HHS Public Access

Author manuscript

Dev Psychopathol. Author manuscript; available in PMC 2016 February 01.

Published in final edited form as:

Dev Psychopathol. 2015 February ; 27(1): 81–95. doi:10.1017/S095457941400131X.

Developmental Mediation of Genetic Variation in Response to the Fast Track Prevention Program

Dustin Albert[1,2], **Daniel W. Belsky**[2,3], **D. Max Crowley**[1], **Conduct Problems Prevention Research Group**[4], **John E. Bates**[5], **Gregory S. Pettit**[6], **Jennifer E. Lansford**[1,2], **Danielle Dick**[7], and **Kenneth A. Dodge**[1]

[1]Center for Child and Family Policy, Duke University

[2]Social Science Research Institute, Duke University

[3]Center for the Study of Aging and Human Development, Duke University Medical Center

[4]The Conduct Problems Prevention Research Group includes (alphabetically): Karen L. Bierman, Ph.D., Pennsylvania State University; Kenneth A. Dodge, Ph.D., Duke University; Mark T. Greenberg, Ph.D., Pennsylvania State University; John E. Lochman, Ph.D., University of Alabama; Robert J. McMahon, Ph.D., Simon Fraser University and Child & Family Research Institute; and Ellen E. Pinderhughes, Ph.D., Tufts University

[5]Indiana University-Bloomington, Department of Psychological and Brain Sciences

[6]Auburn University, Department of Human Development and Family Studies

[7]Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University

Abstract

We conducted a developmental analysis of genetic moderation of the effect of the Fast Track intervention on adult externalizing psychopathology. The Fast Track intervention enrolled 891 children at high risk to develop externalizing behavior problems when they were in kindergarten. Half of the enrolled children were randomly assigned to receive 10 years of treatment with a range of services and resources provided to the children and their families and the other half to usual care (controls). We previously showed that the effect of the Fast Track intervention on participants' risk of externalizing psychopathology at age 25 years was moderated by a variant in the Glucocorticoid Receptor Gene (*NR3C1*). Children who carried copies of the A-allele of the single-nucleotide polymorphism rs10482672 had the highest risk of externalizing psychopathology if they were in the control arm of the trial and the lowest risk of externalizing psychopathology if they were in the treatment arm. In this study, we test a developmental hypothesis about the origins of this for-better-and-for-worse gene-by-intervention interaction (GxI): That the observed GxI effect on adult psychopathology is mediated by the proximal impact of intervention on childhood externalizing problems and adolescent substance use and delinquency. We analyzed longitudinal data tracking the 270 European-American children in the Fast Track RCT with available genetic information (129 intervention children and 141 control-

Corresponding author is: Dustin Albert, Ph.D., Center for Child and Family Policy, Duke University, Durham, NC 27708, Ph.: 919-613-9295, Fax: (919) 668-6923, dustin.albert@duke.edu.

Disclosure: The other authors have no financial relationships to disclose.

group peers, 69% male) from kindergarten through age 25 years. Results show that the same pattern of "for-better-and-for-worse" susceptibility to intervention observed at the age-25 follow-up was evident already during childhood. At the elementary school follow-ups and at the middle/high-school follow-ups, rs10482672 predicted better adjustment among children receiving the Fast Track intervention, and worse adjustment among children in the control condition. In turn, these proximal GxI effects early in development mediated the ultimate GxI effect on externalizing psychopathology at age 25 years. We discuss the contribution of these findings to the growing literature on genetic susceptibility to environmental intervention.

INTRODUCTION

Longitudinal studies of the etiology of externalizing psychopathology suggest that children with early-starting conduct problems are much more likely than their peers to engage in antisocial behavior and alcohol and substance abuse as adults (Moffitt, 1993; Patterson, Reid, & Dishion, 1991). Randomized prevention trials have produced compelling evidence that early intervention can interrupt this developmental progression of externalizing behavior and shift children onto more adaptive trajectories (Conduct Problems Prevention Research Group (CPPRG), 1999, 2002, 2004, 2007, 2009, 2010, 2011). A critical next step for externalizing prevention research is to identify sources of heterogeneity in intervention response, including but not limited to genetic moderators (van IJzendoorn et al., 2011). One impetus for investigating genetic moderation of intervention effects is that identified gene-by-intervention (GxI) interactions can be translated to target "precision" interventions, e.g. genetic testing to determine Warfarin treatment (Epstein et al., 2010). But it remains unclear whether such precision is possible in the case of complex, long-running behavioral interventions. Even if precision targeting is possible, feasibility and ethical challenges remain unresolved.

We propose an alternative reason to examine GxI interactions is that they can elucidate mechanisms through which interventions operate. Identified GxI interactions can be used to examine how risk/susceptibility within a biological substrate manifests over developmental time. Following this logic, we envision a critical role for "developmental backtracking" studies that explicate the meaning of discovered GxI. This approach builds on prior developmental analyses of genetic main effects (D. Belsky et al., 2012; D. Belsky et al., 2013; D. Belsky, Moffitt, & Caspi, 2013). The broad approach we envision involves three steps that follow the initial identification of a GxI effect: (1) Test genetic main effects on pre-treatment manifestations of risk for the intervention target; (2) Test GxI effects on proximal developmental phenotypes measured between the initiation of treatment and the time of final outcome assessment; (3) Test the hypothesis that GxI effects on proximal developmental phenotypes mediate the GxI effect on the long-term outcome. Here, we apply this developmental backtracking approach to study genetic heterogeneity in the effects of the Fast Track Prevention Trial, a 10-year intervention that aimed to prevent kindergarteners with early-starting conduct problems from developing persistent externalizing psychopathology. The Fast Track intervention design was based on evidence that children with early-starting conduct problems are at increased risk for long-term externalizing psychopathology due to a *dynamic cascade* of proximal adjustment problems in childhood

and adolescence (CPPRG, 1992; Dodge, Greenberg, Malone, & CPPRG, 2008). Our aim is to elucidate the proximal processes by which genotype and the Fast Track intervention interact to produce long-term outcomes.

Background: Differential Susceptibility to Intervention

There is emerging evidence that the same children who are most vulnerable to adverse developmental outcomes are also the most likely to benefit from improvements in the quality of their environment (Ellis et al., 2011). These children demonstrate elevated responsiveness to their social environments. In high-risk environments, these children fare poorly. But when environmental conditions are good, they flourish. This "for-better-and-for-worse" phenomenon has been termed "biological sensitivity to context" (Boyce et al., 1995) or "differential susceptibility" (Belsky, 1997). The sensitive/ susceptible child is characterized by difficult temperament and heightened negative emotionality (Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007; Belsky & Pluess, 2009), and by heightened physiological responses to social stressors (Boyce & Ellis, 2005). There is also evidence that sensitivity/susceptibility may be influenced by genetic factors. Polymorphisms in genes related to neurotransmitter function have received substantial attention in this research (Bakermans-Kranenburg & van IJzendoorn, 2006; Bakermans-Kranenburg & van IJzendoorn, 2011; Belsky & Pluess, 2013; Kochanska, Philibert, & Barry, 2009; Mitchell et al. 2011; Mitchell et al. 2014; Sheese, Voelker, Rothbart, Posner, 2007).

A new frontier in genetically-informed differential susceptibility research is the use of randomized trials (van IJzendoorn et al., 2011). Experimental randomization of exposure (i.e., the intervention) overcomes several of the limitations of observational gene-by-environment (GxE) research, including potential confounds arising from gene-environment correlation (e.g., genetically-influenced selection or evocation of environments) and omitted variable bias. Initial support for the utility of the gene-by-intervention (GxI) design comes from studies demonstrating genetic moderation of response to single-domain, time-limited interventions focused on preschool literacy skills (Kegel, Bus, & van IJzendoorn, 2011), positive parenting (Bakermans-Kranenburg et al., 2008), and prevention of alcohol abuse among adolescents (Brody, Chen, & Beach, 2013). Here, we apply the gene-by-intervention design to the Fast Track Prevention Trial, a longer-running, multi-component intervention to prevent the development of externalizing psychopathology in high risk children in kindergarten.

The Glucocorticoid Receptor Gene (*NR3C1*) as a Candidate Moderator of Intervention Response

We focused our investigation on the gene encoding glucocorticoid receptor (to which the hormone cortisol binds) because physiological reactivity to stress has been identified as a hallmark of differential susceptibility. The glucocorticoid receptor plays a critical role in the human stress response; cortisol binding to glucocorticoid receptors in the hippocampus, amygdala, and other limbic structures provides negative feedback to the hypothalamic-pituitary-adrenal axis response (DeRijk, van Leeuwen et al., 2008). Glucocorticoid receptor function influences short- and long-term adaptations of the hypothalamic-pituitary-adrenal (HPA) axis to environmental challenge and stress (Sapolsky et al., 2000; Meaney, 2001;

McEwen, 2012). Dysregulated glucocorticoid signaling has been implicated in child and adult manifestations of externalizing psychopathology (McBurnett et al., 1991; Hawes et al., 2009; Lopez-Duran et al., 2009; Savitz et al., 2009; Stadler et al. 2010; van Zuiden et al. 2011; Fardet et al., 2012). Particularly relevant to the current study is evidence that children exhibiting low cortisol reactivity to experimental challenge respond less favorably than high cortisol-reactive children to an intervention designed to reduce disruptive behavior (van de Wiel et al., 2004).

Polymorphisms in the glucocorticoid receptor gene (hereafter "*NR3C1*") have been associated with glucocorticoid resistance and reduced negative feedback of the HPA-axis (DeRijk et al., 2008, Manenschijn et al., 2009), as well as high cortisol-reactivity to stress (Kumsta et al., 2007; Kumsta et al., 2009; van West et al., 2010). At the level of psychopathology, *NR3C1* variants are associated with child-onset mood disorder (Mill et al., 2009), adolescent alcohol abuse (Desrivieres et al., 2011), and adult major depression (van Rossum et al., 2006; van West et al., 2006; Zobel et al., 2008). *NR3C1* variants have also been associated with differential response to environmental exposure, including greater incidence of depression among individuals exposed to adversity (Bet et al., 2009) and irregular cortisol reactivity and behavior problems among the offspring of mothers with prenatal psychological symptoms (Velders et al., 2012).

Based on this evidence, we hypothesized that *NR3C1* genotypes would differentiate individuals with a "for-better-and-for-worse" sensitivity to Fast Track intervention. Specifically, we hypothesized genotypes would identify children with the lowest rates of externalizing psychopathology in the intervention condition and with the highest rates of externalizing psychopathology in the control condition. We found support for this hypothesis in our previous report, which showed that adult outcomes of the Fast Track intervention varied based on participants' *NR3C1* genotype (Albert et al., 2014). We briefly review this discovery analysis below.

Gene-by-Intervention Discovery Analysis

Our discovery analysis tested whether the Fast Track intervention was more efficacious for children who carried specific *NR3C1* variants. The outcome was externalizing psychopathology at age 25. We defined *Any Externalizing Psychopathology* based on diagnostic assessment of Antisocial Personality Disorder, Attention Deficit Hyperactivity Disorder, Alcohol Abuse Disorder, Marijuana Abuse, and Serious Drug Use. Analyses were conducted separately in European-American and African-American children in the Fast Track RCT to account for allele frequency differences between the two populations.

We selected *NR3C1* test variants based on a haplotype tagging analysis, a hypothesis-free approach that surveys common variation throughout the gene (Dick, 2011; Dick, Latendresse, & Riley, 2011). Haplotype tagging identified 10 single-nucleotide polymorphisms (SNPs) in NR3C1, which were genotyped in the Fast Track sample (Supplemental Figure 1). We used linear probability models to test the intervention-moderating effect of each of these 10 SNPs. An adjusted Bonferroni correction was used to account for multiple testing (Nyholt, 2004).

Across all genotypes, children randomly assigned to the Fast Track intervention were less likely to manifest *Any Externalizing Psychopathology* at age 25 years than children randomized to the control condition (for European-American children, 46% of the treated group as compared to 61% of the control group manifested externalizing psychopathology, p=0.02; for African-American children, 35% in the intervention group as compared to 58% in the control group manifested externalizing psychopathology, p<.001). Among European-American children, the effect of intervention was moderated by variation in the glucocorticoid receptor gene *NR3C1*; intervention was more efficacious in preventing externalizing psychopathology for carriers of the rs10482672 'A' allele. Among carriers of the 'A' allele, 18% of treated children as compared to 75% of control children manifested any externalizing psychopathology at age 25 follow-up. In contrast, for non-carriers of the 'A' allele, 56% of treated children and 57% of control children manifested externalizing psychopathology at follow-up. Among African-American children, there was no evidence that *NR3C1* SNPs moderated Fast Track intervention effects.

In the analyses reported in this article, we test the hypothesis that the GxI between *NR3C1* SNP rs10482672 and Fast Track treatment operates via changes to children's behavior in childhood and adolescence using the 3-step developmental backtracking approach outlined above. In step 1, we test genetic main-effects on the social adjustment of Fast Track participants in kindergarten, prior to their enrollment in the intervention trial. In step 2, we test GxI effects on proximal developmental phenotypes of externalizing psychopathology during primary school and during middle and high school. In step 3, we test our mediation hypothesis-that GxI effects on externalizing phenotypes in primary, middle, and high school mediate GxI effects on externalizing psychopathology at age 25 years. Figure 1 illustrates the conceptual framework. We interpret findings in light of developmental theories of the etiology of externalizing psychopathology and the role of the stress response system in vulnerability and in susceptibility to positive developmental influences.

METHODS

Setting: The Fast Track Prevention Trial

The Fast Track Prevention Trial was implemented in the early 1990's to test whether the developmental outcomes of young children at high risk for long-term antisocial behavior could be improved through random assignment to a sustained, multi-component behavioral intervention (CPPRG, 1999). Intervention design was based on two critical insights derived from longitudinal research on the etiology of persistent externalizing behavior (CPPRG, 1992). First, children at risk for antisocial behavior as adults are identifiable at school entry by their conduct problems in home and school settings; although not all conduct-disordered children will become antisocial adults, almost all antisocial adults have a history of childhood conduct problems (Robins, 1966; CPPRG, 1999). Second, the pathway from early risk to later disorder is comprehensible as a *dynamic cascade* of adjustment problems, as failure at one developmental stage begets failure in the next, and so on, leading to increasing isolation from positive aspects of family, school, and peers (Dodge, Greenberg, Malone, & CPPRG, 2008). High-risk children typically enter school with a risk burden that crosses multiple domains. Socioeconomic disadvantage and dysfunctional parenting contribute to

escalating conduct problems at home (Dodge & McCourt, 2010). Deficits in self-control and emotion regulation undermine social adjustment and academic performance at school (Moffitt, 1993). These early adjustment problems increase risk for social rejection and academic failure in elementary school, association with deviant peers, and delinquency, violence, and substance abuse in adolescence and young adulthood (Dodge et al., 2008). Based on these foundational insights, the creators of the Fast Track intervention reasoned that effective prevention should begin no later than school entry, should be sustained from childhood through early adolescence, and should target the risk factors that are most salient at each developmental period (CPPRG, 1992).

Implemented as a multi-site randomized control trial, the Fast Track trial used a multiple-gating screening procedure to select 891 children with very high levels of conduct problems at the time of school entry, and randomly assigned them to a no-treatment control condition or an intervention condition that provided them with 10 consecutive years of prevention services (grades 1–10; see Figure 2 for further details). Programming during the elementary school years addressed the social cognitive, emotional, and self-control deficits that contribute to aggression toward peers, social rejection, academic failure, and disruptive and oppositional behavior toward authorities. Later programming targeted prevention toward salient issues at critical developmental transitions; for example, programming for the middle-school transition addressed parental supervision and adolescent decision making relevant to alcohol, tobacco, and substance use. Previously published intent-to-treat analyses of Fast Track demonstrated its success in reducing externalizing behavior across the elementary, high school, and young adult years (CPPRG, 1999; 2002; 2004; 2007; 2011; in press), with less robust effects during middle school (CPPRG, 2010). The most pronounced impacts of the Fast Track intervention have been observed in the subgroup of children who displayed the most severe conduct problems at school entry (CPPRG, 2011).

The Fast Track study included both longitudinal study of a community sample and a randomized controlled trial of intervention with high-risk children. The intervention was a comprehensive prevention program for children at high risk for persistent antisocial behavior delivered over a ten-year period, when participating children were in the first through the tenth grades. Three successive cohorts of kindergarten children were enrolled in a randomized controlled trial in 1991, 1992, and 1993 to yield a sample of 891 children (445 in the intervention group and 446 in the control group). Figure 2 illustrates the Fast Track design. Detailed description of Fast Track is available at www.fasttrackproject.org and in published evaluations (CPPRG, 1999; 2002; 2004; 2007; 2011; in press).

Children were selected from each of four geographic sites: Durham, NC; Nashville, TN; rural PA; and Seattle, WA. Elementary schools (n=55) in neighborhoods with very high rates of crime and economic disadvantage were divided into paired sets (one to three sets per site) matched for demographics, and one set was randomly assigned to intervention and one to control.

A multiple-gating screening procedure that combined teacher and parent ratings of aggressive-disruptive behavior was applied to all 9,594 kindergarteners in three cohorts (1991, 1992, and 1993). The first gate relied on teacher-reported classroom conduct

problems, using the Teacher Observation of Child Adjustment-Revised (TOCA-R) Authority Acceptance Score. Children scoring in the highest 40% within cohort and site were solicited for the second gate of screening: parent-rated home behavior problems, using a 22-item instrument based on the Child Behavior Checklist. Teacher and parent scores were standardized within site and summed to yield a *severity-of-risk screen score*.

Children were selected for the study based on this risk score, moving from highest down until desired sample sizes were reached within sites, cohorts, and conditions. 979 children (10% of total) were solicited to yield a sample of 891 participating children (91% consent; intervention $n = 445$; control $n = 446$). At selection, participant mean age was 6.58 years ($SD = 0.48$). Ethnicity varied (51% African American, 47% European American, and 2% other ethnicity), and 69% were boys. The mean externalizing-problem score for the Teacher's Report Form of the Child Behavior Checklist was 1.6 standard deviations above the national mean. The sample was high-risk in many ways: 58% had single parents, 29% of parents had not completed high school, and 35% of families were in the lowest socioeconomic class.

Written informed consent from parents and oral assent from children were obtained. Parents were paid for completing interviews, and intervention-group parents were paid for group attendance. All procedures were approved by the Institutional Review Boards of participating universities.

Elementary school phase (grades 1–5)—During grades 1–5, intervention families were offered group intervention during a 2-hour “enrichment program” that included children’s social-skill “friendship groups”, parent-training groups, guided parent-child interaction sessions, and paraprofessional tutoring in reading. Tutors provided three additional 30-minute sessions per week in reading and peer-pairing to improve friendships with classmates. Teacher consultation and a Fast Track adaptation of the teacher-implemented PATHS curriculum which addresses social-cognitive skill development were implemented universally in grade 1–5 classrooms in intervention schools (except Durham, where it was prohibited) to promote social-emotional competence. Enrichment programs were held weekly during grade 1, biweekly during grade 2, and monthly during grades 3–5. In addition, home visiting helped parents generalize their skill learning and address individual needs. After grade 1, criterion-referenced assessments adjusted the prescribed dosage to match need.

Middle and early high school phase (grades 6–10)—During grades 5 and 6, children received a middle school transition program and four parent-youth groups on topics of adolescent development; alcohol, tobacco, and drugs; and decision-making. In grades 7 and 8, eight Youth Forums addressed vocational opportunities, life skills, and summer employment opportunities. In grades 7–10, individualized interventions addressed parent monitoring, peer affiliation, academic achievement, and social cognition. All children received Oyserman’s School-to-Jobs (STJ) possible-selves intervention aimed at examining emerging identity.

Intervention participation—96% of parents and 98% of children attended at least one group session during grade 1. Of these families, 79% of parents and 90% of children attended at least 50% of prescribed group sessions. Participation decreased modestly across years, primarily due to residential moves. In grades 7–10, intervention continued with at least 80% of all children.

High intervention fidelity was ensured by manualization, regular cross-site training, and weekly clinical supervision. Outside interventions were neither encouraged nor discouraged. The full protocol can be found at: fasttrackproject.org.

Genotyping

Fast Track collected DNA from participants at the age-21 follow-up. DNA was obtained from buccal cells collected using a cytology brush. DNA extraction was performed by Penn State University. Genotyping was performed by the Virginia Institute for Psychiatric and Behavioral Genetics. Genotyping was conducted using commercially available primer and probe sequences from TaqMan Assays-on-Demand (Applied Biosystems, Foster City, CA). Duplicate genotyping produced concordance rates of 100 percent. rs10482672 was successfully genotyped for 94.6% of the sample and was in Hardy-Weinberg equilibrium (p=1.0).

Sample

We analyzed data from all European-American Fast Track participants with available NR3C1 genotype data. (This same sample formed the basis of our earlier report, Albert et al. 2014.) Of 439 European-American participants enrolled in the Fast Track RCT, 62% (n=270) provided a DNA sample that was successfully genotyped at NR3C1; 98% (N=260) of this genetic sample provided data on time 1 measures of psychosocial functioning, and 90% (N=242) were interviewed at age 25 (Treatment n=114; Control n=128). Attrition analyses comparing the age 25 analytic sample of N=242 to the complete European-American Fast Track sample of N=439 on the pre-intervention severity-of-risk score used to screen children into the Fast Track RCT found no statistically significant differences between the full Fast Track sample and the analytic sample for either treated or control children (p-values=0.835). Pre-intervention severity-of-risk score did not differ between control and treated children within the analytic sample (p=0.237).

Measures

Interviews were conducted annually with participants and their parents and teachers during the school years of the trial, and at age-25 with participants and a peer who knew the participant well.

Pre-Intervention Measures of Psychosocial Functioning—Parent ratings on the Child Behavior Checklist and teacher ratings on the parallel Teacher Report Form (Achenbach, 1991) were collected in the summer following kindergarten, before the start of the Fast Track RCT in first grade. We utilize t-scores for the Externalizing and Internalizing broadband scales and the following eight subscales: Anxious/Depressed, Social Problems, Somatic Complaints, Withdrawn, Thought Problems, Delinquency Attention Problems, and

Aggression. Scores were computed as the average of parent- and teacher-report. We also utilize the *severity-of-risk screen* score described above.

Diagnostic Assessments of Child Externalizing Psychopathology—We used the Parent Interview version of th**e** NIMH Diagnostic Interview Schedule for Children (DISC) to assess *Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition* (*DSM-IV*) disorders in the summers following children's completion of Grades 3 and 6. The DISC is a highly structured, laptop-administered, clinical interview that is well-validated for assessing disorders in children and adolescents aged 6–18 years. We used Version 2.3 after Grade 3, following the published anticipated DSM-IV criteria for diagnosis, and Version IV after Grade 6 (Shaffer, Fisher, Lucas, & Comer, 2003). Condition-blind lay interviewers were trained in clinical methods and scoring accuracy by an expert clinical psychologist until demonstrating proficiency. Parent interviews were administered in child's home with the primary parent, usually the mother.

Following recommendations, at the Grade 3 assessment, criteria were solicited for the past 6 months for Oppositional Defiant Disorder (ODD) and Attention-Deficit Hyperactivity Disorder (ADHD), and for the past 12 months for Conduct Disorder (CD). Criteria were solicited for the past 12 months for all disorders at all subsequent assessments. The ADHD variable omitted DSM criteria based on age of onset and criteria in more than one setting. Criteria counts were computed for each of the three disorders (ODD, CD, ADHD) at each assessment.

Measures of Adolescent Problem Behavior—We assessed delinquent behaviors and alcohol and cannabis use at follow-ups from grade 7 through two years post high school. *Delinquency* was measured using the Self-Reported Delinquency Scale from the Denver Youth Study (Huizinga & Elliott, 1987), which measures involvement in property damage, theft, assault, and substance use. The score indexes the proportion of 25 general delinquency behaviors in which the child was involved. *Alcohol Use* and *Cannabis Use* were measured using the *Tobacco, Alcohol and Drugs (grades 7–12) and Tobacco, Alcohol and Drugs-Revised (years 1 and 2 post-high school)* assessment instruments (Bureau of Labor Statistics, 2002). For alcohol, the instrument measured the number of days consuming 5+ drinks and number of days drunk in the past year. These two numbers were averaged to calculate *days of problem drinking*. For cannabis, the instrument measured *days of cannabis consumption in the past month*. Each score was computed as an average of the eight annual reports; participants were required to have non-missing data for at least 50% of the assessments.

Diagnostic Assessment of Externalizing Psychopathology at Age 25 years—Externalizing psychopathology was assessed at age 25 using three standardized instruments administered to participants by condition-blind interviewers. Each participant was also invited to nominate a peer for an independent, confidential interview about the participant. 702 participants (81% of those living) and 535 peers (76% of participants, net 62% of total) provided data. Participation did not differ significantly by condition (n's= 352 control and 350 intervention). For each problem indicator defined below, we coded the problem as present (1) if either the participant or the informant interview responses met criteria, and not

present (0) otherwise. The outcome of *Any Externalizing Psychopathology* was defined as having any of the following externalizing mental health problems: *Antisocial Personality Disorder* and *ADHD* were defined by DSM-IV criterion items from the *Adult Self Report (ASR)* (Achenbach & Rescorla, 2003) instrument used for participant interviews and the parallel *Adult Behavior Checklist-Friend (ABCL-F)* (Achenbach & Rescorla, 2003) used for peer interviews.

Alcohol Abuse Disorder was defined according to the Alcohol and Drug Module of the NIMH Diagnostic Interview Schedule (DIS) (Robins et al., 1981) completed by participants and nominated peers. *Marijuana Abuse* (defined as 27 or more days of use in the past month) and *Serious Substance Use* (cocaine, crack, inhalants, heroin, LSD, PCP, ecstasy, mushrooms, speed and other pills not prescribed by a physician in the past month) were defined from participant responses to the *Tobacco, Alcohol and Drugs Version-III*, a 57-item open-ended and forced-choice instrument based on measures from the National Longitudinal Study of Adolescent Health (Bureau of Labor Statistics, 2002) and from peer responses to an identical instrument adapted for this study.

Detailed documentation of all Fast Track measures is provided on the Fast Track website (www.fastrackproject.org/data-instruments.php).

Analyses

We conducted intent-to-treat analyses, i.e. we treated all children randomized into the intervention condition as if they had received the full dosage of Fast Track intervention. Analyses proceeded in the three developmental backtracking steps outlined in the introduction. In Step 1, we tested genetic main-effects on the social adjustment of Fast Track participants in kindergarten, prior to their enrollment in the intervention trial. In Step 2, we tested GxI effects on proximal developmental phenotypes of childhood externalizing psychopathology (grades 3–6) and adolescent problem behavior (grade 7 through 2 years following high school). In Step 3, we tested mediation of GxI effects on age-25 externalizing psychopathology by the developmental phenotypes analyzed in Step 2. Analyses for Step 1 were conducted using linear regression models. Analyses for Steps 2 and 3 were conducted using structural equation modeling approaches.

The structural equations used in analysis Steps 2 and 3 modeled the childhood and adolescent outcomes as latent variables. The latent variable for childhood externalizing psychopathology was identified by six indicators corresponding to grade 3 and grade 6 parent-reported symptoms of CD, ODD, and ADHD. After freeing three pairs of error terms to covary (grade 3 ADHD with grade 6 ADHD; grade 6 ADHD with grade 6 ODD; and grade 3 ADHD with grade 6 CD), the measurement model showed adequate fit (CFI=0.99, RMSEA=0.07). The latent variable for adolescent problem behavior was identified by indicators for alcohol use, cannabis use, and delinquency. Each indicator was calculated as the mean of self-reported behavior from annual assessments collected from grade 7 through two years post-high school. Because the measurement model included only 3 indicators, fit statistics could not be calculated. All indicators demonstrated large standardized factor loadings (>.6). We standardized the scales of both latent variables to support interpretation

of effects in terms of number of standard deviations (i.e., factor mean = 0; factor variance =1).

Step 2 structural equations modeled proximal developmental phenotypes as a function of main effects terms for genotype and intervention condition, a product term testing the GxI interaction, and a covariate for pre-intervention severity-of-risk. A simplified version of the model for a given proximal outcome is

$$Proximal\ Developmental\ Phenotype = i + a_1 Treatment + a_2 Genotype + a_3 Treatment \times Genotype + \nu X + \varepsilon \quad \text{Eq 1}$$

where *i* is an intercept and *X* is a vector of covariates. GxI hypothesis tests were conducted with the a_3 coefficient. All analyses are reported using an additive genetic model (effects in terms of each additional susceptibility allele carried).

We probed significant GxI interactions to determine whether treatment effects were significantly different from zero for each of the three rs10482672 genotypes (0/1/2 'A' alleles). We estimated conditional treatment effects following the simple slopes approach described by Aiken and West (1991):

$$Conditional\ Treatment\ Effect = a_1 + a_3 * Genotype \quad \text{Eq 2}$$

Step 3 structural equations modeled the extent to which GxI effects on proximal developmental phenotypes mediated the GxI effect on age-25 externalizing psychopathology. Because this mediation analysis focused on an interaction effect, the model can formally be described as testing mediated moderation (Preacher, Rucker, & Hayes, 2007). To test mediated moderation, we fitted structural equation models that simultaneous analyzed two equations. The first equation models the mediator. In our case, this equation is identical to Eq. 1, which estimates the GxI effect on a *proximal developmental phenotype* as a_3. The second equation estimates the effect of the GxI interaction and the proximal developmental phenotype on variable on Age-25 psychopathology as

$$\begin{aligned} &Age25\,Psychopathology \\ &\quad = i + \text{Ä}\breve{\text{G}}_1\, Treatment + \text{Ä}\breve{\text{G}}_2\, Genotype + \text{Ä}\breve{\text{G}}_3\, Treatment \times Genotype \\ &\quad + b_1\, Developmental\ Phenotype + \nu X + \varepsilon \end{aligned} \quad \text{Eq 3}$$

where b_1 is the effect of the proximal developmental phenotype – the second path in the indirect effect – and the *ć* coefficients are the "un-mediated" direct effects of *Treatment*, *Genotype*, and *Treatment* × *Genotype*. We computed point estimates of indirect effects based on coefficient estimates derived from these equations, using the product-of-coefficients method (MacKinnon, 2008). The point estimate for mediated moderation is $a_3 \times b_1$, which is the product of the *Treatment* × *Genotype* effect on the Mediator (a_3) and the Mediator effect on *Age-25 Psychopathology* (b_1) (Preacher et al., 2007). We evaluated the statistical significance of indirect effect estimates using bias-corrected bootstrap confidence intervals (95% CIs) based on 5000 draws with replacement (Preacher & Hayes, 2008). We

estimated the magnitude of each significant indirect effect as a mediation ratio (indirect/total effects; Ditlevsen, Christensen, Lynch, Damsgaard, & Keiding, 2005).

Structural equation models were estimated in Mplus version 7.1 (Muthén & Muthén, 2010). Models testing effects on the binary *Any Externalizing Psychopathology* outcome used the weighted least squares (WLSMV) estimator with a probit link function. All other models used the maximum likelihood estimator. All structural equation models evaluated below showed excellent fit to the data ($\chi 2$ $p>0.3$, CFI>0.99, TLI>0.98, RMSEA<0.02). Complete goodness-of-fit statistics are available in the Supplemental Table 1.

RESULTS

In our original GxI analysis, rs10482672 genotype moderated the effect of Fast Track intervention on externalizing psychopathology at age 25 years in a for-better-and-for-worse fashion: in the treatment arm of the trial, carriers of the 'A' allele were less likely to manifest externalizing psychopathology as compared to non-carriers; in the control arm of the trial, carriers of the rs10482672 'A' allele were more likely to manifest externalizing psychopathology as compared to non-carriers. Across all genotype-trial arm combinations, A-carriers in the control arm had the highest rates of externalizing psychopathology and A-carriers in the treatment arm had the lowest rates of externalizing psychopathology (Figure 1).

Step 1. Test genetic main effects on pre-treatment manifestations of risk for the intervention target

To begin our developmental analysis, we looked 20 years back in time to the initial Fast Track assessments. We asked whether children's rs10482672 genotype predicted differences in their psychosocial function at kindergarten entry, before the Fast Track intervention began. We tested whether children who carried more copies of the rs10482672 'A' alleles exhibited worse psychosocial function as measured by the severity-of-risk score used to select children into the Fast Track trial and ten target subscales of the Achenbach family of instruments. Because measurements were taken prior to randomization, analyses included the full Fast Track RCT sample (N=260). Before the intervention began, children who carried more copies of the rs10482672 A allele were similar to their peers who carried fewer copies on the severity-of-risk score used to screen children into the Fast Track trial and on eight of the ten Achenbach scales, although in all cases, the children who carried two copies of the A allele had the highest scores. Children who carried more copies of the A allele did differ from peers who carried fewer copies on two Achenbach scales. As rated by their parents and their teachers, children who carried more A alleles exhibited more Anxious/Depressed behavior ($\beta=0.17$, $p=0.008$) and more Thought Problems ($\beta=0.14$, $p=0.023$) as compared to peers who carried fewer copies. Full regression results are included in Table 1.

Because this main-effect of genotype on children's anxious/depressed behavior and thought problems was not anticipated and because we conducted a relatively large number of tests, we sought to replicate the result in an independent sample, the Child Development Project (CDP; N=363, 50% male; Dodge, Bates, & Pettit, 1990). We analyzed rs10482672 genotype associations with psychosocial function in kindergarten, also measured via the Achenbach

scales. In replication of what we observed in the Fast Track sample, CDP children who carried more copies of the rs10482672 A allele exhibited more Anxious/Depressed behavior as compared to peers who carried fewer copies (β=0.15, p=0.005). CDP children who carried more copies of the rs10482672 A allele also exhibited more Withdrawn behavior (β=0.11, p=0.033) and were rated higher on the broadband Internalizing scale (β=0.13, p=0.011) as compared to peers who carried fewer copies. Full details of this replication analysis are included in Supplemental Table 2). Thought Problems were not measured in the CDP study.

Step 2. Test GxI effects on proximal developmental phenotypes measured between the initiation of treatment and the time of final outcome assessment

The second step in our developmental analysis examined GxI effects on proximal developmental phenotypes measured during childhood and adolescence. We began with an analysis of externalizing psychopathology in elementary school (grades 3–6). In parallel to our analysis of age-25 psychopathology, we observed a for-better-and-for-worse GxI interaction between rs10482672 genotype and Fast Track treatment predicting children's externalizing psychopathology (Figure 3, Panel A). For each additional copy of the susceptibility allele a child carried, the Fast Track treatment decreased childhood externalizing psychopathology by 0.88 standard deviations (p=0.003). We quantified treatment effects for each genotype as described in Equation 2. There was no effect of Fast Track treatment on childhood externalizing psychopathology for children who carried no copies of the susceptibility allele (p=0.278). For children who carried one susceptibility allele, the Fast Track treatment decreased childhood externalizing psychopathology by 0.71 standard deviations (p=0.007). For children who carried two susceptibility alleles, the Fast Track treatment decreased childhood externalizing psychopathology by 1.59 standard deviations (p=0.003). Full regression results are included in Table 2 Panel 1.

Next, we followed children in the Fast Track trial through their adolescent years. We again observed a for-better-and-for-worse GxI interaction between rs10482672 genotype and Fast Track treatment predicting adolescents' problem behavior from grade 7 through the two years following the end of high school (Figure 3, Panel B). For each additional copy of the susceptibility allele a child carried, the Fast Track treatment decreased adolescent problem behavior by 1.33 standard deviations (p<.001). There was no effect of Fast Track treatment on adolescent problem behavior for children who carried no copies of the susceptibility allele (p=0.293). For children who carried one susceptibility allele, the Fast Track treatment decreased adolescent problem behavior by 1.14 standard deviations (p<.001). For children who carried two susceptibility alleles, the Fast Track treatment decreased adolescent problem behavior by 2.47 standard deviations (p<.001). Full regression results are included in Table 2 Panel 2.

Step 3. Test the hypothesis that GxI effects on proximal developmental phenotypes mediate the GxI effect on the long-term outcome

The third step in our developmental analysis tested the hypothesis that the GxI effects on proximal developmental outcomes analyzed in steps 2 and 3 mediated the ultimate GxI effect on age-25 externalizing psychopathology. We estimated the mediated moderation

equations (i.e., Eq 1 and 3) using both proximal developmental outcomes as mediators in turn. Developmental phenotypes measured in childhood and adolescence were statistically significant mediators of the GxI effect on age-25 externalizing psychopathology, accounting accounted for 16% and 49% of the total GxI effect, respectively (Table 3 Panels 1–2).

The final step in our developmental analysis tested the hypothesis that a portion of the GxI effect on age-25 externalizing psychopathology was mediated by a unique GxI effect on adolescent problem behavior, net of the GxI effect on childhood externalizing psychopathology. We estimated a structural equation model that simultaneously evaluated mediation of GxI effects on age-25 externalizing psychopathology by the two proximal developmental mediators, as illustrated in Figure 4. A portion of the GxI effect on the adolescent developmental phenotype was independent of any GxI effect on the childhood developmental phenotype. In turn, this independent GxE effect on the adolescent developmental phenotype accounted for 40% of the total GxI effect on age-25 externalizing psychopathology. Point estimates and confidence intervals for direct and indirect effects are included in Table 3 Panel 3.

GENERAL DISCUSSION

We conducted a 3-step developmental backtracking analysis to investigate mechanisms mediating genetic heterogeneity in the effects of the Fast Track intervention. We previously observed that carriers of the rs10482672 “A” allele responded to Fast Track in a for-better-and-for-worse fashion: at age 25 years, A carriers had the lowest risk of externalizing psychopathology if they were randomized to the Fast Track trial treatment arm and the highest risk of externalizing psychopathology if they were randomized to the control arm. Our developmental backtracking analyses revealed some evidence that A-carriers were at increased risk at baseline, before the intervention began, although this risk manifested not as externalizing psychopathology, but as anxious-depressed and thought problems symptoms. As we followed the children forward in time, the GxI effect emerged early on in the course of the intervention. GxI effects were detected for externalizing psychopathology measured at grades 3–6. These effects persisted and grew larger in adolescence. During these intermediate developmental stages, A-carriers in the treatment arm manifested the lowest levels of the developmental externalizing phenotypes while A-carriers in the control arm manifested the highest levels of the developmental externalizing phenotypes. In turn, GxI effects on child and adolescent developmental phenotypes mediated over half of the total GxI effect observed at the age-25 follow-up.

These findings have implications for how interventions to prevent externalizing psychopathology are theorized, designed, and evaluated, and for future research into the differential susceptibility hypothesis. The primary implication of our study for theories of early childhood intervention is that significant heterogeneity exists in how children at risk to develop externalizing psychopathology respond to a complex, long-running intervention like Fast Track, and that this heterogeneity has something to do with stress biology, specifically glucocorticoid signaling. Glucocorticoid signaling is traditionally studied in relation to internalizing psychopathology (e.g., Owens et al., 2014). And consistent with this literature, we find that in two independent samples of intervention naïve kindergarteners, those who

carried the *NR3C1* susceptibility allele experienced elevated anxiety-depression and thought problem symptoms. It is a novel contribution of our study that *NR3C1* variation and, by implication, glucocorticoid signaling, may represent an important dimension in the responsiveness of childhood externalizing psychopathology to preventive intervention.

With respect to the design and evaluation of interventions, our study offers two lessons. First, effective intervention takes time. Although a portion of the GxI effect was manifest already during elementary school, the majority was not detected until later in adolescence, when the full 10 years of intervention had been delivered. One implication of this finding is that population delivery of improved early-childhood education may not fully address the needs of the children who benefited from the Fast Track intervention. Second, intervention effects may grow even beyond the completion of treatment. The total GxI effect on age-25 externalizing psychopathology exceeded the portion that was mediated by childhood and adolescent developmental phenotypes. This result is broadly consistent with other randomized trials of early childhood interventions that show effects of increasing magnitude over developmental time—even beyond the end of intervention delivery (Campbell et al., 2014; Eckenrode et al., 2010; Heckman, 2006). Evaluations of the effectiveness and, in particular, the cost-effectiveness of intervention with young children may not have full information until those children have grown to adulthood.

In terms of the translational significance of our finding, we wish to be clear that our data do not indicate that genetic testing can discern a child's susceptibility to interventions like Fast Track. The value of our genetic analysis is instead to point toward a dimension of children's physiology that may provide clues as to whether they are likely to benefit from intervention and why. Important next steps are to evaluate exactly how the polymorphism we studied relates to glucocorticoid signaling in children at risk to develop externalizing psychopathology and how glucocorticoid signaling phenotypes, such as cortisol response, may forecast outcomes for children receiving interventions to prevent or treat externalizing symptoms.

With respect to differential susceptibility research, our findings offer provocative supporting evidence for the hypothesis that heightened sensitivity of the stress response system is at the core of the susceptibility phenotype. *NR3C1* is established as a gene encoding individual differences in the HPA-axis response to social stressors (DeRijk et al., 2008, Manenschijn et al., 2009, Kumsta et al., 2007; Kumsta et al., 2009; van West et al., 2010). We found evidence that a common *NR3C1* variant modified Fast Track intervention response in the classic for-better-and-for worse pattern. Future differential susceptibility research should incorporate *NR3C1* genotypes alongside those of the traditional neurotransmitter genes.

We acknowledge limitations. First, our sample was small and included only European-American Fast Track participants. We focused our analysis on this group because this is the group within which we detected the original GxI effect. Now that we have documented the GxI effect within the context of a randomized trial, larger-scale analyses relying on observational data can be conducted to evaluate the robustness of the finding. Second, our mediation analyses focused on behavioral outcomes in development (children's externalizing symptoms, adolescents' problem behaviors), not psychological processes.

Further research is needed to uncover specific effects of the GxI on socio-emotional development that gave rise to these behavioral changes. Third, our study is right-censored at age 25 years. We do not know if the reduction in externalizing psychopathology at age-25 will persist. Fast Track participants are now aging out of the developmental period during which externalizing symptoms are most common in the general population. An important further test of the GxI will be whether it extends to the prevention of the most damaging and costly life-course-persistent cases (Moffitt, 1993). Finally, our study is not able to specify which component of the Fast Track intervention interacted with genotype to influence behavioral outcomes. GxI studies based on single-component interventions are needed to test hypotheses regarding genetic susceptibility to specific environmental exposures.

Differential susceptibility research is still in its early stages. Studies testing differential-susceptibility hypotheses in the context of randomized trials serve as an acid test of the hypothesis because they enforce strict independence between an individual's susceptibility and the environment to which they are exposed. There is an interest in using differential-susceptibility research to design precision interventions. At least insofar as long-running, high-cost interventions such as Fast Track are concerned, such precision targeting is, at best, ethically fraught. A great deal more research is needed to develop and refine the screening necessary to even consider such a project. More realistic, in our view, is the use of differential susceptibility research to inform developmental theories of how environments affect children's development. We know that children respond to their environments in different ways. As our study begins to illustrate, a powerful contribution of differential susceptibility research is to elucidate how and why these divergent responses come about.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

WDA was supported in part by NICHD HD007376-22 and NIDA DA16903. DWB was supported in part by NIA AG000029. The Fast Track project was supported by National Institute of Mental Health (NIMH) grants R18 MH48043, R18 MH50951, R18 MH50952, R18 MH50953, and by National Institute on Drug Abuse (NIDA) grant 1 RC1 DA028248-01. The Center for Substance Abuse Prevention and NIDA also provided support for Fast Track through a memorandum of agreement with the NIMH. This work was also supported in part by Department of Education grant S184U30002, NIMH grants K05MH00797 and K05MH01027, and NIDA grants DA16903, DA015226, DA017589, and DA023026.

Dr. Greenberg is an author of the PATHS curriculum and has a royalty agreement with Channing-Bete, Inc. Dr. Greenberg is a principal in PATHS Training, LLC. Dr. McMahon is a coauthor of Helping the Noncompliant Child and has a royalty agreement with Guilford Publications, Inc.; he is also a member of the Treatments That Work Scientific Advisory Board with Oxford University Press.

REFERENCES

Achenbach, TM. Manual for the Child Behavior Checklist and 1991 Profile. Burlington, VT: University of Vermont Department of Psychiatry; 1991.

Achenbach, TM.; Rescorla, LA. Manual for ASEBA Adult Forms & Profiles. Burlington: University of Vermont, Research Center for Children, Youth, & Families; 2003.

Albert WD, Belsky DW, Crowley DM, Latendresse SJ, Aliev F, Riley B, Sun C, Conduct Problems Prevention Research Group. Dick DM, Dodge KR. Can genetics predict response to complex

behavioral interventions? Evidence from a genetic analysis of the Fast Track randomized control trial. 2014

Bakermans-Kranenburg MJ, van IJzendoorn MH. Gene-environment interaction of the dopamine D4 receptor (DRD4) and observed maternal insensitivity predicting externalizing behavior in preschoolers. Developmental Psychobiology. 2006; 48(5):406–409. [PubMed: 16770765]

Bakermans-Kranenburg MJ, van IJzendoorn MH. Differential susceptibility to rearing environment depending on dopamine-related genes: New evidence and a meta-analysis. Development and psychopathology. 2011; 23(01):39–52. [PubMed: 21262038]

Bakermans-Kranenburg MJ, Van IJzendoorn MH, Pijlman FTA, Mesman J, Juffer F. Experimental evidence for differential susceptibility: Dopamine D4 receptor polymorphism (DRD4 VNTR) moderates intervention effects on toddlers' externalizing behavior in a randomized controlled trial. Developmental Psychology. 2008; 44:293–300. [PubMed: 18194028]

Belsky DW, Moffitt TE, Baker T, Biddle AH, Evans JP, Harrington HL, Houts R, Meier MH, Sugden K, Williams B, Poulton R, Caspi A. Polygenic risk and the developmental progression to heavy, persistent smoking and nicotine dependence: Evidence from a 4-decade longitudinal study. JAMA Psychiatry (formerly Archives of General Psychiatry). 2013; 70:534–542.

Belsky DW, Moffitt TE, Caspi A. Genetics in population health science: Strategies and opportunities. American Journal of Public Health. 2013; 103:S73–S83. [PubMed: 23927511]

Belsky DW, Moffitt TE, Houts RH, Bennett GG, Biddle AH, Blumenthal J, Evans JP, Houts RH, Sugden K, Williams B, Poulton R, Caspi A. Polygenic risk, rapid childhood growth, and the development of obesity: Evidence from a 4-decade longitudinal study. Archives of Pediatrics & Adolescent Medicine (now JAMA Pediatrics). 2012; 166:515–521.

Belsky J. Variation in susceptibility to rearing influence: An evolutionary argument. Psychological Inquiry. 1997; 8:182–186.

Belsky J, Bakermans-Kranenburg M, van IJzendoorn M. For Better and For Worse: Differential Susceptibility to Environmental Influences. Current Directions in Psychological Science. 2007; 16:305–309. [PubMed: 20300493]

Belsky J, Pluess M. Beyond diathesis stress: Differential susceptibility to environmental influences. Psychological Bulletin. 2009; 135:885–908. [PubMed: 19883141]

Belsky J, Pluess M. Beyond Risk, Resilience and Dysregulation: Phenotypic Plasticity and Human Development. Development and Psychopathology. 2013; 25:1243–1261. [PubMed: 24342838]

Bet PM, Penninx BW, Bochdanovits Z, Uitterlinden AG, Beekman AT, van Schoor NM, Deeg DJ, Hoogendijk WJ. Glucocorticoid receptor gene polymorphisms and childhood adversity are associated with depression: New evidence for a gene-environment interaction. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2009; 150B:660–669.

Boyce WT, Chesney M, Alkon A, Tschann JM, Adams S, Chesterman B, et al. Psychobiologic reactivity to stress and childhood respiratory illnesses: Results of two prospective studies. Psychosomatic Medicine. 1995; 57:411–422. [PubMed: 8552730]

Boyce WT, Ellis BJ. Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. Development and Psychopathology. 2005; 17:271–301. [PubMed: 16761546]

Brody GH, Chen YF, Beach SR. Differential susceptibility to prevention: GABAergic, dopaminergic, and multilocus effects. Journal of Child Psychology and Psychiatry. 2013; 54:863–871. [PubMed: 23294086]

Bureau of Labor Statistics. National Longitudinal Survey of Youth 1997 Cohort, 1997–2001. Produced by the National Opinion Research Center, University of Chicago, and distributed by the Center for Human Resource Research, The Ohio State University; 2002.

Campbell F, Conti G, Heckman JJ, Moon SH, Pinto R, Pungello E, Pan Y. Early childhood investments substantially boost adult health. Science. 2014; 343(6178):1478–1485. [PubMed: 24675955]

Conduct Problems Prevention Research Group. A developmental and clinical model for the prevention of conduct disorders: The Fast Track Program. Development and Psychopathology. 1992; 4:509–527.

Conduct Problems Prevention Research Group. Initial impact of the Fast Track prevention trial for conduct problems: I. The high-risk sample. Journal of Consulting and Clinical Psychology. 1999; 67:631–647. [PubMed: 10535230]

Conduct Problems Prevention Research Group. Using the Fast Track randomized prevention trial to test the early-starter model of the development of serious conduct problems. Development and Psychopathology. 2002; 14:925–943. [PubMed: 12549710]

Conduct Problems Prevention Research Group. The effects of the Fast Track program on serious problem outcomes at the end of elementary school. Journal of Clinical Child and Adolescent Psychology. 2004; 33:650–661. [PubMed: 15498733]

Conduct Problems Prevention Research Group. Fast Track randomized controlled trial to prevent externalizing psychiatric disorders: Findings from grades 3 to 9. Journal of the American Academy of Child & Adolescent Psychiatry. 2007; 46:1250–1262. [PubMed: 17885566]

Conduct Problems Prevention Research Group. The difficulty of maintaining positive intervention effects: A look at disruptive behavior, deviant peer relations, and social skills during the middle school years. Journal of Early Adolescence. 2009; 30:593–624.

Conduct Problems Prevention Research Group. Fast Track intervention effects on youth arrests and delinquency. Journal of Experimental Criminology. 2010; 6:131–157. [PubMed: 20577576]

Conduct Problems Prevention Research Group. The effects of the Fast Track preventive intervention on the development of conduct disorder across childhood. Child Development. 2011; 82:331–345. [PubMed: 21291445]

Conduct Problems Prevention Research Group. Impact of early intervention on psychopathology, crime, and well-being at age 25. American Journal of Psychiatry. 2014

DeRijk RH, van Leeuwen N, Klok MD, Zitman FG. Corticosteroid receptor-gene variants: Modulators of the stress-response and implications for mental health. European Journal of Pharmacology. 2008; 585:492–501. [PubMed: 18423443]

Desrivieres S, Lourdusamy A, Muller C, Ducci F, Wong CP, Kaakinen M, Pouta A, Hartikainen AL, Isohanni M, Charoen P, Peltonen L, Freimer N, Elliott P, Jarvelin MR, Schumann G. Glucocorticoid receptor (NR3C1) gene polymorphisms and onset of alcohol abuse in adolescents. Addiction Biology. 2011; 16:510–513. [PubMed: 20731635]

Dick DM. Gene-environment interaction in psychological traits and disorders. Annual review of clinical psychology. 2011; 7:383.

Dick DM, Latendresse SJ, Riley B. Incorporating genetics into your studies: a guide for social scientists. Frontiers in Psychiatry. 2011; 2:1–11. [PubMed: 21556272]

Ditlevsen S, Christensen U, Lynch J, Damsgaard MT, Keiding N. The mediation proportion: A structural equation approach for estimating the proportion of exposure effect on outcome explained by an intermediate variable. Epidemiology. 2005; 16:114–120. [PubMed: 15613954]

Dodge KA, Bates JE, Pettit GS. Mechanisms in the cycle of violence. Science. 1990; 250:1678–1683. [PubMed: 2270481]

Dodge KA, Greenberg MT, Malone PS. Conduct Problems Prevention Research Group. Testing an idealized dynamic cascade model of the development of serious violence in adolescence. Child Development. 2008; 79:1907–1927. [PubMed: 19037957]

Dodge KA, McCourt SN. Translating models of antisocial behavioral development into efficacious intervention policy to prevent adolescent violence. Developmental Psychobiology. 2010; 52:277–285. [PubMed: 20175096]

Eckenrode J, Campa M, Luckey DW, Henderson CR, Cole R, Kitzman H, Olds D. Long-term Effects of Prenatal and Infancy Nurse Home Visitation on the Life Course of Youths: 19-Year Follow-up of a Randomized Trial. Archives of Pediatrics and Adolescent Medicine. 2010; 164:9–15. [PubMed: 20048236]

Ellis BJ, Boyce WT, Belsky J, Bakermans-Kranenburg MJ, van IJzendoorn MH. Differential Susceptibility to the Environment: A Neurodevelopmental Theory. Development & Psychopathology. 2011; 23:7–28. [PubMed: 21262036]

Epstein RS, Moyer TP, Aubert RE, O'Kane DJ, Xia F, Verbrugge RR, Gage BF, Teagarden JR. Warfarin genotyping reduces hospitalization rates, results from the MM-WES (Medco-Mayo

Warfarin Effectiveness study). Journal of the American College of Cardiology. 2010; 55(25): 2804–2812. [PubMed: 20381283]

Fardet L, Petersen I, Nazareth I. Suicidal behavior and severe neuropsychiatric disorders following glucocorticoid therapy in primary care. American Journal of Psychiatry. 2012; 169:491–497. [PubMed: 22764363]

Hawes DJ, Brennan J, Dadds MR. Cortisol, callous-unemotional traits, and pathways to antisocial behavior. Current Opinions in Psychiatry. 2009; 22:357–362.

Heckman JJ. Skill formation and the economics of investing in disadvantaged children. Science. 2006; 312:1900–1902. [PubMed: 16809525]

Huizinga D, Elliott DS. Juvenile offenders: Prevalence, offender incidence, and arrest rates by race. Crime & Delinquency. 1987; 33:206–223.

Kegel CAT, Bus AG, van IJzendoorn MH. Differential susceptibility in early literacy instruction through computer games: The role of the dopamine D4 receptor gene (DRD4). Mind Brain and Education. 2011; 5:71–78.

Kochanska G, Philibert RA, Barry RA. Interplay of genes and early mother–child relationship in the development of self-regulation from toddler to preschool age. Journal of Child Psychology and Psychiatry. 2009; 50(11):1331–1338. [PubMed: 19207629]

Kumsta R, Entringer S, Koper JW, van Rossum EF, Hellhammer DH, Wust S. Sex specific associations between common glucocorticoid receptor gene variants and hypothalamus-pituitary-adrenal axis responses to psychosocial stress. Biological Psychiatry. 2007; 62:863–869. [PubMed: 17716631]

Kumsta R, Moser D, Streit F, Koper JW, Meyer J, Wust S. Characterization of a glucocorticoid receptor gene (GR, NR3C1) promoter polymorphism reveals functionality and extends a haplotype with putative clinical relevance. American Journal of Medical Genetics Part B-Neuropsychiatric Genetics. 2009; 150B:476–482.

Lopez-Duran NL, Olson SL, Hajal NJ, Felt BT, Vazquez DM. Hypothalamic pituitary adrenal axis functioning in reactive and proactive aggression in children. Journal of Abnormal Child Psychology. 2009; 37:169–182. [PubMed: 18696227]

MacKinnon, D. Introduction to statistical mediation analysis. Routledge: 2008.

Manenschijn L, van den Akker EL, Lamberts SW, van Rossum EF. Clinical features associated with glucocorticoid receptor polymorphisms. An overview. Ann N Y Acad Sci. 2009; 1179:179–198. [PubMed: 19906240]

McBurnett K, Lahey BB, Frick PJ, Risch C, Loeber R, Hart EL, Christ MA, Hanson KS. Anxiety, inhibition, and conduct disorder in children: II. Relation to salivary cortisol. Journal of the American Academy of Child and Adolescent Psychiatry. 1991; 30:192–196. [PubMed: 2016221]

McEwen BS. Brain on stress: How the social environment gets under the skin. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109:17180–17185. [PubMed: 23045648]

Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annual Review of Neuroscience. 2001; 24:1161–1192.

Mill J, Wigg K, Burcescu I, Vetro A, Kiss E, Kapornai K, Tamas Z, Baji I, Gadoros J, Kennedy JL, Kovacs M, Barr CL. International Consortium for Childhood-Onset Mood, Disorders. Mutation screen and association analysis of the glucocorticoid receptor gene (NR3C1) in childhood-onset mood disorders (COMD). American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2009; 150B:866–873.

Mitchell C, Hobcraft J, McLanahan SS, Siegel SR, Berg A, Brooks-Gunn J, Notterman D. Social disadvantage, genetic sensitivity, and children's telomere length. Proceedings of the National Academy of Sciences. 2014; 111(16):5944–5949.

Mitchell C, Notterman D, Brooks-Gunn J, Hobcraft J, Garfinkel I, Jaeger K, McLanahan S. Role of mother's genes and environment in postpartum depression. Proceedings of the National Academy of Sciences. 2011; 108(20):8189–8193.

Moffitt TE. Adolescence-limited and life-course-persistent antisocial behavior: A developmental taxonomy. Psychological Review. 1993; 100:674–701. [PubMed: 8255953]

Muthén, LK.; Muthén, BO. Mplus: Statistical analysis with latent variables: User's guide. Muthén & Muthén; 2010.

Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. American Journal of Human Genetics. 2004; 74:765–769. [PubMed: 14997420]

Owens M, Herbert J, Jones PB, Sahakian BJ, Wilkinson PO, Dunn VJ, Goodyer IM. Elevated morning cortisol is a stratified population-level biomarker for major depression in boys only with high depressive symptoms. Proceedings of the National Academy of Sciences. 2014; 111(9):3638–3643.

Patterson, GR.; Reid, JB.; Dishion, TJ. Antisocial boys. Eugene, OR: Castalia; 1991.

Robins, LN. Deviant children grown up. Baltimore: Williams & Wilkins; 1966.

Robins LN, Helzer JE, Croughan J, Ratcliff KS. National Institute of Mental Health Diagnostic Interview Schedule: Its history, characteristics, and validity. Archives of General Psychiatry. 1981; 38:381–389. [PubMed: 6260053]

Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews. 2000; 21:55–89. [PubMed: 10696570]

Savitz J, Lucki I, Drevets WC. 5-HT1A receptor function in major depressive disorder. Progress in Neurobiology. 2009; 88:17–31. [PubMed: 19428959]

Shaffer, D.; Fisher, P.; Lucas, C.; Comer, J. Scoring manual: Diagnostic interview schedule for children (DISC-IV). New York: Columbia University; 2003.

Sheese BE, Voelker PM, Rothbart MK, Posner MI. Parenting quality interacts with genetic variation in dopamine receptor D4 to influence temperament in early childhood. Development and Psychopathology. 2007; 19(04):1039–1046. [PubMed: 17931433]

Stadler C, Poustka F, Sterzer P. The heterogeneity of disruptive behavior disorders - implications for neurobiological research and treatment. Frontiers in Psychiatry. 2010; 1:1–14. [PubMed: 21451740]

Van IJzendoorn M, Bakermans-Kranenburg M, Belsky J, Beach S, Brody G, Dodge K, Greenberg M, Posner M, Scott S. Gene by environment experiments: A new approach to find missing heritability. Nature Reviews Genetics. 2011; 12:881.

van West D, Del-Favero J, Deboutte D, Van Broeckhoven C, Claes S. Associations between common arginine vasopressin 1b receptor and glucocorticoid receptor gene variants and HPA axis responses to psychosocial stress in a child psychiatric population. Psychiatry Research. 2010; 179:64–68. [PubMed: 20472303]

van West D, Van Den Eede F, Del-Favero J, Souery D, Norrback KF, Van Duijn C, Sluijs S, Adolfsson R, Mendlewicz J, Deboutte D, Van Broeckhoven C, Claes S. Glucocorticoid receptor gene-based SNP analysis in patients with recurrent major depression. Neuropsychopharmacology. 2006; 31:620–627. [PubMed: 16192984]

van de Wiel NM, van Goozen SH, Matthys W, Snoek H, van Engeland H. Cortisol and treatment effect in children with disruptive behavior disorders: a preliminary study. Journal of the American Academy of Child and Adolescent Psychiatry. 2004; 43:1011–1018. [PubMed: 15266196]

van Rossum EF, Binder EB, Majer M, Koper JW, Ising M, Modell S, Salyakina D, Lamberts SW, Holsboer F. Polymorphisms of the glucocorticoid receptor gene and major depression. Biological Psychiatry. 2006; 59:681–688. [PubMed: 16580345]

van Zuiden M, Geuze E, Willemen HLDM, Vermetten E, Maas M, Heijnen CJ, Kavelaars A. Pre-existing high glucocorticoid receptor number predicting development of posttraumatic stress symptoms after military deployment. American Journal of Psychiatry. 2011; 168:89–96. [PubMed: 21078706]

Velders FP, Dieleman G, Cents RA, Bakermans-Kranenburg MJ, Jaddoe VW, Hofman A, Van IJzendoorn MH, Verhulst FC, Tiemeier H. Variation in the glucocorticoid receptor gene at rs41423247 moderates the effect of prenatal maternal psychological symptoms on child cortisol reactivity and behavior. Neuropsychopharmacology. 2012; 37:2541–2549. [PubMed: 22781842]

Zobel A, Jessen F, von Widdern O, Schuhmacher A, Hofels S, Metten M, Rietschel M, Scheef L, Block W, Becker T, Schild HH, Maier W, Schwab SG. Unipolar depression and hippocampal

volume: impact of DNA sequence variants of the glucocorticoid receptor gene. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2008; 147B:836–843.

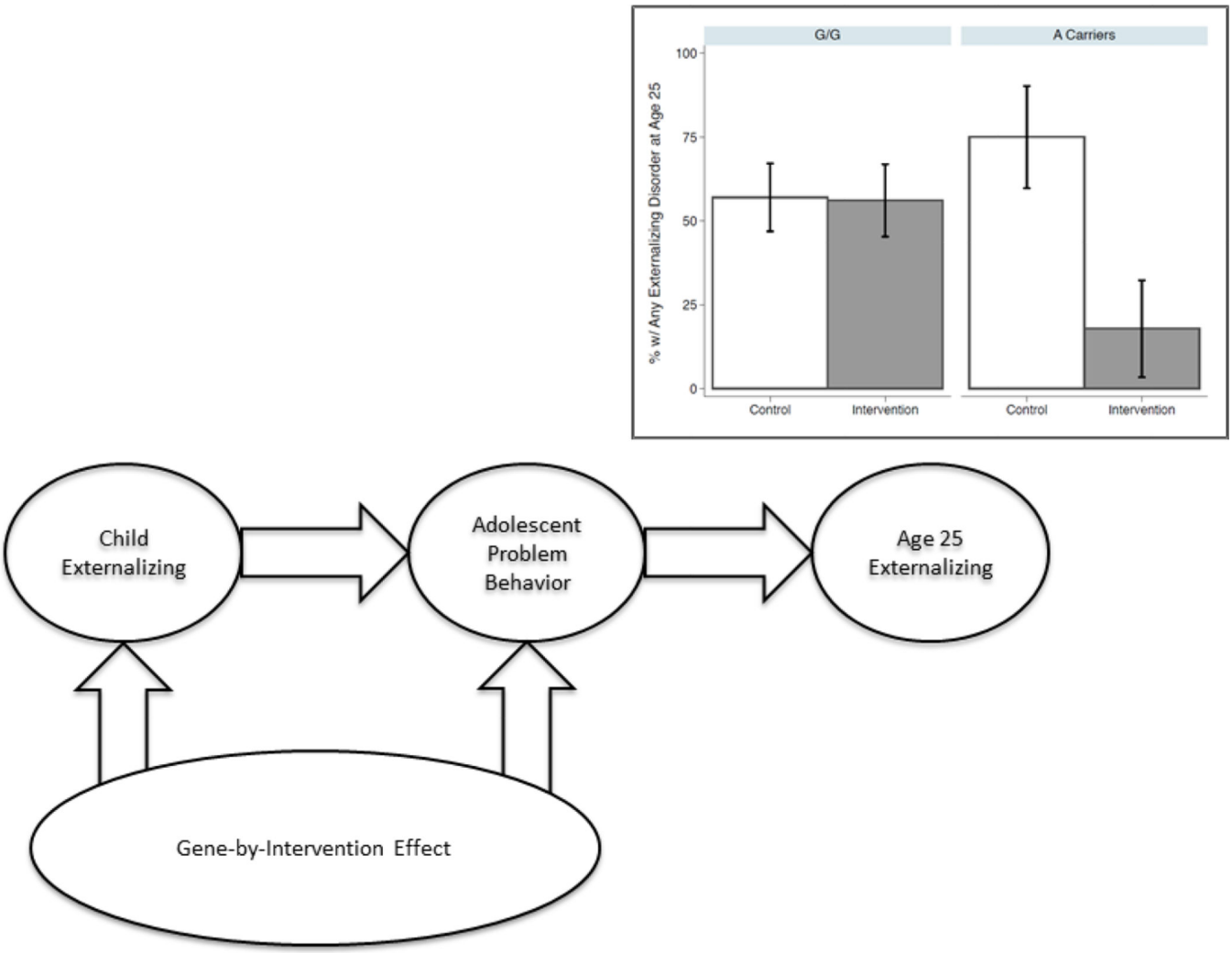

Figure 1. Conceptual Framework for Tests of Direct and Indirect Prevention Effects on Adult Externalizing

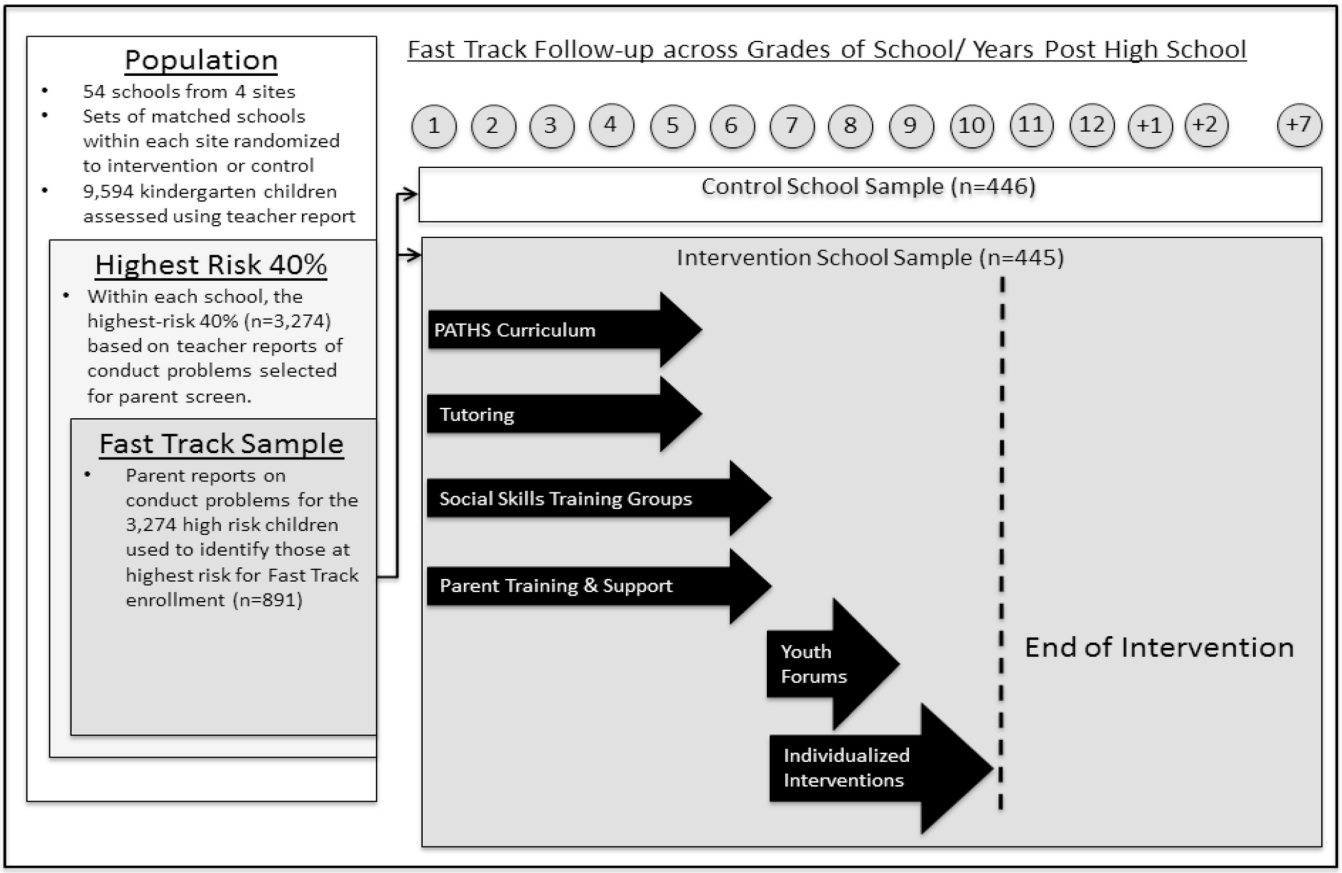

Figure 2. Fast Track Randomized Controlled Trial Design

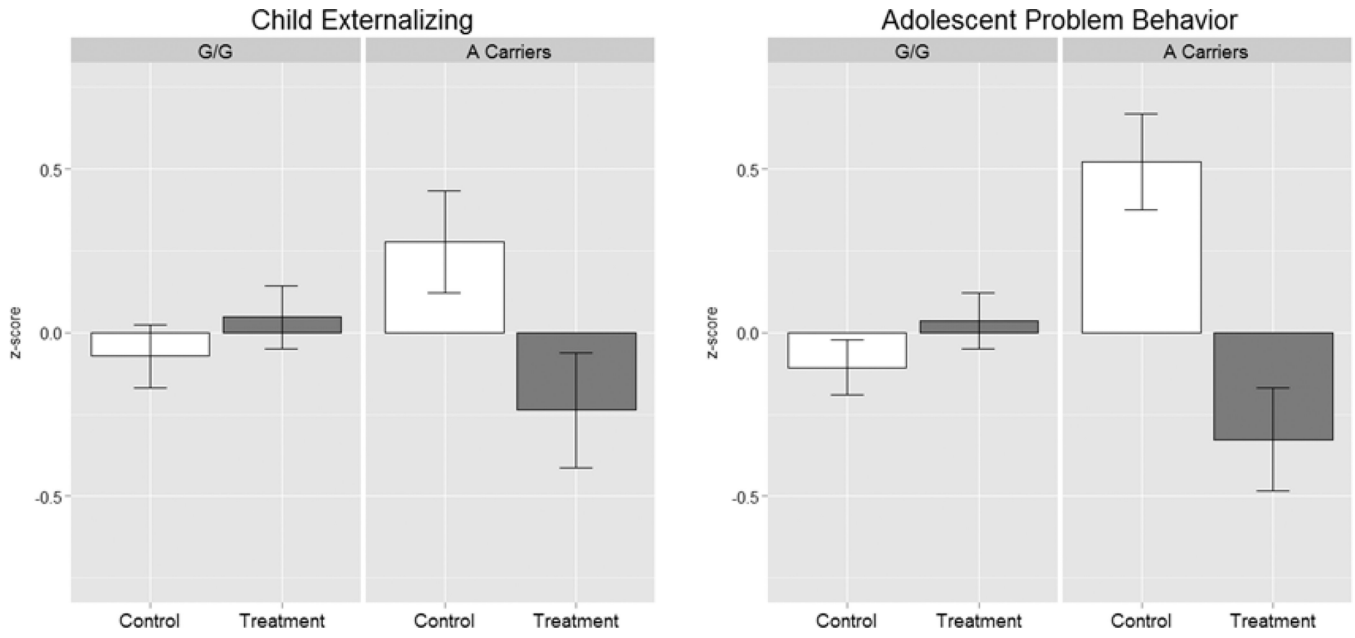

Figure 3. Gene-by-Intervention Differences in Latent Factor Means for Child Externalizing Psychopathology and Adolescent Problem Behavior

Standardized factor scores were extracted from unconditional confirmatory factor analysis models of Child Externalizing Psychopathology and Adolescent Problem Behavior, respectively. Factor indicators for the Child Externalizing factor include parent-reported symptom counts for Conduct Disorder, Oppositional Defiant Disorder, and Attention/ Deficity Hyperactivity Disorder, ascertained via in-person diagnostic interviews in the summers following the child's 3rd and and 6th grade years. Factor indicators for the Adolescent Problem Behavior factor include 3 child-report scales, each of which aggregates reports across 8 assessment years spanning the grade 7 and 2 years post-highschool. The 3 scales are (1) Alcohol Use, operationalized as number of past year binge-drinking days; (2) Cannabis Use, as number of past month days of any use; and (3) Self-Reported Delinquency (General). Measurement details are provided in the methods section of this article.

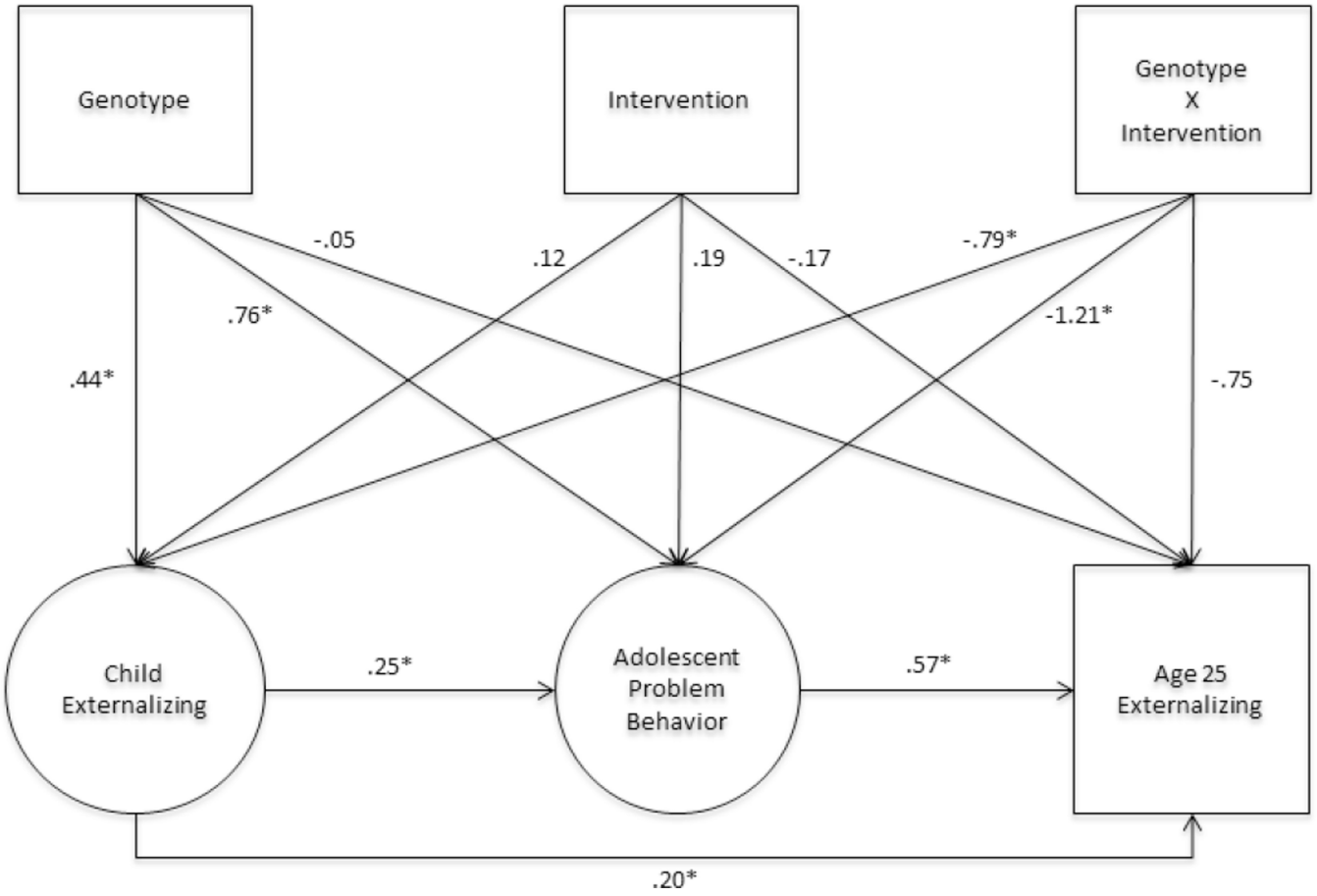

Figure 4. Path Estimates of Unique Effects of Genotype, Intervention, and GxI Effects on Child, Adolescent, and Adult Outcomes

The structural equation model showed strong fit to the data (χ^2=64.20, df=58, CFI=0.99, RMSEA=0.02). All regressions covaried for the pre-intervention risk score used to screen children into the intervention. Latent variables for child externalizing psychopathology and adolescent problem behavior are standardized (factor mean=0; variance=1). Age-25 externalizing is modeled as a latent variable (mean=0; variance=1); scores represent the probability of positive case status on the binary *Any Externalizing Psychopathology* outcome variable. *p<0.05.

Table 1

rs10482672 Associations with Psychosocial Functioning at Kindergarten Entry

Standardized beta coefficients can be interpreted as Pearson's r. Mean scores for Achenbach instruments are reported as T-scores (population mean=50 and standard deviation=10). Because the Fast Track sample was selected from a high-risk segment of the population, the means of Achenbach externalizing scales for the Fast Track sample were above the general population mean of 50. The Severity of Risk Score is mean-centered according to the scale mean observed in a normative sample drawn from the same schools as the Fast Track participants. In the normative sample, the centered scale has mean=0 and SD=1.5.

	High-Risk Kindergarteners Prior to Intervention (n=260)				
	Regression on Genotype		Mean Scores by rs10482672 'A' Alleles		
	Standardized β	p-value	0 (N=196)	1 (N=57)	2 (N=6)
Achenbach T-Scores					
Broadband					
Externalizing	0.03	0.580	61.7	61.6	65.1
Internalizing	0.10	0.110	55.0	56.3	59.9
Subscales					
Anxious/Depressed	0.17	0.008	57.1	58.7	62.9
Social Problems	0.09	0.133	59.5	60.4	63.3
Somatic Complaints	0.10	0.123	55.0	55.6	58.0
Withdrawn	0.00	0.985	56.8	56.4	58.7
Thought Problems	0.14	0.023	55.7	57.4	58.8
Delinquency	0.00	0.984	59.9	59.4	61.9
Attention Problems	0.04	0.479	60.3	60.7	63.0
Aggression	0.06	0.344	62.5	62.7	67.0
Severity-of-Risk Score	0.05	0.459	2.1	2.1	2.9

Table 2

Gene by Intervention Interaction (GxI) Analyses of Proximal Developmental Phenotypes

The table reports coefficient estimates, standard errors, and p-values estimated from structural equations modeling GxI effects on child externalizing psychopathology and adolescent problem behavior. Models were adjusted for the baseline severity of risk score.

Outcome	Predictor	β	*SE*	*p*
Child Externalizing	GxI Effect	−0.884	0.298	0.003
Psychopathology	G: rs10482672	0.467	0.183	0.011
	I: Fast Track Treatment	0.178	0.164	0.278
Adolescent Problem Behavior	GxI Effect	−1.332	0.346	0.000
	G: rs10482672	0.841	0.220	0.000
	I: Fast Track Treatment	0.193	0.183	0.293

Table 3

Mediation of Gene by Intervention Interaction (GxI) Effects on Age-25 Externalizing Psychopathology by GxI Effects on Proximal Developmental Phenotypes

Models tested mediated moderation. Direct effects refer to the GxI effects on age-25 externalizing psychopathology that was independent of any GxI effect on the proximal developmental phenotype. Indirect effects refer to GxI effects on age-25 externalizing psychopathology that were mediated by the proximal developmental phenotypes.

Model		Effect Estimate		[95% CI]	Mediation Ratio
(1)	Mediator: Child Externalizing Psychopathology				
	Direct Effect	−1.44	*	[−2.36,−0.63]	---
	Indirect Effect	−0.27	*	[−0.64,−0.05]	0.16
(2)	Mediator: Adolescent Problem Behavior				
	Direct Effect	−0.87		[−1.87,0.10]	---
	Indirect Effect	−0.84	*	[−1.52,−0.38]	0.49
(3)	Mediator: Child Externalizing Psychopathology & Adolescent Problem Behavior				
	Direct Effect	−0.75		[−1.73,0.24]	---
	Indirect Effect 1: GxI-->Child Ext.-->Age25 Ext.	−0.16		[−0.46,0.02]	0.09
	Indirect Effect 2: GxI-->Child Ext.-->Adolescent Problems-->Age25 Ext.	−0.11	*	[−0.34,−0.02]	0.07
	Indirect Effect 3: GxI-->Adolescent Problems-->Age25 Ext.	−0.69	*	[−1.39,−0.28]	0.40
	Total Indirect Effects	−0.96	*	[−1.70,−0.56]	0.56

For ease of interpretation, effect estimates that are statistically significant are denoted with a *. Models 1 and 2 tested mediated moderation for the two developmental phenotypes in turn. Model 3 tested mediated moderation when both developmental phenotypes were included simultaneously. In Model 3, indirect effect 3 can be interpreted as portion of the GxI effect on age-25 externalizing psychopathology that is attributable to GxI effects on adolescent problem behavior only (i.e. not accounted for by GxI effects on child externalizing psychopathology).