented (PAE=yes if number of drinks > 0, and no if number of drinks = 0) and the ORs were estimated. These ORs and 95% CIs for PAE and binge drinking are provided in Table 5; all effects and the direction of the effects can be seen in Table 6.

**PAE – quantitative measure**

**PAE-ADHD**: Although PAE as an effect by itself increased the risk for ADHD (z-score p=0.019), it was not significantly associated with increased risk for ADHD after adjusting for other variables (p=0.95). MOMAD significantly increased the risk for ADHD (OR=1.88, 95% CI=[1.14, 3.11], p=0.019). As seen in Figure 3A, for low values of PAE, there was no difference in risk due
to MOMAD. However when the mother drank more during the pregnancy, there was an increased risk of ADHD, which was enhanced if the mother was also alcohol dependent (PAE*MOMAD (p>0.056). This effect translated into a 1.5 times increase in risk for INATT if the mother drank one drink per day every day of the pregnancy. This risk was diminished if the mother also smoked during the pregnancy (Figure 3C; PAE*sex*smoking p=0.056). Having a father with ADHD substantially increased the risk for ADHD in the offspring (p<0.0001). As seen in Figure 3C, this risk was increased if the mother was also alcohol dependent (DAD_ADHD*MOMAD p=0.038). Having a mother with ADHD did not increase the risk of ADHD in the offspring (p=0.42).

**PAE – HYPIMP:** There was no significant increase in risk for HYPIMP due to PAE (p=0.58), maternal alcohol dependence (p=0.68) or the interaction (p=0.71) as shown in Figure 4A. There was a substantial increase in risk for HYPIMP if the father was also HYPIMP (p<0.0001), which unlike ADHD, was not different by maternal alcohol dependence (Figure 4B). Maternal HYPIMP diagnosis was not associated with HYPIMP in the offspring (p=0.18).

**PAE – INATT:** PAE significantly increased the risk for INATT ( p=0.0025). Although maternal alcohol dependence did not increase the risk (p=0.21), the interaction PAE*MOMAD increased the risk (p=0.022), as seen in Figure 5A. This effect translated into a 3.9 times increase in risk for INATT if the mother drank one drink per day every day of the pregnancy. Having a father with INATT also increased the risk for INATT (p<0.0001), but as seen in Figure 5B, this was only for low to moderate alcohol exposure. When the number of drinks during the pregnancy, was high, there was no increased risk to INATT due to the father’s INATT diagnosis. The was a slight increase in risk if the mother smoked during pregnancy (OR=1.41, 95% CI=[0.96, 2.06], p=0.09). Maternal INATT diagnosis did not increase the risk of INATT in the offspring (p=0.73).

**PAE – CD:** Neither PAE nor MOMAD significantly increased the risk of CD (p=0.33, p=0.79 respectively. Although Figure 6A depicts an increase in risk for CD if MOMAD=yes as the number of drinks increases, this interaction was not significant (p=0.38). Having a father with CD significantly increased the risk for CD in the offspring (p<0.0001). However, as seen in Figure 6B, this was true for lower levels of PAE during the pregnancy; there was no increased risk due to paternal CD for high levels of PAE (interaction p=0.11). A higher education of the father marginally decreased the risk for CD (p<0.072). During the process of removing demographic effects to arrive at the final model, paternal CD diagnosis was removed. Results for PAE without the father’s diagnosis of CD indicated that PAE significantly increased the risk for CD (p=0.024).
**PAE – ODD**: There was no association of PAE (p>0.69), MOMAD (p>0.70), or PAE*MOMAD (p>0.63) with ODD (Figure 7A). After accounting for other demographic variables, both paternal ODD diagnosis (p=0.0002) and maternal ODD diagnosis (p=0.02) increased the risk for ODD in the offspring (dad OR=12.01, 95% CI=[4.94, 33.70]; mom OR=4.68, 95% CI=[1.66, 13.16]). There was an increased risk for ODD if the father was ODD and the mother was AD (interaction p=0.0003, Figure 7B). A higher education of the father was a significant protective effect of ODD in the offspring (p=0.012).

**BINGE – qualitative measure**

*Binge – ADHD*: Binge drinking did not increase the risk of ADHD (p=0.22). Although maternal alcohol dependence did increase the risk (p=0.039), the interaction PAE*MOMAD was not significant (p>0.15). Having a father with ADHD increased the risk for ADHD in the offspring (p<0.0001). As seen in Figure 8, binge drinking and paternal ADHD increased the risk relative to no binge drinking and no ADHD in the father (p=0.013).

*Binge – HYPIMP*: There was no increased risk for HYPIMP due to binge drinking (p=0.52), maternal alcohol dependence (p=0.41), or the interaction between the two effects (p=0.15). Having a father with HYPIMP increased the risk for HYPIMP in the offspring (p=0.0001; Figure 9). Sex was the only other significant risk effect for HYPIMP (p=0.0011).

*Binge – INATT*: Similar to the results for HYPIMP, there was no increased risk for INATT due to binge drinking (p=0.89), maternal alcohol dependence (p=0.29), or the interaction (p=0.72). Having a father with INATT increased the risk for INATT (<0.0001). The primary effects are depicted in Figure 10.

*Binge – CD*: Binge drinking did not significantly increase the risk of CD after adjusting for other effects (p=0.23). Similarly, having a mother who was alcohol dependent did not increase the risk for CD (p=0.79). The father having a CD diagnosis significantly increased the risk for CD in the offspring (p<0.0001, Figure 11). If paternal CD diagnoses was excluded from the model, binge drinking increased the risk for CD (p=0.05, OR=2.49, 95% CI=[1.25, 4.95]). There was no binge*MOMAD interaction (p=0.74). A higher education of the father decreased the risk for CD (p=0.018).

*Binge – ODD*: There was no significant increase in risk of ODD due to binge drinking (p=0.20). Although MOMAD = yes significantly increased the risk for ODD as an effect on its own
(z-score p=0.002), it was not significant after accounting for other variables (type III SS p=0.24). The binge* MOMAD interaction was also not significant (p=0.46; Figure 12A). There was a significant contribution to the risk of ODD in offspring if the mother was ODD (p=0.011) or if the father was ODD (p=0.0002). Similarly the risk of ODD increased marginally if the father was alcohol dependent (p=0.099) or if both parents were alcohol dependent (p=0.055). There was a substantial increase in risk if the mother was alcohol dependent and the father was ODD (p<0.0001; Figure 12B). A mother who was marijuana dependent increased the risk for ODD before adjusting for other effects (z-score p=0.024) but not after (p=0.67). However, there was a significant increase in risk for ODD if the mother was marijuana dependent and binge drank during pregnancy (p=0.075, Figure 12C).
CHAPTER 4. DISCUSSION

4.1 Overview of results

This is the first study to tease apart the contribution of PAE, parental alcohol dependence, and a broad genetic load for ADHD, to the risk of ADHD, CD and ODD. The COGA sample is unique in that all three factors, and any combined effects (as measured by their interaction terms) can be tested simultaneously. The CIFASD dataset provided a secondary sample to compare overall contributions of PAE and a broad genetic load for ADHD.

As an effect on its own (Aim 1), PAE and binge drinking significantly increased the risk for CD and ODD in both the COGA and CIFASD samples. These results confirm previous reports in the literature, that PAE increases the risk for these externalizing disorders (Mattson et al., 2011; Tsang et al., 2016). While PAE increased the risk for all ADHD-related disorders in CIFASD, contrary to the hypothesis, neither PAE nor binge drinking increased the risk for any ADHD-related diagnosis in COGA, before accounting for any other effects. Also contrary to the hypothesis, a comparison of the logged ORs in the COGA sample indicated the PAE did not convey a higher risk to HYPIMP or INATT compared to CD or ODD. However results from the CIFASD data confirmed the hypothesis that PAE is associated with an increased risk to ADHD, relative to CD and ODD.

Interestingly, PAE and binge drinking were associated with an increased risk to INATT, CD and ODD in COGA after accounting for demographic and environmental effects (Aim 2). However neither increased the risk of any disorder after including parental diagnoses for each specific externalizing disorder in addition to parental diagnoses for alcohol dependence (Aim 4). The parental diagnoses for the disorder being tested were employed as a proxy for genetic risk in COGA, to account for risk variants inherited from a parent with the disorder. In CIFASD, the genetic risk was accounted for by using the polygenic risk scores for ADHD (PRS-ADHD). After accounting for the PRS-ADHD, PAE significantly increased the risk of HYPIMP, INATT, and CD diagnoses in the CIFASD sample.

An interesting outcome in the CIFASD data was the PAE*PRS interaction as a result of the convergence in risk due to PAE and genetic risk for ADHD and ODD, but not for INATT or CD. For ADHD and ODD diagnoses, there was an overall increased risk due to PAE, and a gradual
increase in risk due to genetics for both exposed and non-exposed individuals. The risk in exposed and non-exposed individuals was different for low genetic risk, but almost the same when the genetic risk was highest. This was not true for CD or INATT, and in fact the PAE*PRS interaction was not significant for INATT: genetic risk did not increase the risk for INATT in unexposed individuals. In the final CIFASD models, a genetic risk for ADHD increased the risk only for ADHD, CD and ODD, with the strongest association for CD and ODD, surprisingly. After accounting for this genetic risk and other demographic factors, PAE increased the risk for HYPIMP, INATT, and CD.

The contribution of parental alcohol dependence to the five disorders was examined in the COGA dataset (Aim 5). Paternal alcohol dependence was associated with an increased risk of CD but no other diagnoses. Maternal alcohol dependence was associated with an increased risk of INATT and ODD.

The final analyses in COGA included PAE, MOM AD the interaction term PAE* MOMAD, and a proxy for genetic risk represented by (for most disorders) the father’s diagnosis of the disorder (Aim 6). For all disorders, having a father with the specific disorder (i.e., a proxy for genetic predisposition for the disorder) greatly increased the risk of having the disorder, although this was moderated by maternal alcohol dependence for INATT. PAE was a significant factor of increased risk only for INATT after adjusting for parental alcohol dependence, genetic risk, and other demographic characteristics. Furthermore, by parsing out the variability attributable to these other effects, the strength of the association of PAE with risk for INATT increased. Binge drinking did not increase the risk for any disorder, after accounting for all other effects. There was a significant association of MOMAD with the risk of ADHD, after accounting for risk factors, and the combined interactive effect of an AD mother and PAE increased the risk for only ADHD and INATT.

The interaction effect of maternal drinking and alcohol dependence was exhibited differently for ADHD and INATT. For ADHD, there was a minimal difference in risk due to maternal alcohol dependence at very low number of drinks during the pregnancy. However as the number of drinks increased, the risk for ADHD increased at a much higher rate for individuals with an AD mother compared to those who mother was not AD. This was also true for CD; however, after including paternal CD diagnosis, this effect was no longer significant. On the other hand, regardless of number of drinks during the pregnancy, there was a substantial increased risk
for INATT if the mother was also AD and the father had an INATT diagnosis. However, if the mother was not alcohol dependent, the risk for INATT was greatest if there were low levels of PAE and the father was not INATT. At high levels of PAE, the risk for INATT was similar, regardless of paternal INATT diagnosis or maternal alcohol dependence.

### 4.2 Interpretation of results

After accounting for all effects of interest, including demographic and genetic effects, PAE increased the risk most consistently for INATT in both CIFASD and COGA samples. Without paternal CD, PAE also increased the risk for CD in COGA, consistent with results in CIFASD. Moreover, having an AD mother further increased the risk for INATT and ADHD for higher drinking levels during pregnancy. Paternal alcohol dependence did not increase risk for any disorders after accounting for all other effects. These results taken together support twin studies showing that having an alcohol dependent mother (Knopik et al., 2006), and heavy PAE exposure was a consistent predictor of ADHD in offspring of twins (Knopik, Heath, et al., 2009; Knopik et al., 2005), with evidence showing that the genetic contribution of an AD father was less important (Knopik, Jacob, et al., 2009).

Although PAE increased the risk for HYPIMP in CIFASD, it did not contribute to risk in COGA. However PAE did increase the risk for INATT in both CIFASD and COGA, which was one of two consistent results across both datasets. This could imply that the mechanism of action of PAE on the developing fetus is different for inattention compared to hyperactivity. Individuals with PAE and ADHD have trouble with sustained attention more than exhibiting hyperactivity (Aragon et al., 2008; Brown et al., 1991; Furtado & Roriz, 2016; Infante et al., 2015; Kodituwakku, 2007). These difficulties with attention are different, depending on PAE. Individuals with PAE and ADHD have trouble responding to new information, attending to the new information if necessary and keeping it in working memory, while those without PAE but with ADHD have trouble focusing on appropriate stimuli and sustaining attention (Coles, 2001). These differences in attention could be attributed to PAE exposure, while the hyperactive or impulsive behavior could be moderated by other factors such as HYPIMP or alcohol dependence in the parent. It is also possible that there are different neural activation bases of inhibition vs inattention. A recent study demonstrated that individuals with FAS exhibited different neural activation patterns in a Go/NoGo task compared to non-exposed controls (Kodali et al., 2017). These results potentially
extend the emerging studies implicating a different etiology of ADHD in those with PAE (Glass et al., 2013; Khoury, Jamieson, & Milligan, 2018; Khoury et al., 2015; Kingdon, Cardoso, & McGrath, 2015; Wetherill et al., 2018) to implicate one mechanism of action affecting hyperactive and impulsive symptoms but another PAE-related mechanism of action affecting inattention symptoms.

If PAE differentially moderates inattentive vs hyperactive/impulsive behavioral characteristics, this could account for the lack of an increase in risk to ADHD due to PAE alone, in both COGA and CIFASD samples. The ADHD group included individuals diagnosed with both hyperactivity/impulsivity and inattention, by definition. Thus if PAE is acting on different mechanisms to affect hyperactivity and impulsivity differently from inattention, combining individuals with both types of behavior could dilute the effect, thereby reducing the power to detect increased risk of PAE on ADHD. On the other hand, several studies report an association between PAE and ADHD, and the lack of association between PAE and ADHD in this study is contrary to the literature showing that ADHD is the most prevalent externalizing disorder in individuals with PAE (Bhatara et al., 2006; Burd et al., 2003; Fryer, McGee, et al., 2007; Lange, Rehm, Anagnostou, & Popova, 2018; Mattson et al., 2011; Pagnin, Zamboni Grecco, & Furtado, 2018; Reid, Shelton, Warner, O'Callaghan, & Dawe, 2017; Weyrauch, Schwartz, Hart, Klug, & Burd, 2017), and in particular, ADHD was 10 times higher the rate expected based on the general population (Weyrauch et al., 2017). However, some of these studies had higher sample sizes than either COGA or CIFASD, and the lack of association between PAE and ADHD in this study could be due to decreased power as a result of the smaller sample size. Furthermore the majority of these studies report on prevalence of ADHD in individuals with PAE compared to individuals without PAE, whereas the current study tested the specific hypothesis that PAE increased the risk for PAE after accounting for genetic and environmental contributions. Finally, although there was not a direct effect of PAE on the risk for ADHD, there was a significant interaction of PAE*MOMAD in COGA and of PAE and the polygenic risk scores in CIFASD. This could indicate that the combined effect of PAE with maternal alcohol dependence and/or genetic risk accounted for more variability in risk than PAE alone, which is a novel finding of this study not addressed in any previous study.

Another consistent result across all definitions of PAE and for both COGA and CIFASD samples, was that PAE significantly increased the risk of CD. However this was no longer
significant after accounting for paternal CD diagnosis in COGA. In the CIFASD data, PAE increased the risk of CD across the range genetic risk scores. The contribution of PAE and not the combination of PAE*maternal alcohol dependence could explain the meta-analysis results comparing the LORs of PAE and COA literature, which found no difference in LORs for CD (all permutation p>0.21).

Finally, ODD seemed to only be associated with PAE in the context genetic risk in CIFASD. PAE marginally increased risk in CIFASD, however the interaction of PAE and genetic risk indicated an elevated risk for ODD due to PAE for low values of genetic risk. Individuals with high genetic risk but no PAE had about the same risk for ODD as those with PAE. This was the only disorder where a maternal ODD diagnosis contributed to risk of ODD in the offspring, and a diagnosis in both parents substantially increased the risk for ODD. It is possible that a home life in which both parents exhibit traits of ODD (e.g., such as losing their tempers, blaming others, being spiteful), and other environmental effects that would likely accompany these problems, such as low socioeconomic status, education, and income, accentuate the effect of PAE. Thus PAE contributes to risk of ODD in studies where such environmental factors and parental diagnoses are unavailable, such as CIFASD, but does not contribute to ODD in studies such as this one, which could account for these environmental contributions.

**4.3 Strengths and limitations**

This study has several strengths, with the most important one being the ability to examine the contribution of both PAE and parental alcohol dependence, especially an AD mother. This has been the major weakness of most PAE studies, due to the difficulty of obtaining information about maternal behavior during the time of the pregnancy, since most participants in these studies are adopted. The inclusion of maternal alcohol dependence allowed for the simultaneous evaluation of the effect of an AD mother, PAE, and if there was additional risk due to the combination of PAE and maternal alcohol dependence. The inclusion of paternal alcohol dependence was not a significant contributing factor after adjusting for other effects, a finding supported by twin studies (Knopik, Jacob, et al., 2009), thus the reduction in sample size by including DADAD in the model was not a weakness. There was no information on parental alcohol dependence or specific maternal alcohol consumption during pregnancy for the majority of the CIFASD participants.
Although one weakness was the inability to include information on timing of alcohol exposure in COGA, as this was not captured by the SSAGA, we were able to implement a quantitative measure of PAE, as well as a binge measure of exposure. Implementing a quantitative variable increased statistical power, and enabled us to examine effects across the range of exposure, which was quite helpful in interpreting several results, especially interactions of PAE with other effects. The ability to include data from the CIFASD study was beneficial, as this allowed the comparison of results in COGA to CIFASD results.

Another strength of this study was the ability to implement polygenic risk scores using GWAS data in CIFASD, and parental diagnosis of the specific disorder, as a proxy for genetic risk to the disorder, in COGA. This allowed for the inclusion of a measure of broad genetic risk for ADHD, based on PGC results. Thus this study was able to implement the most significant effects that contribute to externalizing disorders in offspring: parental alcohol dependence, PAE, the interaction of maternal alcohol dependence and PAE, and genetic risk.

The ability to include demographic and environmental factors was a strength for both datasets, which is important due to their moderation of genetic effects on externalizing disorders (Hamdi, Krueger, & South, 2015; South & Krueger, 2011). On the other hand, the limitation due to a reduced sample size when paternal factors were included in COGA analyses was a weakness, as stated above, due to reduced sample size. Moreover, other than sex and race, the demographic and environmental factors that were included in the analyses were quite different between COGA and CIFASD datasets. COGA data allowed for environmental factors of the mothers and fathers, but did not include more detailed information of the children such as head injuries, learning disabilities, and other similar variables that were included in the CIFASD data. These are important covariates as they are likely to increase the risk for most of the disorders.

Due to the detailed SSAGA questions in COGA which asked about consumption of drugs, we were able to include DSM-IV diagnoses of alcohol dependence and marijuana in the mother. Sample sizes for other drugs precluded evaluation of other drugs. Furthermore, to enable better interpretation of results, maternal dependence on the drug was not coded to be “yes” unless she met the diagnosis before or during the year of birth of the child. This approach excluded mothers who may have been taking the drug during the pregnancy but had not yet met criteria for dependence, or who may have had a genetic load for dependence but started consumption of the drug after the pregnancy. Thus this exclusion conservatively included children with exposure
and/or a genetic load to be analyzed as though there was no prenatal exposure to the drug, or there was no risk to the disorder due to a drug dependence in the mother. This also reduced the sample size of potentially exposed children, which was a further conservative outcome of this strategy.

On the other hand, this study did not account for comorbidity of drug dependence in the mother, or for any drug dependence in the father other than alcohol dependence. The sample size for maternal alcohol dependence was the largest, with numbers decreasing for dependence on other drugs. Including comorbidity would have further reduced the sample sizes. Similarly, this study did not account for comorbidity of diagnoses in the offspring for similar reasons. There was no diagnosis data for parents in the CIFASD data.

While it was beneficial to have two comparison samples, the recruitment strategies were quite different for these two samples. COGA probands were ascertained through alcohol treatment centers, and focused on families with 3 or more AD first-degree relatives. As a result, about 85% of COGA families have a high density of alcohol dependence while only about 15% are comparison families. This does not imply that there are high rates of alcohol dependence in females in COGA, since in general females are less likely to be AD than males, and mothers included in these analyses could be anyone in the families, e.g., mates of AD males as well as sisters or mates of non-AD males, or daughters. Therefore the incidence of maternal drinking during pregnancy is lower in COGA than in CIFASD. Ascertainment in CIFASD was focused on including individuals with PAE and/or with a fetal alcohol spectrum disorder. The controls were carefully screened for PAE, and excluded if the absence of PAE could not be verified. These individuals are therefore likely to be more severely affected from PAE. On the other hand, it is possible that individuals included in the no PAE group were not completely unexposed to alcohol prenatally (i.e., difficult to ascertain with 100% certainty that there was no exposure at all). In addition, there are higher rates of these disorders in the CIFASD sample as this sample was more balanced between those with PAE and controls.

COGA participants spent several hours answering SSAGA questions and those included in this study also provided a blood sample. Thus these individuals were likely to truthfully answer questions about drug use and maternal alcohol consumption during pregnancy, after so much time and effort was spent to participate. However, if the mother or relative answering the questions related to maternal drinking during pregnancy felt a negative stigma regarding drinking while pregnant, there could be a bias in down-playing drinking patterns during pregnancy. In any event,
maternal consumption measures were retrospective, and could be unreliable. However, if there was an attempt to answer the questions truthfully, the retrospective nature is not necessarily a weakness, as one study demonstrated that a mother’s recall after 14 years, of her drinking pattern during the pregnancy, was a better predictor of externalizing issues in her offspring, than her drinking pattern reporting during the pregnancy (Hannigan et al., 2010). Another strength of the COGA sample in particular was due to the wealth of SSAGA data, and therefore, the ability to include demographic information on both parents, as well as alcohol dependence, and diagnosis of the specific externalizing disorder to account for heritability of the disorders in the offspring.

Another weakness of the COGA data was the assumption that the children lived with and/or were influenced by their parents, which is not necessarily true. With high rates of alcohol dependence in some families, it would be expected that some children may be raised by another family member or be in foster care. Another weakness was the inability to confirm that the mother and/or the father was the biological mother and father of the child in a small proportion of children included in this study. The ability to identify this parent-offspring relationship was only available when the offspring and parents had GWAS data. This was true for the majority of subjects, but not all. These analyses were applied to individuals living in the United State and may not be generalizable to other cultures. The final obvious weakness is the lack of correction for multiple testing. This study went through 6 aims, 5 disorders, 2 measures of PAE in COGA = 60 sets of analyses in COGA alone. The number of analyses performed in the CIFASD data was 4 (aims) x 5 diagnoses = 20, with a total of 80 statistical models analyzed in total. While this could lead to a bias in effects being included through various stages of the aims, the final models were limited in scope. But even in the final stage of this project, there were 5 CIFASD models and 10 COGA models. Therefore all results and interpretations should be regarded with caution. Replication of these results is warranted.

4.4 Future directions

Much work has been done to show that metabolism of alcohol is highly dependent on genetic variants in the alcohol dehydrogenase genes. Specifically, one variant in ADH1B catalyzes alcohol 80 times more quickly for one allele compared to the other allele, which results in a protective effect for various phenotypes for the fetus (Warren & Li, 2005). The protective effect for cognitive deficits, attention problems, and aggression is also seen even in offspring with the
allele (Jacobson et al., 2006). Another study has shown a gene by environment effect in that a genotype score based on risk alleles of ADH polymorphisms was positively associated with conduct disorder scores if the mother consumed alcohol during the pregnancy, but not if the mother did not drink (Murray et al., 2016). A similar interaction found that this variant protected against attention problems, hyperactivity, and aggression if the mother drank and had the high-metabolizing variant (Dodge, Jacobson, & Jacobson, 2014). Therefore the \textit{ADH1B} genotype should be included and these analyses repeated to test for similar protective effects.

It is possible that the different mechanisms affecting INATT and HYPIMP are due in part, to activation of gene pathways via PAE, or to protective effects of genes other than the alcohol hydrogenase genes. This could be explored by examining the entire genome using GWAS data for an interaction effect of genotype*PAE. While the sample sizes in COGA and CIFASD are quite small, relative to typical GWAS studies, they could be used as a discovery and replication sample, respectively. The scope of testing could be reduced to only examine results in genes or pathways identified in animal models, for example GABA-related (Skorput et al., 2015), serotine-related (Workman et al., 2015), glucocorticoids (Allan et al., 2014; Ngai et al., 2015), or the corticotropin-releasing hormone (Lan et al., 2015). Pathways implicated and verified could inform the field on the development of pharmacological treatment and behavioral interventions.

\section*{4.5 Summary}

This study demonstrated PAE significantly increased the risk for INATT and CD in COGA, and increased the risk for HYPIMP, INATT, and CD in the CIFASD sample. Genetic risk increased the risk for all disorders in both COGA and CIFASD, with the exception of HYPIMP in CIFASD. Maternal alcohol dependence increased the risk ADHD, while the combination of an AD mother and PAE increased the risk for ADHD and INATT. There was modest evidence for a different etiology of INATT and HYPIMP attributable to PAE.

While PAE increased the risk for CD for all definitions of PAE and in both COGA and CIFASD, it is not clear what proportion of the risk is due to PAE alone and what proportion is due to genetics or environmental effects. Contrary to the growing literature contrasting ADHD traits between those with and without PAE, there are no studies comparing CD traits between the two groups. This could be due to a more homogenous set of criteria for CD and therefore there is nothing to compare, or potentially because the risk to CD is the same for those with an AD mother.
who likely drinks while pregnant, as for those with known PAE, regardless of whether or not the mother is AD. One study which found that maternal alcohol dependence increased the risk for both CD and ODD, reported that these results did not change after accounting for measures of maternal alcohol consumption during pregnancy (Malone, McGue, & Iacono, 2010). The other study, which compared the LORs between PAE and COA literature, reported no differences in the LORs for CD between the two domains for CD (Wetherill et al., 2018). A recent meta-analysis demonstrated a large effect size of PAE for conduct problems (Khoury et al., 2018), while an earlier meta-analysis reported a prevalence of 91% for CD in those with PAE (Popova et al., 2016). Finally, a large proportion of genetic variability is shared between alcohol dependence and CD (Edwards & Kendler, 2012; Slutske et al., 2002). These results might imply that CD could be a genetic disorder inherited from an AD parent, and the fact that an AD mother is likely to drink during pregnancy could account for the effect of PAE. One study has demonstrated that binge drinking but not other drinking patterns, led to conduct problems in the Avon Longitudinal Study of Parents and Children (Sayal et al., 2014).

4.6 Conclusion

These are the first results to disentangle the effects of genetics, environment and maternal alcohol dependence from PAE. An early editorial recognized that learning disabilities occur in offspring of alcoholic parents as well as in individuals exposed to alcohol prenatally (Lippmann, 1980). This delineation between ADHD-like symptoms of those with PAE vs those without was a charge from many years ago (Weinberg, 1997). While some studies have started to address this issue (O’Brien & Hill, 2014; Sharma & Hill, 2017), they have not encompassed the complete array of genetic contribution and parental diagnoses, in addition to PAE. The contribution of PAE to INATT and CD were the only consistent results across all definitions of alcohol exposure and in both datasets, indicating that PAE is a veritable risk for INATT and CD. This finding further supports the trending evidence of a unique etiology of ADHD in those with PAE, and more specifically, presents evidence that INATT and HYPIMP are affected according to two different mechanisms of action, independent of a genetic contribution due to either ADHD or alcohol dependence, both of which also were associated with a risk for these disorders. While there are no pharmacological treatments for CD, understanding that a potential cause of CD in a percentage of the population is due to PAE could help direct interventions or therapy in families where the
mother is alcohol dependent. Finally, there is a growing evidence of lack of response of those with PAE to ADHD-related medications (Koren, 2015; O’Malley, Koplin, & Dohner, 2000; Premji, Benzies, Serrett, & Hayden, 2007). These results hinting at a different etiology of INATT symptoms in those with PAE emphasize the conclusion from a recent review showing that ADHD medication relieved hyperactive and impulsive behavior in individuals with PAE but did not help accentuate attention (Koren, 2015). This gap in PAE studies to identify effective treatment strategies for those with INATT due to PAE will hopefully be addressed.
REFERENCES


# TABLES

Table 1: Summary of demographic information for all diagnoses

## Table 1A COGA sample

<table>
<thead>
<tr>
<th></th>
<th>ADHD-combined</th>
<th>ADHD-hyperactivity</th>
<th>ADHD–inattention</th>
<th>CD</th>
<th>ODD</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (% affected)</td>
<td>2,482 (20.7)</td>
<td>1,862 (8.9)</td>
<td>2,687 (12.6)</td>
<td>2,745 (11.6)</td>
<td>2,745 (15.9)</td>
</tr>
<tr>
<td>% of disorder</td>
<td>62.8*</td>
<td>64.8*</td>
<td>62.8*</td>
<td>69.5*</td>
<td>57.5*</td>
</tr>
<tr>
<td>male (vs female)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of disorder</td>
<td>28.1</td>
<td>23.5</td>
<td>27.9</td>
<td>29.4</td>
<td>33.0</td>
</tr>
<tr>
<td>with PAE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of disorder</td>
<td>9.5</td>
<td>9.0</td>
<td>9.4</td>
<td>11.6</td>
<td>13.5</td>
</tr>
<tr>
<td>with binge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of EA/AA</td>
<td>20.3/21.5</td>
<td>9.4/8.5</td>
<td>12.4/11.7</td>
<td>10.5/12.8</td>
<td>14.7/18.8</td>
</tr>
<tr>
<td>with disorder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Table 1B CIFASD sample

<table>
<thead>
<tr>
<th></th>
<th>ADHD-combined</th>
<th>ADHD-hyperactivity</th>
<th>ADHD–inattention</th>
<th>CD</th>
<th>ODD</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (% affected)</td>
<td>220 (44.5)</td>
<td>85 (16.0)</td>
<td>188 (35.3)</td>
<td>50 (9.4)</td>
<td>154 (28.9)</td>
</tr>
<tr>
<td>% recruited for</td>
<td>100</td>
<td>15.0</td>
<td>85.4</td>
<td>4.9</td>
<td>39.0</td>
</tr>
<tr>
<td>ADHD with disorder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of disorder</td>
<td>61.8*</td>
<td>67.1*</td>
<td>61.7</td>
<td>60.0</td>
<td>60.4</td>
</tr>
<tr>
<td>male (vs female)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of disorder</td>
<td>65.4</td>
<td>81.9</td>
<td>65.2</td>
<td>83.3</td>
<td>64.4</td>
</tr>
<tr>
<td>with PAE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of EA/AA</td>
<td>44.2/33.9</td>
<td>18.9/10.7</td>
<td>38.1/26.0</td>
<td>11.3/4.6</td>
<td>32.1/18.5</td>
</tr>
<tr>
<td>with disorder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

COGA = collaboration on the genetics of alcoholism, CIFASD = collaborative initiative on fetal alcohol spectrum disorders, ADHD = attention deficit hyperactivity, CD = conduct disorder, ODD = oppositional defiant disorder, PAE = prenatal alcohol exposure, EA = European American, AA = African American. Tests with p<0.05 are indicated with an *. 
Table 1C CIFASD sample

<table>
<thead>
<tr>
<th>Site</th>
<th>% of EA recruited at site</th>
<th>% of AAs recruited at site</th>
<th>% of Others recruited at site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlanta</td>
<td>11.9</td>
<td>75.6</td>
<td>11.5</td>
</tr>
<tr>
<td>Los Angeles</td>
<td>11.3</td>
<td>3.4</td>
<td>15.9</td>
</tr>
<tr>
<td>Minneapolis</td>
<td>25.9</td>
<td>6.9</td>
<td>38.0</td>
</tr>
<tr>
<td>San Diego</td>
<td>50.8</td>
<td>11.1</td>
<td>34.6</td>
</tr>
</tbody>
</table>

CIFASD = collaborative initiative on fetal alcohol spectrum disorders, EA = European American, AA = African American.
Table 2: Aim 1 Odds ratios for prenatal alcohol exposure

Table 2A in COGA

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>OR</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>z-score</th>
<th>p-value</th>
<th>Type 3 SS p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAE ADHD</td>
<td>0.65</td>
<td>0.39</td>
<td>1.078</td>
<td>0.74</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Binge ADHD</td>
<td>0.76</td>
<td>0.33</td>
<td>1.74</td>
<td>0.52</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>PAE HYPIMP</td>
<td>1.03</td>
<td>0.56</td>
<td>1.15</td>
<td>0.85</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Binge HYPIMP</td>
<td>1.05</td>
<td>0.63</td>
<td>1.18</td>
<td>0.84</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>PAE INATT</td>
<td>1.14</td>
<td>0.78</td>
<td>1.26</td>
<td>0.18</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Binge INATT</td>
<td>1.18</td>
<td>0.81</td>
<td>1.72</td>
<td>0.38</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>PAE CD</td>
<td>1.24</td>
<td>0.81</td>
<td>1.42</td>
<td>0.015</td>
<td>0.083</td>
<td></td>
</tr>
<tr>
<td>Binge CD</td>
<td>1.49</td>
<td>1.02</td>
<td>2.18</td>
<td>0.042</td>
<td>0.077</td>
<td></td>
</tr>
<tr>
<td>PAE ODD</td>
<td>1.24</td>
<td>1.04</td>
<td>1.59</td>
<td>0.0051</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>Binge ODD</td>
<td>1.94</td>
<td>1.40</td>
<td>2.69</td>
<td>0.0001</td>
<td>0.0015</td>
<td></td>
</tr>
</tbody>
</table>

Table 2B in CIFASD

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>OR</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>Type 3 SS p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAE ADHD</td>
<td>10.79</td>
<td>7.60</td>
<td>15.32</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAE HYPIMP</td>
<td>7.43</td>
<td>4.85</td>
<td>11.39</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAE INATT</td>
<td>9.61</td>
<td>6.73</td>
<td>13.72</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAE CD</td>
<td>7.49</td>
<td>4.23</td>
<td>13.19</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAE ODD</td>
<td>4.19</td>
<td>3.03</td>
<td>5.80</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

COGA = collaboration on the genetics of alcoholism, CIFASD = collaborative initiative on fetal alcohol spectrum disorders, ADHD = attention deficit hyperactivity, HYPIMP = hyperactive/impulsive, INATT = inattentive, CD = conduct disorder, ODD = oppositional defiant disorder, PAE = prenatal alcohol exposure, OR = odds ratio, CI = confidence interval, SS = sum of squares.
Table 3: Aim 2 Odds ratios for PAE, after accounting for demographic effects

Table 3A in COGA

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>OR</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>z-score</th>
<th>p-value</th>
<th>Type 3 SS p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAE ADHD</td>
<td>1.08</td>
<td>0.79</td>
<td>1.49</td>
<td>0.64</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Binge ADHD</td>
<td>1.29</td>
<td>0.72</td>
<td>2.31</td>
<td>0.21</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>PAE HYPIMP</td>
<td>0.94</td>
<td>0.63</td>
<td>1.41</td>
<td>0.63</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Binge HYPIMP</td>
<td>1.00</td>
<td>0.56</td>
<td>1.80</td>
<td>0.98</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>PAE INATT</td>
<td>0.89</td>
<td>0.56</td>
<td>1.42</td>
<td>0.0054</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Binge INATT</td>
<td>0.57</td>
<td>0.27</td>
<td>1.24</td>
<td>0.16</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>PAE CD</td>
<td>1.32</td>
<td>0.88</td>
<td>1.97</td>
<td>0.018</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Binge CD</td>
<td>2.36</td>
<td>1.22</td>
<td>4.56</td>
<td>0.011</td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td>PAE ODD</td>
<td>1.91</td>
<td>0.85</td>
<td>4.29</td>
<td>0.97</td>
<td>0.067</td>
<td></td>
</tr>
<tr>
<td>Binge ODD</td>
<td>2.56</td>
<td>1.03</td>
<td>6.36</td>
<td>0.54</td>
<td>0.074</td>
<td></td>
</tr>
</tbody>
</table>

Table 3B in CIFASD

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>OR</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>Type 3 SS p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAE ADHD</td>
<td>3.62</td>
<td>2.32</td>
<td>5.64</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAE HYPIMP</td>
<td>6.99</td>
<td>4.22</td>
<td>11.58</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAE INATT</td>
<td>10.79</td>
<td>5.08</td>
<td>21.73</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAE CD</td>
<td>7.89</td>
<td>4.16</td>
<td>14.97</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAE ODD</td>
<td>3.23</td>
<td>2.05</td>
<td>5.09</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

COGA = collaboration on the genetics of alcoholism, CIFASD = collaborative initiative on fetal alcohol spectrum disorders, ADHD = attention deficit hyperactivity, HYPIMP = hyperactive/impulsive, INATT = inattentive, CD = conduct disorder, ODD = oppositional defiant disorder, PAE = prenatal alcohol exposure, OR = odds ratio, CI = confidence interval, SS = sum of squares.
Table 4: Aim 4 Odds ratios for PAE, accounting demographics, parental diagnosis, PRS

**Table 4A in COGA**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>OR</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>z-score</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAE ADHD</td>
<td>1.01</td>
<td>0.71</td>
<td>1.44</td>
<td>0.016</td>
<td>0.99</td>
</tr>
<tr>
<td>Binge ADHD</td>
<td>1.36</td>
<td>0.75</td>
<td>2.48</td>
<td>0.27</td>
<td>0.36</td>
</tr>
<tr>
<td>PAE HYPIMP</td>
<td>1.06</td>
<td>0.63</td>
<td>1.76</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>Binge HYPIMP</td>
<td>0.95</td>
<td>0.48</td>
<td>1.89</td>
<td>0.89</td>
<td>0.89</td>
</tr>
<tr>
<td>PAE INATT</td>
<td>1.16</td>
<td>0.84</td>
<td>1.60</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Binge INATT</td>
<td>0.79</td>
<td>0.35</td>
<td>1.76</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>PAE CD</td>
<td>1.01</td>
<td>0.63</td>
<td>1.61</td>
<td>0.089</td>
<td>0.21</td>
</tr>
<tr>
<td>Binge CD</td>
<td>1.95</td>
<td>0.90</td>
<td>4.22</td>
<td>0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>PAE ODD</td>
<td>1.69</td>
<td>0.65</td>
<td>4.41</td>
<td>0.50</td>
<td>0.38</td>
</tr>
<tr>
<td>Binge ODD</td>
<td>2.23</td>
<td>0.83</td>
<td>6.04</td>
<td>0.43</td>
<td>0.20</td>
</tr>
</tbody>
</table>

**Table 4B in CIFASD**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>OR</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>Type 3 SS p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAE ADHD</td>
<td>6.68</td>
<td>3.45</td>
<td>12.94</td>
<td>0.25</td>
</tr>
<tr>
<td>PAE HYPIMP</td>
<td>16.46</td>
<td>7.45</td>
<td>36.35</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAE INATT</td>
<td>10.51</td>
<td>5.08</td>
<td>21.73</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAE CD</td>
<td>14.54</td>
<td>5.51</td>
<td>38.37</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAE ODD</td>
<td>5.49</td>
<td>3.38</td>
<td>8.91</td>
<td>0.13</td>
</tr>
</tbody>
</table>

COGA = collaboration on the genetics of alcoholism, CIFASD = collaborative initiative on fetal alcohol spectrum disorders, ADHD = attention deficit hyperactivity, HYPIMP = hyperactive/impulsive, INATT = inattentive, CD = conduct disorder, ODD = oppositional defiant disorder, PAE = prenatal alcohol exposure, OR = odds ratio, CI = confidence interval, SS = sum of squares.
Table 5: Aim 6 Odds ratios for PAE, accounting for all effects (COGA)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>OR</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>z-score</th>
<th>p-value</th>
<th>Type 3 SS p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAE ADHD</td>
<td>1.17</td>
<td>0.71</td>
<td>1.92</td>
<td>0.019</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Binge ADHD</td>
<td>1.65</td>
<td>0.70</td>
<td>3.90</td>
<td>0.29</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>PAE HYPIMP</td>
<td>0.84</td>
<td>0.49</td>
<td>1.47</td>
<td>0.55</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Binge HYPIMP</td>
<td>0.82</td>
<td>0.44</td>
<td>1.53</td>
<td>0.41</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>PAE INATT</td>
<td>1.06</td>
<td>0.64</td>
<td>1.77</td>
<td>0.061</td>
<td>0.58</td>
<td>0.0025</td>
</tr>
<tr>
<td>Binge INATT</td>
<td>1.04</td>
<td>0.57</td>
<td>1.90</td>
<td>0.74</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>PAE CD</td>
<td>1.17</td>
<td>0.64</td>
<td>2.16</td>
<td>0.34</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Binge CD</td>
<td>1.70</td>
<td>0.76</td>
<td>3.76</td>
<td>0.26</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>PAE ODD</td>
<td>0.98</td>
<td>0.55</td>
<td>1.77</td>
<td>0.95</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Binge ODD</td>
<td>2.77</td>
<td>0.79</td>
<td>9.68</td>
<td>0.81</td>
<td>0.20</td>
<td></td>
</tr>
</tbody>
</table>

Odds ratios for PAE are for the binary variable (yes/no). COGA = collaboration on the genetics of alcoholism, PRS = polygenic risk scores, ADHD = attention deficit hyperactivity, HYPIMP = hyperactive/impulsive, INATT = inattentive, CD = conduct disorder, ODD = oppositional defiant disorder, PAE = prenatal alcohol exposure, OR = odds ratio, CI = confidence interval, SS = sum of squares.
Table 6: Depiction of risk (+ = increase, - = decrease) in the final models

<table>
<thead>
<tr>
<th></th>
<th>ADHD (PAE, binge, CIFASD)</th>
<th>HYPIMP (PAE, binge, CIFASD)</th>
<th>INATT (PAE, binge, CIFASD)</th>
<th>CD (PAE, binge, CIFASD)</th>
<th>ODD (PAE, binge, CIFASD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAE (with CIFASD)</td>
<td>NS, NS, +</td>
<td>NS, NS, +</td>
<td>+, NS, +</td>
<td>NS, NS, +</td>
<td>NS, NS, NS</td>
</tr>
<tr>
<td>mom AD</td>
<td>+, +</td>
<td>NS, +</td>
<td>NS, NS</td>
<td>NS, NS</td>
<td>NS, NS</td>
</tr>
<tr>
<td>PAE*mom AD</td>
<td>+, NS</td>
<td>NS, NS</td>
<td>+, NS</td>
<td>NS, NS</td>
<td>NS, NS</td>
</tr>
<tr>
<td>DAD dx (COGA) or PRS (CIFASD)</td>
<td>+, +, +</td>
<td>+, +, NS</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
</tr>
<tr>
<td>Mom dx (COGA)</td>
<td>+, +</td>
<td>+, +, NS</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
</tr>
<tr>
<td>Dad dx* Mom AD</td>
<td>+, NA</td>
<td>+, NA</td>
<td>+, NA</td>
<td>+, NA</td>
<td>+, NA</td>
</tr>
<tr>
<td>PAE<em>Dad dx (COGA) or PAE</em>PRS (CIFASD)</td>
<td>NA, +, +</td>
<td>+, NA, NS</td>
<td>+, NA, +</td>
<td>+, NA, +</td>
<td>+, NA, +</td>
</tr>
<tr>
<td>dad AD</td>
<td></td>
<td></td>
<td></td>
<td>+, NA</td>
<td>+, NA</td>
</tr>
<tr>
<td>mom AD* dad AD</td>
<td></td>
<td></td>
<td></td>
<td>+, NA</td>
<td>+, NA</td>
</tr>
<tr>
<td>sex (males)</td>
<td>+, +, NS</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, NA, +</td>
<td>+, NA, +</td>
</tr>
<tr>
<td>smoke-preg (did )</td>
<td>+, NA</td>
<td>+, NA</td>
<td>+, NA</td>
<td>+, NA</td>
<td>+, NA</td>
</tr>
<tr>
<td>smoke-preg*mom AD (yes/yes vs no/no)</td>
<td>+, NA</td>
<td>+, NA</td>
<td>+, NA</td>
<td>+, NA</td>
<td>+, NA</td>
</tr>
<tr>
<td>race (black vs white)</td>
<td>NA, NA, -</td>
<td>-</td>
<td>NA, NA</td>
<td>-</td>
<td>NA, NA, -</td>
</tr>
<tr>
<td>mom MJ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NA, +</td>
</tr>
<tr>
<td>pae*mom MJ (yes, yes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NA, +</td>
</tr>
<tr>
<td>pae<em>sex</em>smoke (yes-male vs no female)</td>
<td>-</td>
<td>NA, NA</td>
<td>-</td>
<td>NA, NA</td>
<td>NA, -</td>
</tr>
<tr>
<td>dad grade</td>
<td>-</td>
<td>NA, NA</td>
<td>-</td>
<td>NA, NA</td>
<td>-</td>
</tr>
</tbody>
</table>

+ in red if p<0.05 for type 3 sum of squares. First 2 symbols are for COGA PAE and binge. CIFASD results are provided as the 3rd set of symbols for PAE, prs-ADHD, and PAE*prs-ADHD. Primary effects of interest are highlighted in yellow (PAE, Mom AD) and blue (PRS). NS = not significant (p>0.05), NA = not applicable, COGA = collaboration on the genetics of alcoholism, PRS = polygenic risk scores, AD = alcohol dependent, AlcDep = alcohol dependence, ADHD = attention deficit hyperactivity, HYPIMP = hyperactive impulsive, INATT = inattentive, CD = conduct disorder, ODD = oppositional defiant disorder, PAE = prenatal alcohol exposure, smoke = maternal smoking during pregnancy, MJ = marijuana dependent.
FIGURES

1A PRS-ADHD and attention deficit hyperactivity disorder risk in CIFASD

![Graph showing predicted probabilities for ADHD risk](image)

1B PRS-ADHD and inattention disorder risk in CIFASD

![Graph showing predicted probabilities for inattention disorder risk](image)

Figure 1: Probability of diagnosis as a function of genetic risk of ADHD in CIFASD
Figure 1 continued

1C PRS-ADHD and conduct disorder risk in CIFASD

![Graph showing predicted probabilities for CD risk in CIFASD.]

1D PRS-ADHD and oppositional defiant disorder risk in CIFASD

![Graph showing predicted probabilities for ODD risk in CIFASD.]

Fit computed at rate group 2 AA
Figure 2A Race and attention deficit hyperactive disorder risk in CIFASD

Figure 2B Race and inattentive disorder risk in CIFASD

Figure 2: Probability of diagnosis as a function of prenatal alcohol exposure (exp) and race
Figure 2 continued

Figure 2C Race and oppositional defiant disorder risk in CIFASD

Predicted Probabilities for odd = 1
With 95% Confidence Limits

Fit computed at prs  odd  p05=-0

(EA=European American, AA = African American)
Figure 3A ADHD - PAE and maternal alcohol dependence (mom_alcdep) in COGA

Figure 3B ADHD – PAE and maternal alcohol dependence by smoking during pregnancy in COGA

Figure 3: Probability of attention deficit hyperactivity disorder (ADHD) in COGA
Figure 3C ADHD – Maternal alcohol dependence and Paternal ADHD diagnosis in COGA

Predicted Probabilities for adhd = 1
With 95% Confidence Limits

Num_drinks_total = PAE = number drinks total reported during pregnancy, pregsmoke = maternal smoking during pregnancy, ADHD = attention deficit hyperactivity disorder.
Figure 4A HYPIMP - PAE and maternal alcohol dependence (mom_alcdep) in COGA

Figure 4B HYPIMP – Maternal alcohol dependence and Paternal HYPIMP diagnosis in COGA

Num_drinks_total = PAE = number drinks total reported during pregnancy

Figure 4: Probability of hyperactive/impulsive diagnosis (HYPIMP) in COGA
Figure 5A INATT - PAE and maternal alcohol dependence (mom_alcdep) in COGA

Figure 5B INATT – PAE and paternal INATT (dad_inatt) diagnosis by maternal INATT (mom_inatt) diagnosis in COGA.

Num_drinks_total = PAE = number drinks total reported during pregnancy.

Figure 5: Probability of Inattentive (INATT) diagnosis in the COGA
Figure 6A CD - PAE and maternal alcohol dependence (mom_alcdep) in COGA

Figure 6B CD – PAE and paternal CD (dad_cd) diagnosis in COGA

Figure 6: Probability of conduct disorder (CD) in COGA
Figure 7A ODD - PAE and maternal alcohol dependence (mom_alcdep) in COGA

![Graph showing predicted probabilities for ODD dx = 1 with 95% confidence limits]

Figure computed at:
- dad: grade=13.14
- sex=2
- mom_odd=2
- dad_odd=2
- no

Figure 7B ODD – maternal alcohol dependence (mom_alcdep) and paternal ODD diagnosis (dad_odd) in COGA

![Graph showing predicted probabilities for ODD dx = 1 with 95% confidence limits]

Figure computed at:
- dad_odd: 0, 1, yes, 2, no
- mom_alcdep: 1, yes, 2, no

Figure 7: Probability of oppositional defiant disorder (ODD) in COGA
Mom_alcdep = maternal alcohol dependence, dad_adhd = paternal ADHD diagnosis, ADHD = attention deficit hyperactivity diagnosis

Figure 8: Probability of BINGE drinking and ADHD in the COGA
Figure 9: Probability of BINGE drinking and HYPIMP in COGA

Mom_alcdep = maternal alcohol dependence, dad_hypimp = paternal hyperactive/impulsive diagnosis, HYPIMP = hyperactive/impulsive diagnosis
Mom_alcdep = maternal alcohol dependence, dad_inatt = paternal inattentive diagnosis, INATT = inattentive diagnosis

Figure 10: Probability of BINGE drinking and INATT in COGA
Figure 11: Probability of BINGE drinking and CD in COGA

Mom_alcdep = maternal alcohol dependence, dad_cd = paternal conduct disorder, CD = conduct disorder
Figure 12: Probability of oppositional defiant disorder (ODD) in COGA
Figure 12 continued

Figure 12C ODD – binge drinking and maternal marijuana dependence in COGA
VITA

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Indiana University, Bloomington IN
MA, 1994, Cognitive Psychology

IUPUI, Indianapolis IN
MS, 1995, Applied Statistics

Purdue University, West Lafayette IN
MS, 1998, Statistical Genetics

Indiana University Purdue University, Indianapolis IN
PhD, 2018, Addiction Neuroscience

Positions:
1986-88 Math Department Assistant, Olivet Nazarene University
1987 Supplemental Course Instructor, Olivet Nazarene University
1988 Math Lab Assistant, Olivet Nazarene University
1988-90 Teaching Assistant, Indiana University, Department of Psychology
1994&96 Part-time faculty, IUPUI, Department of Mathematics
1998- Applied Statistician, Dept of Medical & Molecular Genetics, Indiana University
School of Medicine

Honors:
1985 Honor Scholar
1986-88 President’s Scholar
1988 Graduation cum laude, with departmental (psychology) honors
1988 Who’s Who
1988 Outstanding Young Women
1996-98 Frederick N. Andrews Fellowship

Memberships in Professional Organizations:
   International Genetic Epidemiology Society
   Research Society on Alcoholism

University Service:
2016- Instructor, Indiana University School of Medicine, Biostatistics for Health Care
Researchers: A Short Course, Design of Genetic Studies
2018- Lecturer, Basic Human Genetics Q580, Department of Medical and Molecular
Genetics
2018- Lecturer, Introduction to Genetic Counseling Research Q608, Department of Medical
and Molecular Genetics
2018- Lecturer, Special Topics in Human Genetics Q640, Department of Medical and
Molecular Genetics
2017-18 Masters Student Genetic Counseling Committee member, Annie Nyberg, Department
of Medical and Molecular Genetics
2018-19 Masters Student Genetic Counseling Committee member, Cara Hein, Department of
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2018-19 Masters Student Genetic Counseling Committee member, Kathryn Imrie, Department of Medical and Molecular Genetics
2018-19 Masters Student Genetic Counseling Committee member, Kayla Quirin, Department of Medical and Molecular Genetics
2018-19 Masters Student Genetic Counseling Committee member, Rebecca Steele Baud, Department of Medical and Molecular Genetics
2018- Masters Student Genetic Counseling Committee member, Lauren Churchill, Department of Medical and Molecular Genetics
2018- Masters Student Genetic Counseling Committee member, Courtney Brown, Department of Medical and Molecular Genetics
2018- Masters Student Genetic Counseling Committee member, Alexis McEntire, Department of Medical and Molecular Genetics
2018- Masters Student Genetic Counseling Committee member, Allie Hentschell, Department of Medical and Molecular Genetics

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Alcoholism: Clinical and Experimental Research

American Journal of Medical Genetics – Neuropsychiatric Genetics, Part B

BMC Genomics

Drug and Alcohol Dependence

Journal of Studies on Alcohol and Drugs

Molecular Psychiatry

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Psychiatric Genetics

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