Serotonin Functioning and Adolescents' Alcohol Use:

A Genetically Informed Study Examining Mechanisms of Risk

by

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ABSTRACT

The current study utilized data from two longitudinal samples to test mechanisms in the relation between a polygenic risk score indexing serotonin functioning and alcohol use in adolescence. Specifically, this study tested whether individuals with lower levels of serotonin functioning as indexed by a polygenic risk score were vulnerable to poorer self-regulation, and whether poorer self-regulation subsequently predicted the divergent outcomes of depressive symptoms and aggressive/antisocial behaviors. This study then examined whether depressive symptoms and aggressive/antisocial behaviors conferred risk for later alcohol use in adolescence, and whether polygenic risk and effortful control had direct effects on alcohol use that were not mediated through problem behaviors. Finally, the study examined the potential moderating role of gender in these pathways to alcohol use.

Structural equation modeling was used to test hypotheses. Results from an independent genome-wide association study of 5-hydroxyindoleacetic acid in the cerebrospinal fluid were used to create serotonin (5-HT) polygenic risk scores, wherein higher scores reflected lower levels of 5-HT functioning. Data from three time points were drawn from each sample, and all paths were prospective. Findings suggested that 5-HT polygenic risk did not predict self-regulatory constructs. However, 5-HT polygenic risk did predict the divergent outcomes of depression and aggression/antisociality, such that higher levels of 5-HT polygenic risk predicted greater levels of depression and aggression/antisociality. Results most clearly supported adolescents' aggression/antisociality as a mechanism in the relation between 5-HT polygenic risk and later alcohol use. Deficits in self-regulation also predicted depression and

aggression/antisociality, and indirectly predicted alcohol use through aggression/antisociality. These pathways to alcohol use might be the most salient for boys with low levels of socioeconomic status.

Results are novel contributions to the literature. The previously observed association between serotonin functioning and alcohol use might be due, in part, to the fact that individuals with lower levels of serotonin functioning are predisposed towards developing earlier aggression/antisociality. Results did not support the hypothesis that serotonin functioning predisposes individuals to deficits in self-regulatory abilities. Findings extend previous research by suggesting that serotonin functioning and self-regulation might be transdiagnostic risk factors for many types of psychopathology.

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Alcohol use among adolescents and young adults is a major public health concern. Alcohol use and disorder increase the risk for violence, HIV, injuries, and other negative outcomes (CDC, 2010). Moreover, excessive alcohol use is a leading cause of preventable death and is associated with over $200 billion in economic costs (Bouchery, Harwood, Sacks, Simon, & Brewer, 2011; Mokdad, Marks, Stroup, & Gerberding, 2004). It is therefore a priority to understand the determinants of alcohol use in youth in order to elucidate factors salient to prevention. There is some evidence that serotonin functioning is a risk factor for alcohol use, such that lower levels of serotonin functioning tend to predict greater levels of alcohol use. This evidence has been provided by experimental studies that manipulate serotonin, studies that measure serotonin levels through drug challenge, metabolite levels, or receptor binding, and studies of measured serotonergic genes (see LeMarquand, Pihl, & Benkelfat, 1994a, for a review).

However, the mechanisms through which serotonin functioning increases the risk for alcohol use are less clear. Although many diverse mechanisms have been proposed (e.g., Canli & Lesch, 2007; Carver, Johnson & Joorman, 2008; Hariri & Holmes, 2006; Heinz, Mann, Weinberger, & Goldman, 2001; Lesch, 2005), an integrated model that coherently outlines the pathways from serotonin functioning to alcohol use has not yet been established. Additionally, few longitudinal studies have been utilized to prospectively examine these possible mechanisms. Investigating the mechanisms underlying serotonin functioning and alcohol use in a longitudinal framework would

inform the etiology of alcohol use and disorder and guide prevention and intervention efforts.

The Serotonergic System and Methods for Measuring Serotonin Functioning

The serotonergic system is one of the most widely distributed neurotransmitter systems in the brain, with the majority of serotonin neurons located along the midline of the brainstem. Long axons from these neurons project out to many areas of the nervous system (van Goozen, Fairchild, Snoek, & Harold, 2007). Although not fully understood, many factors can influence the overall functioning of the serotonergic system. These include the levels of available serotonin, serotonin receptor density and sensitivity, the efficiency of serotonin reuptake and metabolism, the recency of cell firing, and the consumption of amino acids (Carver, Johnson, & Joorman, 2008; Neumeister et al., 2006). Genetic variation also influences serotonin functioning, possibly through several of these processes.

Various methods have been used to measure serotonin functioning in the literature. Although it would be optimal to measure levels of serotonin in the brain directly, this is often not possible with human subjects because of its invasive nature. One common method that is less invasive is to measure the amount of 5-hydroxyindoleacetic acid (5-HIAA), which is a major metabolite of serotonin, present in the cerebrospinal fluid (CSF). Lower levels of CSF 5-HIAA might indicate decreased serotonin metabolism in the central nervous system and, therefore, have been interpreted to reflect lower levels of serotonin in the brain. Interestingly, studies have shown that levels of 5-HIAA in the CSF are correlated with levels of 5-HIAA in the brains of post-mortem human subjects and non-human primates (Huggins et al., 2012; Stanley et al., 1985). This

suggests that CSF 5-HIAA may be a good proxy for serotonergic system processes in the brain. Researchers have also used the drug challenge method. In this method, a drug that is a precursor, agonist, or releaser of serotonin is administered and subsequent changes in hormones that are sensitive to changes in serotonin are assessed (such as prolactin or cortisol). Blunted hormonal responses to drug challenges are interpreted to indicate lower levels of serotonin functioning. Another common method is called acute tryptophan depletion or enhancement. It involves temporarily lowering or heightening levels of serotonin by depleting or enhancing, respectively, levels of the serotonin precursor, tryptophan.

Finally, researchers have studied serotonin functioning by examining the role of genetic polymorphisms thought to play a role in the serotonin system. For example, one of the most widely studied serotonin polymorphisms is the functional insertion-deletion polymorphism in the 5-HTT linked promotor region (5-HTTLPR). This polymorphism is tri-allelic, although only two alleles have been commonly studied (i.e., the short and long alleles). Many other genetic polymorphisms thought to influence serotonin functioning have also been studied, although an extensive review of these polymorphisms is beyond the scope of this manuscript (see Dick & Foroud, 2003 for a review; see McHugh, Hofmann, Asnaani, Sawyer, & Otto, 2010 for a meta-analysis and review).

The current study will utilize genomic data in order to measure serotonin functioning. It is now widely recognized that serotonin functioning is polygenic in nature (i.e., involving many genes of small effect size). Also, genetic polymorphisms located within serotonin genes are not solely responsible for overall serotonin functioning, but the current literature has mainly used single serotonin variants to index this construct.

Therefore, the current study will improve upon these previous studies by creating a polygenic risk score to index serotonin functioning. Specifically, a polygenic risk score consisting of the additive effect of multiple single nucleotide polymorphisms (SNPs) will be created using results from an independent genome-wide association study (GWAS; Luykx et al., 2014). This GWAS examined the relations among thousands of common variants and CSF 5-HIAA concentrations in a sample of European adults. Thus, the polygenic score will index genetic risk for levels of CSF 5-HIAA functioning, which has been posited to index levels of serotonin functioning in the brain. For simplicity, this genetic risk score will be referred to as polygenic risk for serotonin functioning throughout the text, such that greater levels of polygenic risk reflect lower levels of serotonin functioning.

Unlike most other studies that have created polygenic risk scores indexing specific psychological *disorders*, this polygenic risk score attempts to explain variation in an *endophenotype* of a disorder (i.e., serotonin functioning). Endophenotypes have been defined as neurobiological, cognitive, or psychological constructs that are heritable and might be intermediate in the relation between genes and more distal outcomes, such as alcohol use and disorder (Gottesman & Gould, 2003). Gottesman and Gould (2003) highlighted the importance of examining endophenotypes to better understand the genetic architecture of psychological problems. Indeed, psychological disorders as they are defined in current classification systems do not map onto specific genes in a one-to-one fashion. This is likely because psychological disorders have been defined by observable symptom clusters that are heterogeneous, share many commonalities with other disorders, and simply do not "carve nature at its joints" (Mendelsohn et al., 1982, pp. 1168-1169).

Given this information, is seems important to examine polygenic risk for an endophenotype of alcohol use (i.e., serotonin functioning) to help elucidate the genetic underpinnings of alcohol use and disorder. In addition, most polygenic risk scores created to date do not directly inform how genes *functionally* influence these psychological problems. The current study will fill these gaps in the literature by creating a polygenic risk score for an endophenotype of alcohol use, serotonin functioning. This score will have direct implications for understanding the functional influence of genes on psychological problems. Moreover, using this methodology might shed light on the mechanisms in the relation between serotonin functioning and greater alcohol use.

Serotonin Functioning and Alcohol Use

An extensive literature of both animal and human studies suggests that serotonin functioning in the brain is linked with alcohol consumption and dependence (see LeMarquand et al., 1994a, 1994b for a review). For instance, the depletion of brain serotonin levels in rodents increases their alcohol consumption, and the restoration of serotonin levels reverses this effect (Naranjo, Sellers, & Lawrin, 1986). Interestingly, rodent strains bred with an alcohol preference tend to have lower serotonin levels than rodents without an alcohol-preference, even prior to alcohol exposure (Gongwer et al., 1989; Murphy et al., 1982). This suggests that animals with lower levels of brain serotonin are at premorbid risk for alcohol consumption.

In addition, human studies show that CSF 5-HIAA concentrations are lower in alcoholics compared to controls after a period of abstinence (Ballenger et al., 1979; Banki, 1981; Borg et al., 1985) and lower in individuals with early onset alcoholism (prior to the 25 years of age) compared to individuals with late-onset alcoholism (Füs-

Aime et al., 1996). In the latter study, CSF 5-HIAA levels also did not differ between alcoholics who did or did not have depression, antisocial behavior, or history of suicide attempts (Füs-Aime et al., 1996). This suggests that the relation between serotonin function and alcoholism may not be conditional upon the presence or absence of these co-occurring conditions. Drug challenge studies also found similar results. These studies have shown that male alcoholics with no other Axis I diagnoses and 2 weeks of abstinence, adult alcoholics with 2 weeks of abstinence, and non-abstinent heavy drinkers had blunted hormonal responses to serotonin agonists or releasers (Balldin et al., 1994; Farren et al., 1995; Lee & Meltzer, 1991). Ernouf et al., (1993) also found that abstinent adult alcoholics had increased serotonin uptake compared to controls, indicating less serotonin in the synaptic cleft available for neurotransmission.

In addition to showing that serotonin functioning and alcoholism have a cross-sectional or correlational relation, human studies have shown that serotonin functioning might be a heritable, premorbid risk factor for alcohol consumption and dependence. Indeed, studies showed that individuals with a family history of alcoholism had lower levels of serotonin functioning than individuals without, even prior to chronic exposure to alcohol. For instance, both children of alcoholics who had never had alcohol and non-abstaining adult male offspring of alcoholics who did not have any DSM-III Axis I disorders had higher platelet serotonin uptake compared to control participants (Ernouf et al., 1993; Rausch et al., 1991). Interestingly, one study found that abstinent alcoholics who had both an alcoholic mother and father had lower CSF 5-HIAA concentrations compared to alcoholics who had only one alcoholic parent (Füs-Aime et al., 1996). This suggests that serotonin functioning could be a heritable risk factor because alcoholics

with denser family histories of alcoholism are likely to have greater genetic vulnerabilities for alcoholism compared to alcoholics with less dense family histories.

Genetic studies also point to the possibility that serotonin functioning is a premorbid, heritable risk factor. Indeed, genotypic variation should rarely be influenced by an individual's alcohol consumption (although the *expression* of genotypic variation might be influenced). Along these lines, in a meta-analysis on the 5-HTTLPR polymorphism, Feinn, Nellissery & Kranzler (2005) found that the frequency of the short allele was significantly associated with alcohol dependence. The short allele has been associated with lower levels of serotonin uptake. This functional difference results in *greater* amounts of serotonin in the synapse for neurotransmission (Lesch et al., 1996). This finding may seem counterintuitive to the notion that individuals with *lower* levels of serotonin functioning have greater rates of alcoholism. However, an extensive review posited that excess levels of serotonin present during early central nervous system development might inhibit the ultimate outgrowth, size, and capacity of the serotonin system through negative feedback processes (Nordquist & Oreland, 2010). Note that we did not include the 5-HTTLPR polymorphism in the current polygenic risk score because we had no information about how it relates with 5-HIAA in the CSF. Numerous studies including one meta-analysis also found links between other serotonin SNPs and alcohol phenotypes (e.g., Cao, LaRocque & Li, 2013; Enoch, Gorodetsky, Hodgkinson, Roy, & Goldman, 2011; Polina, Contini, Hutz, & Bau, 2009; Zlojutro et al., 2011). Collectively, these studies suggest that serotonin functioning might represent a heritable biological risk factor for alcohol consumption and dependence, such that lower levels of serotonin functioning predict greater alcohol use.

Despite the volume of research on the link between serotonin functioning and alcohol phenotypes, the mechanism(s) underlying this relation are less clear. Among others, researchers have posited that social cognition (Canli & Lesch, 2007), negative emotionality (Heinz, Mann, Weinberger, & Goldman, 2001), impulsivity (Soubriè, 1986), low behavioral inhibition (Depue & Spoont, 1986; Gray, 1982), a low level of response to the acute effects of alcohol (Heinz et al., 2001), and emotion and self-regulation (Carver, Johnson & Joorman, 2008; Hariri & Holmes, 2006; Lesch, 2005) might be mechanisms that mediate the relation between serotonin functioning and alcohol use and dependence. Unfortunately, few studies have utilized longitudinal models to directly test the mechanisms through which serotonin functioning influences the risk for alcohol use over development. Indeed, if serotonin functioning is a *premorbid* risk factor for alcohol problems, there are likely earlier-appearing behaviors or traits in youth (i.e., occurring prior to alcohol initiation) that would help explain this relation. Testing an integrated, longitudinal model that coherently outlines the pathways from serotonin functioning and alcohol use is needed to better understand the etiology of alcohol use and disorders.

The Role of Serotonin Functioning in Aggressive/Antisocial and Depressive Symptoms

Some of the most consistently replicated associations in the literature on serotonin function are those between serotonin function and impulsive aggression and serotonin function and depression (see Carver et al., 2008). The pattern of these relations is such that lower levels of serotonin are associated with greater levels of depression and impulsive aggression. This body of work might be useful in further understanding the relation between serotonin functioning and alcohol use for several reasons. First,

aggressive/antisocial and depressive symptoms generally appear at younger ages than does alcohol use and both types of problem behavior increase the risk for later alcohol use and disorder. Thus, youth with lower levels of serotonin functioning might be at risk for aggressive/antisocial behaviors and depressive symptoms. These same youth may begin to use alcohol because they affiliate with deviant peers and/or to self-medicate (see Sher, 1991). Furthermore, it is possible that aggressive/antisocial behaviors, depressive symptoms, and alcohol use could actually represent age-specific expressions of serotonin functioning (i.e., heterotypic continuity). Thus, it may be critical to pay close attention to the vast literatures on serotonin functioning, aggressive/antisocial behaviors and depressive symptoms to better understand the etiology and development of alcohol use and disorder.

Multiple lines of evidence indicate that serotonin functioning may be associated with aggressive/antisocial behavior, such that lower levels of serotonin functioning are associated with greater levels of aggression/antisocial behavior. Studies have shown that adults and children who displayed aggressive and violent behavior and children who had disruptive behavior disorders and/or attention deficit/hyperactivity disorder (ADHD) had lower levels of CSF 5-HIAA compared to control groups (Brown et al., 1982; Brown, Goodwin, Ballenger, Goyer, & Major, 1979; Kruesi et al., 1990, 1992; Lidberg, Tuck, Asberg, Scalia-Tomba, & Bertilsson, 1985; Limson et al., 1991; Roy, Adinoff, & Linnoila, 1988; Virkkunen, Nuutila, Goodwin, & Linnoila, 1987). A series of studies also found that CSF 5-HIAA concentrations were lower in infants with a family history of antisocial personality compared to infants without a family history, and predicted infants'

externalizing behaviors at 30 months of age (Clarke, Murphy, & Constantino, 1999; Constantino, Morris, & Murphy, 1997).

In addition, conduct-disordered children with less dense serotonin receptors had greater aggressive behaviors (Birmaher et al., 1990; Stoff, Pollock, Vitiello, Behar, & Bridger, 1987). Compared to controls, serotonin receptor density was also found to be lower in delinquent adolescents and lower in boys whose parents had a history of substance abuse or incarceration (Blumensohn et al., 1995; Pine et al., 1996). Twitchell et al. (1998) found that lower whole blood serotonin levels were associated with higher behavioral problems in children of alcoholics. Furthermore, blunted hormonal responses to drug challenge were associated with greater aggressive behaviors in a group of boys with ADHD, prospectively predicted antisocial personality disorder in boys with ADHD 9 years later, and prospectively predicted greater aggression and antisocial behavior in adolescent males 7 years later (Flory, Newcorn, Miller, Harty & Halperin, 2007; Halperin et al.,2006).

A number of genetic variants associated with reduced serotonin, such as the short allele of the 5-HTTLPR polymorphism and the low activity form of the MAO-A gene, have also been implicated in antisocial/violent behavior and intermittent explosive aggression (Brunner, Nelen, van Zandvoort, et al., 1993; Brunner, Nelen, Breakefield, Ropers, & van Oost, 1993; Liao, Hong, Shih, & Tsai, 2004; Lyons-Ruth et al., 2007). It is important to note that studies of adults, as opposed to studies of children, have been more consistent in demonstrating associations between serotonin functioning and aggressive/antisocial behaviors (see van Goozen et al., 2007 for a comprehensive review). Although the data are less consistent for children, researchers have posited that

these inconsistencies might be due to differences in the composition of antisocial samples across studies such as using only ADHD children with comorbid aggression and antisociality vs. a more homogeneous aggressive group (van Goozen et al., 2007).

Research also suggests that serotonin functioning contributes to depressive symptoms, such that lower levels of serotonin functioning are associated with greater depressive symptoms. Studies demonstrated that CSF 5-HIAA levels were reduced in depressed patients compared to healthy controls, reduced in depressed patients who had more frequent hospitalizations, and increased after recovery from depression (Asberg et al., 1976; Dencker, Malm, Roos, & Werdinius, 2006; Traskman-Bendz et al., 1984; van Praag & de Haan, 1979). Blunted hormonal response to drug challenge was also found among depressed adults and children and among children with a high familial loading of major depressive disorder (Birmaher et al., 1997; Flory et al., 1998; Lichtenberg et al., 1992; Siever, Murphy, Slater, de la Vega, & Lipper, 1984). Studies also showed that depressed patients had lower plasma tryptophan levels and decreased platelet serotonin uptake compared to controls (Coppen et al., 1973; Cowen et al., 1989; Healy & Leonard, 1987).

Moreover, acute tryptophan depletion has been shown to temporarily induce several different symptoms of depression. Among these include temporary increases in depressive symptoms in individuals with histories of major depressive disorder, cognitive deficits, memory impairments, and, in those with a family history of major depressive disorder, increased negative affect and vegetative symptoms (Benkelfat et al., 1994; Booij & Van der Does, 2007; Booij et al., 2002; Delgado, Charney, Price, & Aghajanian, 1990; Delgado et al., 1999; Klaassen et al., 1999; Lam et al., 1996; Moreno et al., 1999;

Munafò, Hayward, & Harmer, 2006; Neumeister et al., 2002, 2006; 2004; Porter et al., 2005; Sambeth et al., 2007; Smith, Clifford, Hockney, Clark, & Cowen, 1997). A number of studies have also found that the 5-HTTLPR polymorphism is implicated in depression (see Clarke, Flint, Attwood & Munafo, 2010 for a review and meta-analysis). In addition, the 5-HTTLPR polymorphism has been shown to have a main effect on, and to interact with adverse life events to predict, depressive symptoms in children and adolescents (Eley et al., 2004; Kaufman et al., 2004, 2006). Finally, studies have shown that other serotonin gene variants are associated with depression phenotypes as well (see Levinson, 2006).

However, the fact that aggressive/antisocial and depressive symptoms co-occur at a higher rate than would be expected by chance in both community and clinical samples could confound the associations mentioned previously (Angold & Costello, 1993; Zoccolillo, 1992). The relation between serotonin functioning and aggressive/antisocial symptoms could be confounded by its co-occurrence with depression if serotonin functioning has a true influence on only depression, and vice versa. However, at least some studies indicate that this is not the case. For example, studies show that serotonin mediated hormonal responses were blunted in those with only major depressive disorder and no other DSM-III diagnoses (Cleare, Murray, & O'Keane, 1996, 1998). Also, depressed patients with no other major psychiatric or personality disorders had a reduced sensitivity of 5-HT_{1D} receptor binding when compared with controls (Cleare, Murray, Sherwood, & O'Keane, 1998). Birhmaher et al. (1990) found that the density of serotonin binding was associated with lower externalizing problems in a sample of children with a history of aggression and/or impulsivity but who did not have DSM-III affective

disorders. Moreover, in a review, Coccaro (1992) established that greater levels of aggressive/antisocial behaviors in adults were associated with lower levels of serotonin functioning, independent of co-occurring depression.

Along similar lines, alcohol use and disorder, aggressive/antisocial behaviors and depression also co-occur at a high rate (Kendler, Heath, Neale, Kessler, & Eaves, 1993; Krueger et al., 2002). Thus, serotonin function might have a true influence on alcohol consumption only, and might be spuriously associated with aggressive/antisocial behaviors or depression due to co-occurrence. However, the available evidence does not support this hypothesis either. For example, in one meta-analysis, adults with antisocial behavior had lower levels of CSF 5-HIAA compared to adults without antisocial behavior. This effect was not moderated by alcoholism, suggesting that this relation was not solely due to co-occurrence with alcoholism. Also, indices of serotonin functioning predicted aggressive/antisocial behaviors in children even prior to the development of alcohol problems or alcohol initiation (e.g., Birmaher et al., 1990; Clarke, Murphy, & Constantino, 1999; Constantino, Morris, & Murphy, 1997; Pine et al., 1996; Stoff, Pollock, Vitiello, Behar, & Bridger, 1987; Twitchell et al., 1997). Studies have also shown that depressed individuals with no history of substance dependence or past year substance abuse had lower 5- HT functioning as measured by receptor binding potential, decreased uptake, and blunted prolactin response when compared with controls (Cannon et al., 2007; Drevets et al., 1999; Mann et al., 1995; Tuomisto, Tukianen, & Ahlfors, 1979). In summary, it appears that serotonin functioning is a common biological diathesis, such that lower levels of serotonin functioning may increase the risk for greater levels of aggressive/antisocial and depressive symptoms. These effects also appear to be

independent of alcoholism, co-occurring depressive symptoms and/or co-occurring aggressive/antisocial behaviors.

Mechanisms to Explain a Common Biological Diathesis in the Divergent Outcomes of Aggressive/Antisocial and Depressive Symptoms

The fact that serotonin functioning predicts both aggressive/antisocial and depressive symptoms might be surprising given that these two types of problem behavior lie on distinct spectra of psychopathology (i.e., internalizing vs. externalizing; Achenbach & Edelbrock, 1978). However, one relatively recent theory might help reconcile this apparent discrepancy. Carver et al. (2008) posited that individuals with lower levels of serotonin functioning are predisposed towards lower levels of top-down, reflective self-regulation, which in turn increases the relative dominance of bottom-up, reflexive control. As the name suggests, *reflective* regulation describes an individual's ability to voluntarily and flexibly regulate and plan behaviors, emotions, and thoughts. On the other hand, *reflexive* control describes an individual's automatic and relatively involuntary predisposition towards approach *or* avoidance. Individuals may regulate their reflexive control tendencies by voluntarily mobilizing reflective self-regulatory abilities. For example, an individual predisposed towards approaching rewarding stimuli due to a relatively reflexive approach tendency might nonetheless have the ability to regulate this desire by mobilizing top-down self-regulatory skills. Conversely, an individual predisposed towards avoiding novel situations in spite of the potential rewards might have the ability to regulate this tendency by mobilizing self-regulation.

Parallel constructs have been described in the literature on temperament. Effortful control is the regulatory component of temperament defined as, "the efficiency of

executive attention-including the ability to inhibit a dominant response and/or to activate a subdominant response, to plan, and to detect errors" (Rothbart & Bates, 2006, p. 129). Effortful control is a voluntary form of regulation that allows individuals to respond adaptively to the situation at hand (Eisenberg, Spinrad, & Morris, 2002) and is an analogue of the top-down regulation described by Carver et al. (2008). Unlike effortful control, reactive control is an aspect of temperament that describes relatively involuntary and automatic control (Eisenberg & Morris, 2002). Reactive control describes an individual's tendency to approach rewards *or* to avoid and withdraw, especially in novel situations, and is an analogue of the bottom-up control described by Carver et al. (2008).

Carver et al. (2008) posited that individuals with lower levels of serotonin functioning experience breakdowns in reflective self-regulation/effortful control, which then results in the expression of pre-existing vulnerabilities to either approach or avoidance (i.e., one's bottom-up, or reactive, control tendencies). Therefore, they hypothesized that deficits in serotonin and subsequent declines in voluntary regulation would predict depression, but only if the individual was also predisposed to reflexive inhibition, withdrawal, or avoidance. Conversely, deficits in serotonin and subsequent declines in voluntary regulation would predict aggressive/antisocial behaviors, but only if the individual was also predisposed to reflexive approach and reward sensitivity. In sum, this model suggests that serotonin functioning predicts the divergent outcomes of depression and aggressive/antisocial behaviors due its role in producing a common vulnerability to low effortful control/voluntary self-regulation. Although reflexive/reactive control is an important aspect of this theory, the goal of the current study is to elucidate the common vulnerability produced by serotonin functioning.

Therefore, the subsequent review of the literature will focus on the role of serotonin functioning in the development of reflective self-regulation/effortful control.

The Role of Serotonin Functioning in Reduced Self-Regulation/Effortful Control

In support of Carver et al. (2008)'s theory, studies suggest that individuals with lower levels of serotonin functioning might have lower levels of effortful control and related self-regulatory capacities (also see Hariri & Holmes, 2006; Lesch, 2005; Lucki, 1998). Corticolimbic circuits that are responsible for mediating emotional behaviors are densely innervated by serotonin neurons (Hariri & Holmes, 2006). The underlying neurobiology of the higher-order regulatory temperament system likely involves the dorsal and lateral prefrontal cortex and anterior cingulate cortex (Carver, Johnson, & Joorman, 2008; White, Lamm, Helfinstein, & Fox, 2012). Studies showed that the experimental depletion of serotonin levels via tryptophan depletion attenuated activity in the dorsolateral/medial prefrontal cortex during a working memory task and in the anterior cingulate cortex (Allen et al., 2006; Smith, Morris, Friston, Cowen, & Dolan, 1999). Such effects appeared to be especially prominent among individuals with the short allele of the 5-HTTLPR polymorphism, which is implicated in serotonin functioning (Neumeister et al., 2006). Another study found that carriers of the short allele of the 5-HTTLPR polymorphism had smaller gray matter volume in a region of the prefrontal cortex (Pezawas et al., 2005). Furthermore, serotonin genes appear to influence the executive attention network, which is involved in regulating brain networks implicated in thought and emotion and is empirically related to effortful control in childhood (Canli et al., 2005; Gerardi-Caulton, 2000; Posner, Sheese, Odludas, & Tang, 2006; Reuter, Ott, Vaitl, & Hennig, 2007; Rothbart, Ellis, Rueda, & Posner, 2003). Thus, individuals with

lower levels of serotonin functioning may have attenuated activity of brain regions involved in higher-order self-regulation.

Serotonin functioning has also been associated with individuals' ability to inhibit their emotional and behavioral responses, which might reflect self-regulatory abilities. For instance, the reduction of serotonin availability via tryptophan depletion made it more difficult for individuals to inhibit responses to cues that were formerly rewarded, especially among individuals with high baseline levels of impulsivity, and increased aggression only in those with pre-existing aggressive tendencies (Cleare & Bond, 1995; Cools, Blackwell, et al., 2005; Finn, Young, Pihl, & Ervin, 1998). Tryptophan depletion also caused greater responsivity to negative emotional cues in formerly depressed individuals but not in individuals with no history of depression (Hayward, Goodwin, Cowen, & Harmer, 2005). Similarly, tryptophan depletion elevated the risk for negative affect and vegetative depressive symptoms in individuals with a family history of major depression but not for individuals with no such family history (Benkelfat et al., 1994; Klaassen et al., 1999; Neumeister et al., 2002). Thus, experimental studies produce divergent effects, such as increases in depressive symptoms or aggression, but only or more strongly for those who are predisposed to such traits. This pattern of results suggests that the depletion of serotonin influences one's ability to regulate *underlying* reflexive or reactive emotions and behaviors.

Regarding the role of serotonin genetic polymorphisms, Kochanska, Philibert, and Barry (2009) found that children who carried the short allele of the 5-HTTLPR polymorphism and were also insecurely attached developed poorer effortful control when compared with children who were securely attached. Similarly, Nederhof et al. (2010)

found that childhood stressful events predicted lower effortful control only in children with the short allele of the 5-HTTLPR polymorphism and the *Met* allele of the *BDNF* Val66Met polymorphism. Collectively, these studies suggest that serotonin functioning might be related to impairments in the regulation of emotion and behavior on both a neurological and behavioral level. Note that if this is true, serotonin functioning should also relate to ADHD symptoms through lowered effortful control, given that ADHD is a disorder characterized by executive inhibition deficits (Nigg, 2001). However, this is beyond the scope of the current study, which aims to understand pathways to alcohol use, and ADHD is not as robust a predictor of alcohol use and disorder after considering aggressive/antisocial behaviors (Disney, Elkins, McGue, & Iacono, 1999).

The Role of Effortful Control in Aggressive/Antisocial and Depressive Symptoms

Studies also show that poor effortful control is a common mechanism that predisposes to both aggressive/antisocial and depressive symptoms, providing further support for Carver et al (2008)'s theory. For example, studies showed that poorer effortful control predicted adolescents' greater conduct problems both cross-sectionally and prospectively (Loukas & Roalson, 2006; Loukas & Robinson, 2004; Wang, Chassin, Eisenberg, & Spinrad, 2015a). Furthermore, deficits in effortful control have been related to peer reports of children's involvement in anger and conflict situations (Murphy & Eisenberg, 1997), higher levels of proactive and reactive aggression (de Castro, Merk, Koops, Veerman, & Bosch, 2005; Xu, Farver, & Zhang, 2009), broad measures of aggression (Dennis & Brotman, 2003; Muris, Van Der Pennen, Sigmond, & Mayer, 2008), and more rapid expressions of anger (Kochanska, Murray, & Harlan, 2000).

Similarly, cross-sectional studies have found that effortful control is related to depressive symptoms, such that lower levels of effortful control are associated with greater depressive symptoms (Loukas & Robinson, 2004; Muris, van der Pennen, Sigmond, & Mayer, 2008; Verstraeten, Vasey, Raes, & Bijttebier, 2009). Wang et al., (2015a) found that individuals with lower levels of effortful control had greater depressive symptoms over time, but other studies either found marginally significant or no significant effects of effortful control on depressive symptoms (Loukas & Roalson, 2006; Verstraeten, Vasey, Raes, & Bijttebier, 2009). Moriya and Tanno (2008) found that depressive symptoms were related to inhibitory, but not attentional, control after accounting for comorbidity with other internalizing problems. On the other hand, in a review of the literature Compas, Connor-Smith, and Jaser (2004) concluded that attentional control was likely implicated in child and adolescent depression. Interestingly, a recent meta-analysis by Snyder (2013) showed in a synthesis of 113 studies that adult major depressive disorder was associated with deficits on a host of neuropsychological measures of executive functioning likely involved in effortful control. Thus, it appears that self-regulatory capacities might be important in the development of depressive symptoms.

In summary, multiple lines of evidence suggest that serotonin functioning might predict the divergent outcomes of aggressive/antisocial behaviors and depression because it creates a common vulnerability to poor effortful control.

Relevance to the Etiology of Alcohol Use

As stated earlier, researchers have proposed a multitude of mechanisms that might be involved in the relation between serotonin functioning and alcohol use and disorder.

However, increasing the specificity and clarity of these mechanisms is obviously needed to better understand the link between serotonin functioning and adolescents' alcohol use. Because research suggests that serotonin functioning is a premorbid risk factor for alcohol use and disorder, it would be surprising if this pre-existing biological vulnerability did not influence other types of psychological problems prior to the onset of alcohol problems. Alternatively, this pre-existing biological vulnerability could simply influence the development of certain types of adolescent symptomatology that, in turn, increase the risk for alcohol use. Therefore, it would be useful to understand the mechanisms in the relation between serotonin function and alcohol problems that appear earlier in youth.

Indeed, aggressive/antisocial and depressive symptoms are phenomena that increase sharply in prevalence during adolescence, and effortful control is evident even earlier (Loeber & Keenan, 1994; Murphy, Eisenberg, Fabes, Shepherd, & Guthrie, 1999). In addition, these constructs have been shown to predict later alcohol use outcomes. For instance, poorer effortful control has been associated with a variety of substance use outcomes (Cheetham, Allen, Yücel, & Lubman, 2010; Creemers et al., 2010; Willem et al., 2011). In addition, adolescents' aggressive/antisocial and depressive symptoms are robust predictors of later substance outcomes over and above other types of psychopathology. (e.g, Hussong & Chassin 1994; Fleming, Mason, Mazza, Abbot, & Catalano, 2008; Pardini, White & Stouthamer-Loeber, 2007). Finally, it is evident from the previous review that effortful control, aggressive/antisocial, and depressive symptoms might share a common biological vulnerability with alcohol problems (i.e., serotonin functioning).

Thus, an integrated, longitudinal model of the development of alcohol use in adolescence might involve the following. First, individuals with a pre-existing heritable vulnerability for lower levels of serotonin function might have lower levels of effortful control. This common vulnerability to poorer effortful control might then lead to the divergent outcomes of aggressive/antisocial or depressive symptoms in adolescence, a time when these behaviors become more prominent. Adolescents with aggressive/antisocial symptoms might subsequently use alcohol because they affiliate with deviant peers (Sher, 1991), or due to shared personality traits (e.g., sensation seeking). Alternatively, aggressive/antisocial behaviors and alcohol use might both simply be age-specific manifestations of serotonin functioning and effortful control. Similarly, individuals with depressive symptoms might use alcohol to alleviate negative emotions or because they affiliate with rejected/deviant peers (Sher, 1991). Alternatively, depressive symptoms and alcohol use might both simply be age-specific manifestations of serotonin function and/or effortful control. Finally, any direct effects observed from serotonin functioning to alcohol use may indicate that other untested mechanisms are at play in this relation. The current study will test this integrated, longitudinal model of adolescents' alcohol use using a polygenic risk score indexing CSF 5-HIAA concentrations as a marker of a heritable predisposition for serotonin functioning (See Figure 1).

Potential Moderating Role of Gender

There are gender differences in effortful control, aggressive/antisocial symptoms, depressive symptoms, and alcohol problems. Numerous studies indicate that males generally have lower levels of effortful control and depressive symptoms and higher

levels of aggressive/antisocial symptoms and alcohol use and disorders than do females (Bongers, Koot, van der Ende, & Verhulst, 2003; Else-Quest et al., 2006; Lemery, Essex, & Smider, 2002; Nolen-Hoeksema & Girgus, 1994). These gender differences might lead some of the paths in the proposed models to be stronger for males or females to the extent that males or females have greater *variability* in these constructs. For example, it is possible that the links among effortful control, aggressive/antisocial symptoms and alcohol use would be stronger in males than in females. However, previous studies investigating these relations provide mixed evidence. For instance, research suggested that effortful control predicted externalizing problems similarly for boys and girls, such that lower levels of effortful control predicted greater levels of externalizing problems (Eisenberg et al., 2005, 2009). This suggests that lowered self-regulatory abilities influence the development of externalizing problems equally for boys and girls. Also, several studies suggest that behavioral undercontrol, externalizing behaviors, and antisociality predicted alcohol phenotypes equally in males and females (Moffitt, Caspi, Rutter, & Silva, 2001; Slutske et al., 2002). On the other hand, other studies found that antisociality and behavioral undercontrol predicted alcohol phenotypes more strongly for males than for females (Caspi, Moffit, Newman, & Silva, 1996; Hussong, Curran, & Chassin, 1998). Thus, although there is little evidence that effortful control would more strongly, or only, predict aggressive/antisocial behaviors in males when compared to females, it is possible that aggressive/antisocial behaviors would more strongly, or only, predict alcohol problems in males when compared to females.

Females are almost twice as likely to be diagnosed with depression when compared with males (Nolen-Hoeksema & Girgus, 1994). In addition, researchers have

found gender differences in the links between depression and alcohol phenotypes. When measured longitudinally (over the span of a year or more), researchers have generally found that the link between depression and alcohol use and disorder was stronger for women than for men in community samples, high-risk samples, and among alcoholics (Chassin et al., 2002; Fillmore et al., 1997; Helzer & Pryzbeck, 1988; Olenick & Chalmers, 1991; Rubonis et al., 1994). However, despite the higher prevalence of depression in females, there is little evidence to suggest that effortful control predicts depressive symptoms more strongly, or only, for females when compared with males (Eisenberg et al., 2005; 2009). Thus, lowered self-regulatory abilities might contribute equally to the development of depressive symptoms in males and females, whereas depressive symptoms might predict alcohol use more strongly, or only, for females when compared with males.

Finally, few studies have specifically investigated gender differences in the relation of serotonin functioning to effortful control, aggressive/antisocial symptoms, depressive symptoms, or alcohol use. However, some studies have shown that early onset/Cloninger's Type II alcoholics might have lower levels of serotonin functioning when compared with late onset/Type I alcoholics (Virkkunen & Linnoila, 1990). Early onset/Type II alcoholics have been classified as a primarily male group that is particularly prone to antisocial and violent behaviors and that often has a family history of alcoholism. Late onset/Type I alcoholics have been classified as a group that uses alcohol primarily to relieve anxiety and that has high harm avoidance and low novelty seeking. Because alcohol use will be measured in late adolescence through emerging adulthood in the current study, it is possible that serotonin functioning might be more

strongly related to alcohol use for males than for females to the extent that the alcohol use measure captures the early onset/Type II group. Because of the possibility that proposed paths will differ by gender, all paths were tested for moderation by gender.

Implications and Importance of the Proposed Model

The pattern of relations elucidated by this model will inform the mechanisms underlying alcohol use and disorder. If all hypothesized paths in the model are confirmed, this would suggest that serotonin functioning predicts alcohol use through aggressive/antisocial and depressive symptoms, and that all of these problem behaviors are the result of a common deficit in effortful control (See Figure 1). However, other patterns are possible as well. For instance, the polygenic risk score indexing serotonin functioning might predict alcohol use only through effortful control and not through adolescent psychopathology. This would suggest that serotonin functioning influences alcohol use through deficits in self-regulation; however, in this case the link between self-regulation and alcohol use would likely not be due to the dysregulation of mood, impulse, or aggression. Instead, perhaps deficits in self-regulation impede the ability to resist alcohol-related cues, to resist alcohol cravings, or to regulate anxiety symptoms, thus leading to higher levels of alcohol use. Alternatively, it is possible that the polygenic risk score indexing serotonin functioning predicts alcohol use only through depression or aggressive/antisocial behaviors and not through effortful control. This would suggest that deficient effortful control is not the common vulnerability caused by serotonin functioning and that effortful control does not mediate the influence of serotonin functioning on the divergent outcomes of depression, aggressive/antisocial behaviors, and alcohol use. In addition, if the polygenic risk score indexing serotonin functioning

predicts alcohol use directly, this would suggest that mechanisms other than effortful control, depression, and aggressive/antisocial behavior mediate this relation. For example, researchers have hypothesized a compensatory mechanism whereby individuals with lower levels of serotonin functioning misuse alcohol because alcohol consumption induces acute *increases* in brain serotonin levels (LeMarquand et al., 1994a). In addition, some research suggests that individuals with lower levels of serotonin functioning are at-risk for alcohol use and disorder because they have lower levels of response to alcohol (Heinz et al., 2001).

Findings may also inform the developmental pathways to alcohol use and disorder. If findings indicate that alcohol use is a common outcome resulting from different types of psychopathology, this would provide evidence for equifinality to adolescents' alcohol use. If the polygenic risk score indexing serotonin functioning predicts alcohol use through effortful control, aggressive/antisocial behaviors, and depression, this would provide evidence of heterotypic continuity. In other words, effortful control, aggressive/antisocial behavior, depression, and alcohol might simply be different manifestations of deficits in serotonin functioning. Results could also suggest another pathway to alcohol use aside from the deviance proneness and stress dampening pathways (Sher, 1991). Specifically, an alternate pathway to alcohol use might be a common vulnerability towards low effortful control. Note, however, that although this model may provide evidence for both heterotypic continuity and for an alternate pathway to alcohol use (i.e., effortful control), the model will be unable to distinguish between these possibilities.

Finally, these results could have implications for the development of a more etiologically-based diagnostic system. Existing classification systems are not in concordance with current knowledge in biological and psychological research. Psychological disorders as they are currently defined do not map onto specific genes or neurotransmitter systems in a one-to-one fashion. Instead, genes and neurotransmitter systems show relations with a number of putatively distinct mental disorders, suggesting that the current diagnostic categories may not very accurately reflect nature. Thus, if serotonin functioning predicts depression, aggressive/antisocial behaviors, and alcohol use through effortful control, then effortful control would be a useful transdiagnostic phenotype that cuts across all of these problem behaviors. This transdiganostic phenotype could be used to better classify individuals, tailor research, and guide treatment efforts. In summary, results from the current study will inform prevention and intervention efforts, elucidate developmental pathways to alcohol problems, and inform more etiologically-based diagnostic and research classification.

The Proposed Study

The current study used three time points to test whether a polygenic risk score indexing serotonin functioning prospectively predicted alcohol use (T3) through effortful control (T1) and/or aggressive/antisocial and depressive symptoms (T2). Because some of the proposed relations may differ by gender, gender moderation was tested for all of the paths in the model. A high-risk longitudinal sample was utilized to test these hypotheses, in which approximately half of the participants have a parent with a lifetime substance disorder (Adult and Family Development Project; AFDP; Chassin, et al., 1992). This sample is particularly well-suited to test the proposed model of genetic

liability to alcohol use. In addition, the current study replicated these associations in an independent longitudinal community sample of adolescents (Child Development Project; CDP; Dodge, Bates, & Pettit, 1990).

It was hypothesized that the polygenic risk score indexing serotonin functioning would directly predict effortful control, such that high levels of the polygenic risk score (i.e., lower levels of serotonin functioning) would be associated with lower levels of effortful control. It was not hypothesized that the polygenic risk score would directly predict aggressive/antisocial behaviors and depressive symptoms after accounting for effortful control. However, it was expected that polygenic risk would predict these two problem behaviors indirectly, through effortful control. Next, it was hypothesized that one pathway to greater levels of alcohol use starts with polygenic risk, which predicts alcohol use through effortful control and aggressive/antisocial behaviors. A second pathway starts with polygenic risk, which predicts alcohol use through effortful control and depressive symptoms. It was not expected that effortful control would have a direct influence on alcohol use after accounting for the effects of depression and aggressive/antisocial behaviors. Finally, a direct path from polygenic risk to greater levels of alcohol use was hypothesized. This was based on previous findings showing that serotonin functioning had a unique influence on alcohol phenotypes even after considering co-occurrence with depression and aggressive/antisocial behaviors.

Regarding gender moderation, it was expected that the first pathway involving polygenic risk, effortful control, aggressive/antisocial behaviors, and alcohol use might be stronger, or only present, in males when compared to females. Finally, due to the potential moderating role of gender in the relation between depressive symptoms and

alcohol use and disorder, it was expected that the second pathway involving polygenic risk, serotonin functioning, effortful control, depressive symptoms, and alcohol use might be stronger, or only present, in females when compared to males.

Method

The Adult and Family Development Project (AFDP)

AFDP participants. The first sample of participants for the current study was drawn from a larger three-generational longitudinal study of familial alcoholism (Chassin et al., 1992). The larger longitudinal study collected data at three annual waves and three additional follow-ups which were each separated by five years. Wave 1 commenced in 1988 and collected data from 454 adolescents (Generation 2s, G2s) and their parents. 246 of these adolescents had at least one biological custodial parent with alcoholism (COAs) and 208 adolescents were demographically matched controls who did not have a parent with alcoholism. At wave 4, siblings of G2s were interviewed if they fell within the same age range as the original G2s (also referred to as G2s). At wave 5, any remaining non-interviewed siblings of G2s were recruited to participate in the study, regardless of their age, but only if they had a child who was at least 5 years old (also referred to as G2s). For the first time at wave 5 and again at wave 6, the children of all G2s (referred to as G3s; N = 695), their children's "other" biological parents, and the children's teachers were interviewed. At wave 5 only, G3s and their parents were invited to participate in a laboratory visit. Finally, three follow-up assessments were conducted after wave 6 for the G3s only. The first occurred 18 months after wave 6, the second occurred approximately three years after wave 6, and the third occurred approximately four years after wave 6. See Figure 2 for a visual display of the data collection timeline.

Sample retention across all follow-ups was excellent. 449 (99%) original adolescent G2s were interviewed at wave 2, 444 (98%) were interviewed at wave 3. 734 (90%) G2s were interviewed at wave 4, 818 (91%) G2s were interviewed at wave 5, and 816 (90%) G2s were interviewed at wave 6. Of the original 695 G3s interviewed in wave 5, 606 (87%) G3s were interviewed in wave 6, 554 (78%) G3s were interviewed at the wave 6 18-month follow up, 622 (89%) G3s were interviewed at the wave 6 3-year follow up, and 515 (74%) G3s were interviewed at the wave 6 4 year follow up. At least some data were collected for 471 G3s at the wave 5 laboratory visit.

AFDP recruitment. COA families were recruited using court records, community telephone surveys, and health maintenance organization (HMO) wellness questionnaires. Individuals who were convicted of driving while intoxicated were identified by using records from seven court systems from 1985-1988. The court records were examined for potential indicators of alcohol problems, such as whether the individual had a blood alcohol content of at least 0.15 during the time of their arrest, prior alcohol-related arrests, a score of seven or above on the Michigan Alcohol Screening Test (Selzer, 1971) or a probable diagnosis of alcoholism as determined by a substance abuse screening center. Individuals were only invited to participate if they lived in Arizona, were non-Hispanic Caucasian or Hispanic, and were born between 1926 and 1960. One hundred and three COA families were recruited using these methods.

Next, HMO wellness questionnaire responses of members who had joined between 1986 and 1988 were screened. The questionnaires of new members who had been arrested between 1964 and 1988 and who met the demographic criteria mentioned above were examined for several indicators of alcoholism. These included whether the

individual self-reported having consumed 26 or more alcoholic drinks per week, experienced three or more alcohol-related social consequences, or whether they self-identified as an alcoholic. Twenty two additional COA families were recruited using these methods.

Community telephone surveys were also used to identify potential participants. Families who met the demographic criteria were assessed for alcoholism using several indicators. These included whether they attended Alcoholics Anonymous meetings, whether they had been hospitalized for a drinking problem, or whether they reported that their spouse was an alcoholic. One hundred and twenty COA families were recruited using these methods. One COA family was referred by a local Veteran's Administration outpatient treatment program. Finally, 80 families who had been screened to participate as part of the demographically matched control group (see below) met diagnostic criteria for alcoholism and were re-classified as COA families.

Structured face-to-face interviews using the DIS-III (Robins, Helzer, Croughan, & Ratcliff, 1981) were used to directly verify whether parents had lifetime alcohol abuse or dependence using DSM-III criteria. These interviews were conducted with the parent suspected of alcoholism, when possible. However, if he/she refused to participate, his/her spouse reported on their lifetime alcohol abuse or dependence symptoms using the Family History-Research Diagnostic Criteria (FH-RDC; Endicott, Andreasen & Spitzer, 1975). Of those interviewed, 219 biological fathers and 59 biological mothers were diagnosed with DSM-II lifetime alcohol abuse or dependence.

Reverse directories were used to recruit families who lived in the same neighborhoods as the recruited COA families and were matched to the COA families on

children's age, family composition, ethnicity, and socioeconomic status. Non-alcoholic families were included if neither biological nor custodial parent met DSM-III or FH-RDC criteria for lifetime alcohol abuse or dependence. Seventeen of these families reported sub-threshold levels of lifetime alcohol abuse or dependence. These families were eliminated from the study to decrease the likelihood that the parent would be diagnosed with alcoholism later in the project.

AFDP recruitment biases. Two main sources of potential recruitment bias were observed for the larger longitudinal study. First, among the potential COA families, bias could have been incurred if those who were successfully contacted differed systematically from those who were not (Chassin, et al., 1992). Thus, the court and HMO records of successful contacted participants were compared to those who were not. These records were specifically examined because they provided archival data on potential participants. T-test and chi-square analyses revealed that participants who were successfully contacted did not differ from those who were not contacted on blood alcohol level at the time of arrest, number of prior alcohol-related arrests, self-identification as an alcohol, or MAST scores. However, those who were not successfully contacted were significantly more likely to be younger, to be of Hispanic ethnicity, to be unmarried, and to have a lower socioeconomic status (SES) compared to contacted participants.

Second, bias could have been incurred if those who refused to participate differed systematically from those who agreed. Of the families who were screened using telephone surveys, 73% of the COA families and 77% of the control families participated. Individuals from COA families who refused to participate did not differ from those who agreed on their age, sex, SES or alcoholism indicators. However,

individuals from COA families who refused to participate more significantly more likely to be Hispanic and married. For the control sample, no differences were found between those who participated vs. those who refused on family composition or SES. However, mothers and fathers from control families who refused to participate were more likely to be Hispanic than mothers and fathers from control families who agreed. For more details on possible biases see Chassin et al. (1992).

AFDP procedure. At wave 6 (hereafter referred to as T1), in-state G3 children and their parents were interviewed in their homes or at Arizona State University. At the wave 6 18-month follow up (hereafter referred to as T2) and the four-year follow-up (hereafter referred to as T3), children were interviewed via the telephone. At all waves, out-of-state children and their parents were interviewed through mailed surveys or the telephone. Written informed consent was obtained from parents and written informed assent was obtained from adolescents at every interview. These consent and assent forms described the nature of the interview, including that information about substance use would be asked, emphasized the voluntary nature of participation, and described limits of confidentiality.

AFDP genotyping. Extraction of DNA, standardization and plating were completed in the Department of Psychiatry at the Washington University School of Medicine and genotyping through the Washington University Genome Sequencing Center. 1536 SNPs were designed for genotyping using the Illumina Golden Gate technology that draws on a previous collaboration illustrated in Hodgkinson et al. (2008) with substitutions reflecting advances in the literature. Quality control analyses included examining cluster plots to rule out ambiguous genotype calls, checking for Mendelian

inconsistencies, incorrect gender assignments and sample swaps, cryptic relatedness, flagging SNPs with low call rates (<95%), and deviations from Hardy-Weinberg equilibrium (HWE). SNPs were removed if they had a call rate < 95%, deviated from HWE ($p<10^{-6}$), or if they had a minor allele frequency (MAF) < 2%. In addition, participants whose genetic data showed Mendelian inconsistencies or were suspected of incorrect gender assignments, cryptic relatedness, and/or sample swaps were excluded from the study ($N = 5$). Note that cryptic relatedness refers to the presence of close relatives within a sample who were seemingly unrelated based on information collected in the study. Participants who actually were related to one another based on records were retained and their relatedness accounted for (see Data Analytic plan).

Child Development Project

CDP participants. The second sample for the current study was drawn from a longitudinal, multi-site community sample (Dodge, Bates, & Pettit, 1990). Data were collected annually for 20 years from Nashville, TN, Knoxville, TN, and Bloomington, IN. These sites were demographically diverse and included rural, semi-rural, small- and midsize-urban communities. Families were recruited in 1987 and 1988, forming two cohorts of participants. The annual attrition rate is approximately 3.3%.

CDP recruitment. Families were randomly approached during their child's kindergarten preregistration (5 years old) and asked to participate in the study. Across the two cohorts, 585 (75%) of the parents who were approached in this manner agreed to participate in the study. Of these 585 families, 477 (81.5%) were non-Hispanic Caucasian, 97 (16.5%) were African American, and 11 (2%) were Asian or Middle-Eastern.

CDP procedure. In the summer before children entered kindergarten, mothers were interviewed in their homes and filled out questionnaires regarding their child's developmental history. Each year, mothers, fathers, and children were interviewed and filled out questionnaires. Children's teachers filled out questionnaires yearly. Written informed consent was obtained from parents and written informed assent was obtained from children at every interview. The current study used data from years 8 (12-13 years old), 10 (14-15 years old) and 11 (15-16 years old), hereafter referred to as T1, T2, and T3, respectively.

CDP genotyping. The CDP sample was genotyped using the Axiom Biobank array, which contains rare exome/loss of function variants (~75,000), eQTLs markers (16,000), imputation GWAS grid (246,000 SNPs), and cSNPs and InDels Variants (264,000).

The Present Study

AFDP participants. Data were drawn from three waves of AFDP when the participants were 10-17.99 (T1), 11-18.99 (T2) and 13-20.99 (T3). Further inclusion criteria for the AFDP sample were non-Hispanic Caucasian ethnicity (to reduce concerns related to population stratification), no genotyping errors or suspicion of sample swaps (excluded $N = 5$) and were genotyped ($N = 254$). These age restrictions were utilized to limit the considerable age heterogeneity within each wave and to capture the relative developmental period of adolescence while still maximizing the sample size. Compared to excluded participants, included participants were significantly younger at T1 ($t = 2.75$, $p = 0.01$), had significantly higher parental education (i.e., SES; $t = -4.78$, $p < 0.001$) and lower levels of Hispanic ancestry ($t = -16.38$, $p < 0.001$). Included and excluded

participants did not differ with respect to their gender, parental substance use disorder (SUD), T1 effortful control, T1 or T2 depressive symptoms, T1 or T2 aggressive/antisocial behavior, or T2 or T3 alcohol problems. Of those who had genetic data, included and excluded participants also did not differ with regards to polygenic risk for serotonin functioning. The younger ages and lower levels of Hispanic ancestry of included compared to excluded participants were due the inclusion criteria, which aimed to reduce age heterogeneity and concerns of population stratification. The differences in SES between included and excluded participants likely reflect the inclusion of only non-Hispanic Caucasian participants.

CDP participants. Data were drawn from three waves of CDP when the participants were in 7th grade (T1; 12-13 years old), 9th grade (T2; 14-15 years old), and 10th grade (T3; 15-16 years old). These ages were chosen for several reasons. These age ranges approximately reflect the mean ages captured in AFDP so that similar developmental periods could be captured across studies. However, mismatch in the developmental periods during which constructs were assessed across both studies was unavoidable because AFDP has considerable age heterogeneity within waves, while CDP has very little age heterogeneity within waves. In addition, the age ranges chosen in CDP were dictated in part by measure availability. The only CDP measure that adequately reflects effortful control was measured when participants were in 7th grade. Moreover, the latest alcohol use assessment was administered in 10th grade. This was also the only alcohol use assessment that was measured at two waves to allow prospective prediction. Note that although an alcohol *problems* measure was administered one year after high school, many of the participants were in college by that time. Because alcohol use in

college is theoretically different from alcohol use initiated in adolescence, we chose not to use this measure to facilitate reproducibility across the samples.

Further inclusion criteria for the CDP sample were that participants were non-Hispanic Caucasian (to reduce concerns related to population stratification) and had genetic data (N = 348). Compared to excluded participants, included participants had significantly higher SES (t = -6.85, $p < 0.001$) and significantly lower T1 conduct problems (t = 2.79, $p < 0.01$). Included and excluded participants did not differ significantly with respect to their gender, parental alcohol problems, T1 conscientiousness, T1 or T2 depressive symptoms, T2 aggressive/antisocial behaviors, or T2 or T3 alcohol use. The differences in SES between included and excluded participants likely reflect the inclusion of only non-Hispanic Caucasian participants. Note that genetic data were only obtained for included participants and, therefore, no comparisons to excluded participants could be made.

Measures

Descriptive statistics for all variables are shown in Table 1. Correlations among all variables for AFDP are shown in Table 2 and for CDP in Table 3.

Demographic information. In both studies, adolescents self-reported their gender, age, and ethnicity. Gender was dummy coded such that 0 represented females and 1 represented males. Only children who self-identified as non-Hispanic Caucasian were included in both samples to reduce concerns that population stratification confounded results. The CDP analyses did not use age as a covariate because all children were virtually the same age at each wave.

Socioeconomic status (SES). The highest level of education achieved by the adolescents' mother or father indexed SES in both the AFDP and CDP samples and was used as a continuous covariate. This variable has been shown by previous researchers to be the most stable and sensitive indicator of SES risk for adolescents (Krieger, Williams, & Moss; Williams & Collins, 1995).

At T1 in AFDP, parents self-reported their highest level of education [1 = 8th grade or less, 2 = some high school, 3 = GED (high school equivalency diploma), 4 = high school graduate, 5 = some vocational or technical school, 6 = completed vocational or technical school, 7 = some college, 8 = AA degree (2 year college), 9 = BA or BS (4 year college degree), 10 = some graduate or professional school, 11 = completed graduated or professional school). In this sample, 0.4% completed 8th grade or less, 3.2% completed some high school, 2.8% completed a GED, 6.9% completed high school, 2.4% completed some vocational or technical school, 6.5% completed vocational or technical school, 27.0% completed some college, 15.7% completed an Associate's degree, 21.4% completed a 4 year college degree, 1.6% completed some graduate or professional school, and 12.1% completed graduate or professional school. Overall, the sample was of moderate levels of SES, given that parents on average completed some college or an associate degree.

In CDP, parents self-reported their highest level of education [1 = 1-6 years, 2 = 7-9 years, 3 = 10-11 years, 4 = 12 years, 5 = 13-15 years, 6 = 16-17 years, 7 = 18+ years]. In this sample, 0.6% of parents' obtained 7-9 years of education, 2.9% had 10-11 years of, 30.7% had 12 years, 19.0% had 13-15 years, 27.8% had 16-17 years, and 19% had 18

or more years. Overall, the sample was of moderate levels of SES, given that parents on average completed 13-15 years of education.

Parent substance use disorder (SUD). At T1 in AFDP, adolescents' biological parents reported their lifetime alcohol/drug abuse or dependence by *DSM-IV* criteria using the Computerized Diagnostic Interview Schedule (C-DIS; Robins et al., 2000). Spousal reports of the Family History Research Diagnostic Criteria (Endicott, Andreasen, & Spitzer, 1975) were used for non-interviewed parents. Adolescents were classified as having a parent with an SUD if one biological parent had a lifetime alcohol or drug disorder. This measure was used as covariate in the AFDP analyses. 54.9% of adolescents had at least one parent with an SUD, demonstrating the high-risk nature of this sample.

During the 12th year of the CDP study (11th grade), adolescents' parents reported their lifetime alcohol problems [0 = no, 1 = yes] using 12 problem drinking items from the Short Michigan Alcohol Screening Test (MAST; Selzer, Vinokur, & van Rooigen, 1975). These items were summed for each parent. Cronbach's alpha was 0.82 for fathers' alcohol problems and 0.67 for mothers' alcohol problems. The highest alcohol problem score across mother and father was used to represent parents' alcohol problems. Although it would have been ideal to utilize a measure of parents' lifetime alcohol problems that was assessed prior to or at the same wave as adolescents' temperament and/or problem behaviors, these data were not available in the CDP. This measure was used as a covariate in the CDP analyses. Overall, adolescents' parents had low levels of lifetime drinking problems, given that parents reported only 1.71 alcohol problems in their lifetime on average and 54.5% of the adolescents' parents reported no lifetime alcohol problems.

Adolescent ancestry. Thirty-seven of the SNPs genotyped in the AFDP dataset are ancestry informative markers. Previous research demonstrated that these SNPs distinguished between non-Hispanic Caucasian and Mexican/Mexican-American ancestry (Tian, Gregersen, & Seldin, 2008). The two most represented ethnic groups in this sample and in Arizona are Non-Hispanic Caucasian and Mexican/Mexican-American. It is important to include ancestry as a covariate in analyses despite the inclusion of only self-identified non-Hispanic Caucasian participants. This is because there is likely population admixture between non-Hispanic Caucasian and Mexican/Mexican-American individuals in this sample due to geographical location.

A principal components analysis was performed on these SNPs. Results indicated that the first component explained 18.99% of the variance (eigenvalue = 7.03), the second explained 3.36% (eigenvalue = 1.24), and the third explained 3.11% (eigenvalue = 1.02). 32 SNPs that had loadings greater than 0.30 on the first principal component were used as indicators of a one-factor model using maximum likelihood estimation. The model fit the data well: $\chi^2(464) = 824.99$, $p < 0.001$, RMSEA = 0.03, CFI = 0.94, SRMR = 0.03. Factor scores were saved and used a covariate in the analyses. These scores were highly correlated with adolescents' self-reported ethnicity in the combined non-Hispanic Caucasian and Mexican/Mexican-American sample ($r = -0.83$, $p < 0.001$), confirming their validity. Higher factor scores indicate higher levels of Caucasian (as opposed to Mexican/Mexican-American) ancestry. A measure of genetic ancestry was unavailable for the CDP sample.

Adolescents' prescription medication use. At T1 in AFDP, parents reported on whether their child was currently taking any prescription medications other than

antibiotics or allergy medication (1 = Yes, 0 = No). 14.7% of adolescents were currently taking prescription medications. This was used as a covariate to ensure that psychotropic medication use did not confound or obscure the relation between polygenic risk and outcomes. Note that this variable was unavailable in CDP.

Time between assessments. The time between assessments was calculated for use as a covariate in AFDP analyses. This is because the time between T1 and T2, and between T2 and T3, was not uniform across participants, and participants were also heterogeneous in age (see Table 1 for descriptive statistics). For example, a participant with a shorter period of time in between their T2 and T3 assessments may not exhibit as much drinking behavior at T3 when compared to a same-aged participant with a longer period of time between their T2 and T3 assessments. Controlling for this variable may further elucidate paths of interest, especially to alcohol use. The same was not done for CDP because there was much less heterogeneity in time between assessments, given that participants were assessed annually by grade.

Polygenic risk score. A polygenic risk score indexing 5-HIAA (serotonin metabolite) concentrations in the cerebrospinal fluid was created in both AFDP and CDP. Greater scores on the polygenic risk score index one's presumed genetic risk for lower levels of serotonin functioning (hereafter referred to as the 5-HT polygenic risk score for simplicity). Results were drawn from a GWAS that examined 414 participants from outpatient preoperative screening services in four hospitals in the Netherlands (Luykx et al., 2014). Participants were included if they were 18-60 years old, had four grandparents born in the Netherlands, Belgium, Germany, United Kingdom, France, and Denmark (i.e., of European ancestry), and were undergoing spinal anesthesia for minor elective

surgeries. Patients were excluded from the study if they had a history of psychotic or major neurological disorders or if they were not of European ancestry as determined by genetic analyses. Six ml of CSF were obtained from each participant and concentrations of 5-HIAA were measured using high-performance liquid chromatography and electrochemical detection. Genotyping was performed using the Illumina HumanOmniExpress Beadchip (730,525 SNPs). Relevant covariates were included in the GWAS computation (see Luykx et al., 2014 for details).

1191 SNPs were genotyped in both AFDP and Luykx et al. (2014)'s study. Of these 1191 SNPs, 1119 SNPs were genotyped in CDP. The current study drew from these overlapping SNPs to create 5-HT polygenic risk scores in both AFDP and CDP. This study analyzed a smaller number of SNPs in the CDP despite the availability of GWAS data to achieve the greatest possible concordance between AFDP and CDP analyses and to facilitate replication.

To prepare the data, quality control and data preparation steps were performed. For instance, during the genotyping process, some genes are read on the forward strand while others are read on the reverse strand. Thus, it is possible that some genes were read on the forward strand in the GWAS but on the reverse strand in AFDP or CDP. For non-palindromic SNPs (i.e., *not* C/G or A/T SNPs), these strand issues are typically easily reconciled because it is always the case that A binds with T (e.g., A on the forward strand corresponds to T on the reverse strand and vice versa) whereas C binds with G. Thus, if the GWAS specified their reference allele as 'A', but our study genotyped 'C' and 'T' alleles, it would be clear that the reference allele for our SNP should be specified as 'T'. When observed, these types of strand issues were reconciled. However, palindromic

SNPs (i.e., C/G and A/T SNPs) that have MAFs > 45% might be difficult to reconcile with respect to which is the reference allele (Arpana Agrawal, personal communication). Therefore, any C/G or A/T SNPs with MAFs > 45% were deleted. SNPs that were in strong linkage disequilibrium with another SNP were pruned to ensure that the score represented the effect of independent (not redundant) SNPs. A pairwise r^2 threshold of 0.25 within a 200-SNP sliding window was employed using the PLINK command, –indep-pairwise 200 5 .25, based on recommendations by Purcell et al. (2009).

Currently, there are no firmly established criteria for using GWAS results to create polygenic risk scores or for choosing the GWAS *p*-value threshold to create maximally informative polygenic risk scores. Including only those SNPs that pass the genome-wide significance threshold in the discovery GWAS will likely fail to capture the polygenicity of the trait, especially because GWAS are typically underpowered. Some researchers created polygenic risk scores by including SNPs that passed an a priori liberal threshold of $p < 0.50$ in the GWAS discovery sample (Hamshere et al., 2013; Purcell et al., 2009). However, another more recent study suggested that this threshold could be too liberal (Evans et al., 2013) because it increased the likelihood of spurious associations due to the pleiotropic effects of many SNPs and increased the chance of Type I error for certain constructs (Evans et al., 2013). Therefore, Evans and colleagues suggested employing the strictest *p*-value threshold from the GWAS discovery set that maximizes the amount of variance explained in the construct it was created to index. Unfortunately, however, neither AFDP nor CDP measured serotonin functioning. Furthermore, it is likely not a defensible approach to choose the significance threshold by creating polygenic risk scores using the GWAS test-statistics and *p*-value thresholds and then examining how

much variance they explain in the very same CSF 5-HIAA scores that were used to create the polygenic risk scores. Any spurious associations arising from idiosyncrasies in the GWAS sample would appear to contribute meaningfully to the phenotype, and therefore may bias the selection of the polygenic risk score *p*-value threshold. Likewise, examining which *p*-value threshold maximizes the variance in the current study variables would also not be defensible due to its reliance on circular reasoning (e.g., testing whether polygenic risk for serotonin functioning predicts alcohol use by choosing the genetic risk score that best predicts alcohol use).

Therefore, the current study included SNPs in the 5-HT polygenic risk score if they passed a significance threshold of $p < 0.05$ in the GWAS discovery set (Agrawal, personal communication). Although this threshold is more liberal than typical genome-wide significance thresholds, it may better capture the polygenicity of serotonin functioning. This may be especially true since the current GWAS was underpowered due to a relatively small sample size ($N = 414$). However, a threshold of $p < 0.05$ is also less likely than the liberal threshold of $p < 0.50$ to reflect spurious associations. Finally, by choosing a threshold of $p < 0.05$ *a priori*, we further reduce the likelihood of chance findings. Of those SNPs that met the $p < 0.05$ threshold, only those that were genotyped in both AFDP and CDP (23 SNPs) were used to facilitate replication. Thus, a 5-HT polygenic risk score using 23 SNPs that were associated with CSF 5-HIAA levels at $p < 0.05$ were created by weighting the number of risk alleles for each SNP by its GWAS

test-statistic and averaging these figures[1]. See Table 4 for a list of the SNPs included in the AFDP and CDP polygenic risk scores.

Methods for addressing item overlap between effortful control and adolescent symptomatology. Temperament/personality and psychopathology measures often contain item overlap (Lemery, Essex, & Smider, 2002). Therefore, preliminary analyses were conducted in order to reduce potential confounding of AFDP temperament and psychopathology measures using previously established methods by Eisenberg and colleagues (e.g., Eisenberg et al., 2004). Specifically, 16 expert raters (8 faculty members, 6 post-doctoral fellows and 2 graduate students) rated each problem behavior and temperament item used in the current study using a questionnaire that asked the extent to which each item reflected either temperament or psychopathology [1 = Much better measure of temperament than symptoms, 3 = Not a better measure of temperament or symptoms, substantial content for both, 5 = Much better measure of symptoms than temperament]. Scores were averaged across each item. Effortful control/conscientiousness items with an average score greater than 3 (rated as a better measure of symptoms than temperament) were deleted. Problem behavior items with an average score less than 3 (rated as a better measure of temperament than symptoms) were deleted. Using this procedure, it was found that no temperament or problem behavior items should be deleted and all items were retained for the analyses. See Appendices A-C for all the effortful control and symptomatology items used in AFDP and CDP.

[1] Another approach to creating polygenic risk scores is to unit-weight the SNPs and take their average. The unit-weighted polygenic risk scores were correlated very highly and significantly with the test-statistic weighted polygenic risk scores (r_{AFDP} = 0.99, $p < 0.001$; r_{CDP} = 0.99, $p < 0.001$). Very similar results would likely be obtained using either score. Therefore, the test-statistic weighted polygenic risk scores were used in all analyses.

T1 effortful control. In AFDP at T1, mothers, fathers, and adolescents reported [1 = almost always untrue; 5 = almost always true] on adolescents' effortful control using the attentional control, activation control, and inhibitory control subscales of the Early Adolescent Temperament Questionnaire (EATQ; Capaldi & Rothbart, 1992). These subscales were averaged to form mother-, father-, and adolescent-reported composite scales of effortful control. Cronbach's alpha for mother-, father-, and adolescent-reported effortful control were 0.83, 0.91, and 0.91, respectively. Mother-, father-, and adolescent-reported effortful control composites were used as indicators of a one-factor model in Mplus v. 7.2 (Muthén & Muthén, 1998-2012) using maximum likelihood estimation with robust standard errors. Model fit was not available because the model was just-identified. However, standardized factor loadings for mother-, father-, and adolescent-reported effortful control were significant ($p < 0.001$) and high, ranging from 0.64-0.86. Effortful control factor scores were saved in Mplus to reduce complexity and used in all subsequent analyses.

In CDP at T1, adolescents self-reported [1 = hardly at all, 5 = extremely much] their conscientiousness using a shortened version of the Big Five Personality Questionnaire (Costa & McCrae, 1992). Conscientiousness involves emotional and behavioral control (Shiner & Caspi, 2003), is associated with effortful attention (Rothbart & Ahadi, 1992) and has been theorized to be the dimension of personality that corresponds to the effortful control superfactor of temperament (Ahadi & Rothbart, 1994). Furthermore, in a review of the literature, Eisenberg, Duckworth, Spinrad, and Valiente (2014) argued that effortful self-regulation in childhood fosters conscientiousness later in life. Based on this information, this measure of

conscientiousness may reflect effortful control (note that the CDP did not have a measure of effortful control). These items were averaged to form an adolescent-reported measure of conscientiousness. Cronbach's alpha was 0.63. Unfortunately, no other raters reported on adolescents' conscientiousness and therefore a multiple reporter model of conscientiousness was not possible.

T1 and T2 symptomatology. In both AFDP and CDP, adolescents' reports of their symptomatology were chosen for several reasons. First, only adolescents reported on the same symptomatology items at two different waves in AFDP which allowed for longitudinal prediction in both samples. Second, research suggests that adolescents may be more informative reporters of behaviors intentionally hidden from parents, such as rule-breaking behaviors. Third, adolescents are considered more valid reporters of their depressive symptoms than are parents (Grills & Ollendick, 2002).

At T1 and T2 in both AFDP and CDP, adolescents self-reported [0 = Not True, 2 = Somewhat True, 3 = Very True/Often True] their aggressive/antisocial behaviors and depressive symptoms using the DSM-oriented Conduct Problems Scale and Affective Problems scale from the CBCL (Achenbach & Rescorla, 2001). The 15 aggressive/antisocial behavior items reported during T1 and T2 were summed to form two separate composites representing adolescents' aggressive/antisocial behaviors at T1 or T2. In AFDP, Cronbach's alpha for these measures was 0.82 at T1 and 0.82 at T2. In CDP, Cronbach's alpha was 0.72 at T1 and 0.75 at T2. The depressive symptom items reported during T1 and T2 were summed to form two separate composites representing adolescent depressive symptoms at T1 or T2. In AFDP, Cronbach's alpha for the 13-item measures was 0.79 at T1 and 0.81 at T2. In CDP, Cronbach's alpha for the 12-item

measures was 0.66 at T1 and 0.77 at T2 (note that one item, "There is very little that I enjoy" was not assessed in CDP but was assessed in AFDP). Adolescents' self-reported problem behaviors at T1 were used as covariates in predicting the corresponding problem behavior at T2 to allow for prospective prediction. On average, AFDP and CDP participants had low-to-moderate levels of symptomatology.

T3 alcohol use. In AFDP at T2 and T3, adolescents self-reported [0 = Never; 1 = 1-2 times; 2 = 3-5 times; 3= More than 5 times but less than once a month; 4 = 1-3 times per month; 5 = 1-2 times per week; 6 = 3-5 times per week; 7 = Every day] how often they drank alcohol in the past year. Adolescents' self-reported alcohol use at T2 was used as a covariate in predicting their self-reported alcohol use at T3 to allow for prospective prediction. 74.4% of participants had never used alcohol at T3.

In the CDP at T2 and T3, adolescents self-reported [0 = Never; 1 = Once in a while; 2 = Sometimes; 3 = Fairly often; 4 = Very often] how often they drank alcohol in the past year. Adolescents' self-reported alcohol use at T2 (9^{th} grade) was used as a covariate in predicting their self-reported alcohol use at T3 (10^{th} grade) to allow for prospective prediction. 66.4% of participants had never used alcohol.

Data from the Monitoring the Future study showed that 76.5% of 10^{th} graders had not had an alcoholic beverage in the past 30 days (Monitoring the Future 2014 Survey Results, 2014). Assuming that alcohol use in the past 30 days is at least somewhat reflective of alcohol use in the past year, this suggests that the frequency of past year alcohol use in both the AFDP and CDP samples are fairly age-typical. An alcohol frequency variable was selected for use in both samples because it provides the best concordance across studies in terms of content and ages of assessment, thus facilitating

reproducibility. Moreover, this was the only alcohol use variable in the CDP data that was assessed at multiple time points. Adolescents' self-reports of alcohol use were chosen because research shows that parents tend to be less accurate reporters of adolescents' alcohol use (Fisher et al., 2006).

Data Analytic Plan

AFDP-specific information. Analyses for AFDP were conducted in Mplus v.7.2 (Muthén & Muthén, 1998-2012) using maximum likelihood with robust standard errors and full information maximum likelihood to estimate missing data which provide unbiased estimates when data are missing completely at random (MCAR) or missing at random (Schafer & Graham, 2002; $N = 254$). Little's MCAR test suggested that the data were MCAR ($\chi^2(59) = 66.34$, $p = 0.24$). Because participants are nested within families, the TYPE=COMPLEX function in Mplus was used to account for the non-independence of the data. Covariates included adolescents' age, ancestry, SES, parent SUD, time between assessments, prescription medication use, T1 aggressive/antisocial behaviors and depressive symptoms (symptomatology), and T2 alcohol use. Predictor variables included adolescent gender (hypothesized moderator), 5-HT polygenic risk, T1 effortful control, and T2 symptomatology. T3 alcohol use was the outcome variable.

CDP-specific information. Analyses for CDP were conducted in Mplus v.7.2 (Muthén & Muthén, 1998-2012) using maximum likelihood with robust standard errors and full information maximum likelihood to estimate missing data ($N = 348$). Little's MCAR test suggested that the data were MCAR ($\chi^2(25) = 32.08$, $p = 0.16$). Covariates included SES, parents' alcohol problems, T1 symptomatology, and T2 alcohol use. Predictor variables included adolescents' gender (hypothesized moderator), 5-HT

polygenic risk, T1 conscientiousness, and T2 symptomatology. T3 alcohol use was the outcome variable.

Data plan common to both samples. Paths were estimated from all covariates and the 5-HT polygenic risk score to all mediators (T1 effortful control/conscientiousness and T2 symptomatology) and the outcome variable (T3 alcohol use). Next, paths were estimated from T1 effortful control/conscientiousness to T2 symptomatology and T3 alcohol use. Paths were also estimated from adolescents' T2 symptomatology to their T3 alcohol use. Autoregressive paths from T1 to T2 symptomatology and from T2 to T3 alcohol use were also estimated. Note that this allowed prospective prediction of these constructs at T2 and T3, respectively. Correlations among all exogenous variables were modeled. In addition, within-wave correlations among T1 symptomatology measures and T1 effortful control/conscientiousness as well as among T2 symptomatology measures and T2 alcohol use were modeled.

A separate set of analyses tested this same model except without the autoregressive paths of T2 symptoms on T1 symptoms and of T3 alcohol use on T2 alcohol use. These analyses were conducted to examine whether genetic influences on change in symptoms over time (i.e., with autoregressive paths) differed from genetic influences on more trait-like measures of symptoms (i.e., without autoregressive paths). Note that it was not of primary interest whether the effects of other predictors changed after removing autoregressive paths.

Preliminary analyses determined which predictor-by-covariate and predictor-by-predictor interactions to include in the final models. Although predictor-by-covariate interactions were not hypothesized, they were still tested by entering their cross-products.

Indeed, it would be incorrect to assume that genetic effects are necessarily constant across age or SES. The exception was that gender-by-covariate interactions were not tested since gender is only considered a predictor due to its hypothesized *moderating* influence on the main paths of interest. Due to their non-hypothesized nature, predictor-by-covariate interactions were only retained if they survived a false-discovery rate (FDR) correction $p < 0.05$ using SAS (fdr option). FDR corrections were chosen because they have greater power to detect truly significant results, are less conservative than Bonferroni corrections, and still maintain adequate control of Type 1 error (Shaffer, 1995). If a polygenic risk-by-covariate interaction survived the FDR correction, all polygenic risk-by-covariate and covariate-by-covariate interactions were included in the model to ensure that the interaction effect was not driven by confounding variables, as recommended by Keller (2014). Any significant interactions involving the 5-HT polygenic risk score were also re-tested following monotone transformations of the interacting variables to ensure that results were not due to scaling-related spuriousness (Young-Wolff, Enoch, & Prescott, 2011).

Gender was considered a predictor in the current study due to its relevance to hypothesis testing. The hypothesized predictor-by-gender interactions were tested by entering their cross-products and retained if their $p < 0.05$. If a polygenic risk-by-gender interaction was significant, all polygenic risk-by-covariate and gender-by-covariate interactions were included to ensure that the interaction effect was not driven by confounding variables (Keller, 2014). In addition, any significant interactions involving the polygenic risk score were re-tested following monotone transformations of the

interacting variables to ensure that results are not due to scaling-related spuriousness (Young-Wolff, Enoch, & Prescott, 2011).

Nonessential multicollinearity was reduced by centering all predictors and covariates prior to computing interaction terms. Interaction effects were tested using simple slope analyses (Aiken & West, 1991). The joint significance test was used to test two- and three-path mediated effects. The joint significance test requires each of the paths in the mediated effect to be significant for the overall mediational effect to be significant. This approach has been suggested by previous researchers to be a good approach for balancing Type 1 error and statistical power for both two- and three-path mediated effects (MacKinnon, Lockwood, Hoffman, West & Sheets, 2002; Taylor, MacKinnon, & Tein, 2007). If any paths in the mediational chain were moderated by gender or demographic variables, moderated mediation was tested. To do so, simple slope analyses were conducted to probe each link in the mediational chain by relevant moderators.

Because the T3 alcohol use outcome measures were zero-inflated variables in both AFDP (74.4%) and CDP (66.4%), preliminary analyses determined the best modeling strategy. Models estimating only the effects of covariates on T3 alcohol use were conducted, with alcohol use alternatively specified as either a zero-inflated poisson, zero-inflated negative binomial, continuous, or categorical variable. The modeling procedure producing the lowest Akaike Information Criteria (AIC), Bayesian Information Criteria (BIC) and the highest -2 log likelihood was chosen.

All variables were examined for out of range values. Model diagnostics were performed for all analyses to identify outliers and influential cases. Cook's D, which measures the influence of an observation on the parameter estimates, and DFBETAS,

which measures the standardized change in the regression coefficient when a case is deleted, were used to identify influential cases (Cohen et al., 2003; Cook, 1977). Research suggests that cases with a Cook's D or DFBETAS greater than an absolute value of one may be influential for moderately sized datasets (Neter, Wasserman, & Kutner, 1989). For cases that may be influential or may be outliers, interview notes were examined to isolate potential explanations. Cases that could be reasonably excluded using these interview notes were dropped.

There was sufficient power (> 0.80) to detect small effect sizes (f^2 =.03) in the full samples of AFDP and CDP. With regards to gender moderation, there was sufficient power (> 0.80) to detect small-moderate effect sizes (f^2 =.10) for both female and male groups in AFDP and to detect small-moderate effect sizes (f^2 =.05) for both female and male groups in CDP.

Results

Zero-Order Correlations Among Study Variables

AFDP. In accordance with hypotheses, adolescents with higher levels of 5-HT polygenic risk (i.e., lower serotonin functioning) had higher levels of T2 aggressive/antisocial behaviors, T2 depressive symptoms, and T2 and T3 alcohol use. Note that 5-HT polygenic risk was not significantly correlated with effortful control. Adolescents with lower levels of T1 effortful control had higher levels of T1 and T2 symptomatology and T2 and T3 alcohol use. All of the symptomatology and alcohol use variables were significantly correlated in the expected direction.

Regarding correlations with demographic variables, females were more likely to have a parent with an SUD and had higher levels of effortful control and T2 depressive

symptoms when compared to males. Older adolescents and adolescents with higher levels of Mexican/Mexican-American ancestry had higher levels of symptomatology and alcohol use at most waves. Unexpectedly, older adolescents also had higher levels of Mexican/Mexican-American ancestry and higher levels of 5-HT polygenic risk. However, 5-HT polygenic risk and ancestry were not significantly correlated. Adolescents whose parents had SUDs had lower levels of SES and effortful control and higher levels of symptomatology and alcohol use at most waves when compared with adolescents whose parents did not have SUDs. Adolescents with lower levels of SES had higher levels of T2 alcohol use.

CDP. Contrary to hypotheses, adolescents with higher levels of 5-HT polygenic risk had *lower* levels of T3 alcohol use. However, adolescents with higher levels of 5-HT polygenic risk also had marginally significantly higher levels of T1 aggressive/antisocial behaviors. No other significant correlations with 5-HT polygenic risk were found. Adolescents with higher levels of T1 conscientiousness had lower levels of T1 and T2 symptomatology, but this trait was uncorrelated with alcohol use. In general, all of the symptomatology and alcohol use variables were significantly correlated in the expected direction, except T1 depressive symptoms did not significantly correlate with T2 or T3 alcohol use and T1 aggressive symptoms did not significantly correlate with T3 alcohol use.

Regarding correlations with demographic variables, females had higher levels of conscientiousness and T1 and T2 depressive symptoms, and lower levels of T1 and T2 aggressive/antisocial behavior when compared with males. Adolescents with lower levels of SES had higher levels of parental alcohol problems (marginal). Finally, adolescents

with higher levels of parental alcohol problems had higher levels of T1 aggressive/antisocial behaviors and T3 alcohol use.

Outliers

Cook's D and DFBETAS were calculated in an OLS regression framework. No cases had a Cook's D or DFBETAS value that exceeded an absolute value of one. No influential cases were identified or removed.

Multicollinearity

The variance inflation factor (VIF) was calculated for each independent variable in both AFDP and CDP to ensure that model estimates were not unreliable or unstable due to one or more independent variable(s) being linear combinations of other independent variables. VIFs exceeding 7 were considered indicative of multicollinearity (Neter, Wasserman, & Kutner, 1989). All VIFs from both AFDP and CDP ranged from 1.01-2.76. Thus, it was concluded that multicollinearity was not an issue and no predictors were removed from the models.

Determining the Appropriate Modeling Strategy for Alcohol Use Outcomes

The AIC, BIC, and log-likelihoods of covariate-only models that alternatively specified the T3 alcohol use outcomes as zero-inflated poisson, zero-inflated negative binomial, categorical, or continuous variables demonstrated that both AFDP and CDP alcohol use outcomes should be modeled as categorical variables. See Table 5 for the relative fit indices of each modeling strategy. Because all analyses modeled alcohol use as a categorical variable, montecarlo integration was used. Absolute fit indices are not available for models using montecarlo integration.

AFDP Results

Preliminary analyses to determine predictor-by-gender and predictor-by-covariate interactions. No predictor-by-gender interactions were significant ($p < 0.05$) and were trimmed. The T2 depressive symptoms-by-ancestry and T2 depressive symptoms-by-SES interactions survived the FDR-corrected $p < 0.05$. All interaction terms were included in the final model in a separate block so that main effects could be examined prior to the addition of interaction terms. Simple slopes were probed at the 1 *SD* below the mean, at the mean, and 1 *SD* above the mean for continuous variables (referred to as low, mean, and high levels, respectively). Standardized regression coefficients are reported to describe the simple slopes of significant interaction effects. Results from the final AFDP model are organized by outcome. See Table 6 and Figure 3.

T1 effortful control. 5-HT polygenic risk did not predict effortful control.[2] Males, adolescents whose parents had SUDs, and adolescents who took prescription medication had lower levels of effortful control.

T2 aggressive/antisocial behaviors. Higher levels of 5-HT polygenic risk predicted higher levels of T2 aggressive/antisocial behaviors. Lower levels of T1 effortful control prospectively predicted higher levels of T2 aggressive/antisocial behaviors.

[2] In AFDP, mother, father, and teacher-reported measures and observer ratings of effortful control were collected at an earlier wave occurring about 5 years before T1. Additional analyses tested whether a latent factor of effortful control including the current T1 effortful control measures and the effortful control measures collected 5 years prior were predicted by the polygenic risk score. This factor might better capture "trait" effortful control. This model fit the data well (χ^2 (18) = 19.5, p = 0.36, RMSA=0.02, CFI=1.0, SRMR=0.05). The polygenic risk score did not predict this more "trait-like" measure of effortful control. Analyses also tested whether the polygenic risk score would relate more strongly with any specific sub-dimension of effortful control (i.e., attentional control, activation control, inhibitory control). The polygenic risk score did not significantly correlate with any of the child-, mother-, father-, teacher-, or observer-rated sub-dimensions of effortful control.

Adolescents whose parents had SUDs and who had higher levels of T1 aggressive/antisocial behaviors had higher levels of T2 aggressive/antisocial behaviors.

T2 depressive symptoms. Higher levels of 5-HT polygenic risk predicted higher levels of T2 depressive symptoms. Lower levels of T1 effortful control prospectively predicted higher levels of T2 depressive symptoms. Females, adolescents whose parents had SUDs, adolescents who were taking prescription medications, and those who had higher levels of T1 depressive symptoms had higher levels of T2 depressive symptoms.

T3 alcohol use. Higher levels of 5-HT polygenic risk predicted higher levels of T3 alcohol use. Effortful control did not predict alcohol use over and above polygenic risk, depressive symptoms, and aggressive/antisocial behaviors (note, however, that there was a significant zero-order correlation between effortful control and alcohol use). Higher levels of T2 aggressive/antisocial behaviors prospectively predicted higher levels of T3 alcohol use. T2 depressive symptoms did not have a main effect on T3 alcohol use. Older adolescents, adolescents who parents had SUDs (marginal), and adolescents with a greater lapse between T2 and T3 had higher levels of T3 alcohol use.

The significant depressive symptoms-by-SES and depressive symptoms-by-ancestry interaction terms were entered in a second block. The patterns of the interactions were such that *lower* levels of depressive symptoms predicted greater levels of T3 alcohol use at mean ($\beta = -0.22$, $p = 0.01$) and low levels of SES ($\beta = -0.38$, $p < 0.001$) and mean ($\beta = -0.22$, $p = 0.01$) and low levels of ancestry ($\beta = -0.32$, $p < 0.001$; i.e., greater levels of Mexican/Mexican American ancestry). However, depressive symptoms did not predict alcohol use at high levels of SES ($\beta = -0.03$, $p = 0.69$) or high levels of ancestry ($\beta = -0.12$, $p = 0.14$; i.e., greater levels of Caucasian ancestry). See Figures 5 and 6.

Models omitting autoregressive paths. The 5-HT polygenic risk score did not predict any outcomes differently after removing autoregressive paths.

Models omitting parental SUD. Having a parent with an SUD likely confers greater genetic and environmental risk for offspring SUD. Because it was possible that including parents' SUD as a covariate weakened the effects of polygenic risk on outcomes, we ran models that omitted parental SUD as well. The 5-HT polygenic risk score did not predict any outcomes differently after removing autoregressive paths.

Mediation. 5-HT polygenic risk significantly and indirectly predicted T3 alcohol use through T2 aggressive/antisocial behaviors (both *a* and *b* paths were significant). T1 effortful control significantly and indirectly predicted T3 alcohol use through T2 aggressive/antisocial behaviors. See Figure 3.

Because T2 depressive symptoms was moderated by SES and ancestry in predicting T3 alcohol use, simple slope analyses tested the *a* and *b* paths at low, mean, and high levels of these moderators. For instance, 5-HT polygenic risk had a main effect on depressive symptoms, and depressive symptoms predicted T3 alcohol use at certain levels of SES. Thus, to examine at which levels of SES this mediational chain significant, a polygenic risk-by-SES interaction term was included in predicting T2 depressive symptoms. By running analyses while centering SES at 1 *SD* below the mean, at the mean, or at 1 *SD* above the mean, both main effect paths in this mediational chain would now refer to the effect for adolescents with low SES, mean SES, and high SES, respectively. This procedure was completed for all possible mediational effects and moderators by re-centering each of the continuous moderating variables at 1 *SD* above and below the mean and at the mean (see Table 8).

These analyses indicated that greater polygenic risk significantly and indirectly predicted lower T3 alcohol use through greater T2 depressive symptoms, but only for adolescents with low and mean levels of SES and ancestry. In addition, greater effortful control significantly and indirectly predicted lower T3 alcohol use through greater T2 depressive symptoms, but only for adolescents with low and mean levels of SES and with mean levels of ancestry.

CDP Results

Preliminary analyses to determine predictor-by-gender and predictor-by-covariate interactions. Gender interacted ($p < 0.05$) with T2 aggressive/antisocial behaviors and depressive symptoms to prospectively predict T3 alcohol use.[3] SES interacted (FDR corrected $p < 0.05$) with 5-HT polygenic risk and with conscientiousness to predict both T2 depressive symptoms and aggressive/antisocial behaviors.

Additional analyses tested the robustness of interactions involving the 5-HT polygenic risk score. Interactions with 5-HT polygenic risk were still significant after adding the extra interaction terms recommended by Keller (2014) and applying logarithmic transformations to both interacting variables. All interaction terms were included in the final model in a separate block so that main effects could be examined prior to the addition of interaction terms. Simple slopes were probed at the 1 *SD* below the mean, at the mean, and 1 *SD* above the mean for continuous variables (referred to as

[3] Because gender was a hypothesized moderator, it might be of interest to test whether there was weak evidence for a similar gender-by-depression or gender-by-aggression/antisociality interaction in predicting alcohol use in AFDP (e.g., marginally significant interaction term, similar simple slopes). Neither of these interactions was marginally significant ($p < 0.10$), and the simple slopes of these non-significant interaction terms were also dissimilar to the patterns seen in CDP. For example, higher levels of depression predicted *lower* levels of alcohol use for girls in AFDP. The only exception was that the standardized regression coefficient of aggression/antisociality on alcohol use was higher for boys than for girls, although the interaction was not statistically significant.

low, mean, and high levels, respectively). Standardized regression coefficients are reported to describe the simple slopes of significant interaction effects. Results from the final CDP model are organized by outcome. See Table 7 and Figure 4.

T1 conscientiousness. 5-HT polygenic risk did not predict T1 conscientiousness[4]. Females had higher conscientiousness than males.

T2 aggressive/antisocial behaviors. 5-HT polygenic risk and conscientiousness did not have main effects on T2 aggressive/antisocial behaviors. Adolescents with higher levels of T1 aggressive/antisocial behaviors had higher levels of T2 aggressive/antisocial behaviors.

The significant polygenic risk-by-SES and conscientiousness-by-SES interactions were entered in a second block. The pattern of the 5-HT polygenic risk interaction was such that higher levels of 5-HT polygenic risk predicted higher levels of T2 aggressive/antisocial behaviors at low levels of SES ($\beta = 0.16$, $p = 0.01$), but predicted *lower* levels of T2 aggressive/antisocial behaviors at high levels of SES ($\beta = -0.19$, $p = 0.01$). 5-HT polygenic risk did not predict T2 aggressive/antisocial behaviors at mean levels of SES ($\beta = -0.03$, $p = 0.67$). The pattern of the conscientiousness interaction was such that lower levels of conscientiousness significantly predicted higher levels of T2 aggressive/antisocial behaviors at low levels of SES ($\beta = -0.16$, $p = 0.01$), marginally

[4] The item "How forgetful do you think you are?" in the Conscientiousness scale might be the least reflective of effortful control of all items in the scale. Thus, conscientiousness was recomputed by dropping this item. Dropping this item reduced Cronbach's alpha from 0.64 to 0.60. In addition, preliminary zero-order correlations suggested that polygenic risk, aggression/antisociality, depression, and alcohol use all related *less* strongly with the new conscientiousness composite score. Thus, the original conscientiousness scale was retained for all analyses.

significantly at mean levels of SES (β = -0.08, p = 0.08), but did not predict at high levels of SES (β = 0.02, p = 0.70). See Figures 7 and 8.

T2 depressive symptoms. 5-HT polygenic risk did not have a main effect on T2 depressive symptoms. Lower levels of T1 conscientiousness predicted higher levels of T2 depressive symptoms. Females and adolescents with higher levels of T1 depressive symptoms had higher levels of T2 depressive symptoms.

The significant polygenic risk-by-SES and conscientiousness-by-SES interactions were entered in a second block. The pattern of the 5-HT polygenic risk interaction was such that higher levels of polygenic risk predicted higher levels of T2 depressive symptoms at low levels of SES (β = 0.17, p = 0.01), but marginally significantly predicted *lower* levels of T2 depressive symptoms at high levels of SES (β = -0.13, p = 0.07). 5-HT polygenic risk did not predict T2 depressive symptoms at mean levels of SES (β = 0.06, p = 0.29). The pattern of the conscientiousness interaction was such that lower levels of conscientiousness predicted higher levels of T2 depressive symptoms at low (β = -0.30, p < 0.001) and mean levels of SES (β = -0.16, p = 0.003), but not at high levels of SES (β = 0.004, p = 0.96). See Figures 9 and 10.

T3 alcohol use. Lower levels of 5-HT polygenic risk (i.e., higher levels of serotonin functioning) marginally significantly predicted higher levels of T3 alcohol use. Conscientiousness did not predict T3 alcohol use. T2 aggressive/antisocial behaviors and depressive symptoms did not have main effects on T3 alcohol use. Females and adolescents with higher levels of SES, parental alcohol problems, and T2 alcohol use had higher levels of T3 alcohol use.

The significant gender-by-T2 aggressive/antisocial behaviors and gender-by-T2 depressive symptoms interactions were entered in a second block. The pattern of the former interaction was such that higher levels of T2 aggressive/antisocial behaviors only prospectively predicted higher levels of T3 alcohol use for boys (β = 0.34, p = 0.01, OR = 1.30), but not for girls (β = -0.06, p = 0.60, OR = 0.96). The pattern of the latter interaction was such that higher levels of T2 depressive symptoms marginally significantly predicted higher levels of T3 alcohol use for girls (β = 0.15, p = 0.06, OR = 1.10), but did not predict for boys (β = -0.23, p = 0.12, OR = 0.90). See Figures 11 and 12.

Models omitting autoregressive paths. The 5-HT polygenic risk score did not predict any outcomes differently after removing autoregressive paths.

Moderated mediation. Because the *a* and *b* paths of several of the mediational chains in the final CDP model were moderated by different variables, simple slope analyses tested the *a* and *b* paths at every possible combination of the relevant moderators. For instance, 5-HT polygenic risk predicted T2 aggressive/antisocial behaviors, but only at certain levels of SES, and T2 aggressive/antisocial behaviors subsequently predicted T3 alcohol use, but only for males. To examine at which combinations of SES and gender this mediational chain was significant, a polygenic risk-by-gender interaction term was included in predicting T2 aggressive/antisocial behaviors and an aggressive/antisocial behavior-by-SES interaction term was included in predicting T3 alcohol use. By assigning males a code of '0' and centering SES at 1 *SD* below the mean, both main effect paths in this mediational chain would now refer to the effect for males with low SES. This procedure was completed for all possible mediational effects

and combinations of moderators by re-centering each of the continuous moderating variables at 1 *SD* above and below the mean and at the mean, and by re-centering each of the dichotomous moderators such that different categories were equal to zero (see Table 9).

These analyses showed that higher levels of 5-HT polygenic risk indirectly predicted higher levels of T3 alcohol use through greater T2 aggressive/antisocial behaviors, but only for males with low levels of SES. However, *lower* levels of 5-HT polygenic risk indirectly predicted higher levels of T3 alcohol use through greater T2 aggressive/antisocial behaviors for males with high levels of SES. In addition, lower levels of conscientiousness indirectly predicted higher levels of T3 alcohol use through greater T2 aggressive/antisocial behaviors, but only for males with low levels of SES.

Comparing Results from AFDP and CDP

Because of the complex patterns of moderation found in both studies, it was informative to organize the main results of interest in a side-by-side comparison format. See Table 10.

Discussion

The purpose of the current study was to test an integrated, developmental model to better understand the relation between serotonergic functioning and alcohol use. This study tested the hypothesis that 5-HT polygenic risk (i.e., reflecting serotonin functioning) would create a vulnerability for poor effortful control, which would, in turn, predict the divergent outcomes of depressive symptoms and aggressive/antisocial behaviors. The hypothesis further posited that depressive symptoms and aggressive/antisocial behaviors would subsequently predict later alcohol use, but that

direct effects of 5-HT polygenic risk and/or effortful control on alcohol use might still remain. This hypothesis was tested in two independent longitudinal samples and, therefore, the first sections will focus on replicated findings across samples.

5-HT Polygenic Risk and Effortful Control/Conscientiousness

The first major finding was that the polygenic risk score created to index serotonergic functioning (i.e., 5-HIAA in the cerebrospinal fluid) did not predict effortful control in AFDP or conscientiousness in CDP. Therefore, the current data do not support the hypothesis that adolescents with lower levels of serotonin functioning have a vulnerability for deficits in effortful and trait like components of self- regulation. Further, the data do not support the notion that self-regulation is the mechanism through which serotonin functioning creates risk for multiple types of psychopathology. Of course, one reason for this finding might be due to methodological limitations of the 5-HT polygenic risk scores. Indeed, only 23 SNPs were included in both scores, and many more genetic variants are involved in the complex process of serotonin functioning.

However, this concern might be mitigated by the other evidence of predictive validity demonstrated by the 23-SNP 5-HT polygenic risk score. Consistent with the previous literature, individuals with greater scores on 5-HT polygenic risk (reflecting lower levels of serotonin functioning) had greater depressive symptoms, aggressive/antisocial behaviors, and alcohol use across samples (albeit for certain subgroups in CDP). This suggests that the 5-HT polygenic risk scores might capture the intended construct, and that the limitations of the scores likely do not account for the lack of association between 5-HT polygenic risk and self-regulation (see Carver et al., 2008; LeMarquand et al., 1994a, 1994b; Van Goozen et al., 2007).

Alternatively, another reason that the 5-HT polygenic risk score did not predict effortful control or conscientiousness could be because of inadequate measurement of these latter two constructs. However, similar to the 5-HT polygenic risk score, these self-regulatory constructs demonstrated predictive validity. As expected, effortful control and conscientiousness were related to adolescents' depressive symptoms and aggression/antisociality in both samples (albeit for certain subgroups in CDP). Effortful control was also associated with adolescents' alcohol use in AFDP.

Moreover, the measurement of effortful control in AFDP was particularly strong, which further decreases the likelihood that inadequate measurement was the reason that polygenic risk effects were not detected. Effortful control reflected what was common across multiple observers of adolescents' effortful control because mother-, father-, and adolescent-reported effortful control were used to create the measure. Moreover, the Early Adolescent Temperament Questionnaire, from which the effortful control scale was derived, has been shown to be reliable and valid based on previous work (Capaldi & Rothbart, 1992). Conscientiousness in the CDP was somewhat less robustly measured because only adolescents self-reported on the measure and it contained only 5 items. However, the fact that this measure of self-regulation produced similar results to the stronger measure of effortful control suggests that inadequate measurement of self-regulation is likely not the reason that polygenic risk was not related to effortful control or conscientiousness.

Finally, perhaps no associations were found between 5-HT polygenic risk and self-regulatory constructs because the effect of 5-HT polygenic risk is actually non-linear. Indeed, the current study did not propose to test non-linear effects of 5-HT polygenic

risk. This possibility is buttressed by our findings that, in some cases, CDP adolescents with *lower* levels of 5-HT polygenic risk had higher levels of depression, aggression/antisociality, and alcohol use (but, see later sections for more details about these findings). Thus, perhaps individuals with both higher levels and lower levels of 5-HT polygenic risk have the lowest levels of effortful control, whereas individuals with average levels of 5-HT polygenic risk have the highest levels of effortful control. However, post-hoc analyses that included a quadratic 5-HT polygenic risk term in the final models showed that the quadratic effect of 5-HT polygenic risk was not significant for any of the outcomes.

Because the 5-HT polygenic risk scores demonstrated predictive validity, it is worth elaborating upon the strengths of the scores and the SNPs that were included. First, the promising results found using the 5-HT polygenic risk scores suggest that the use of genome-wide association studies (GWAS) to create polygenic risk scores is a valuable approach. This approach might be especially useful in the service of better understanding the role of genetic risk in developmental models of psychopathology. Second, it is noteworthy that we were able to directly infer the functional significance of the 5-HT polygenic risk score because we selected SNPs that were associated with 5-HIAA concentrations in the cerebrospinal fluid in a previous GWAS. This is an improvement over some previous studies that have created polygenic risk scores to index broad psychological disorders. Indeed, this broader method does not as easily allow researchers to form specific hypotheses about the biological mechanisms underlying polygenic risk scores because genetic variants belonging to different biological pathways are often combined into a single score.

It is also potentially interesting to note that some of the SNPs included in the score might play a functional role in gene expression and/or have been associated with substance-related phenotypes in the literature. Notably, rs1799971, located in the OPRM1 gene, has been extensively studied and was recently shown to predict substance dependence in a meta-analysis of 28,000 European American adults (Schwantes-An et al., 2015). This variant has also been shown to functionally influence mu-opioid receptor expression levels, binding affinity, and signaling (Befort, Filliol, Decaillot, Gaveriaux-Ruff, Hoehe, & Kieffer, 2001; Beyer, Koch, Schröder, Schulz, & Hollt, 2004; Wang, Huang, Ung, Blendy, & Liu-Chen, 2012). Similarly, rs760288, located in NRXN3, distinguished between individuals who were dependent on illegal substances from controls across two different samples (Liu, 2005). Interestingly, this SNP forms a haplotype block with rs8019381, which has been posited to influence alternative splicing in exon 23 of this gene (Sasabe & Ishiura, 2010). These SNPs might influence serotonergic functioning through their aforementioned functional effects or through different mechanisms entirely. It is difficult to hypothesize the mechanism of action by which these SNPs influence serotonergic functioning based on current information.

Several other SNPs in the polygenic risk score have also been associated with substance use phenotypes, although little has currently been published on their potential functional effects. These include rs6494212 (CHRNA7), rs135757 (CSNK1E), rs2236256 (IPFCEF1), and rs2272381 (OPRM1; Chen et al., 2011; Levran et al., 2008; Saccone et al., 2010; Smelson et al., 2012). Note that most of these results were derived from candidate gene studies with relatively small sample sizes, which suffer from limitations such as difficulties with replication (Plomin, DeFries, Knopik, & Neiderhiser,

2016). However, it is possible that these SNPs are related to substance use phenotypes through their effects on serotonergic functioning.

5-HT Polygenic Risk and Adolescents' Problem Behaviors

As noted earlier, adolescents with higher 5-HT polygenic risk scores (i.e., lower serotonin functioning) had greater levels of aggressive/antisocial behaviors and depressive symptoms in both AFDP and CDP. However, in CDP, the effects of 5-HT polygenic risk on greater depression and aggression/antisociality only held for adolescents with low levels of SES. Two points are important to note. First, the fact that serotonin functioning as indexed by a polygenic risk score predicted adolescents' symptomatology (for certain subgroups) across two samples is consistent with multiple previous studies. Specifically, other work has found that serotonin functioning as indexed by 5-HIAA in the cerebrospinal fluid, tryptophan depletion/enhancement, and blunted hormonal responses to drug challenges (among others) related to children's and adults' aggression, delinquency, antisociality, and depressive symptoms and disorder (Booij & Van der Does, 2007; Clarke et al., 1999; Dencker, Malm, Roos, & Werdinius, 2006; Flory et al., 1998; Halperin et al., 2006; Kruesi et al., 1990, 1992; Lidberg et al., 1985; Limson et al., 1991; Traskman-Bendz et al., 1984). These results were such that lower levels of serotonin functioning were associated with greater problems. Taken together, this presents strong evidence that individuals with lower levels of serotonergic functioning are more prone towards developing aggressive/antisocial behaviors and depressive symptoms in childhood and adulthood. Note that in the CDP we also found the opposite relation for adolescents with high levels of SES, wherein *lower* levels of

polygenic risk predicted greater levels of aggression/antisociality. Because this was not replicated in AFDP, this finding will be discussed further in a later section.

Second, it is important to understand why 5-HT polygenic risk was moderated by SES in predicting behavioral problems, but only in the CDP. It is possible that the smaller sample size of AFDP (N = 254) compared to CDP (N = 348) resulted in lowered power to detect this moderation effect in AFDP. Alternatively, perhaps polygenic risk only had main effects in AFDP because AFDP is a high-risk sample in which participants likely have greater genetic vulnerabilities for problem behaviors compared to CDP participants who represent a general community sample. Finally, it is possible that differences in SES across samples account for findings. Although the SES variables available in each sample were not identical, comparing the SES across samples suggests that CDP participants more highly represent the upper levels of education than do AFDP participants. For example, 46.8% of CDP participants' parents obtained at least 16-17 years of education or higher, which roughly corresponds to obtaining at least a Bachelor's degree or higher. Fewer AFDP participants' parents (35.1%) obtained a Bachelor's degree or higher. Similarly, 19% of CDP participants' parents obtained 18+ years of education, which roughly corresponds to some graduate or professional schooling or the completion of a graduate degree. Again, a smaller percentage (12%) of AFDP participants' parents obtained some graduate or professional schooling and/or completed a graduate degree. Comparing SES levels at the lower end of the distribution similarly suggests that AFDP participants might be more representative of lower levels of SES than CDP. For example, 6.5% of AFDP participants' parents did not graduate from high

school (some in this group obtained a GED), whereas fewer (3.5%) CDP participants' parents obtained 10-11 years of schooling or less.

Thus, perhaps the effect of serotonin functioning on depressive and aggressive/antisocial symptomatology is truly stronger or only present under conditions of low SES (as was found in CDP), and perhaps this was captured as a main effect in AFDP because low SES participants were more highly represented in this sample. Relatedly, perhaps this effect was only conditional on SES in CDP because CDP participants had greater variability at the upper end of the distribution of SES, facilitating the detection of differential effects at high levels of SES.

If differences in SES variability account for the differential moderation by SES across studies, we might expect to see weak polygenic risk-by-SES interaction effects in predicting AFDP adolescents' aggression/antisociality and/or depression that perhaps did not survive FDR corrections in original analyses. Thus, to further probe and understand this polygenic risk-by-SES effect, post-hoc analyses tested whether a polygenic-risk-by-SES interaction predicted AFDP adolescents' aggression/antisociality or depressive symptoms. Interestingly, the polygenic-risk-by-SES interaction marginally significantly predicted aggression/antisociality ($\beta = -0.08$, $p = 0.099$). The interaction was such that higher levels of 5-HT polygenic risk (lower serotonin functioning) significantly predicted greater aggression/antisociality at low ($\beta = 0.20$, $p = 0.01$) and mean ($\beta = 0.12$, $p = 0.02$) levels of SES, but not at high levels of SES ($\beta = 0.03$, $p = 0.59$). Perhaps this interaction would have survived corrections for multiple testing if AFDP had a larger sample size or more variability in the upper range of SES.

This finding supports the possibility that the effect of 5-HT polygenic risk on greater aggression/antisociality is amplified at low SES. Although this post-hoc analysis does not support the possibility that 5-HT polygenic risk effects are *reversed* at high levels of SES (as was found in CDP), it does not contradict it either. This is because we might not expect to obtain similar effects at high SES in AFDP and CDP given that AFDP has less variability in the upper range of SES than CDP. Finally, note that the same trend towards an interaction was not found for depressive symptoms in AFDP. However, it is possible that corresponding effects were not found for depression because AFDP had lower SES variability and power, especially in light of the fact that the polygenic risk-by-SES interaction in predicting aggression/antisociality was clearly weaker in AFDP compared to CDP.

In sum, there are several reasons to believe that this SES moderation is not spurious, despite the fact that we only found the effect in one sample. The findings that 5-HT polygenic risk only predicted aggression/antisociality and depression for adolescents with low levels of SES in the CDP are consistent with previous research suggesting that genetic risk variants more strongly predicted adolescents' greater externalizing- and internalizing-related outcomes under conditions of low SES or high environmental adversity (Laucht et al., 2009; Nobile et al., 2007, 2010; Sweitzer et al., 2013). This pattern is consistent with the dual-risk or diathesis-stress model, which suggests that the combination of adverse environmental conditions and individual risk factors constitutes the greatest risk for the development of problems (Monroe & Simons, 1991). Indeed, a large body of work has established that low SES is associated with multiple negative

health and socioemotional outcomes, in part because it decreases access to resources and is associated with higher levels of stress (Bradley & Corwyn, 2002).

Interestingly, this polygenic risk-by-SES interaction could also be due to the fact that one's SES can influence the *expression* of genetic material through DNA methylation, histone modification, or other processes (i.e., epigenetic influences). For example, one previous study showed that lower levels of SES were associated with genome-wide hypomethylation in adults after controlling for their gender, age, and other lifestyle factors related to SES such as health and housing (McGinnis et al., 2012). Lower levels of SES also predicted genome-wide changes in methylation for adolescents who carried the *s* allele of the 5-HTTLPR polymorphism, after controlling for age and gender (Beach, Brody, Lei, Kim, & Cui, 2014). Thus, it is possible that low SES conditions, and/or some correlate of low SES, altered the epigenomic state of one or several genes in the 5-HT polygenic risk score (i.e., altered gene expression), and this could explain why polygenic risk effects were conditional on SES.

The fact that high SES adolescents with *lower* levels of 5-HT polygenic risk (i.e., *higher* serotonin functioning) had greater aggression/antisociality is more difficult to interpret due to a lack corresponding effects in AFDP. That is, adolescents with lower levels of polygenic risk (i.e., higher serotonin functioning) did not have greater levels of any symptom measure in AFDP. It is important to briefly note that other studies in the literature have found that higher levels of serotonin functioning were associated with greater aggression and hostility in children, adolescents, and adults (i.e., Castellanos et al., 1994; Pine et al., 1997; Halperin et al., 1994; Hughes et al., 1996).

Despite evidence for the opposite direction of effect in the current study and previous studies, a recent meta-analysis of 175 independent samples that included most of the aforementioned studies (i.e., Castellanos et al., 1994; Pine et al., 1997; Halperin et al., 1994) found an overall small effect of serotonin functioning on aggression-related phenotypes, such that lower levels of serotonin functioning predicted greater levels of aggression (Duke, Begue, Bell, & Eisenlohr-Moul, 2013). These authors also explored potential moderating variables that might account for differences in results across the 175 studies (e.g., why some studies found positive, and others negative, correlations). They found that the differences in outcomes were not explained by moderators such as experimental design, sample size, effect size, assessment instruments and methods used, presence of control conditions, age, gender, race/ethnicity, and other psychopathology. However, the use of self- vs. other-reported measures did moderate study outcome variability. The pattern was such that other-reported measures of greater aggression tended to correlate positively with *higher* serotonin functioning whereas self-reported measures of greater aggression tended to correlate positively with *lower* serotonin functioning. However, this moderator does not explain our reversed findings because we only used self-reported, and not other-reported, measures of aggression/antisociality. Although this meta-analysis did not assess SES as a moderator, there was no systematic evidence that the samples from studies that found the opposite direction of effect had particularly high SES.

Taken together, the previous literature and the current findings suggest the possibility that the observed *reversed* relation (i.e., higher serotonin functioning associated with greater aggression) could be accounted for by some other untested

moderator, or could likely be spurious. The most reliable information gleaned from the current study is that *higher* scores on the 5-HT polygenic risk score (which reflects *lower* levels of serotonin functioning) represents risk for adolescents' depression and aggression/antisociality, and that this association might be amplified under adverse economic conditions.

In summary, the current findings suggest that adolescents with lower levels of serotonin functioning as indexed by this 5-HT polygenic risk score have higher levels of depressive and aggressive/antisocial behaviors (perhaps more strongly under low SES conditions). However, this 5-HT polygenic risk score does not appear to be associated with self-regulatory constructs. Thus, one interesting avenue of future research would be to better understand whether there is a common underlying trait, physiological marker, or other biological phenotype intermediate between serotonergic functioning and these divergent forms of problem behavior in adolescence. Moreover, results suggest that serotonin functioning could be a valuable candidate for future research on transdiagnostic risk factors.

Aggression/Antisociality and Alcohol Use

In both samples, greater levels of aggressive/antisocial behaviors prospectively predicted greater levels of later alcohol use. However, this effect only held for boys in the CDP sample. The CDP gender differences did not appear to be related to differences in variability for boys versus girls. Indeed, Levene's test of the homogeneity of variances suggested that the variances of aggressive/antisocial behaviors ($F(1) = 0.02$, $p = 0.88$) and alcohol use ($F(1) = 1.78$, $p = 0.18$) did not differ for boys and girls. This finding mirrors that of other studies, which showed that antisociality, conduct disorder, novelty seeking,

and behavioral undercontrol (i.e., externalizing-related traits and outcomes) predicted alcohol phenotypes more strongly for males than for females (e.g., Caspi, Moffit, Newman, & Silva, 1996; Hussong, Curran, & Chassin, 1998; Kendler, Edwards, & Gardner, 2015). The inconsistency between CDP and AFDP with regards to this finding could be due to several reasons.

First, as stated previously, perhaps AFDP had lower power to detect gender moderation than CDP due to a smaller sample size. Second, a recent review of the literature showed that women's drinking rates began to catch up with men's drinking rates in the time spanning 1989-2009, possibly due to differences in the societal structure of gender roles or social norms (Keyes, Li, and Hasin, 2011). Because the CDP participants self-reported alcohol use over a decade earlier (1997-1998) than AFDP participants (2012-2013), perhaps aggression/antisociality only predicted alcohol use for boys in the CDP because there were greater negative social sanctions against drinking for females than males during CDP data collection. If this is true, alcohol use could have been a less likely consequence or correlate of females' externalizing problems in the CDP, but not in AFDP. Third, this finding could be due to the fact that AFDP is a high-risk sample, in which over half of all participants had a parent with an SUD, whereas the CDP was a community sample. Moreover, in the subsample of adolescents we selected from AFDP, there happened to be a significantly greater percentage of girls (55.4%) than boys (44.6%) whose parents had an SUD ($\chi^2(1) = 5.68$, $p = 0.02$; also see Table 2). Perhaps the greater percentage of females with a high genetic and familial loading for substance use in the AFDP sample resulted in aggression/antisociality being equally predictive of alcohol use in boys and girls.

The Indirect Effect of 5-HT Polygenic Risk on Alcohol Use

In both AFDP and CDP, 5-HT polygenic risk prospectively and indirectly predicted later alcohol use through adolescents' aggression/antisociality, such that greater levels of 5-HT polygenic risk (i.e., lower levels of serotonin functioning) predicted greater aggression/antisociality, which predicted greater alcohol use. However, this path was only significant for boys with low SES in the CDP sample (i.e., moderated mediation).[5] This finding is a novel contribution to the literature. Although studies have found associations between indices of serotonin functioning and aggression, antisociality, and alcohol use and disorder (e.g., Halperin et al., 2006; Kruesi et al., 1990, 1992; LeMarquand et al., 1994a, 1994b Lidberg et al., 1985; Limson et al., 1991), this is the first study to our knowledge to show that aggression/antisociality might be one mechanism underlying the relation between serotonergic functioning and alcohol consumption.

Perhaps individuals with lower levels of serotonin functioning have a predisposition towards aggressive, delinquent, and/or deviant behaviors, which prompts affiliation with deviant peer groups and, subsequently, increases alcohol use (e.g., Sher, 1991). This might be especially true for individuals who are already at high risk for these behaviors, such as boys with low SES (e.g., Bradley & Corwyn, 2002; Else-Quest et al., 2006). Findings are also consistent with the possibility that serotonergic functioning manifests as different behavioral phenotypes across development (i.e., heterotypic

[5] In the CDP, *lower* polygenic risk also indirectly predicted greater alcohol use through greater aggression/antisociality for boys with high SES. Because the *a* path in the aforementioned mediated effect could be spurious due to a lack of replication (i.e., lower polygenic risk predicting greater aggression/antisociality at high SES), this mediated effect will not be elaborated upon further.

continuity). To illustrate, since we did not find that serotonergic functioning predicted effortful control, perhaps it predicts some facet of behavioral disinhibition. Indeed, research suggests that behavioral disinhibition presents a generalized vulnerability for developing externalizing problems (Young et al., 2009). If this were true, it is possible that a greater vulnerability for behavioral disinhibition is primarily expressed as aggression and delinquency in earlier adolescence and later expressed as greater alcohol use due to increases in autonomy, access to substances and peer alcohol use.

Effortful Control/Conscientiousness and Adolescents' Problem Behaviors

Similarly, in both AFDP and CDP, effortful control/conscientiousness prospectively predicted aggressive/antisocial behaviors and depressive symptoms, such that lower levels of effortful control/conscientiousness predicted greater levels of problem behaviors. However, in CDP the effect on aggression/antisociality only held for adolescents with low levels of SES and the effect on depression only held for adolescents with low and mean levels of SES. The fact that SES was found to moderate the effects of two virtually unrelated, independent risk factors (i.e., 5-HT polygenic risk and conscientiousness; see Tables 2 and 3) further suggests that the SES moderation found in CDP was not spurious. It also indicates that CDP participants' SES is an important environmental factor that protects against or exacerbates the effects of individual differences in predicting problem behaviors. Note that many of the same reasons that SES moderated the effects of 5-HT polygenic risk in the CDP and not in AFDP likely apply to these findings (i.e., SES variability differences, power differences, high-risk vs. community samples). Similar to the polygenic risk-by-SES interaction, perhaps we might expect to see weak effortful control-by-SES interactions in predicting problem behaviors

in AFDP that did not survive original FDR corrections. Although post-hoc analyses showed that effortful control did not significantly or marginally significantly interact with SES to predict problem behaviors in the AFDP sample, the lack of such an interaction in AFDP could still be due to lowered power or SES variability.

Taken together, findings suggest that impairments in self-regulatory abilities prospectively predict greater change in adolescents' aggression/antisociality and depression. These results are consistent with multiple previous studies (e.g., Loukas & Robinson, 2004; Loukas & Roalson, 2006; Muris et al., 2008; Verstraeten et al., 2009; Wang et al., 2015a). Although not replicated in AFDP, results also provide preliminary evidence that deficient self-regulation is especially detrimental for economically disadvantaged adolescents. Perhaps a lack of self-regulation did not predict problem behaviors for adolescents from higher SES families because these adolescents have greater access to resources from parents, neighborhoods, and schools (Bradley & Corwyn 2002). This greater access might help these adolescents develop compensatory strategies to deal with their poor regulatory abilities and this could curb the development of problem behaviors in this subgroup. In contrast, without these resources, adolescents with low levels of SES might not learn new ways of dealing with their self-regulatory deficits and might manifest problem behaviors as a result.

Other studies have similarly found interactions between self-regulatory constructs and other aspects of the environment in predicting problem behaviors. Specifically, adolescents with both lower levels of self-regulation (i.e., conscientiousness or effortful control) and greater coercive parenting, lower parental emotional expressivity, negative parental control, or lower maternal guidance had the greatest externalizing problems or

depressive symptoms (Prinzie et al., 2003; Kiff, Lengua, & Bush, 2011; Van Leeuwen, Mervielde, Braet, & Bosmans, 2004; Wang, Eisenberg, Valiente, & Spinrad, 2015b). These interaction effects could be due to the cumulative risk of regulatory deficits and poor parenting. These interactions could also arise if adolescents with low self-regulatory abilities have trouble inhibiting problematic behaviors or emotions that occur in response, or as a reaction to, harsh or poor parenting practices. Given that distress among parents with low SES might contribute to their over-use of negative control, low warmth, and low monitoring (McLoyd, 1990), perhaps the moderating role of SES operates indirectly through these parenting behaviors

The Indirect Effect of Effortful Control/Conscientiousness on Alcohol Use

Deficits in self-regulation (i.e., effortful control and conscientiousness) also indirectly predicted greater levels of alcohol use through aggressive/antisocial behaviors. However, this mediated effect only held for boys with low SES in the CDP. Interestingly, in AFDP, the effect of effortful control on alcohol use appeared to be entirely mediated through antisociality/aggression because there was no longer a direct effect of effortful control on alcohol use after accounting for this mediator. Multiple previous studies have linked effortful control/self-regulation with aggression/antisociality and substance use phenotypes separately (Cheetham et al., 2010; Creemers et al., 2010; Loukas & Roalson, 2006; Loukas & Robinson, 2004; Wang et al., 2015a; Willem et al., 2011). This study is the first to our knowledge to link these constructs together in a developmental and longitudinal framework. Specifically, it appears that deficits in self-regulation are related to changes in alcohol use over time because this trait predisposes adolescents to an earlier risk factor for alcohol use, namely, aggression/antisociality. Adolescents with high levels

of aggression/antisociality might drink more alcohol because they affiliate with deviant peers, or as a way to dampen negative affectivity associated with aggression (e.g., anger). It could also be that deficits in effortful control manifest as different correlated phenotypes at different points in development (i.e., aggression/antisociality and alcohol use).

Results also point to the possibility that deficits in effortful control represent another pathway to alcohol problems, in addition to the more common deviance proneness and stress dampening pathways (see Sher, 1991). That is, in addition to one pathway characterized by sensation seeking, reward sensitivity, and impulsivity (i.e., deviance proneness), and a second pathway characterized by negative affectivity and self-medication (stress dampening), a third pathway might be characterized by a more global, top-down inability to regulate behaviors, thoughts and feelings. On the other hand, it is likely that effortful control is implicated in both the deviance proneness and stress dampening pathways because each of these pathways also involve difficulties in regulating behaviors, thoughts, or feelings. Thus, effortful control might simply contribute to deviance proneness and/or stress dampening processes.

Finally, results suggest that self-regulation is an important transdiagnostic risk factor because it predicts multiple types of problem behaviors, including aggression/antisociality, depression, and alcohol use (indirectly). Prevention and intervention efforts to increase effortful control/conscientiousness might be helpful and effective in reducing many kinds of psychopathological problems in adolescence.

Study Specific Findings and Cross-Study Comparisons

The link between adolescents' depression and alcohol use exhibited some consistency across studies. Namely, there was replication at the zero-order correlation level, wherein higher levels of depression correlated positively with higher levels of alcohol use. However, after adding all of the covariates and predictors to the models, these paths were no longer the same across the two studies. In CDP, there was no main effect of depression on alcohol use. However, depression and gender interacted to predict alcohol use such that higher levels of depressive symptoms marginally significantly predicted greater levels of alcohol use for girls but not for boys. In AFDP, there was also no main effect of depression on alcohol use. However, depression interacted with both SES and ancestry to predict alcohol use, such that higher levels of depressive symptoms predicted *lower* levels of alcohol use for adolescents with low and mean levels of SES and ancestry (i.e., greater Mexican/Mexican-American ancestry), but not for adolescents with high levels of SES or ancestry. [6] Note that removing aggression/antisociality from the model, which was highly correlated with depressive symptoms (r = 0.68), did not change the negative direction of the effect of depression on alcohol use in AFDP. This suggests that some combination of multiple predictors and covariates, rather than only aggression/antisociality, accounts for this reversal effect in AFDP.

[6] Because of this moderation effect, depression also served as a mediator in the link between effortful control and alcohol use and between polygenic risk and alcohol use in AFDP. Specifically, greater effortful control and polygenic risk indirectly predicted lower alcohol use through greater depressive symptoms for adolescents low and mean levels of SES and/or ancestry. These significant moderated mediation effects are not elaborated on further because the *b* path in this mediational chain (i.e., depression to alcohol use) might be spurious and was not replicated.

Our findings mirror the previous literature investigating the link between adolescents' depression and later alcohol use, which has also been equivocal. For example, some previous research showed that depressive symptoms in adolescence raised the risk for later alcohol use, abuse, and substance use disorder in adolescence even after controlling for co-occurring externalizing problems in most cases (e.g., Conway et al., 2016; Henry et al., 1993; Hussong & Chassin, 1994; King, Iacono, & McGue, 2004; Sung et al., 2004). However, other studies that controlled for externalizing risk found that adolescents' depression and internalizing symptoms did not predict later heavy drinking in adolescence or alcohol use disorders in young adulthood (Hussong, Curran, & Chassin, 1998; Pardini, Raskin White, & Stouthamer-Loeber, 2007). Finally, studies have also found that internalizing problems, particularly in the absence of externalizing problems, protected against substance use (i.e., Colder et al., 2013; Kaplow, Curran, Angold, & Costello, 2001). Taken together, this suggests that the unique effect of adolescents' depression on alcohol use after controlling for relevant covariates and other robust predictors (i.e., aggression/antisociality) is complex not easily interpretable. Several researchers have suggested that the effect of adolescents' internalizing symptoms on alcohol use might vary based on other important moderating variables not tested in the current study (e.g., impulsivity, coping styles, externalizing problems; Hussong, Curran, & Chassin, 1998; Pardini et al., 2007) or might not be as strong or relevant of a predictor as is adulthood depression on alcohol use outcomes (Hussong, Curran, & Chassin, 1998). Although beyond the scope of the current study, these possibilities deserve further study.

Finally, in AFDP, 5-HT polygenic risk had a significant direct effect on alcohol use after accounting for earlier symptoms and effortful control, such that individuals with

higher 5-HT polygenic risk scores had greater alcohol use. In contrast, individuals with *lower* 5-HT polygenic risk scores had marginally significantly greater levels of alcohol use in CDP after controlling for similar variables. AFDP's findings are more consistent with the previous literature on this topic. Indeed, multiple studies found that serotonergic functioning as measured by 5-HIAA in the cerebrospinal fluid, drug challenge, and platelet serotonin uptake was lowered in adults with alcohol use disorders and in children and adults with family histories of alcohol disorder vs. controls (Balldin et al., 1994; Ballenger et al., 1979; Banki, 1981; Borg et al., 1985; Ernouf et al., 1993; Farren et al., 1995; Füs-Aime et al., 1996; Lee & Meltzer, 1991; Rausch et al., 1991).

The conflicting direct effects found in AFDP vs. CDP are difficult to understand, especially in light of the fact that the indirect effect of polygenic risk on alcohol use through aggression/antisociality was in the same direction in both samples. However, it is possible that the expected direction of effect (wherein lower levels of serotonin functioning are associated with greater levels of alcohol use) only holds for certain subgroups in CDP, similar to its effects on depression and aggression/antisociality in this sample. Perhaps 5-HT polygenic risk interactions were not detected in predicting alcohol use in CDP because of the developmental timing of the alcohol use assessment (15-16 years old). Indeed, some adolescents who were at genetic risk might not have had the opportunity to initiate alcohol use by that time. However, this hypothesis is speculative and the current data cannot inform the validity of this hypothesis. More work is needed to understand the direct effect of serotonin functioning on alcohol use in adolescence, especially after controlling for potential earlier mediating variables such as aggression/antisociality and depression.

Implications for Theory and Practice

The present findings have several implications for theory and clinical practice. One important theoretical implication is in better understanding causes of co-occurrence. Indeed, research shows that depressive symptoms, aggression/antisociality, and alcohol use all co-occur with one another at a high rate (Bukstein, Brent, & Kaminer, 1989). Moreover, in both of the current samples the T2 and T3 measures of these problem behaviors were moderately-to-highly correlated.

Several causes of co-occurrence have been outlined in the literature. First, some explanations for the observed rates of co-occurrence between disorders are artifactual. For instance, high rates of co-occurrence might be observed if only clinical samples are obtained, because these individuals are more likely to have multiple disorders (i.e., ascertainment bias). Co-occurrence could also be artifactually due to the presence of symptom overlap in the measurement of two disorders or could be due to random chance (Neale & Kendler, 1991). However, multiple previous studies have established that the observed co-occurrence between depressive symptoms and aggressive/antisocial behaviors, and between externalizing and internalizing disorders more broadly are not due to these factors (see Lilienfeld, 2003; Zoccolillo. 1992).

There are also substantive reasons for co-occurrence. For example, psychological disorders might co-occur because they share the same underlying continuum of liability, their risk factors are correlated and/or shared, or one disorder causes the other disorder (i.e., direct causal model). Moreover, it is also possible that the co-occurring form is a third and separate disorder apart from pure forms or that the co-occurring form shares a similar etiology with one of the pure forms (Caron & Rutter, 1991; Neale & Kendler,

1995). Although the current study findings cannot determine the exact causes of co-occurrence, they can point towards likely explanations to be followed up in future studies.

The current finding that 5-HT polygenic risk and deficits in self-regulation predicted depression and aggression/antisociality across two samples provides evidence that these are shared risk factors that might account for the co-occurrence between these two problem behaviors. To illustrate, perhaps because deficits in self-regulation make it more difficult to inhibit negative affectivity, such as sadness and anger, this could result in the eventual manifestation of both depressive symptoms and aggressive/antisocial behaviors within the same individual. It is also possible that polygenic risk and self-regulation are truly only risk factors for one of the two disorders, but are correlated with some other unmeasured risk factors that contribute to risk for the other disorder. For example, deficits in self-regulation are often correlated with poor social competence (Spinrad et al., 2007). Perhaps deficits in self-regulation contribute to the risk for aggression/antisociality only, and the correlated risk factor of poor social competence predicts depression within the same individual.

The present findings are also in line with previous work suggesting that the co-occurrence between depression and conduct problems is due, in part, to a common genetic liability (O'Connor, McGuire, Reiss, Hetherington, & Plomin, 1998; Rowe et al., 2008; Subbarao et al., 2007). Specifically, results suggest that 5-HT polygenic risk might be one component that operates within this larger shared genetic risk liability. Interestingly, the present results also suggest that mechanisms of co-occurrence might vary depending on contextual factors. Indeed, our results suggested that 5-HT polygenic

risk and self-regulation might be stronger predictors of aggression/antisociality and depression at lower levels of SES. Thus, the extent to which these shared risk factors might contribute to the co-occurrence between these problem behaviors might be especially robust when SES is low.

There was less evidence that 5-HT polygenic risk and self-regulation were risk factors for alcohol use because these variables showed somewhat contradictory direct effects across the two samples in the current study. Thus, the co-occurrence of alcohol use with adolescents' aggression/antisociality and depression might not be due these common risk factors. Instead, results suggested that aggression/antisociality might cause later alcohol use even after controlling for relevant covariates and earlier alcohol use. Thus, the direct causal model of co-occurrence could partially account for the co-occurrence between aggression/antisociality and alcohol use. In contrast, the current data do not very clearly demonstrate why adolescents' depressive symptom might co-occur with alcohol use.

These results also have implications for improving clinical practice. Given the transdiagnostic nature of deficient self-regulation, results suggest that intervention and/or prevention programs that target self-regulatory abilities will be helpful in reducing risk for depressive symptoms, aggressive/antisocial behaviors, and possibly their co-occurrence. These types of programs might also exert downstream effects in reducing adolescents' alcohol use and later disorder by curbing earlier aggression/antisociality. Consistent with this notion, prevention programs such as the PATHS curriculum (Riggs, Greenberg, Kusché, & Pentz, 2006) and the Rochester Resilience Project Intervention (Wyman et al., 2010) that are specifically designed to enhance self-control and emotion

regulation have demonstrated promising effects, including increasing children's inhibitory control and reducing internalizing and externalizing behaviors.

Similarly, results are consistent with previous work suggesting that medications used to alter serotonergic neurotransmission might reduce psychopathological symptoms. For example, Selective Serotonin Reuptake Inhibitors (SSRIs), which serve to increase the amount of serotonin available to bind to the postsynaptic receptor, are some of the most commonly prescribed medications to treat a variety of mental health issues. SSRIs have been shown to be effective in treating depression in children and adolescents, severe depression in adults, and impulsive aggression in adolescents and adults (Bond, 2005; Cherek, Lane, Pietras, & Steinberg, 2002; Kamarck et al., 2009; Kirsch, Deacon, Huedo-Medina, Scoboria, Moore, & Johnson, 2008; Usala, Clavenna, Zuddas, Bonati, 2008). Studies also showed mild and transient effects of SSRIs in reducing alcohol consumption for moderate drinkers, but not for individuals with alcohol dependence (Litten et al., 1996).

Although very preliminary, it is possible that the methods and ideas used to create the current polygenic risk scores could have clinical utility, especially because they demonstrated predictive validity and replicability across studies. Perhaps more well-refined and comprehensive polygenic risk scores indexing serotonergic functioning could be used to identify individuals who would benefit the most from SSRI treatments. Indeed, because SSRIs lead to initial increases in serotonin neurotransmission, perhaps they would be the most effective for individuals with genetic risk for lower baseline serotonergic functioning.

Finally, findings contribute to current efforts aligned with the Alcohol Addiction Research Domain Criteria (AARDoC; Litten et al., 2015). The AARDoC seeks to better understand the considerable heterogeneity in the presentation and etiology of alcohol use disorders across individuals. To do so, AARDoC calls for future research to investigate whether several broader domains of functioning, such as reward, stress and affect regulation, incentive salience, executive function, and social processes, might differentiate among individuals with alcohol use disorder and subsequently inform the application of differential treatments. Moreover, this framework calls for an integration of multiple levels of analysis to understand these broad domains, including genetics, neuroscience, physiology, and behavior.

Although preliminary, our results suggest that serotonergic functioning might not play a role in an executive functioning domain due to its lack of association with effortful control/conscientiousness, whose definitions overlap with executive functioning. Because aggression/antisociality mediated the relation between polygenic risk and alcohol use in the current study, the present results also call for future research that examines whether 5-HT polygenic risk might be involved in other broader domains related to aggression and delinquency, such as incentive salience or reward pathways. This could help to identify the domain (i.e., dysregulated incentive salience or reward) that would require the most attention in treatment for individuals with lower levels of serotonergic functioning, and this biological phenotype might act as an early identification factor for at-risk individuals as well.

Strengths and Limitations

The current study had several limitations that should be noted. First, as stated earlier, the polygenic risk scores created in this study only contained 23 SNPs. Without a doubt, many more genetic variants and genomic processes are involved in serotonergic functioning that we were unable to include in this score. Future studies that create polygenic risk scores might consider including more SNPs as well as other forms of genetic variation, such as insertions/deletions and variable number tandem repeats. Similarly, it would be very informative for future studies to examine other genetic processes to gain the most comprehensive picture of genetic risk for serotonergic deficits, such as epigenetic mechanisms, functional human nonprotein-coding RNAs, and rare variants. Additionally, neither study used in these analyses collected measures of serotonergic functioning. Thus, we were unable to confirm whether the polygenic risk scores created to index serotonergic functioning actually captured the intended construct. A measure of serotonergic functioning would also have been useful to include in the longitudinal models. Doing so could have provided even more robust evidence that serotonergic functioning is a mechanism that underlies risk pathways to adolescents' alcohol use.

Relatedly, it will be important for future studies with larger sample sizes to replicate the effects found in this study. Both of the samples used in this study were only powered to detect between small and moderate effect sizes separately for boys and girls (the only hypothesized moderator in the study). Thus, the current samples were underpowered to detect small polygenic risk effects that differed by one or more moderating variables. The samples were also limited to adolescents who self-reported

their ethnicity to be non-Hispanic Caucasian. Although this was advantageous for several reasons (see below), it also does not address the important question of whether the same risk processes operate in individuals from other ethnic groups. This limits the generalizability of the findings and limits the advancement of knowledge and research with minority groups.

Another limitation of the study was the heavy reliance on adolescents' self-report data. Variables in the CDP model were all self-reported by adolescents except for polygenic risk. The same is true for AFDP, except that effortful control was a multi-reporter construct. Although adolescents' self-reports of depression, aggression/antisociality, and alcohol use are likely more reliable and accurate than reports made by parents (e.g., Grills & Ollendick, 2002), perhaps also including peer ratings of these constructs (particularly alcohol use) could have increased confidence in the findings. However, peer-reports of adolescents' symptoms were not available in either study.

The AFDP and CDP samples and measures were also different in several ways, which could be viewed as a limitation. For example, the AFDP sample had considerably greater age heterogeneity than the CDP. Thus, even though the mean ages at each time point were somewhat similar across samples, the age ranges were quite discrepant. Given that the effects of problem behaviors and temperament on alcohol use are developmentally sensitive, the differences in ages across samples could have accounted for the non-replication of certain paths across models. Similarly, AFDP is a high-risk sample whereas CDP is a community sample and we did not have the exact same measures across both studies. These factors also might have rendered replication more

difficult. On the other hand, Rosenbaum (2001) suggested that observing similar phenomena across different situations (i.e., replication in samples that differ) might provide even stronger evidence that the phenomena observed are real. Indeed, discovering the same effects across different situations helps to eliminate the possibility that the same third variable is causing spurious relations. Because of the differences between studies, the findings that were replicated across studies might be even more trustworthy. Thus, the presence of differences across studies has both advantages and disadvantages.

The current study also had several strengths that increase confidence in the findings. The 5-HT polygenic risk scores were created using GWAS results from an independent sample (Luykx et al., 2014). We were able to test and replicate the results from this work in two independent, longitudinal samples of adolescents. Using this approach yielded some consistent polygenic risk effects across the two studies. This is noteworthy in light of the replication difficulties that have challenged the genetic literature (Plomin et al., 2016). Similarly, it was a major strength of the study to have two very well characterized longitudinal samples of adolescents with good measurement of adolescents' problem behaviors and relevant demographic and environmental information. Thus, in both studies, adolescents' problem behaviors were assessed using well-validated and reliable measures and several potential confounding variables were controlled for, therefore increasing confidence in the present findings.

Another strength of the study was the focus on capturing genetic risk for an endophenotype of alcohol use risk. This methodology provided insight into the mechanisms underlying risk for alcohol use in adolescence. It is also more in line with

current efforts to advance personalized treatment of alcohol use disorders by understanding the unique biological and behavioral mechanisms of the disorder (e.g., AARDoC; Litten et al., 2015). The 5-HT polygenic risk scores also demonstrated predictive validity. Although it was not possible to test associations with serotonergic functioning, the5-HT polygenic risk scores related with constructs expected to be related to serotonergic functioning, such as aggression/antisociality, depression, and alcohol use. By including only non-Hispanic Caucasian adolescents, it was less likely that population stratification accounted for the results. Moreover, it was important to choose this subgroup because the independent GWAS was performed with European participants and, therefore, at least some of the genetic risk variants identified might be most salient for Caucasian populations.

A major strength of this study was the use of baseline measures of psychopathology and alcohol use as covariates so that analyses were prospective. For instance, it was possible to establish that T3 aggression/antisociality predicted change in alcohol use from T3-T4 in both studies, and that this relation was not spuriously caused by the concurrent association between T3 alcohol use and aggression.

Conclusion

The current study provided several insights into developmental risk pathways for alcohol use and other problem behaviors. This study found robust evidence that aggression/antisociality is one mechanism that underlies the well-established association between serotonergic functioning and alcohol consumption. This pathway might be especially salient for boys from economically disadvantaged homes. This study also provided evidence to suggest that individuals with lower levels of serotonin functioning

might not be predisposed towards deficits in effortful control/conscientiousness. Therefore, self-regulation is likely not the mechanism through which serotonergic functioning exerts risk for multiple different types of psychopathology. 5-HT polygenic risk scores and effortful control/conscientiousness were both risk factors for later aggression/antisociality, depression, and alcohol use (either for certain subgroups or indirectly). This suggests that these two constructs are very good candidates for future work on transdiagnostic risk factors. Clarifying the common and unique roles that these more basic risk factors play in psychopathology could aid in the creation of better diagnostic tools, advance our understanding of the etiology of psychopathology, and greatly enhance prevention and intervention efforts.

Table 1

Descriptive Statistics for Study Variables

Continuous/Count Variables	Mean	*SD*	Min.	Max.	Skewness	Kurtosis
T1 Age	12.40 (12.5)	1.66 (n/a)	10.02 (n/a)	17.27 (n/a)	0.76 (n/a)	-0.50 (n/a)
T2 Age	13.65 (14.5)	1.75 (n/a)	11.01 (n/a)	18.89 (n/a)	0.80 (n/a)	-0.30 (n/a)
T3 Age	16.69 (15.5)	2.09 (n/a)	13.14 (n/a)	20.95 (n/a)	0.18 (n/a)	-1.05 (n/a)
SES[a]	7.50 (5.27)	2.25 (1.20)	1.00 (2.00)	11.00 (7.00)	-0.58 (-0.06)	0.19 (-1.05)
Parents' Alcohol Problems[b]	n/a (0.98)	n/a (1.71)	n/a (0.00)	n/a (12.00)	n/a (3.26)	n/a (14.35)
Ancestry Factor Scores	0.54 (n/a)	0.33 (n/a)	-1.00 (n/a)	1.32 (n/a)	-0.78 (n/a)	1.80 (n/a)
Time between T1 and T2	1.27 (n/a)	0.38 (n/a)	0.10 (n/a)	3.40 (n/a)	2.95 (n/a)	11.75 (n/a)
Time between T2 and T3	2.85 (n/a)	1.04 (n/a)	0.52 (n/a)	4.64 (n/a)	-0.30 (n/a)	-0.88 (n/a)
Polygenic Risk Score	0.40 (0.46)	0.30 (0.30)	-0.36 (-0.44)	1.37 (1.47)	0.28 (0.08)	0.48 (0.43)
T1 Effortful Control (Conscientiousness)[c]	0.00 (3.49)	0.57 (0.63)	-1.54 (1.20)	1.31 (5.00)	0.09 (-0.21)	-0.57 (0.34)
T1 Aggressive/Antisocial Behavior	2.09 (2.40)	2.97 (2.49)	0.00 (0.00)	17.00 (13.00)	2.05 (1.26)	4.73 (1.33)
T1 Depressive Symptoms	3.01 (3.70)	3.25 (2.76)	0.00 (0.00)	22.00 (12.00)	2.10 (0.81)	6.32 (0.27)
T2 Aggressive/Antisocial Behavior	1.86 (2.73)	2.93 (2.93)	0.00 (0.00)	18.00 (23.00)	2.56 (2.29)	7.93 (9.69)
T2 Depressive Symptoms	2.39 (3.56)	3.21 (3.40)	0.00 (0.00)	21.00 (16.00)	2.49 (1.24)	8.84 (1.43)
T2 Alcohol Use	0.23 (0.28)	0.80 (0.65)	0.00 (0.00)	4.00 (4.00)	3.68 (2.87)	12.96 (9.55)
T3 Alcohol Use	0.74 (0.51)	1.48 (0.87)	0.00 (0.00)	7.00 (4.00)	2.00 (1.91)	3.03 (3.52)
Dichotomous Variables						
Gender	51.5% males (47.9% males) 48.5% females (52.1% females)					
Parents' SUD[b]	45.1% no parent with SUD (n/a) 54.9% parent with SUD (n/a)					
Medication Use	84.3% do not use prescription medications (n/a) 14.7% do use prescription medications (n/a)					

Note. N = 254 for AFDP and N = 348 for CDP. Descriptive statistics for AFDP presented outside of parentheses and descriptive statistics for CDP presented in parentheses. Values of "n/a" indicate that this variable was not assessed in that particular sample or that they are not relevant. [a]The SES variable used for AFDP and CDP is the highest level of parental education [b]A continuous measure of parents' lifetime alcohol problems was used for CDP analyses whereas a dichotomous measure of parents' lifetime diagnosis of alcohol or drug dependence was used for AFDP analyses. [c]AFDP assessed effortful control at T1 whereas CDP assessed conscientiousness at T1.

Table 2

Zero-Order Correlations Among AFDP Study Variables

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
1. Polygenic Risk Score	1															
2. Gender	-0.11†	1														
3. SES	-0.01	0.07	1													
4. Parents' Substance Use Disorder	0.01	-0.15*	-0.23**	1												
5. Ancestry	-0.09	0.02	0.10	-0.08	1											
6. Age	0.14*	-0.06	0.01	0.10	-0.18**	1										
7. Prescription Medication Use	-0.01	0.11†	0.03	0.09	-0.12†	0.10	1									
8. Time between T1 and T2	-0.02	-0.02	-0.04	0.10	-0.01	0.17*	0.02	1								
9. Time between T2 and T3	0.17*	-0.15*	0.07	0.003	-0.06	0.23**	0.06	-0.36**	1							
10. Effortful Control	-0.03	-0.16**	0.004	-0.12*	0.03	-0.05	-0.25**	-0.05	0.01	1						
11. T1 Aggressive/Antisocial Behaviors	0.12†	0.10	-0.12†	0.13*	-0.14*	0.33**	0.32**	0.01	0.09	-0.40**	1					
12. T1 Depressive Symptoms	0.06	-0.04	-0.01	0.10	-0.06	0.15*	0.29**	-0.07	0.16*	-0.32**	0.64**	1				
13. T2 Aggressive/Antisocial Behaviors	0.20**	0.04	-0.09	0.20**	-0.18**	0.27**	0.33**	-0.02	0.17*	-0.37**	0.66**	0.53**	1			
14. T2 Depressive Symptoms	0.24**	-0.15*	-0.03	0.20**	-0.08	0.17**	0.26**	-0.03	0.17*	-0.26**	0.38**	0.56**	0.68**	1		
15. T2 Alcohol Use	0.28**	-0.09	-0.14*	0.16*	-0.19**	0.29**	0.14*	0.11†	0.09	-0.23**	0.46**	0.28**	0.55**	0.28**	1	
16. T3 Alcohol Use	0.35**	-0.04	-0.03	0.02	-0.19**	0.35**	0.14*	0.06	0.25**	-0.18**	0.45**	0.16*	0.34**	0.22**	0.43**	1

Note. $^{\dagger}p \leq 0.10$. $^{*}p \leq 0.05$. $^{**}p \leq 0.01$. $N = 254$. Higher scores on the Polygenic Risk Score represent lower concentrations of CSF 5-HIAA. Gender is coded 0 for Females and 1 for Males. Parents' Substance Use Disorder is coded 0 for parents without SUD and 1 for parents with SUD. Higher levels of ancestry indicate greater levels of Caucasian ancestry. Higher levels of all other variables indicate higher levels of the construct.

Table 3

Zero-Order Correlations Among CDP Study Variables

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
1. Polygenic Risk Score	1										
2. Gender	-0.003	1									
3. SES	0.03	0.10	1								
4. Parents' Alcohol Problems	-0.02	-0.03	-0.10†	1							
5. T1 Conscientiousness	-0.03	-0.13*	-0.004	-0.02	1						
6. T1 Aggressive/Antisocial Behavior	0.10†	0.16*	-0.09	0.16*	-0.14*	1					
7. T1 Depressive Symptoms	0.09	-0.12†	-0.04	0.04	-0.17**	0.35**	1				
8. T2 Aggressive/Antisocial Behavior	-0.01	0.13*	-0.06	0.01	-0.12†	0.36**	0.15*	1			
9. T2 Depressive Symptoms	0.06	-0.21**	-0.08	0.04	-0.17**	0.13*	0.46**	0.43**	1		
10. T2 Alcohol Use	-0.03	0.00	0.01	-0.03	-0.03	0.20**	0.05	0.47**	0.18**	1	
11. T3 Alcohol Use	-0.12*	-0.10†	0.09	0.11†	0.01	0.06	0.03	0.28**	0.15*	0.37**	1

Note. $^{\dagger}p \leq 0.10$. $^{*}p \leq 0.05$. $^{**}p \leq 0.01$. $N = 348$. Higher scores on the Polygenic Risk Score represent lower concentrations of CSF 5-HIAA. Gender is coded 0 for Females and 1 for Males. Higher levels of all other variables indicate higher levels of the construct.

Table 4

Single Nucleotide Polymorphisms (SNPs) Included in the AFDP and CDP Polygenic Risk Scores

SNP	**Gene**	***p*-value**[a]
rs4863731	MAML3	0.001815
rs1544623	NRXN3	0.003153
rs9847748	FAM19A4	0.003487
rs5753625	EIF4ENIF1	0.01117
rs7219247	GRIN2C	0.01206
rs6494212	CHRNA7	0.01219
rs2823662	MIR99AHG	0.01595
rs4953262	PRKCE	0.01805
rs135757	CSNK1E	0.0192
rs636842	AVEN	0.0192
rs2611605	CHRNA7	0.01929
rs1799971	OPRM1	0.0225
rs760288	NRXN3	0.02268
rs2236256	IPFCEF1	0.02273
rs2272381	OPRM1	0.02965
rs1931059	DLGAP3	0.03011
rs782444	MGLL	0.03569
rs1869237	NRP1	0.03846
rs10213647	NPY2R	0.04387
rs4782262	GRIN2A	0.04626
rs1861957	NRXN3	0.04736
rs1426223	GABRB3	0.04805
rs1151523	FOSL1	0.04948

Note. [a]The *p*-value of each SNP in the discovery GWAS (i.e., Luykx et al., 2014).

Table 5

Comparison of the Relative Model Fit Indices to Determine the Appropriate Modeling Strategy for Alcohol Use Outcomes

	AFDP Alcohol Use Outcome			
	Categorical	Continuous	Zero-Inflated Negative Binomial	Zero-Inflated Poisson
Akaike Information Criteria	3357.05	3684.53	3388.92	3392.03
Bayesian Information Criteria	3470.24	3780.04	3487.97	3487.54
Loglikelihood	-1646.52	-1815.27	-1666.46	-1669.02
	CDP Alcohol Use Outcome			
	Categorical	Continuous	Zero-Inflated Negative Binomial	Zero-Inflated Poisson
Akaike Information Criteria	3669.14	3841.41	3878.43	3876.42
Bayesian Information Criteria	3711.45	3876.03	3916.89	3911.04
Loglikelihood	-1823.57	-1911.71	-1929.21	-1929.21

Table 6

AFDP Final Model

	Outcomes				
	First Block				
	T1 Effortful Control	T2 Depressive Symptoms	T2 Aggressive/ Antisocial Behaviors	T3 Alcohol Use	
Predictors	β(*SE*)	β(*SE*)	β(*SE*)	β(*SE*)	*OR*
Ancestry	-0.01(0.06)	-0.01(0.07)	-0.07(0.07)	-0.05(0.07)	0.66
Gender	-0.16(0.05)**	-0.13(0.06)*	-0.03(0.04)	-0.04(0.08)	0.80
Age	-0.02(0.07)	0.04(0.07)	0.04(0.06)	0.31(0.08)**	1.62
Parents' SUD	-0.13(0.06)*	0.12(0.06)*	0.10(0.04)*	0.16(0.09)†	2.27
Parents' Education	-0.003(0.08)	0.03(0.04)	-0.01(0.04)	-0.15(0.09)	0.84
Prescription Medication Use	-0.22(0.06)**	0.12(0.05)*	0.11(0.07)†	0.01(0.08)	0.09
Time 1→Time 2	--	-0.02(0.03)	-0.04(0.03)	--	--
Time 2→Time 3	--	--	--	0.22(0.08)*	1.71
Polygenic Risk Score	-0.05(0.06)	0.19(0.05)**	0.11(0.05)*	0.24(0.09)*	8.06
T1 Effortful Control	--	-0.09(0.04)*	-0.10(0.04)*	-0.06(0.09)	0.75
T1 Depressive Symptoms	--	0.42(0.08)**	--	--	--
T1 Aggressive/ Antisocial Behaviors	--	--	0.53(0.06)**	--	--
T2 Depressive Symptoms	--	--	--	-0.17(0.13)	0.87
T2 Aggressive/ Antisocial Behaviors	--	--	--	0.23(0.09)*	1.23
T2 Alcohol Use	--	--	--	0.11(0.11)	1.42
	Second Block				
T2 Depression-by-SES	--	--	--	0.16(0.05)*	--
T2 Depression-by-Ancestry	--	--	--	0.14(0.05)*	--

Note. $^{\dagger}p \leq 0.10$. $^{*}p \leq 0.05$. $^{**}p \leq 0.01$. $N = 254$. Higher scores on the Polygenic Risk Score represent lower concentrations of CSF 5-HIAA. Gender is coded 0 for Females and 1 for Males. Parents' SUD is coded 0 for parents without SUD and 1 for parents with SUD. Higher levels of ancestry indicate greater levels of Caucasian ancestry. Higher levels of all other variables indicate higher levels of the construct.

Table 7

CDP Final Model

	Outcomes				
	First Block				
	T1 Conscientiousness	T2 Depressive Symptoms	T2 Aggressive/ Antisocial Behaviors	T3 Alcohol Use	
Predictors	β(*SE*)	β(*SE*)	β(*SE*)	β(*SE*)	*OR*
Gender	-0.13(0.06)*	-0.17(0.06)*	0.06(0.06)	-0.18(0.08)*	0.49
Parents' Education	0.01(0.06)	-0.04(0.05)	-0.02(0.06)	0.17(0.07)*	1.33
Parents' Alcohol Problems	-0.02(0.09)	0.01(0.05)	-0.04(0.05)	0.13(0.07)*	1.17
Polygenic Risk Score	-0.03(0.06)	0.02(0.05)	-0.02(0.05)	-0.12(0.07)†	0.43
T1 Conscientiousness	--	-0.12(0.05)*	-0.05(0.05)	-0.02(0.06)	0.95
T1 Depressive Symptoms	--	0.40(0.05)**	--	--	--
T1 Aggressive/ Antisocial Behaviors	--	--	0.34(0.07)**	--	--
T2 Depressive Symptoms	--	--	--	0.03(0.08)	1.02
T2 Aggressive/ Antisocial Behaviors	--	--	--	0.11(0.12)	1.08
T2 Alcohol Use	--	--	--	0.31(0.08)**	2.61
	Second Block				
Polygenic Risk-by-SES	--	-0.15(0.05)**	-0.18(0.05)**	--	--
Polygenic Risk-by-Gender	--	0.03(0.06)	0.04(0.06)	--	--
Polygenic Risk-by-Parents' Alcohol Problems	--	-0.06(0.06)	-0.07(0.05)	--	--
SES-by-Gender	--	-0.02(0.05)	-0.03(0.06)	--	--
SES-by-Parents' Alcohol Problems	--	-0.02(0.08)	0.04(0.05)	--	--
Conscientiousness-by-SES	--	0.18(0.05)**	0.11(0.04)*	--	--
T2 Depression-by-Gender	--	--	--	-0.17(0.08)*	--
T2 Aggressive/ Antisocial-by-Gender	--	--	--	0.20(0.09)*	--

Note. $^{\dagger}p \leq 0.10$. $^{*}p \leq 0.05$. $^{**}p \leq 0.001$. $N = 348$. Higher scores on the Polygenic Risk Score represent lower concentrations of CSF 5-HIAA. Gender is coded 0 for Females and 1 for Males. Higher levels of all other variables indicate higher levels of the construct. Gray text refers to interaction terms included based on recommendations by Keller (2014).

Table 8

Moderated Mediation Coefficients for AFDP Model

	Mediational Chain	
Combinations of Moderators	Polygenic Risk→Depressive Symptoms→Alcohol Use	
	a	*b*
Low Levels of SES	**0.15(0.06)***	**-0.39(0.11)****
Mean Levels of SES	**0.19(0.05)****	**-0.22(0.08)***
High Levels of SES	0.23(0.07)**	-0.03(0.08)
Low Levels of Ancestry	**0.16(0.06)***	**-0.32(0.09)****
Mean Levels of Ancestry	**0.20(0.06)***	**-0.22(0.08)***
High Levels of Ancestry	0.24(0.08)**	-0.12(0.08)
	Effortful Control→Depressive Symptoms→Alcohol Use	
	a	*b*
Low Levels of SES	**-0.13(0.06)***	**-0.39(0.11)****
Mean Levels of SES	**-0.10(0.04)***	**-0.22(0.08)***
High Levels of SES	-0.09(0.05)†	-0.03(0.08)
Low Levels of Ancestry	-0.08(0.07)	-0.32(0.09)**
Mean Levels of Ancestry	**-0.10(0.04)***	**-0.22(0.08)***
High Levels of Ancestry	-0.11(0.06)*	-0.12(0.08)

Note. †$p < 0.10$. *$p < 0.05$. **$p < 0.001$. $N = 254$. Standardized regression coefficients are shown. Standard errors are shown in parentheses. Low, mean and high SES and ancestry refer to 1 *SD* below the mean, at the mean, and 1 *SD* above the mean of SES and ancestry, respectively. Bolded terms refer to a significant mediated effect.

Table 9

Moderated Mediation Coefficients for CDP Model

	Mediational Chain	
	Polygenic Risk→Aggressive/Antisocial→Alcohol Use	
Combinations of Moderators	*a*	*b*
Females with Low SES	0.13(0.09)	-0.02(0.16)
Females with Mean SES	-0.05(0.09)	-0.05(0.12)
Females with High SES	-0.24(0.12)*	-0.08(0.13)
Males with Low SES	**0.21(0.09)***	**0.35(0.15)***
Males with Mean SES	0.03(0.06)	0.34(0.13)*
Males with High SES	**-0.15(0.07)***	**0.31(0.14)***
	Polygenic Risk→Depressive Symptoms→Alcohol Use	
	a	*b*
Females with Low SES	0.14(0.09)	0.16(0.11)
Females with Mean SES	-0.01(0.09)	0.16(0.09)†
Females with High SES	-0.18(0.11)†	0.16(0.14)
Males with Low SES	0.23(0.08)*	-0.19(0.15)
Males with Mean SES	0.07(0.06)	-0.17(0.15)
Males with High SES	-0.09(0.06)	-0.19(0.19)
	Conscientiousness→Aggressive/Antisocial→Alcohol Use	
	a	*b*
Females with Low SES	-0.12(0.08)	-0.02(0.16)
Females with Mean SES	-0.05(0.06)	-0.05(0.12)
Females with High SES	0.05(0.05)	-0.08(0.13)
Males with Low SES	**-0.17(0.07)***	**0.35(0.15)***
Males with Mean SES	-0.10(0.06)	0.34(0.13)*
Males with High SES	-0.01(0.07)	0.31(0.14)*
	Conscientiousness→Depressive Symptoms→Alcohol Use	
	a	*b*
Females with Low SES	-0.32(0.09)**	0.16(0.11)
Females with Mean SES	-0.21(0.08)*	0.16(0.09)†
Females with High SES	-0.04(0.10)	0.16(0.14)
Males with Low SES	-0.24(0.07)**	-0.19(0.15)
Males with Mean SES	-0.10(0.05)†	-0.17(0.15)
Males with High SES	0.08(0.06)	-0.19(0.19)

Note. †$p < 0.10$. *$p < 0.05$. $N = 348$. Standardized regression coefficients are shown. Standard errors are shown in parentheses. Low, mean and high SES refer to 1 *SD* below the mean, at the mean, and 1 *SD* above the mean of SES, respectively. Bolded terms refer to a significant mediated effect.

Table 10

Side-by-Side Comparison of AFDP and CDP Main Paths of Interest

	AFDP	CDP
Polygenic Risk→ Self-Regulation[a]	**ns**	**ns**
Polygenic Risk → Depression	**+**	**+ (at low SES)**
		- (at high SES)
Polygenic Risk → Aggression/Antisociality	**+**	**+ (at low SES)**
		- (at high SES)
Polygenic Risk → Alcohol Use	+	- (marginal)
Self-Regulation → Depression	**-**	**- (at low and mean SES)**
		ns (at high SES)
Self-Regulation → Aggression/Antisociality	**-**	**- (at low SES)**
Self-Regulation → Alcohol Use	**ns**	**ns**
Depression → Alcohol Use	- (low and mean SES and ancestry)	+ (marginally for girls)
Aggression/Antisociality→ Alcohol Use	**+**	**+ (for boys)**

Note. [a]Self-regulation refers to effortful control in AFDP and to conscientiousness in CDP. Bolded text in black print refers to effects that were replicated in both studies. '+' indicates that the effect is positive. '-' indicates that the effect is negative. 'ns' indicates that the effect is not statistically significant. Gray text refers to effects that were not replicated across studies.

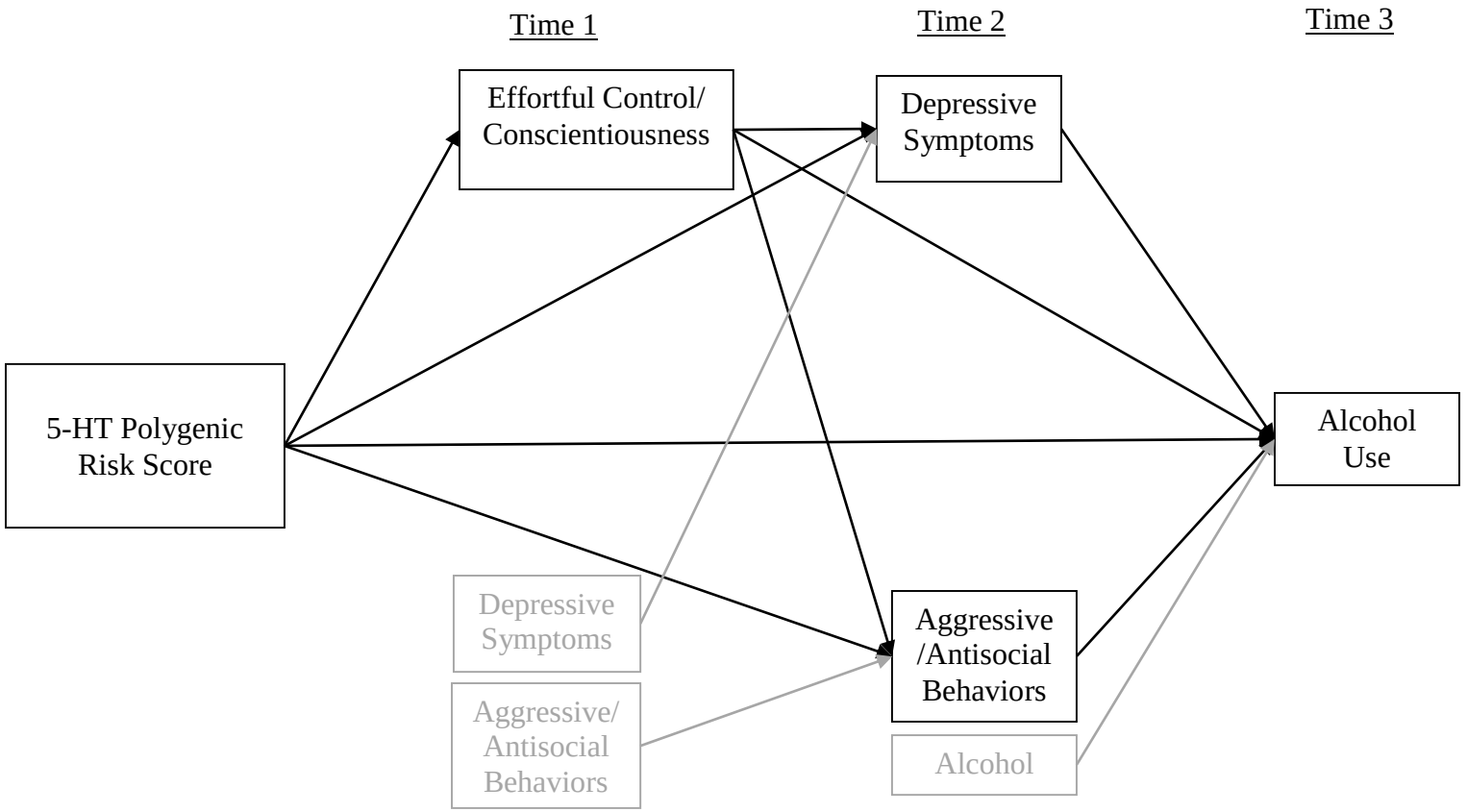

Figure 1. Hypothesized Model. For AFDP, Time 1 = 10-17.99 years old, Time 2 = 11-18.99 years old, and Time 3 = 13-20.99 years old. For CDP, Time 1 = 12-13 years old, Time 2 = 14-15 years old, and Time 3 = 15-16 years old. For CDP, a measure of conscientiousness was used to reflect effortful control. Gray boxes and lines: baseline control variables. All other covariates not shown for ease of presentation. Correlations were estimated among all exogenous variables, among T1 depressive symptoms, aggressive/antisocial behaviors and effortful control/conscientiousness, and among T2 depressive symptoms, aggressive/antisocial behaviors and alcohol use (not shown here). Refer to Methods section for more details about the structural equation modeling.

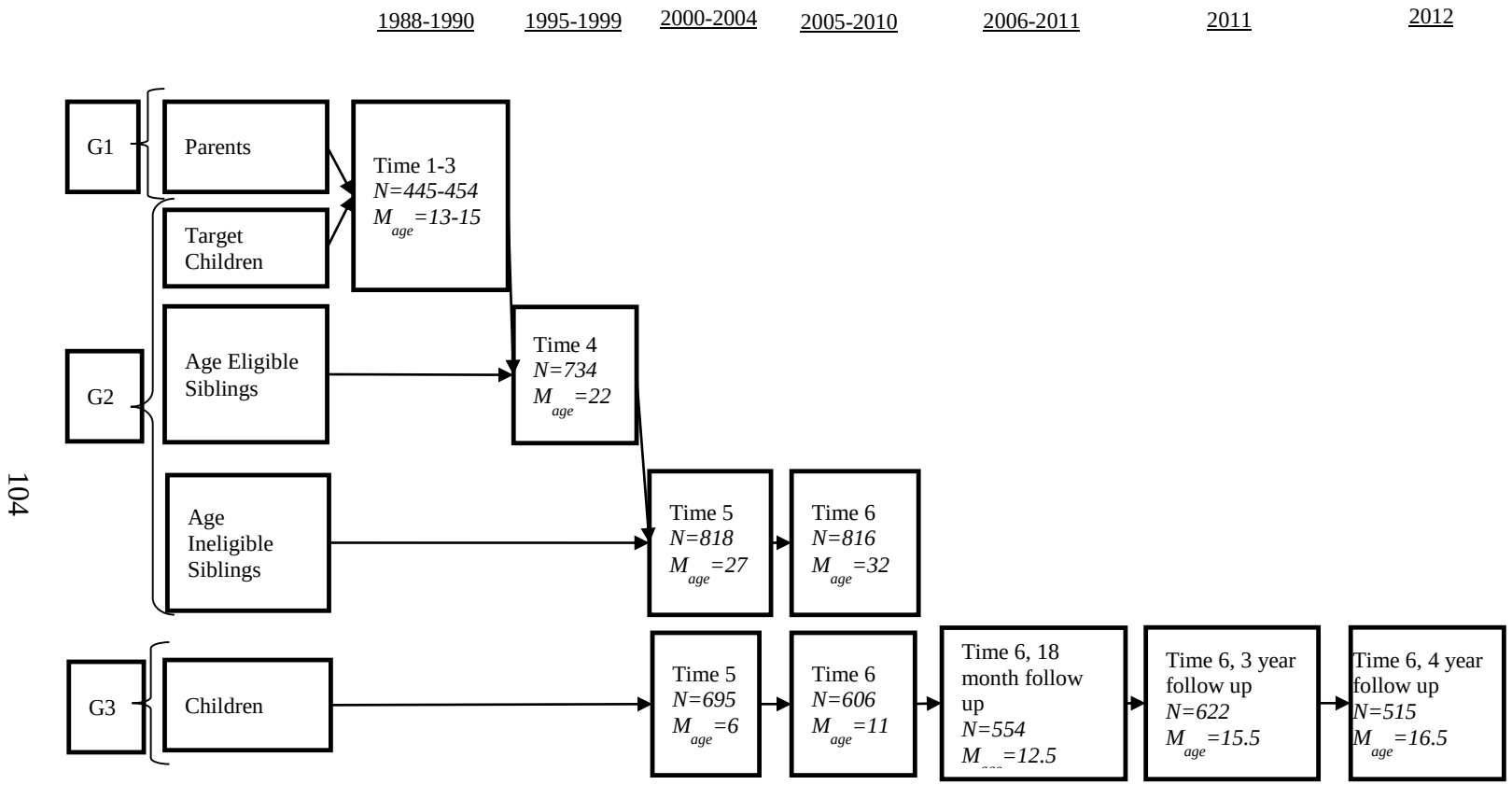

Figure 2. Visual Display of the AFDP Data Collection Timeline. G1: Generation 1. G2: Generation 2. G3: Generation 3.

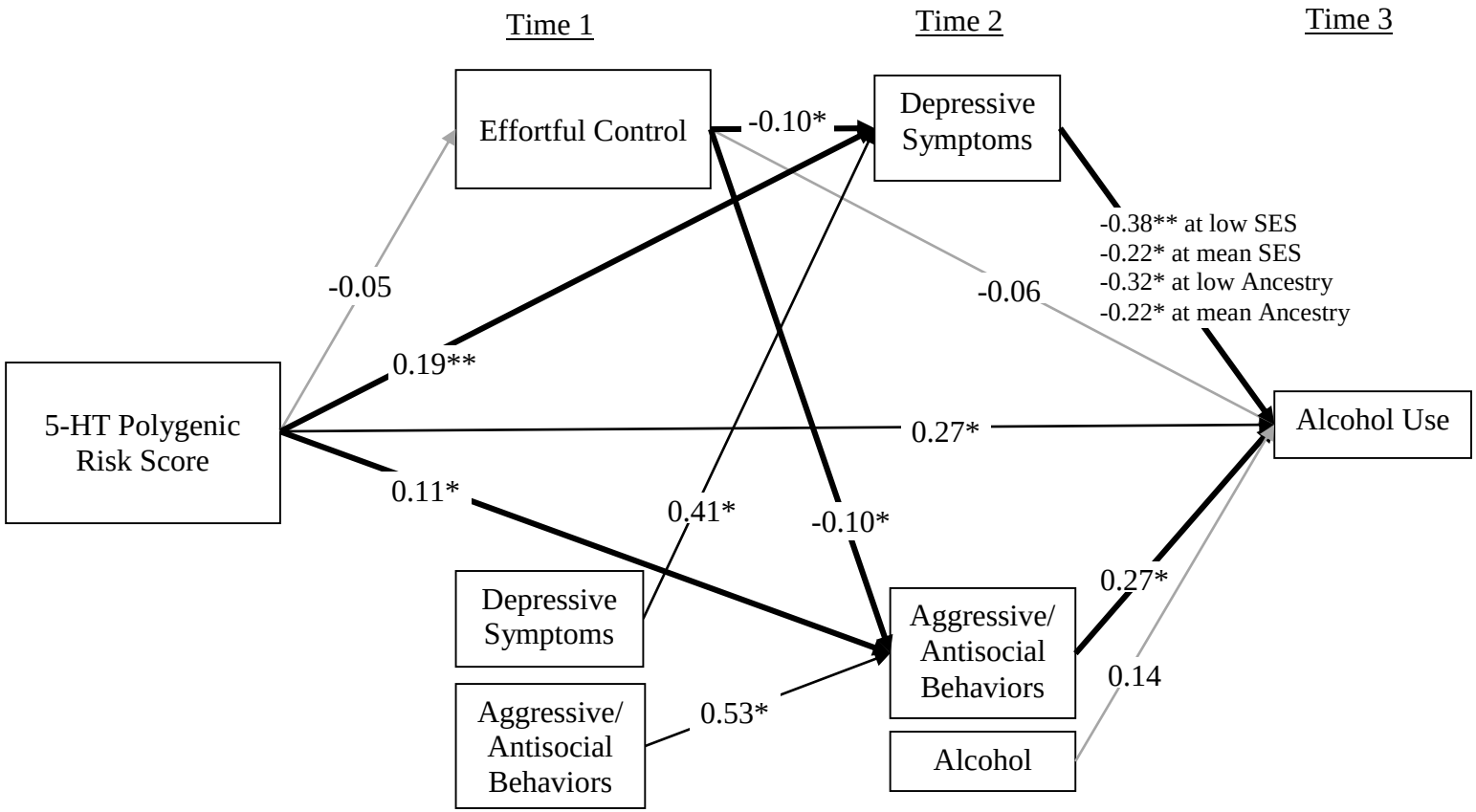

Figure 3. AFDP Final Path Model. $^{\dagger}p < 0.10$. $*p < 0.05$. $N = 254$. Time 1 = 10-17.99 years old, Time 2 = 11-18.99 years old, and Time 3 = 13-20.99 years old. Grayed lines: non-significant paths. Black lines: significant or marginally significant paths. Bolded lines: paths involved in significant mediated effects. All other covariates not shown for ease of presentation. Correlations were estimated among all exogenous variables, among T1 depressive symptoms, aggressive/antisocial behaviors and effortful control, and among T2 depressive symptoms, aggressive/antisocial behaviors and alcohol use (not shown here). 5-HT Polygenic Risk Score coded such that higher scores represent lower levels of CSF 5-HIAA (i.e., lower levels of serotonin functioning).

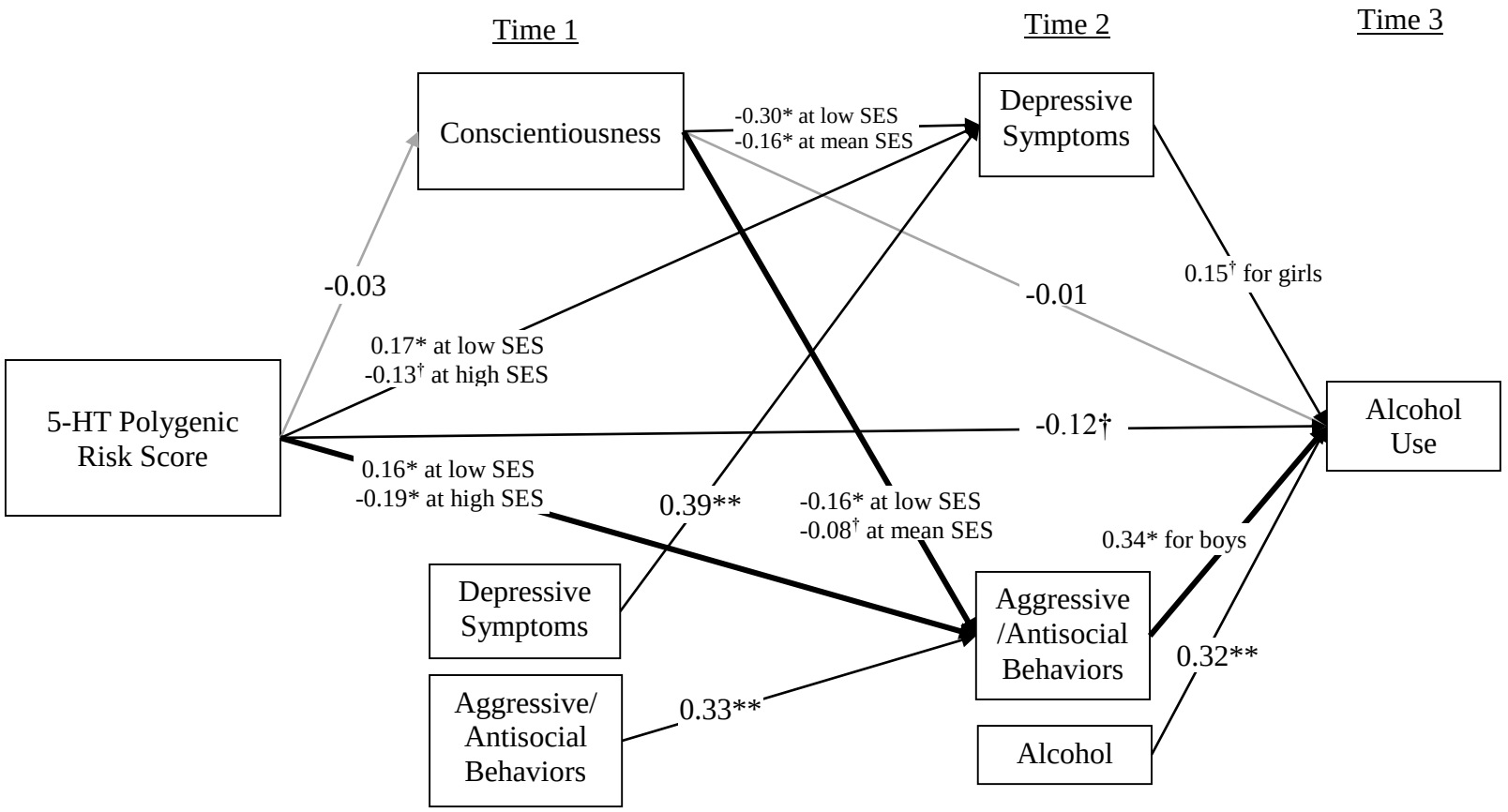

Figure 4. CDP Final Path Model. †$p < 0.10$. *$p < 0.05$. **$p < 0.001$. $N = 348$. Time 1 = 12-13 years old, Time 2 = 14-15 years old, and Time 3 = 15-16 years old. Grayed lines: non-significant paths. Black lines: significant or marginally significant paths. All other covariates not shown for ease of presentation. Bolded lines: paths involved in significant mediated effects. Correlations were estimated among all exogenous variables, among T1 depressive symptoms, aggressive/antisocial behaviors and conscientiousness, and among T2 depressive symptoms, aggressive/antisocial behaviors and alcohol use (not shown here). 5-HT Polygenic Risk Score coded such that higher scores represent lower levels of CSF 5-HIAA (i.e., lower levels of serotonin functioning).

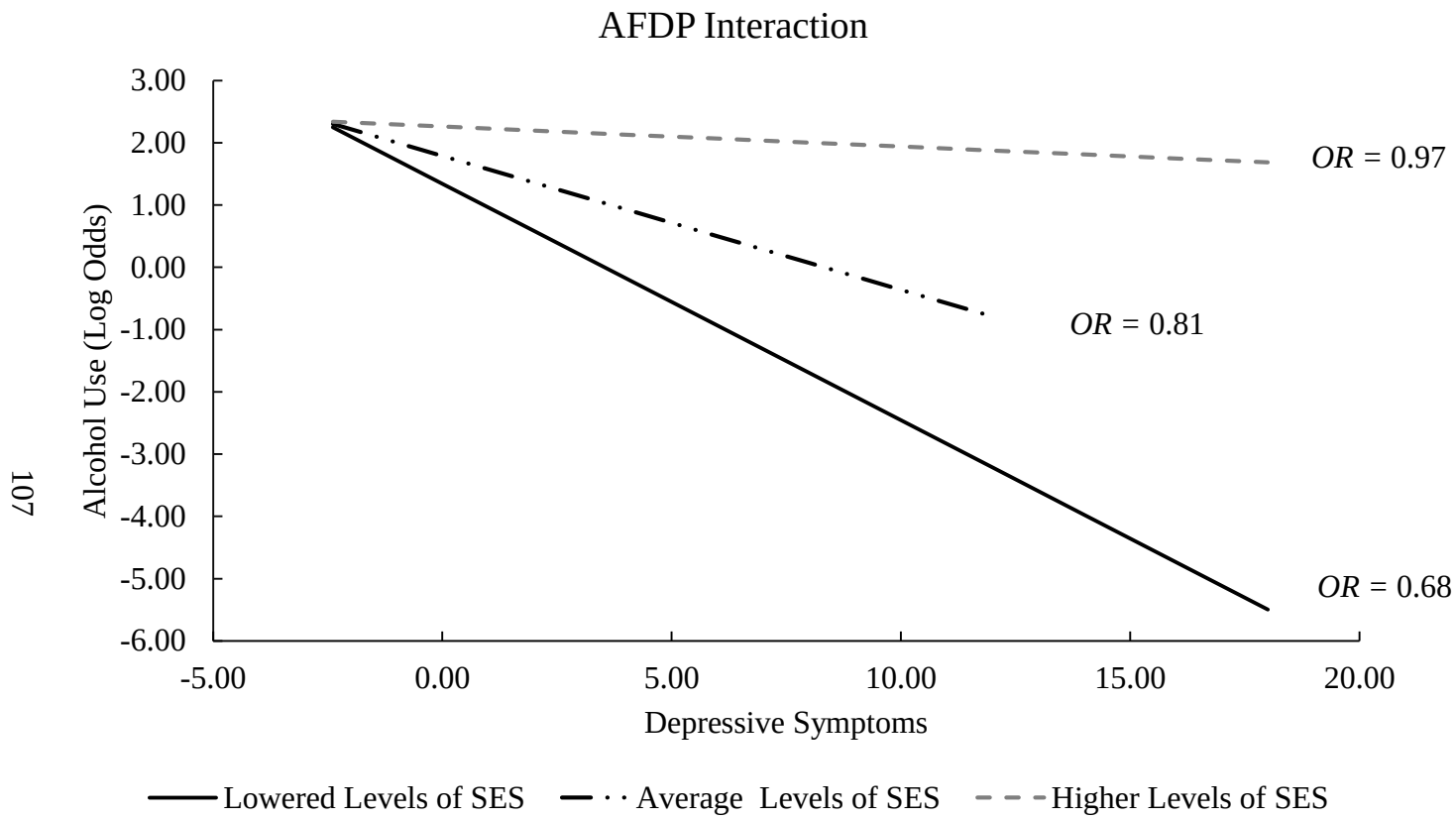

Figure 5. AFDP Interaction between Depressive Symptoms and Socioeconomic Status in Predicting Alcohol Use. Black lines indicate statistically significant simple slopes. Slopes probed at low and average levels of socioeconomic status are statistically significant. Simple slopes represent combinations of socioeconomic status and depressive symptoms that actually exist in the data.

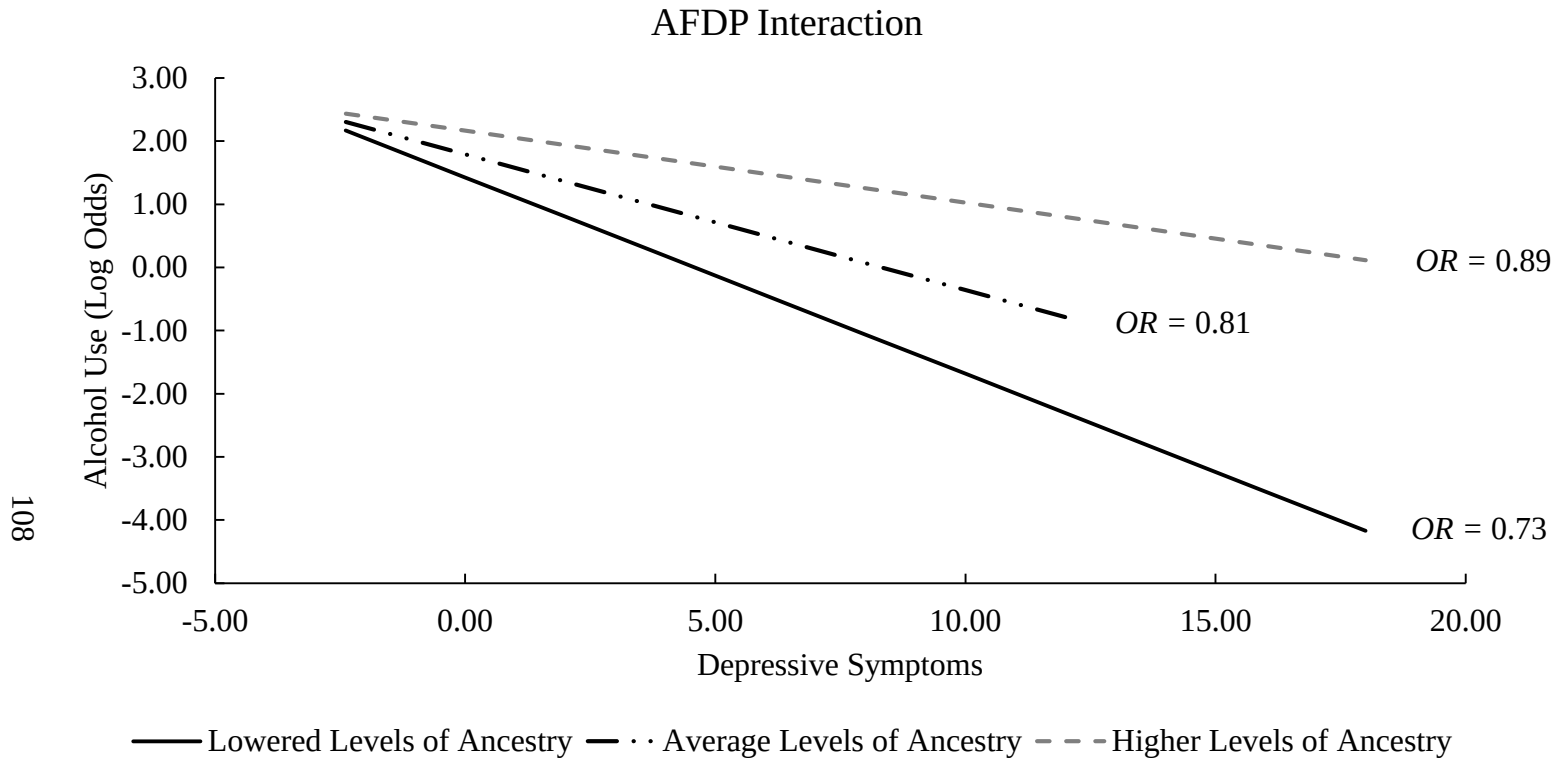

Figure 6. AFDP Interaction between Depressive Symptoms and Ancestry in Predicting Alcohol Use. Black lines indicate statistically significant simple slopes. Slopes probed at low and average levels of ancestry are statistically significant. Simple slopes represent combinations of ancestry and depressive symptoms that actually exist in the data.

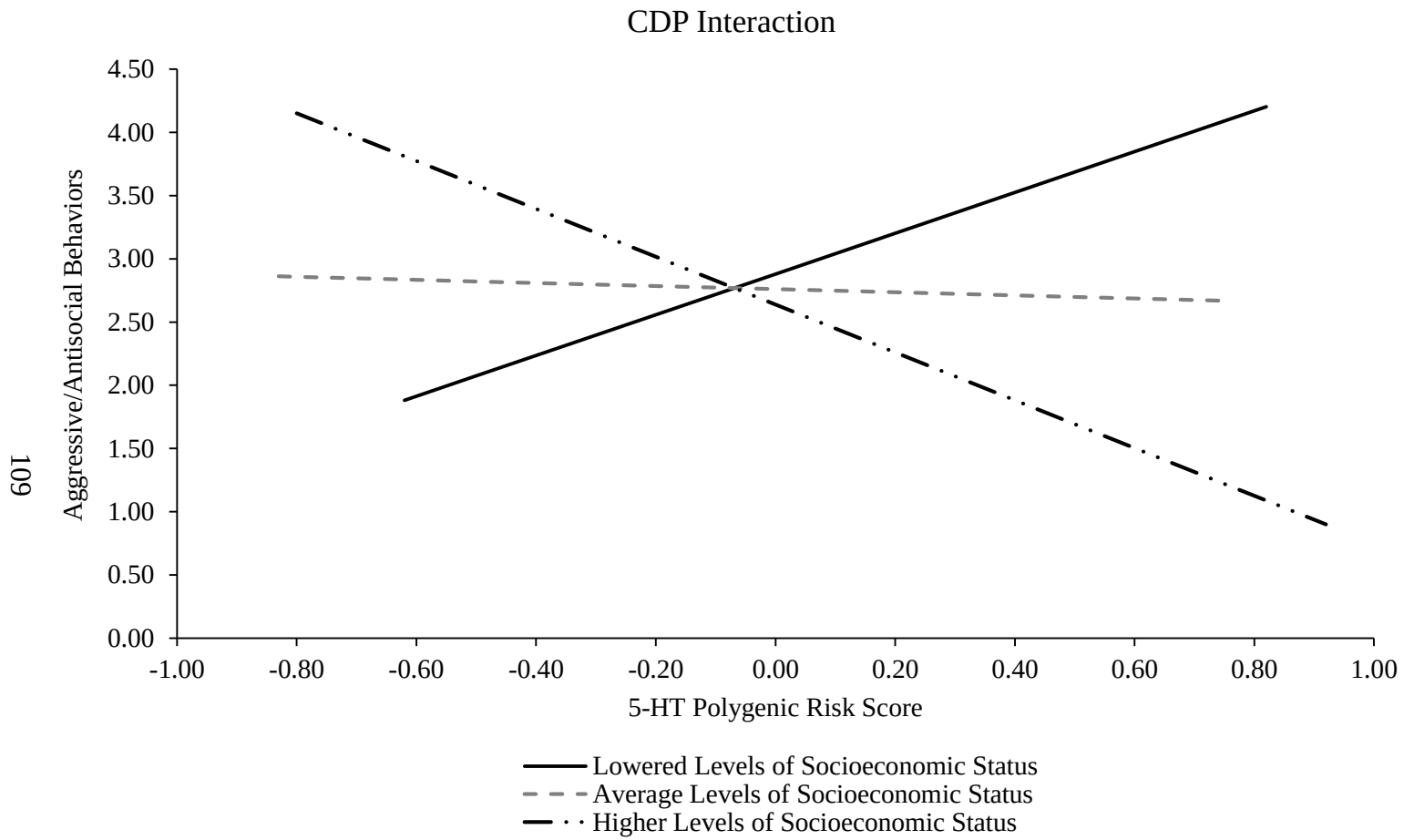

Figure 7. CDP Interaction between Polygenic Risk and Socioeconomic Status in Predicting Aggressive/Antisocial Behaviors. Black lines indicate statistically significant simple slopes. Slopes probed at low and high levels of socioeconomic status are statistically significant. Simple slopes represent combinations of socioeconomic status and polygenic risk that actually exist in the data.

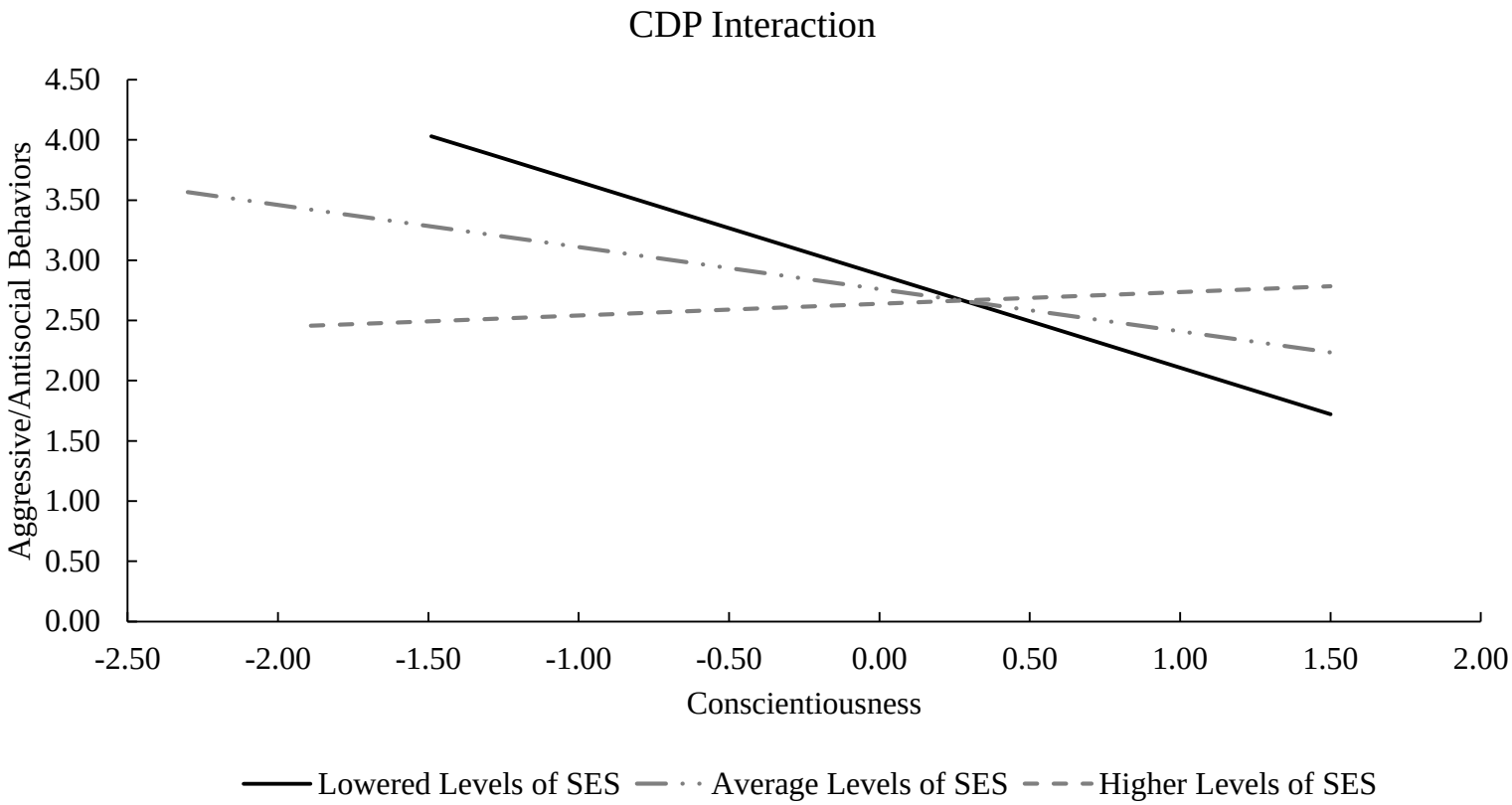

Figure 8. CDP Interaction between Conscientiousness and Socioeconomic Status in Predicting Aggressive/Antisocial Behaviors. Black lines indicate statistically significant simple slopes. Slope probed at low levels of socioeconomic status are statistically significant. Simple slopes represent combinations of socioeconomic status and conscientiousness that actually exist in the data.

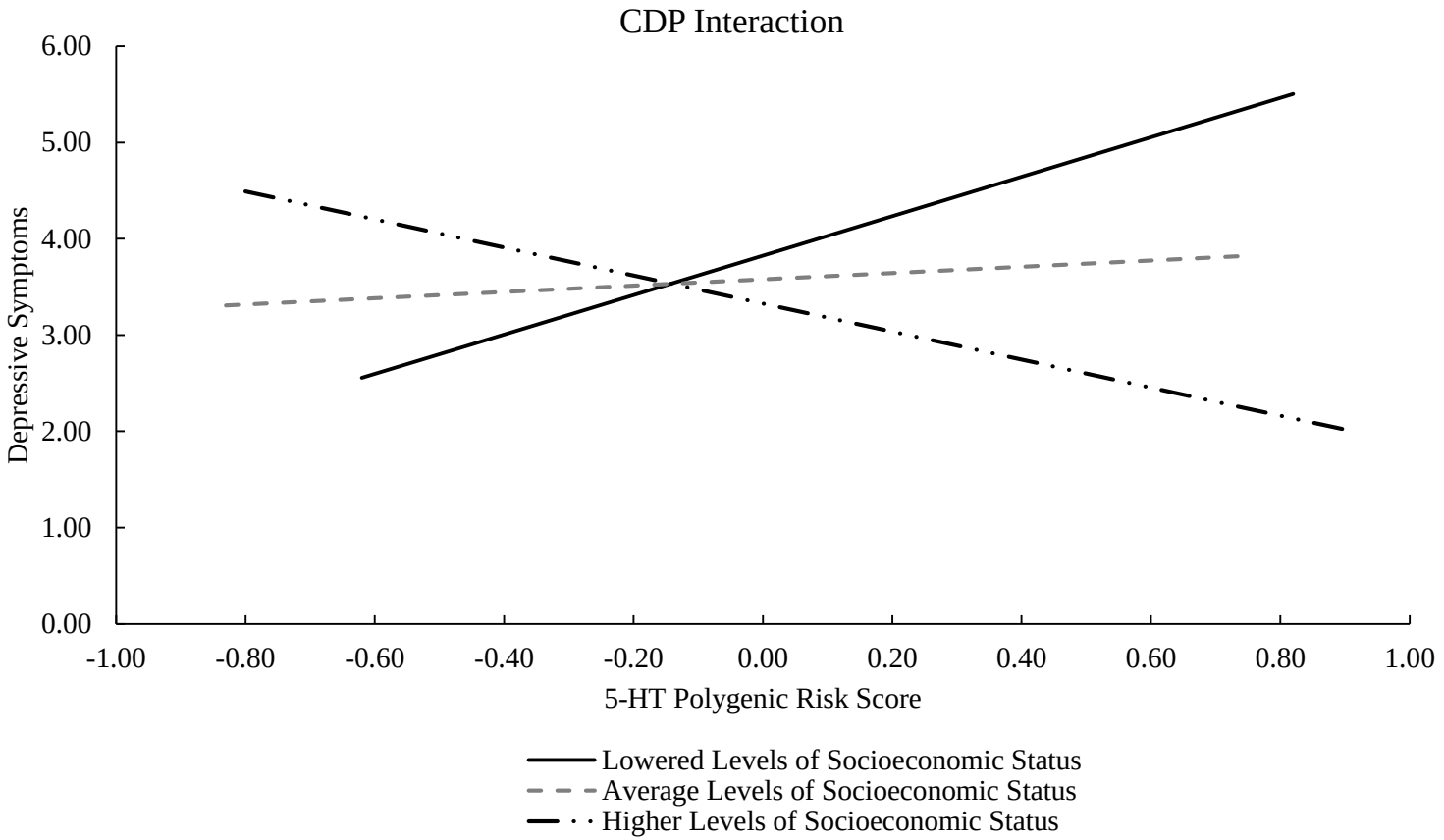

Figure 9. CDP Interaction between Polygenic Risk and Socioeconomic Status in Predicting Depressive Symptoms. Black lines indicate statistically significant simple slopes. Slopes probed at low and high levels of socioeconomic status are statistically significant. Simple slopes represent combinations of socioeconomic status and polygenic risk that actually exist in the data.

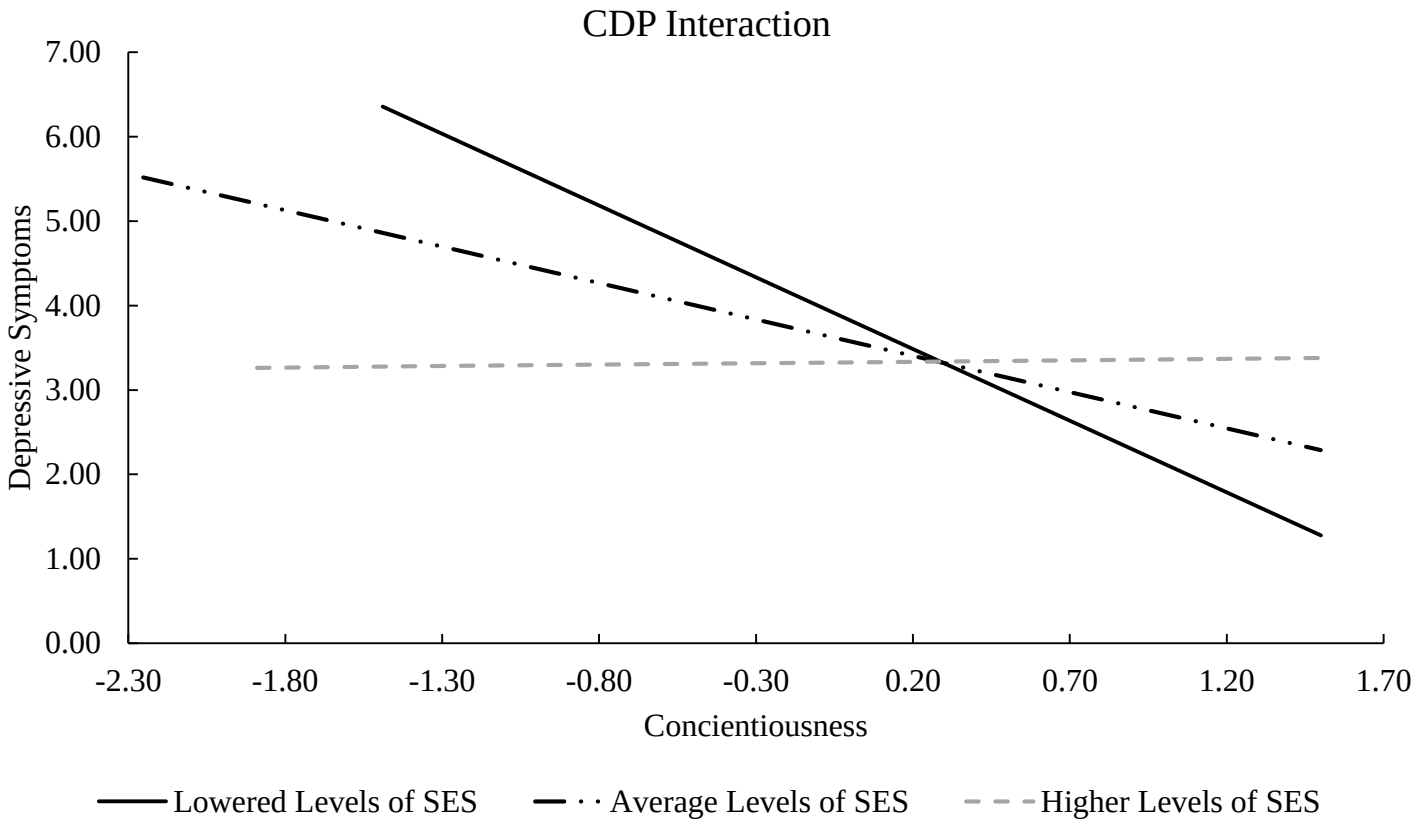

Figure 10. CDP Interaction between Conscientiousness and Socioeconomic Status in Predicting Depressive Symptoms. Black lines indicate statistically significant simple slopes. Slopes probed at low and mean levels of socioeconomic status are statistically significant. Simple slopes represent combinations of socioeconomic status and conscientiousness that actually exist in the data.

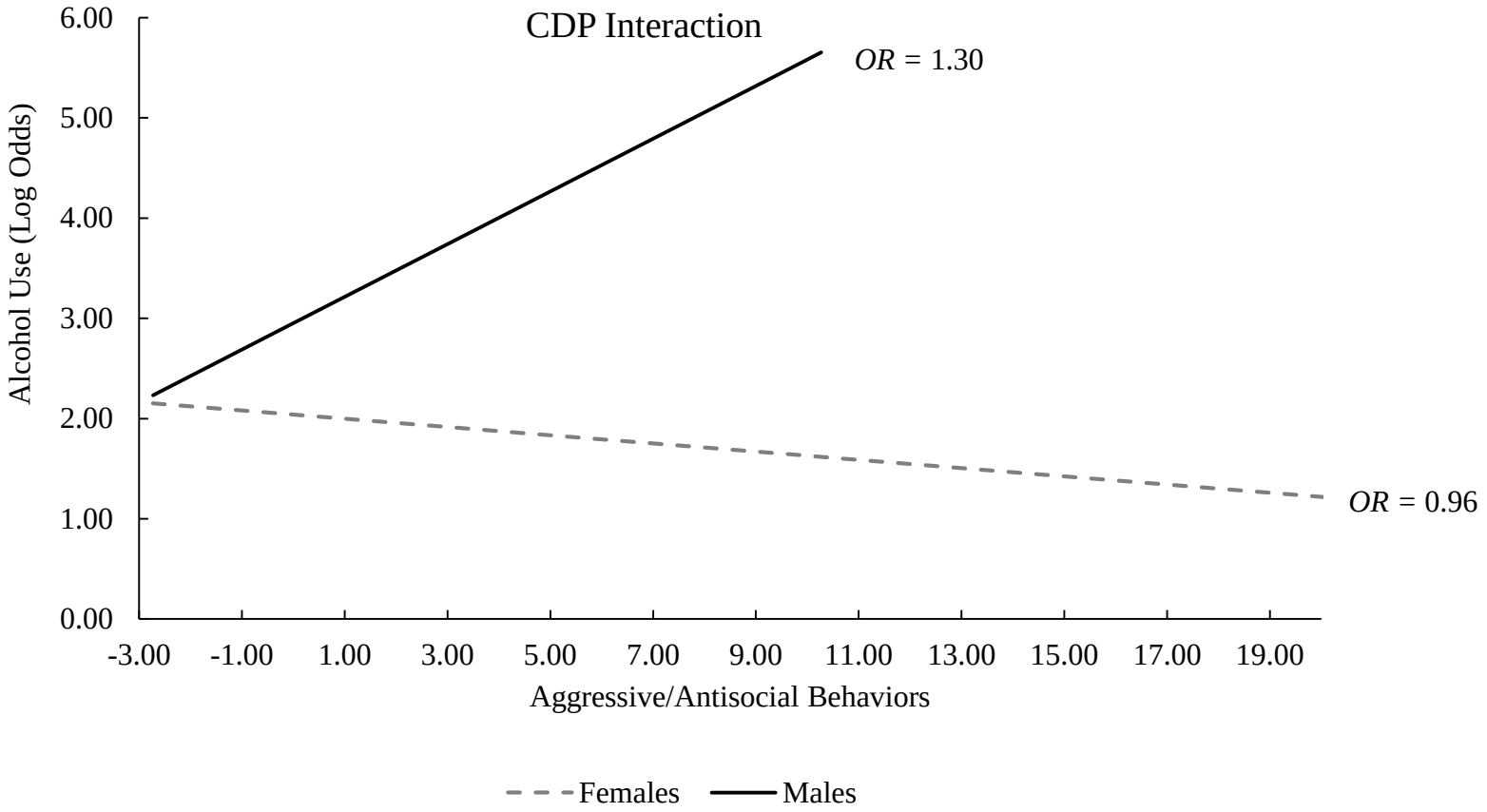

Figure 11. CDP Interaction between Aggressive/Antisocial Behaviors and Gender in Predicting Alcohol Use. Black line indicates statistically significant simple slope. The simple slope for males is statistically significant. Simple slopes represent values of aggressive/antisocial behaviors that actually exist in the data for males or for females.

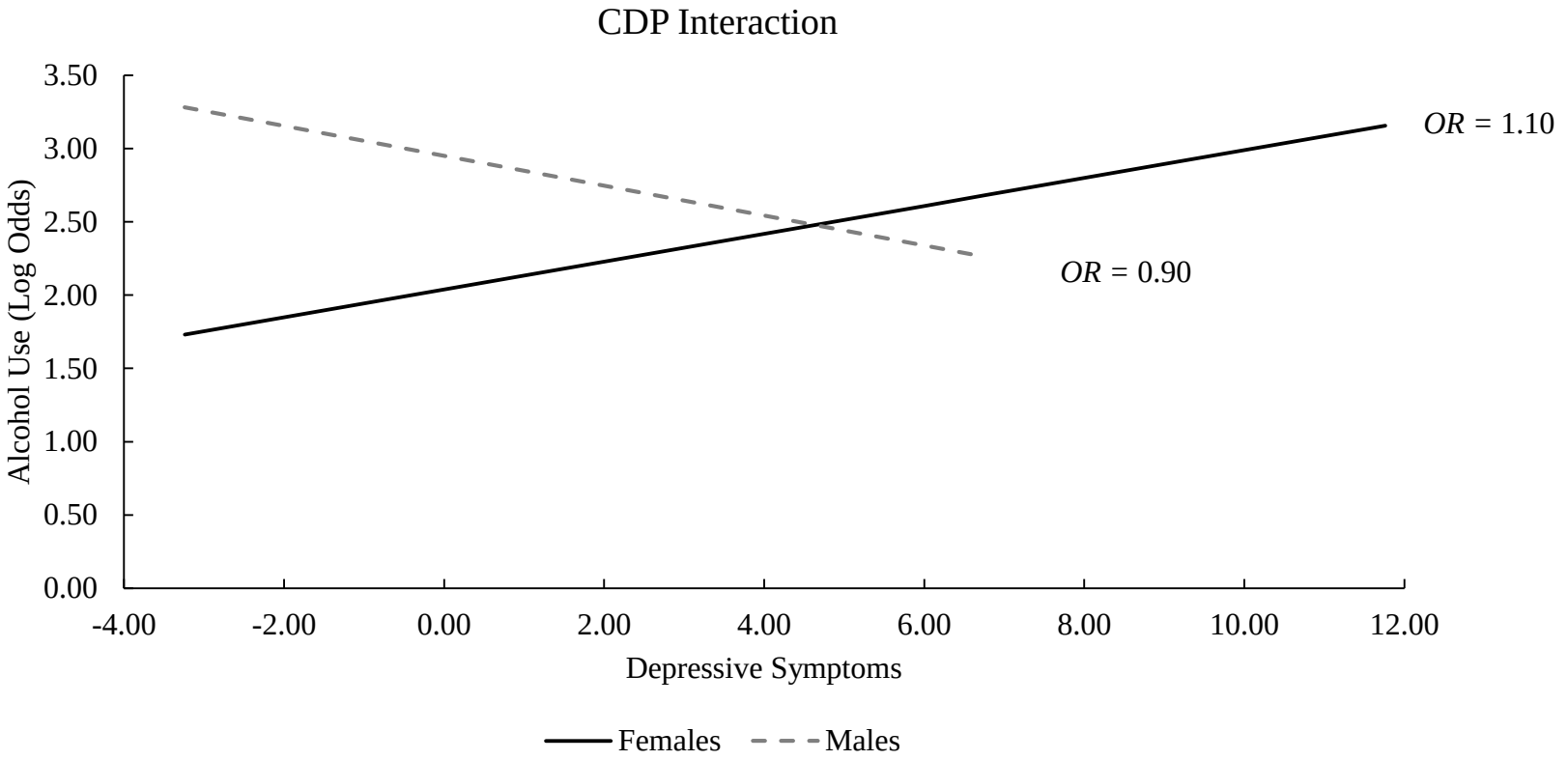

Figure 12. CDP Interaction between Depressive Symptoms and Gender in Predicting Alcohol Use. Black line indicates statistically significant simple slope. The simple slope for females is statistically significant. Simple slopes represent values of depressive symptoms that actually exist in the data for males or for females.

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APPENDIX A

AFDP EFFORTFUL CONTROL ITEMS

Inhibitory Control

1. It's hard for me not to open presents before I'm supposed to.
2. When someone tells me to stop doing something, it is easy for me to stop.
3. The more I try to stop myself from doing something I shouldn't, the more likely I am to do it.
4. It's easy for me to keep a secret.
5. I can stick with my plans and goals.

Attentional Control

1. It is easy for me to really concentrate on homework problems.
2. I find it hard to shift gears when I go from one class to another at school.
3. When trying to study, I have difficulty tuning out background noise and concentrating.
4. I am good at keeping track of several different things that are happening around me.
5. I pay close attention when someone tells me how to do something.
6. I tend to get in the middle of one thing, then go off and do something else.

Activation Control

1. I have a hard time finishing things on time.
2. I do something fun for awhile before starting my homework, even when I'm not supposed to.
3. If I have a hard assignment to do, I get started right away.
4. I finish my homework before the due date.
5. I put off working on projects until right before they're due.

Note. These items were the same for parents but instead of the stem "I..." the stem was "[Child's Name]…"

APPENDIX B

CDP CONSCIENTIOUSNESS ITEMS

1. How organized do you think you are?
2. How lazy do you think you are? (reverse)
3. Some kids are very responsible, they can be counted on to do what they are told to do, but other kids are not very responsible, they often do not remember what they were told to do. How responsible do you think you are?
4. How neat do you think you are about your things?
5. How forgetful do you think you are? (reverse)

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APPENDIX C

AFDP AND CDP SYMPTOMATOLOGY ITEMS

DSM-Oriented Affective Problems

1. There is very little that I enjoy
2. I cry a lot
3. I deliberately try to hurt or kill myself
4. I don't eat as well as I should
5. I feel worthless or inferior
6. I feel too guilty
7. I feel overtired without good reason
8. I sleep less than most kids
9. I sleep more than most kids during the day and/or at night
10. I think about killing myself
11. I have trouble sleeping
12. I am unhappy, sad, or depressed
13. I don't have much energy

DSM-Oriented Conduct Problems

1. I am mean to others
2. I destroy things belonging to others
3. I don't feel guilty after doing something I shouldn't
4. I break rules at home, school, or elsewhere
5. I get in many fights
6. I hang around with kids who get in trouble
7. I lie or cheat
8. I physically attack people

9. I run away from home

10. I set fires

11. I steal at home

12. I steal from places other than home

13. I swear or use dirty language

14. I threaten to hurt people

15. I cut classes or skip school