Specific Aims

The overall goal of this project is to test the biopsychosocial model of childhood disruptive behavior by examining longitudinal trajectories of conduct problems (CP) and symptoms of Attention Deficit Hyperactivity Disorder (ADHD) in school-age children and adolescents. Biopsychosocial models have been proposed to explain development of childhood disruptive disorders associated with ADHD symptoms and CP (Dodge & Petit, 2003), and research has identified both shared environmental and genetic risk factors for these disruptive behavior symptoms (Waschbusch, 2002). However, while ADHD and CP are commonly comorbid, they are not universally comorbid, and often follow a sequential, overlapping pattern whereby early ADHD symptoms precede emergence of later comorbid CP (Lahey & Loeber, 1997). Distinctions among unique and joint developmental trajectories of these symptom clusters may relate to previously identified correlates, including age and puberty, environment (e.g. family stress, peer influences), and genetic risk.

The biopsychosocial model assumes that both biological predisposition (i.e. genetic risk) and environmental factors contribute to disruptive behavior outcomes through additive, interactive, and transactional associations, and that the predictive effects of these associations may vary across development. However, despite the appeal and general acceptance of the biopsychosocial model to explain the emergence of ADHD and CP, to date this theory has not been rigorously tested at multiple time points, across both sexes, and with the inclusion of *a priori* identified genotypes as moderators. Specific candidate genes associated with dopaminergic and serotonergic neural pathways have been repeatedly identified in studies of disruptive behaviors. In particular, interactions between childhood adversity and the low-activity Monoamine oxidase A (MAOA) and promoter region tandem-repeat on dopamine D4 receptor (DRD4) genotypes have been associated with higher levels of disruptive behaviors (e.g., Kim-Cohen et al., 2006; Martel et al., 2011). Similarly, the valene/valene polymorphism at codon 158 on the catechol O-methyltransferase (COMT) gene has been associated with higher levels of CP in children with ADHD (Caspi et al., 2008). However, no single gene has emerged as necessary or sufficient to cause disruptive behavior disorders, and molecular genetic studies examining single genes are often limited by low power and Type 1 error. Thus, in order to test these important developmental and moderating processes across time, it is necessary that 1) this be examined in a representative, community sample of youth covering developmentally salient ages; 2) HI, CP and environmental stress be repeatedly tested in a multi-method, multi-wave design; and 3) the research should aim to replicate previously identified, specific g x e findings for disruptive behavioral outcomes.

The proposed study intends to fill several significant gaps in the literature by conducting secondary analyses of an ongoing, 8-wave, prospective study following 3rd, 6th and 9th grade youths (N=682) and a parent over 21 months (Hankin, Jenness, Abela, & Smolen, 2011). The parent study was originally designed to test development of depression in youth, but contains ample data to address my aims.

Aim 1. Test whether the severity of ADHD in school age youth potentiates the emergence of CP more strongly than CP potentiates the emergence of ADHD. *Hypothesis 1a:* Higher initial levels of ADHD will be associated with higher intercept and slope of CP. *Hypothesis 1b:* ADHD severity will predict subsequent changes in CP, such that ADHD at Time X will predict severity of CP at Time X+1. *Hypothesis 1c:* Development will moderate the predictive effect of ADHD on CP, with the magnitude of association between these two behavioral problems declining with increased age.

Aim 2. Test the effects of environmental risk on patterns of disruptive behavior development. *Hypothesis 2a:* Lower socioeconomic status and inconsistent parenting will be associated with higher intercepts and slopes of CP and ADHD. *Hypothesis 2b:* Stressful life events in the family, peer and school domains will predict subsequent scores of CP and ADHD, such that stress at Time X will predict severities of both ADHD and CP at Time X+1, over and above the effects specified in Hypothesis 2a. *Hypothesis 2c:* The predictive strengths of the stressors in Hypothesis 2b will demonstrate a domain by age interaction, such that the effect of family stressors will decline over age and the effects of peer and school stressors will increase.

Aim 3. Test the main and moderating effects of *a priori* theoretically specified candidate genes on CP and ADHD. *Hypothesis 3a:* Genetic risk will demonstrate main effects on the intercept and slope for development of both ADHD and CP. *Hypothesis 3b:* Genetic risk will increase the strength of behavioral and environmental predictors on disruptive behavior outcomes. *Hypothesis 3c:* Gene x environment interactions will demonstrate developmental and environmental specificity, with MAOA and DRD4 showing interactions with family stress in elementary school, and COMT showing the strongest interactions with peer and school stress during middle and high school ages.

<center>**Research Strategy**</center>

Significance

Conduct problems (CP) and symptoms of Attention Deficit/Hyperactivity Disorder (ADHD) are impairing, salient, and developmentally complex childhood disruptive behaviors. ADHD affects 3 to 5 percent of school-age children in a 6-month period (Pennington, 2002) and is characterized by symptoms of inattention, hyperactivity and impulsivity. Childhood ADHD is frequently comorbid with disorders featuring CP (for review see Jensen, Martin, & Cantwell, 1997; Pennington, 2002), namely Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD). ADHD and CP levels are worse in children exhibiting comorbid ADHD + CP, compared to those showing only ADHD or CP (Waschbusch, 2002) and comorbid ADHD + CP is associated with worse outcomes than either symptom cluster on its own. These sequelae include increased severity and long-term persistence of peer problems, early adult arrest, and low verbal IQ. In 2000, the economic burden of ADHD alone in the United States was estimated at $31.6 billion per year (Birnbaum et al., 2005). However, this annual cost is substantially greater when the child with ADHD exhibits comorbid symptoms of ODD and/or CD (Kessler et al., 2006). The additive effects of co-occurrence can be detrimental; thus, **understanding the etiology of the joint developmental trajectories of these symptom clusters is crucial to the development of more effective, personalized intervention programs.**

The etiologies of ADHD and CP symptoms are thought to be largely genetic, with the heritability of ADHD estimated at .76 (Faraone et al., 2005), and CP symptoms at around .50 (Moffitt, 2005). Reviews of genetic markers of ADHD, ODD and CD indicate that candidate genes for these disorders largely overlap, and are typically implicated in the dopaminergic and serotonergic neural pathways (Moffitt et al., 2008; Nigg, Nikolas, & Burt, 2010). Further, all three disorders are found in higher rates in boys than girls, with ADHD estimated at 3:1, and CD/ODD at 4:1 (Arnold, 1996; Dodge, Coie, & Lynam, 2006). Likewise, environmental risk factors are often common to all three disruptive behavior disorders, and include socioeconomic status, parent marital status, negative parenting, family stress, school achievement, and peer relationship stressors (Larsson, Dilshad, Lichtenstein, & Barker, 2011; Shaw, 2005; Waschbusch, 2002). There is evidence for large shared genetic effects in ADHD and ODD/CD (Nadder, Rutter, Silberg, Maes, & Leaves, 2002), suggesting that other factors besides shared genes, such as unique genes and environmental factors, must differentiate them. Indeed, additive, interactive and transactional associations among these variables are often implicated (Berry, 2012; Kim-Cohen et al., 2006), suggesting a broader, biopsychosocial model of development of disruptive behaviors. In a biopsychosocial model of development, predisposing genetic factors (including gender and candidate risk genes) interact with stable and dynamic environmental factors (e.g. socioeconomic status and peer relationships, respectively) to increase the likelihood of disruptive and antisocial behaviors by adolescence (Nigg, Nikolas, & Burt, 2010). Further, age appears to moderate the impact of negative experiences in each of these environmental domains (Moffitt, 2005; Nigg, et al., 2010).

Despite considerable knowledge of problematic public health outcomes resulting from CP and ADHD symptoms, and evidence of both environmental and genetic etiologies for these disruptive behaviors (Deault, 2010; Loeber, Green, Lahey, Frick, & McBurnett, 2000; Waschbusch, 2002), **several important and significant questions remain**. Developmental, neuropsychological, and clinical science can be enhanced with particular focus on investigating **dynamic, biopsychosocial models of CP and ADHD symptom severity over time**. Specifically, this application seeks to test three key postulates of longitudinal models of CP and ADHD symptoms. First, longitudinal research using annual diagnostic evaluations of psychiatrically referred samples (predominantly boys) suggests that among school-age children, there exists a developmental trajectory and sequential comorbidity pattern in which earlier ADHD, with or without comorbid CP, tends to predict increased severity of later CP symptoms (Beauchaine, Hinshaw, & Pang, 2010; Waschbusch, 2002). Second, the relative predictive strength of environmental factors, such as parenting and deviant peers, varies across childhood, adolescence and pubertal onset. For example, proximal environmental factors, such as family stressors, typically show stronger effects in early childhood, compared to peer influences, which demonstrate increasing predictive effects with age (e.g., Ingoldsby et al., 2006). Finally, considerable behavioral and molecular genetic research demonstrates gene-environment interplay in the development of ADHD and CP. These interactions also appear to be moderated by developmental age. For example, the low-activity Monoamine oxidase A (MAOA) genotype has been shown to interact with exposure to childhood adversity (including family stress and inconsistent parenting) to predict higher levels of disruptive behaviors (e.g., Foley et al., 2004; Kim-Cohen et al., 2006), but similar findings are not reported for exposure to adolescent or adult stress (e.g., Young et al., 2006).

In sum, the etiology of disruptive behavioral symptoms is multifactorial, with environmental, genetic, and g x e risk factors playing distinct roles at different points in development (Dodge & Petit, 2003). These multiple, nonlinear interactions among predictors have not been integrated into a coherent, developmental model. The primary aim and overarching goal of the present proposal is to **advance knowledge on predicting severity of ADHD and CP symptoms over time by longitudinally analyzing data collected from an ongoing multi-wave** (8 waves total across 21 months), **multi-informant, multi-method, multi-cohort** (3rd, 6th, and 9th grades at baseline) **study**. I aim to test and expand the three postulates described above using developmental, biopsychosocial growth curve models of ADHD and CP symptom severity.

Studies of the joint developmental trajectories of ADHD and CP thus far have been significantly limited by their samples, which are almost exclusively clinically-referred, Caucasian boys (Deault, 2010; Lahey & Loeber, 1995; Loeber, Green, Lahey, Frick, & McBurnett, 2000; Shaw, 2005). Given the high prevalence of CP symptoms in 9 to 16 year-olds (2.7%; Costello, Mustillo, & Erkanli, 2004), the rate of comorbid ADHD + CP in girls (1.3%; Waschbusch, 2002), and the increase in parental, peer, and school stress during adolescence for youth with ADHD and CP (Dodge & Petit, 2003; Steinberg, 2001)**, it is essential that this developmental trajectory be tested in a community sample that includes both male and female youth covering developmentally salient ages.** Further, adolescence is characterized by dramatic reorganization of stress-sensitive brain regions, as well as increased levels of stress hormones. These changes have been associated with increases in psychopathology, thus supporting the need for research on age as moderators of genetic and environmental influences on emergence of disruptive behaviors (Faraone et al., 2005; Nigg et al., 2010).

Gene by environment interaction has emerged as a strong candidate in the etiology of childhood disruptive disorders. In particular, three g x e interactions have been identified: 1) the low-activity Monoamine oxidase A (MAOA) genotype has been shown to interact with exposure to childhood adversity (including family stress and inconsistent parenting) to predict higher levels of disruptive behaviors (e.g., Foley et al., 2004; Kim-Cohen et al., 2006); 2) polymorphisms on the dopamine D4 receptor gene (DRD4) have long been associated with ADHD, but recently a tandem-repeat polymorphism in the promoter region of DRD4 was found to increase disruptive behavior symptoms in the presence of inconsistent parenting and marital conflict (Martel et al., 2011); and 3) the valene/valene polymorphism at codon 158 on the catechol O-methyltransferase (COMT) gene, which relates to lower dopamine expression in the prefrontal cortex, has repeatedly been associated with poorer executive functioning and higher levels of conduct problems in children with ADHD (Caspi et al., 2008). All three of these genes are known to relate to behavioral control via expression of enzymes that regulate levels of serotonin, norepinephrine, and dopamine in critical areas of the brain, such as the prefrontal cortex.

Despite ample research implicating genetic markers common to both ADHD and CP, the moderating effects of *a priori* theoretically identified candidate genes on **joint developmental trajectories** of CP and ADHD symptom severity have not been tested adequately. Limitations of previous candidate gene analyses include small and clinically-referred sample sizes, minimal power, and poor replication of specific findings. Notably, each of the previously described g x e interactions is specific to a particular developmental stage, and few have been tested in a wide range of age groups. The current application would address each of these issues by attempting to replicate previous g x e findings for these three relevant, theoretically based genotypes: low activity MAOA, homozygous tandem-repeat polymorphism in the promoter region of DRD4, and homozygous valene polymorphism at codon 158 on COMT. Further, the current study will attempt to replicate the moderating effects of these genotypes in a large, community based sample spanning a wide range of ages, using hierarchical linear modeling (HLM). This method of analysis will improve the detection of main and interaction effects of these genotypes across individuals and age groups.

The results of the proposed biopsychosocial developmental trajectory analyses would have far-reaching implications for decreasing the rates of common and debilitating child disruptive behaviors. Improved identification of specific g x e risk factors in a developmental context will inform early detection, parent education, prevention, and remediation that capitalize on a child's specific genetic and environmental risks for disruptive behavior disorders.

The present study aims to fill several major gaps in the literature by modeling predictive effects of development, environment, stress and genetic risk on severity of child ADHD and CP longitudinally, in cohorts of 3rd, 6th, and 9th graders. Unlike previous large-scale studies that address risk and predictors of ADHD and CP, the proposed study will use a general community sample, multiple time points, and extensive, multi-informant measures of child disruptive behaviors, environmental risk, and child developmental markers. **This level of measurement will permit creation of a more specific, developmental model of interactions and trajectories of behavioral phenotypes, environmental risk, and genetic risk to predict ADHD and CP**

symptom severity. The results of these analyses will directly inform development of individualized treatment programs.

Approach

The design of Dr. Hankin's parent research project purposefully incorporates elements advocated by Rutter (2005) for methodologically rigorous developmental psychopathology research. These include: (1) an 8-wave prospective study, (2) a large-scale sample (N=682 youth, N=682 parents, across 2 sites), (3) a community sample of both boys and girls, (4) a genetically sensitive design with multiple candidate molecular gene markers of various biological systems, and (5) inclusion of meaningful developmental transitions (e.g., school transitions, puberty).

The parent study is a cross-sequential, multi-wave, accelerated longitudinal study of 3 cohorts of youth and a parent: 207 3rd graders, 249 6th graders, and 226 9th graders. The total sample size is 682 participants (55% female, 40% minority) and the current retention rate is 96%. The study follows each cohort every 3 months for 3 years; data collection is currently completed through the 18-month follow-up assessments and is nearly complete through the 21-month follow-up. The study will continue to be funded through a no-cost extension of Dr. Hankin's R01 grant through the end of 2013. The aims of the parent study are to investigate the development of depressive symptoms in youth. My proposed **secondary** analyses will take advantage of this strong study design and the multitude of available measures to study **biopsychosocial processes in the development of disruptive behaviors** in youth.

The proposed analyses will use a combination of self-report measures, parent-report measures, laboratory observations, and DNA samples collected in Dr. Hankin's study to assess disruptive behavior symptoms, environmental risk factors, and genetics. Table 1 shows the measurement collection schedule. At present, Dr. Hankin's study is completely enrolled and data is being collected through the 21 month follow-up assessment (i.e. 8 waves). However, the parent study is not utilizing data related to disruptive behavior in any of these waves, and the coding of the baseline negative parenting observational data was only recently completed. Thus, the first six months of this award will be dedicated toward data entry, cleaning, and preliminary analyses.

Table 1. Schedule of Relevant Measurement Tools

	Baseline	3 mo f/u	6 mo f/u	9 mo f/u	12 mo f/u	15 mo f/u	18 mo f/u	21 mo f/u
Child CP	CBCL	SDQ	SDQ	SDQ	SDQ	SDQ	CBCL	SDQ
Child ADHD	CBCL	SDQ	SDQ	SDQ	SDQ	SDQ	CBCL SNAP-IV	SDQ
Stressful Events	ALEQ ASM	ALEQ ASM	ALEQ ASM	ALEQ ASM	ALEQ ASM	ALEQ ASM	ALEQ ASM	ALEQ ASM
SES	Parent Report						Parent Report	
Puberty	PDS						PDS	
Negative Parenting	APQ, PSS P-C obs.						APQ, PSS	
Genetics	Saliva							

Measurement. Child Conduct Problems and Child ADHD Symptoms are measured via phone using the Strengths and Difficulties Questionnaire, Parent Report (**SDQ**; Goodman, 1997) every 3 months (except Baseline and the 18 month follow-up). The SDQ scales are highly correlated with other established parent-report measures of conduct problems and ADHD symptoms, including the Rutter scales (CP r=.82, ADHD r=.88; Goodman, 1997). Internal validity for the SDQ scales have been established for a large (N=9,878) sample of children age 4-17.The SDQ ADHD scale combines inattentive and hyperactive/impulsive symptoms into one behavioral scale; for example, items include "restless, overactive" and "easily distracted." Recent research has revealed low stability of clinical subtypes of ADHD (i.e. Inattentive, Hyperactive/Impulsive, and Combined), meaning that children frequently meet criteria for multiple ADHD subtypes across childhood and adolescence (Willcutt et al., In Press; Lahey, Pelham, Loney, Lee, & Willcutt, 2005). Further, conduct problems have been associated with both inattentive and hyperactive/impulsive ADHD symptom clusters, and are more severe in children exhibiting the ADHD-Combined subtype (e.g., Willcutt, et al., In Press). Finally, it will be possible to characterize the sample into clinical subtypes using the CBCL and SNAP-IV, described below. Thus, the combined measurement of the ADHD symptom dimensions in the SDQ is not likely to be a limitation.

At baseline and 18 month follow-up, parents complete the externalizing scale of the Child Behavior Checklist 6-18 (**CBCL**; Achenbach, 2001). The CBCL has high test-retest reliability (r=.89) and is correlated

with the Quay and Peterson Revised Behavior Problem Checklist (r=.59 to .88; Quay & Peterson, 1983). The CBCL will be used for sample descriptive purposes, and as an index of validity for the SDQ scales.

Additionally, parent ratings of the Swanson, Nolan and Pelham-IV (**SNAP-IV**) were collected at the 18-month follow up. The SNAP-IV questionnaire targets DSM-IV criteria for ADHD Inattentive, Hyperactive and Combined Types, and is frequently used in research and clinical settings to diagnose ADHD subtypes. The measure is reliable (α=.94) and valid (Bussing et al., 2008). Following clinical guidelines, subjects with six or more items endorsed (receiving a rating of "often" or "very often") on the inattention or hyperactivity/impulsivity subscales will be classified as ADHD-Inattentive Type or ADHD-Hyperactive/Impulsive Type, respectively; subjects with six or more items endorsed on both scales will be classified as ADHD-Combined Type. Diagnostic classification will be used for sample descriptive purposes, and as an index of validity for the SDQ ADHD scale at the 15 month follow-up. Additionally, I will test the possibility that the SDQ ADHD scores at 15 and 21 months are more strongly correlated with either inattention or hyperactivity/impulsivity as measured by the **SNAP-IV** at 18 months. This will allow more accurate interpretations of the results, particularly for the genetic analyses, with regard to the contributions of these individual symptom clusters to the results.

Stressful Events are assessed every 3 months using both child- and parent-report responses to the Adolescent Life Events Questionnaire (**ALEQ**), which measures frequency of negative events in the child's environment. The measure has high internal consistency (.94) and test-retest reliability (.65) (Muris & Meesters, 2009). Second, the Adult Stress Measure (**ASM**) is a 36-item, parent-report checklist based on the Life Events Inventory which indexes major life events in the family context (Cochrane & Robertson, 1973). Prior to analyses, I will create composites using expected items from the ALEQ and ASM to establish three independent factors of family, peer, and school stressful events.

Genetic Data has been analyzed as part of the parent study and includes child DNA samples collected via saliva using Oragene kits. Following previous work (e.g., Foley et al., 2004; Kim-Cohen et al., 2006), 3- and 5-repeat alleles on the MAOA gene were classified as low activity, and 3.5- and 4-repeat alleles were classified as high-activity. The MAOA gene is expressed on the X chromosome, of which females have two copies; thus for females, one gene was selected randomly for inclusion in analysis (Prom-Wormley et al., 2009). DRD4 120-bp tandem repeats were coded according to previous research, with expected "short" allele lengths of 2-5 repeats and "long" lengths of 6-8 repeats (Martel et al., 2011). Subjects with a DRD4 short/short or long/short genotype were coded as "low risk"; long/long genotypes were coded as "high risk." Finally, the COMT valene/valene polymorphism at codon 158 was classified as a risk factor, in contrast with the valene/methionine or methionine/methionine haplotypes (Caspi et al., 2008).

Socioeconomic Status is measured at baseline and at 18 month follow-up using a demographic parent-report of factors relating to education, income, resources, and family size.

Puberty is measured via a brief self-report by the adolescents at baseline and at 18 months using Petersen's self-report scale (**PDS**). The PDS is a well-established measure of pubertal development, with good reliability (α=.77) and validity (.61-.80; Petersen et al., 1988).

Negative Parenting is hypothesized to predict initial levels of disruptive behaviors in this study, thus it is measured at baseline. Literature on parenting (e.g., Prinzie, 2009; Shelton, Frick, & Wootton, 1996) identifies three broad dimensions of negative parenting associated with disruptive behaviors: inconsistent discipline, low involvement, and harsh parenting. In the current study, confirmatory factor analysis will be used to construct these three dimensions out of expected items from two established parent-report measures and one laboratory observation task. The Alabama Parenting Questionnaire (**APQ**) measures three relevant, negative parenting scales that demonstrate internal consistency (unstandardized α=.46 to .67) and are elevated in children with disruptive behaviors (Shelton, Frick, & Wootton, 1996). The Parenting Style Scale (**PSS**) includes three corresponding scales that show high reliability (α=.72 to .82) and predict adolescent school achievement (Steinberg & Lamborn, 1992). The APQ and PSS are measured again at the 18-month follow-up, which will allow me to consider the stability of negative parenting over time. Finally, the parent-child observation (**P-C obs**) comprises a five minute conversation between the parent and child that is later coded for authoritarian, authoritative, and permissive discipline, as well as degrees of conflict, affect, criticism and support. Interrater reliability ranges from α=.71 to .97.

Preliminary Analyses. Preliminary analyses on this sample aimed to determine that there was enough variance in the disruptive behavioral and genetic constructs to go forward with the growth modeling. In a subsample of n=294 subjects at the 18 month follow-up, 20% had CBCL externalizing scale scores greater than 1.5 standard deviations above the sample mean, and SNAP-IV ratings indicated that 16% of participants met diagnostic criteria for any ADHD subtype. SDQ scores for a subsample of 94 participants at the 15 month

follow-up were compared to published norms for a large, U.S. sample of 4-17 year olds (N=9878; CDC, 2004); the number of participants with scale scores greater than 1.5 standard deviations above the published mean was 17% for Conduct Problems and 5% for ADHD. Additionally, the SDQ Conduct Problems and ADHD scales were modestly correlated (r=.520, *p*<.001) in a subsample at the 15 month follow-up (n=94), indicating that these SDQ subscales are measuring related, but independent constructs. In order to determine if enough change occurred across the proposed time period to proceed with analyses, raw change scores were calculated between the 3 month and 21 month follow-ups for SDQ ADHD and CP symptoms, in a subset of n=72 for whom these data were entered. The amount of individual change ranged from 0 to 4 points (out of 10) for ADHD, and 0 to 7 points (out of 8) for CP, indicating adequate variance to detect change across these time points. Finally, in a subset of the current sample for which genetic data has been entered (n=354), 47% had the low activity MAOA polymorphism, 7% had the high risk long/long DRD4 genotype, and 29% had the val/val COMT polymorphism.

Data Analysis and Power. Data analysis will take advantage of hierarchical linear modeling methods (HLM) to model growth curves specific to each of the hypotheses, as described below. To reflect the true shape of change, I will model raw scores (i.e. not age-adjusted) in my analyses (Francis, Fletcher, Stuebing, Davidson, & Thompson, 1991). HLM (also referred to as general linear mixed modeling) is well suited for developmental analyses because it measures both inter-individual and intra-individual patterns of growth. Additionally, HLM permits inclusion of time-varying covariates, meaning that it allows for models of time-dependent associations between outcome and predictor variables (Burchinal, Nelson, & Poe, 2006). Power estimates for HLM are strong, due to the fact that individuals with missing or deviant data can remain in the sample by weighing complete data more heavily in the overall group parameters. In this current study, this is useful because cohorts can be modeled together, with age included as a covariate. General guidelines for statistical power for HLM suggest that the sample size at Level 2 should be larger than that at Level 1 in order to detect small effects. More specifically, following guidelines by Scherbaum & Ferreter (2009), power to estimate effects with a Level 1 covariate (e.g., age) can be estimated using the equations

$$\text{Standard Error}(\gamma_{01}|X)=\sqrt{\frac{4(p+(1-p)/n)}{J}}\left(1+\frac{1}{Jn\text{-}4}\right)$$

and

$$Z_1\text{-}\beta \leq \frac{\text{Effect Size}}{\text{Standard Error }(\gamma_{01})} - Z_{1\text{-}\alpha/2}$$

where p= Interclass Correlation Coefficient; *J*= Level 1 sample size; *n*= Level 2 sample size; $Z_{1\text{-}\alpha/2}$= the z score associated with the chosen level of Type 1 error for a two-tailed test; and $Z_1\text{-}\beta$ = the z score associated with the level of statistical power. Substituting the results of the first equation into the second indicates that I will have 99.9% power to detect a small effect (0.2) with α=.05, using time points (*J*=7) at the first level and individuals (*n*=682) at level two. Estimates of power for Level 2 covariates (e.g. genetic risk) are less easily computed; however, Scherbaum & Ferreter (2009) assert that power to detect small effects of Level 2 covariates will be strong assuming a sample size greater than 30 at Level 2; thus, the current study's Level 2 sample size of 682 should be more than adequate.

Aim 1: Effects of ADHD on CP (and vice versa) will be analyzed using HLM. First, I will examine various growth shapes for the CP and ADHD (Outcome) trajectories over age (i.e., testing significance of the linear, quadratic, and cubic growth parameters). This and all subsequent analyses will include gender as a covariate. Additionally, model invariance across ethnicity will be addressed in this first aim by adding minority status as a covariate of the intercept.

Level 1: *Outcome$_{ti}$ = β$_0$ + β$_1$*Age$_{ti}$ + β$_2$*Age$_{ti}^2$ + e$_{ti}$*
Level 2: *β$_0$* = γ$_{00}$ + γ$_{01}$*Sex$_i$ + γ$_{02}$*Minority$_i$ + u$_0$
   β$_1$ = γ$_{10}$ + γ$_{11}$*Sex$_i$ + u$_1$
   β$_2$ = γ$_{20}$ + γ$_{21}$*Sex$_i$ + u$_2$

Next, I will test ADHD symptoms as predictors of CP scores, and vice versa. For the first analysis, the effects of ADHD will be decomposed into person-level effects (i.e., effects of having high vs. low ADHD relative to the overall sample) and effects of time-varying ADHD scores (i.e., effects of individual ADHD fluctuations over

time). In order to conduct this analysis, I will add both grand-mean-centered time-invariant ADHD scores that reflect between-person variability in ADHD (*GMADHD$_i$*) at Level 2, and lagged person-mean-centered time-varying ADHD scores that reflect fluctuations in ADHD across assessments (*laggedTVADHD$_i$*) at Level1. The opposite effect, whereby person-level and time-varying CP scores predict ADHD, will also be tested in order to address the hypothesis that ADHD severity will predict CP outcomes more strongly than CP will predict ADHD.

Level 1: $CP_{ti} = \beta_0 + \beta_1{}^*Age_{ti} + \beta_2{}^*Age_{ti}{}^2 + \beta_3{}^*laggedTVADHD_i + e_{ti}$
Level 2: $\beta_0 = \gamma_{00} + \gamma_{01}{}^*Sex_i + \gamma_{02}{}^*Minority_i + \gamma_{03}{}^*GMADHD_i + u_0$
$\beta_1 = \gamma_{10} + \gamma_{11}{}^*Sex_i + \gamma_{12}{}^*GMADHD_i + u_1$
$\beta_2 = \gamma_{20} + \gamma_{21}{}^*Sex_i + u_2$

Aim 2: The effects of the three stress factors (i.e. family, peer, and school) will be modeled for CP and ADHD outcomes separately. For each of these outcomes, SES, negative parenting and pubertal stage will be included as covariates of the intercept. The lagged time-varying environmental stressors and their interaction with age will be set to predict the outcome variable.

Level 1: $Outcome_{ti} = \beta_0 + \beta_1{}^*Age_{ti} + \beta_2{}^*Age_{ti}{}^2 + \beta_3{}^*laggedStessor_i + \beta_4{}^*Age_{ti}{}^*laggedStessor_i + e_{ti}$
Level 2: $\beta_0 = \gamma_{00} + \gamma_{01}{}^*Sex_i + + \gamma_{02}{}^*Minority_i + \gamma_{03}{}^*SES_i + \gamma_{04}{}^*NParent_i + \gamma_{05}{}^*Puberty_i + u_0$
$\beta_1 = \gamma_{10} + \gamma_{11}{}^*Sex_i + u_1$
$\beta_2 = \gamma_{20} + \gamma_{21}{}^*Sex_i + u_2$

Aim 3. I will test the main effects of genetic risk (i.e. *a priori* specified candidate genes) on the CP and ADHD intercepts and slopes. I will independently test the SDQ ADHD, SDQ CP, SNAP-IV inattention, and SNAP-IV hyperactivity/impulsivity subscales as outcome measures:

Level 1: $Outcome_{ti} = \beta_0 + \beta_1{}^*Age_{ti} + \beta_2{}^*Age_{ti}{}^2 + e_{ti}$
Level 2: $\beta_0 = \gamma_{00} + \gamma_{01}{}^*Sex_i + + \gamma_{02}{}^*Minority_i + \gamma_{03}{}^*GeneticRisk_i + u_0$
$\beta_1 = \gamma_{10} + \gamma_{11}{}^*Sex_i + \gamma_{12}{}^*GeneticRisk_i + u_1$
$\beta_2 = \gamma_{20} + \gamma_{21}{}^*Sex_i + u_2$

Next, I will examine whether genetic risk moderates the predictive effects of environmental stressors on these CP and ADHD outcomes, with the hypotheses that the low-activity MAOA and DRD4 long/long genotypes will increase the predictive effect of family stress on disruptive behavior (including inattention) during elementary school, and the COMT polymorphism will increase the effects of peer and school stress on disruptive behavior during middle and high school stages:

Level 1: $Outcome_{ti} = \beta_0 + \beta_1{}^*Age_{ti} + \beta_2{}^*Age_{ti}{}^2 + \beta_3{}^*laggedStessor_i + \beta_4{}^*GeneticRisk_i{}^*laggedStessor_i + e_{ti}$
Level 2: $\beta_0 = \gamma_{00} + \gamma_{01}{}^*Sex_i + + \gamma_{02}{}^*Minority_i + \gamma_{03}{}^*GeneticRisk_i + u_0$
$\beta_1 = \gamma_{10} + \gamma_{11}{}^*Sex_i + \gamma_{12}{}^*GeneticRisk_i + u_1$
$\beta_2 = \gamma_{20} + \gamma_{21}{}^*Sex_i + u_2$

The results of these three aims will inform a broader developmental perspective on the environmental, genetic and g x e effects on the development of disruptive behaviors during childhood and adolescence.