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Can Genetics Predict Response to Complex Behavioral Interventions?

Evidence from a Genetic Analysis of the Fast Track Randomized Control Trial

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ABSTRACT

Early interventions are a preferred method for addressing behavioral problems in high-risk children, but often have only modest effects. Identifying sources of variation in intervention effects can suggest means to improve efficiency. One potential source of such variation is the genome. We conducted a genetic analysis of the Fast Track Randomized Control Trial, a ten-year-long intervention to prevent high-risk kindergarteners from developing adult externalizing problems including substance abuse and antisocial behavior. We tested whether variants of the glucocorticoid receptor gene *NR3C1* were associated with differences in response to the Fast Track intervention. We found that in European-American children, a variant of *NR3C1* identified by the single-nucleotide polymorphism rs10482672 was associated with increased risk for externalizing psychopathology in control group children and decreased risk for externalizing psychopathology in intervention group children. Variation in *NR3C1* measured in this study was not associated with differential intervention response in African-American children. We discuss implications for efforts to prevent externalizing problems in high-risk children and for public policy in the genomic era.

INTRODUCTION

Intervention during childhood to promote human capital development has the potential to prevent a cascade of negative outcomes, including poor health, criminal behavior, and overreliance on government services in the future (Anderson et al., 2003; Dodge, 2009; Eckenrode et al., 2010; Garner et al., 2011; Heckman et al., 2010; O'Connell, Boat, & Warner, 2009). Evidence of this has made strategies for investing in youth a policy priority in the United States and globally (America's Promise Alliance, 2013; Barnett & Masse, 2007; Belfield et al., 2006; Heckman, 2006; Obama, 2013). A challenge is that interventions to promote human capital development are complex and expensive and "average treatment effects" are often modest. One reason for modest treatment effects is that intervention response varies across subpopulations (Bloom & Michalopoulos, 2013; Imbens & Angrist, 1994; Kraemer et al., 2002). Opportunities to maximize the fit between people and programs are therefore of keen interest in efforts to bolster intervention impacts (Duncan & Vandell, 2012; Hinshaw, 2002). Research is needed to uncover measureable characteristics of individuals that identify them as likely to respond more or less positively to intervention.

A provocative finding from developmental psychology research into variation in intervention response is that those children most at risk for adverse developmental outcomes are often the ones who benefit most from resources and services. This phenomenon has been termed "biological sensitivity to context" (Boyce & Ellis, 2005) or "differential susceptibility" (Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007). Sensitive/susceptible children are especially responsive to environmental stimuli both "for better AND for worse": Under conditions of ample environmental resources and support, these children achieve better outcomes relative to less sensitive/susceptible peers; under conditions of scarce resources and inadequate support, these

children experience adverse outcomes relative to less sensitive/susceptible peers. Initial formulations of this model focused on temperamental characteristics—personality-like traits of young children—as markers of sensitivity/susceptibility. Current differential susceptibility research is focused on genetic differences between individuals (Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007; Belsky et al., 2009; Belsky & Pluess, 2009). The best evidence for differential susceptibility—in which individuals who carry a particular genotype are both most likely to develop problems under adverse conditions and most likely to benefit from advantaged conditions—comes from studies that use randomized controlled trials to study how behavioral interventions may have different consequences for individuals who carry different variants of certain genes (Bakermans-Kranenburg & van Ijzendoorn, 2011; Brody et al., 2013; van IJzendoorn et al., 2011). To date, this approach has been used primarily to study discrete, short running interventions, including literacy support programs for preschoolers (Kegel, Bus, & van IJzendoorn, 2011) and positive parenting interventions (Bakermans-Kranenburg et al., 2008). An example of GxI research with a broader focus is the genetic analysis of the Strong African American Families intervention, a 7-week, family-based youth risk-behavior prevention program for rural African Americans (Brody et al., 2009). Here, we apply this now established method to study genetic heterogeneity in the effects of a much longer-running trial, the 10-year Fast Track intervention, which aimed to prevent high-risk kindergarteners from developing persistent externalizing psychopathology.

Our analysis focused on the glucocorticoid receptor gene *NR3C1*, which encodes a protein critical to human stress physiology. We studied Fast Track participants who provided genetic data at age 21 and interview reports on externalizing problems at age 25. We tested genetic moderation effects separately in European-American (N=242) and African-American

(N=248) subsamples. We found that a common *NR3C1* variant, identified by the single nucleotide polymorphism (SNP)[1] rs10482672, significantly moderated the effectiveness of the Fast Track intervention among European-American participants. The less common 'A' allele[2] of this SNP identified children who were both (1) especially like to develop externalizing psychopathology if they did not receive the Fast Track intervention; and (2) especially likely to benefit in terms of reduced likelihood of developing externalizing psychopathology if they did receive the Fast Track intervention (18 percent of treated 'A' carriers as compared to 75 percent of control 'A' carriers manifested externalizing psychopathology at age 25 years; in contrast, 56 percent of treated non-carriers and 57 percent of control non-carriers manifested externalizing psychopathology at age 25 years). Analyses examining children's substance use and delinquent behavior during grades 7-12 and the two years following high school indicated that gene-by-intervention effects were apparent already during adolescence. We identified no genetic moderation effects among African-American participants.

Findings have implications for intervention policy and research. First, previously unobservable contours in children's likelihood of responding to a treatment may be revealed through genetic analysis of intervention trials. Fast Track was a ten-year multi-level intervention program delivering services to high-risk children and their families through direct contact and via school-based programming. A formal economic evaluation of Fast Track estimated the total cost of the ten-year intervention at $58,000 per child (Foster, Jones, & Conduct Problems Prevention Research Group (CPPRG), 2006). Targeting these resources at those children most likely to benefit is therefore a priority. Second, genetic prediction of treatment response in the

[1] A single nucleotide polymorphism (SNP) is a single-letter change in the DNA sequence, e.g. from cytosine (C) to thymine (T), that is present in 1 percent or more of a human population (rarer variants are usually referred to as "mutations").
[2] An allele is any naturally occurring variation in DNA sequence. For example, a C/T SNP has two alleles, C and T.

case of complex behavioral interventions such as Fast Track is far from ready for prime time. But proof of concept now exists. Efforts are needed to develop data resources that can support genome-wide investigations of gene-by-intervention interactions. Third, ethical, legal, and social issues surrounding the use of genetic or other biological information to target complex behavioral interventions should receive increasing attention. In addition, our findings further highlight the now established principle that genetic influences on the development of behavioral problems are modifiable. Moreover, they point out that genetically at-risk children may be among those who benefit most from preventive interventions.

BACKGROUND

Children with early-starting conduct problems exhibit elevated risk for persistent externalizing psychopathology in adulthood, including antisocial personality, alcohol, and substance use disorders (Dodge & McCourt, 2010; Jaffee, Strait, & Odgers, 2012; Moffitt, 1993). In turn, externalizing psychopathology leads to poor individual outcomes and substantial public costs. Substance abuse alone is estimated to account for over $600 billion a year in increased costs to health, criminal justice, and government service systems (Centers for Disease Control and Prevention, 2009; National Institute of Drug Abuse, 2012; Rehm et al., 2009). Such costs underscore the critical need to intervene early and effectively with conduct-disordered children.

The Fast Track intervention design was based on evidence that children with early-starting conduct problems are at increased risk for later disorder due to a cascade of social adjustment failures in family, school, and peer contexts (CPPRG, 1992; Dodge et al., 2008). High-risk children typically enter school with a risk burden that crosses multiple domains. Early deficits in emotion regulation and behavioral control are exacerbated by dysfunctional parenting

and other stressors in the home environment (Dodge & McCourt, 2010, Moffitt, 1993). Exposure to harsh parenting contributes to the child's tendency to perceive benign social challenges as threatening and to respond with "reactive aggression" (Dodge, Bates, & Pettit, 1990). For such children, an accidental bump on the playground from a classmate may be perceived as a hostile provocation requiring an aggressive response. Aggressive children are more likely than their peers to experience social rejection and academic failure in elementary school. This in turn increases the likelihood that they will affiliate with deviant peers in middle school and escalate their involvement in delinquency, violence, and substance abuse through high school and young adulthood (Dodge et al., 2008).

The Fast Track prevention program was implemented in the early 1990's as a randomized controlled trial to test the hypothesis that a comprehensive, sustained intervention with children at risk for persistent conduct problems would have a lasting impact on the incidence of externalizing behavior in adulthood. 891 high-risk youth were randomly assigned in kindergarten to receive (or not) a 10-year comprehensive intervention. Previously published intent-to-treat analyses indicate that Fast Track was successful in reducing externalizing behavior across the elementary, high school, and young adult years (CPPRG, 1999; 2002; 2004; 2007; 2011; in press). The goal of the current paper is to investigate whether genetic differences between Fast Track participants affected how they responded to treatment. Our analysis focuses on the glucocorticoid receptor gene *NR3C1*.

Hypothesized Role of the Glucocorticoid Receptor Gene (*NR3C1*) in Regulating Response to the Fast Track Intervention

The Fast Track intervention was designed in part to help children develop capacities for emotion regulation and self-control, specifically in the context of social stress. And there is

evidence Fast Track was successful in this respect. For example, children who received the Fast Track intervention showed improved capacity to accurately appraise benign social stimuli, whereas control children were more likely to appraise these stimuli as threatening (Dodge, Godwin, & CPPRG, 2013). We hypothesized that children who were more biologically sensitive to stress would show the worst outcomes in the control arm of the trial and the best outcomes in the intervention arm of the trial. To identify such children, we turned to a gene encoding a key mediator of the social-stress response, the glucocorticoid receptor *NR3C1*.

The hypothalamus-pituitary-adrenal (HPA) axis is a key mediator of the body's response to stress. In the face of a perceived threat, hormones released from the hypothalamus result in a cascade of signaling processes with the end product being the release of the glucocorticoid cortisol, the paramount stress hormone involved in energy storage and expenditure, digestion, and immune function. Importantly, the HPA axis regulates itself by means of a negative-feedback loop. Cortisol signaling inhibits the release of hormones in the hypothalamus, down-regulating HPA axis activity and allowing the body to return to homeostasis once the perceived threat is resolved. This inhibitory signaling is mediated by glucocorticoid receptor proteins encoded by the gene *NRC31* (DeRijk et al., 2008; McEwen, 2012; Meaney, 2001; Sapolsky, Romero, & Munck, 2000). Dysregulated glucocorticoid signaling disrupts HPA axis response to and recovery from a wide range of stressors, and has been implicated in child and adult manifestations of externalizing psychopathology (Fardet, Petersen, & Nazareth, 2012; Hawes, Brennan, & Dadds, 2009; Lopez-Duran et al., 2009; McBurnett et al., 1991; Savitz, Lucki, & Drevets, 2009; Stadler, Poustka, & Sterzer, 2010; van Zuiden et al., 2011). 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).

Prior research on the glucocorticoid receptor has linked variants in the gene with mental health problems such as mood and substance abuse disorders (Ambroggi et al., 2009; Desrivieres et al., 2011; Mill et al., 2009; van Rossum et al., 2006; van West et al., 2006; Zobel et al., 2008). Studies also identify the glucocorticoid receptor variants as moderators of stress reactivity and of developmental outcomes to environmental adversity (Bet et al., 2009; DeRijk et al., 2008; Kumsta et al., 2007; Kumsta et al., 2009; Manenschijn et al., 2009; van West et al., 2010; Velders et al., 2012). Based on this evidence, we hypothesized that glucocorticoid receptor variants would differentiate individuals with a "for better and for worse" sensitivity to environmental exposure (Belsky & Pluess, 2009), characterized by reduced rates of externalizing psychopathology in response to intervention and elevated rates in the absence of intervention.

Study Overview

We tested whether variants of the glucocorticoid receptor gene affected response to intervention in subsamples of European-American (N=242) and African-American (N=248) Fast Track RCT participants who were followed prospectively from age 5 to 25 years. The outcome was externalizing psychopathology at age 25 years.

Prior research has not clarified exactly which variants in the gene *NR3C1* should be studied (Vandenbergh & Schlomer, 2014**).** Therefore, we undertook a "gene-wide" association study approach (Dick, 2011). This approach surveys variation throughout a gene and conducts comprehensive testing. Results are then subjected to a statistical correction that accounts for increased risk of false positives arising from multiple testing. We surveyed variation throughout

NR3C1 using haplotype tagging analysis[3] (Dick, Latendresse, & Riley, 2011). Haplotype tagging identified 10 single-nucleotide polymorphisms (SNPs), which were genotyped in the Fast Track sample (Appendix Figure A1**)**. We used linear probability models to test the intervention-moderating effect of each of these 10 SNPs. To correct for multiple testing, we applied an adjusted Bonferroni correction (Nyholt, 2004). SNPs demonstrating significant gene-by-intervention effects after correction for multiple testing were further examined for stratified main effects in intervention vs. control groups and subjected to sensitivity analyses.

DATA & METHODS

The Fast Track Intervention Trial

The Fast Track 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 1** illustrates the Fast Track design. Fast Track participants were selected from four geographic sites: Durham, NC; Nashville, TN; rural PA; and Seattle, WA. Fifty-four elementary schools identified from high-crime, low-SES neighborhoods were divided into demographically matched sets within each site, and one set of schools was randomly assigned to intervention and one to control. The nested boxes on the left side of the figure illustrate the multiple-gating screening procedure (Lochman, 1995) applied to kindergarteners in the 54 schools (N=9,594). First, teacher ratings of disruptive behavior using the Teacher Observation of Classroom Adaptation-Revised (TOCA-R) Authority Acceptance score (Werthamer-Larsson, Kellam, & Wheeler, 1991) were used to

[3] A haplotype is a set of DNA sequence polymorphisms that are commonly inherited together. A haplotype tagging analysis is a means of identifying the minimum number of DNA sequence variants required to explain a given threshold of variation in DNA sequence.

identify children scoring in the highest 40 percent within cohort and site (n=3,274). These children were further screened using parent-ratings on the Aggression scales of the Child Behavior Checklist (Achenbach, 1991). Teacher and parent scores were standardized within site and summed to yield a *severity-of-risk screen score*. Children were ranked on this risk score, and selected into the study in order from highest risk to lowest until desired sample sizes were reached within sites, cohorts, and conditions (979 children (10 percent of total) solicited; 891 participants—intervention n=445, control n=446—enrolled). The sample was 61 percent male, 47 percent European-American, 51 percent African-American, and 2 percent of other ethnicity. Mean age was 6.58 years (*SD* = 0.48) at enrollment in the first grade. During the elementary school phase (grades 1 to 5), intervention included a universal classroom-based implementation of the Promoting Alternative Thinking Strategies (PATHS) curriculum (Kusche & Greenberg, 1994), and opportunities to participate in 2-hr. extracurricular "enrichment programs" consisting of child social skills training, parent training/support groups, guided parent-child interaction, and academic tutoring. Enrichment programs were held weekly during grade 1, biweekly during grade 2, and monthly during grades 3 through 5. In addition, paraprofessional tutors provided three additional 30-minute sessions per week in reading and peer-pairing to improve friendships with classmates. Families were offered home visits every other week to promote positive parenting behavior and parental problem solving skills. After grade 1, criterion-referenced assessments adjusted the prescribed dosage to match need. During grades 5 and 6, children received a middle school transition program and four parent-youth group intervention sessions on topics of adolescent development; alcohol, tobacco, and drugs; and decision-making. In grades 7 and 8, eight Youth Forums (Oyserman, 2000) addressed vocational opportunities, life skills, and summer employment opportunities. In grades 7 through 10, individualized

interventions addressed parent monitoring, peer affiliation, academic achievement, and social cognition. Detailed description of Fast Track is available at www.fasttrackproject.org and in published evaluations (CPPRG, 1999, 2000, 2002, 2004, 2007, 2011).

Primary Outcome Measure: Externalizing Psychopathology at Age 25 Years

Externalizing psychopathology was assessed 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 percent of those living) and 535 peers (76 percent of participants, net 62 percent 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 *Attention Deficit/Hyperactivity Disorder* (*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.

Additional Outcome Measures

Fast Track 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 Pittsburgh 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 through12) 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. Detailed documentation of all Fast Track measures is provided on the Fast Track website (www.fastrackproject.org/data-instruments.php).

Genotyping

We hypothesized that alleles of the glucocorticoid receptor gene *NR3C1* would moderate Fast Track treatment effects because of the central role the gene plays in stress physiology. Human studies have not identified common *NR3C1* variants that reliably associate with individual differences in stress physiology. We therefore took a hypothesis-free approach to analyzing variation within *NR3C1*, consistent with current best practice in genetic epidemiology (Dick, Latendresse, & Riley, 2011). There are many common DNA sequence variants within the *NR3C1* gene. Clusters of these variants are in "linkage" (inherited together and therefore non-

independent). We used a reference database (HapMaP version 3; http://hapmap.ncbi.nlm.nih.gov/) to identify variants that "tagged" independent clusters of variants (Dick et al., 2009). The ten single-nucleotide polymorphisms (SNPs) identified through this tagging procedure were at least partially independent in the current samples (maximum linkage disequilibrium[4] as measured by R^2 equal to 0.56 (European-American) and 0.53 (African-American); **Appendix Figure A1**).

Genotyping of the 10 SNPs was conducted using DNA extracted from buccal cells collected at the phase 21 Fast Track follow-up using a cytology brush. DNA extraction was performed by Penn State University. Samples were then shipped to the Virginia Institute for Psychiatric and Behavioral Genetics for genotyping. 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. For samples passing quality control (genotyping success rate of >80 percent), call rates for the 10 *NR3C1* SNPs ranged from 87 percent to 99 percent. All SNPs were in Hardy-Weinberg equilibrium[5] (minimum p-value>0.2 for European-American and African-American samples).

Analysis

The structure of the genome differs across ethnic groups (International HapMap Consortium, 2007) and these differences can confound genetic association analyses (Cardon & Palmer, 2003). Briefly, allele frequencies vary between populations separated in human evolutionary history. These differences can give rise to spurious genetic associations in the case

[4] Linkage describes the tendency of closely-spaced segments of DNA to be inherited together. Linkage disequilibrium (LD) is a measure of the degree to which pairs of alleles occur together at greater-than-chance frequency. High LD indicates that two alleles were likely inherited together.

[5] Hardy-Weinberg equilibrium is a measure of whether the observed distribution of genotypes (homozygotes and heterozygotes) deviates from the expectation based on the frequency of individual alleles. Tests of Hardy-Weinberg equilibrium are used as a quality control measure in genetic studies.

that the outcome of interest is unequally distributed across ethnic groups within a sample. This problem is conventionally referred to as "population stratification" in the genetics literature; an accessible discussion of this issue can be found in Hamer & Sirota (2000). We address this issue in our sample, which included both African American and white children, by conducting analyses separately within ethnically homogenous groups.

Of 439 European-American participants enrolled in the Fast Track RCT, 62 percent (n=270) provided a DNA sample that was successfully genotyped at NR3C1; 90 percent (N=242) of this genetic sample were interviewed at age 25 (Treatment n=114; Control n=128). Attrition analyses comparing the 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 (see Figure 1 caption for details) 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).

Of 452 African-American RCT participants, 62 percent (n=282) were genotyped; 88 percent (N=248) of this genetic sample were interviewed at age 25 (Treatment n=127; Control n=121). This analytic sample did not differ from the full African-American sample on pre-intervention severity-of-risk scores for treatment or control groups (p-values>0.670). Pre-intervention severity-of-risk score did not differ between control and treated children within the analytic sample (p=0.779).

Across the ten SNPs analyzed, frequencies of the less-common alleles ranged from 5 percent to 44 percent. The double-helix structure of DNA means that each individual carries two copies of each nucleotide base in the DNA sequence. Each SNP genotype comprises a nucleotide

from each strand. Therefore, individuals may carry 0, 1, or 2 copies of a given allele. Carriers of 0 or 2 copies are called "homozygotes." Carriers of 1 copy are called "heterozygotes." For several SNPs, there were very few homozygotes for the less-common allele (the expected proportion of homozygotes is the squared proportion of the allele frequency). We conducted primary analyses assuming an additive model (SNPs were coded 0, 1, or 2 according to the number of copies of the test allele). Because of the low frequencies of test-allele homozygotes for some SNPs, we repeated analyses using a dominance model. In the dominance model, a value of '1' indicated the presence of one or two test alleles and a value of '0' indicated the presence of no test alleles.

To test whether variation in *NR3C1* moderated the effects of the Fast Track intervention, we fitted a series of 10 linear probability models (one for each SNP) predicting the outcome of *Any Externalizing Psychopathology* at age 25 as a function of Fast Track treatment condition, SNP genotype, and a product term measuring the interaction between treatment and genotype:

$$prob(Any\ Externalizing\ Psychopathology) = \alpha + \beta Treatment + \gamma Genotype + \delta Treatment \times Genotype + \nu X + \mathcal{E}$$

where *X* is a vector of covariates. All models included sex as a covariate. Sensitivity analyses also included site, cohort and pre-intervention severity-of-risk as covariates.

Hypothesis tests were conducted with the δ coefficient for the product term testing interaction between Fast Track treatment and genotype. We adjusted for multiple testing using a modified Bonferoni correction according to the procedure described by Nyholt (2004). After accounting for the linkage structure (non-0 correlation) among the ten SNPs, the significance criteria approximating α=0.05 for the European-American and African-American samples were p=0.0056 and p=0.0054, respectively. We conducted a series of post-hoc analyses on SNPs

meeting the adjusted significance criterion, including sensitivity analyses and analyses examining the diagnostic specificity of interaction effects. All analyses were conducted using both additive and dominance models. We report coefficient estimates and p-values from the additive model (effects in terms of each additional test allele carried). We report outcome prevalences according to the dominance model (carriers vs. non-carriers of test alleles). All analyses were conducted in SPSS 20.0.

RESULTS

Children in the intervention group of the Fast Track RCT were less likely to manifest *Any Externalizing Psychopathology* at age 25 years as compared to peers randomized to the control condition (prevalence for European-American children was 46 percent in the intervention group and 61 percent in the control group, p=0.013; for African-American children, prevalence was 35 percent in the intervention group and 58 percent in the control group).

Among European-American children, the effect of intervention was moderated by variation in the glucocorticoid receptor gene *NR3C1*; the Fast Track intervention was more efficacious in preventing externalizing psychopathology among carriers of the rs10482672 'A' allele (δ=-0.520, p= 0.00006). Among carriers of the 'A' allele, 18 percent of treated children as compared to 75 percent of control children manifested any externalizing psychopathology at age 25 follow-up. In contrast, for non-carriers of the 'A' allele, 56 percent of treated children and 57 percent of control children manifested externalizing psychopathology at follow-up (**Figure 2**). The remaining 9 SNPs did not show evidence of moderating Fast Track intervention response. Among African-American children, there was no evidence that any of the measured SNPs moderated Fast Track intervention effects. Full results for European-American and African-American samples for all ten tested SNPs are reported in **Appendix Tables A1 and A2.**

We conducted a series of sensitivity analyses to evaluate the robustness of the rs10482672 finding among European-American participants. First, to test whether the gene-by-intervention interaction was sensitive to the coding of SNP genotypes, we repeated the analysis assuming a dominance model (in which the SNP was coded as 0 or 1 according to the absence or presence of one or more 'A' alleles). Results were unchanged (δ=-0.548, p=0.00018). As a further check, because there were few AA homozygotes for rs10482672 (n=5 treated, n=1 control), we excluded these individuals and repeated the analysis. Again, results were unchanged (δ=-.499, p=0.00111). Second, we tested whether the gene-by-intervention interaction was sensitive to whether externalizing psychopathology status was reported by participants as compared to peer informants. Results were similar regardless of the source of outcome information (for self-reports, δ=-0.415, p= 0.00106; for peer-reports, δ=-0.466, p= 0.00670). Third, we repeated analyses with adjustment for site, cohort, and pre-intervention severity-of-risk for externalizing psychopathology, which was found to moderate intervention effectiveness for some outcomes in previous evaluations of Fast Track (e.g., CPPRG 2011). This analysis also included a severity-of-risk by intervention product term (Keller, 2013). Results from this model were unchanged (δ=-0.551, p< 0.00004).

Finally, we conducted a series of analyses to examine the specificity of the gene-by-intervention interaction to the components of the *Any Externalizing Psychopathology* indicator used in our discovery analysis. **Figure 3** shows that, across components, carriers of the rs10482672 'A' allele experienced a more beneficial effect of intervention as compared to non-carriers, but this difference was statistically significant only in the cases of *Alcohol Abuse* (δ=-0.427, p= 0.00086) and *ADHD* (δ=-0.191, p= 0.03399).

We next asked whether the result we observed at the age-25 follow-up was already apparent earlier in life. We examined summary measures of participants' self-reported delinquency, problem alcohol use, and problem cannabis use across an 8-year window spanning grade 7 through two years post high school. Analyses were limited to the European-American subsample. Parallel to results at age 25 follow-up, Fast Track intervention was associated with lower levels of delinquency, alcohol use, and cannabis use during the adolescent period for rs10482672 'A' carriers; for non-carriers of the 'A' allele, the intervention had no effect (for delinquency, δ=-0.034, p=0.00200; for alcohol, δ=-4.165, p= 0.00185; for cannabis, δ=-2.390, p= 0.00714). Complete model results are reported in **Appendix Tables A3 and A4.**

Finally, we tested how the gene-by intervention interaction effect manifested over the adolescent period. Fast Track intervention children who carried A-alleles exhibited a slower progression of substance use behavior during adolescence as compared to A-carriers in the control condition; children who did not carry an A allele exhibited similar patterns of change in substance use during adolescence (i.e., gene-by-intervention-by-time effects in repeated measures ANCOVA analysis for alcohol use, ηp^2=0.012, p=0.014; for cannabis use, ηp^2=0.017, p=0.010). Gene-by-intervention effects on delinquency did not change over the 8-year period (ηp^2=0.007, p=0.178). Between-subjects ANCOVA analysis indicated statistically significant differences in mean levels of externalizing behaviors (for delinquency, ηp^2=0.042, p=0.002; for alcohol use, ηp^2=0.042, p=0.002; for cannabis use, ηp^2=0.051, p=0.001). Complete model results are reported in **Appendix Table A5**. **Figure 4** shows average levels of each of the externalizing measures from grade 7 through 2-years post high school in the intervention and control groups for carriers and non-carriers of the rs10482672 'A' allele.

DISCUSSION AND POLICY IMPLICATIONS

We conducted a genetic analysis of treatment response in the Fast Track randomized control trial, a 10-year-long intervention to prevent externalizing psychopathology. We tested whether carrying certain alleles of the glucocorticoid receptor gene *NR3C1* predisposed children to benefit more or less from the Fast Track intervention. We found that European-American children who carried one or two copies of the rs10482672 'A' allele benefitted from Fast Track intervention more than did their age- and sex-matched peers who did not carry a copy of this allele. The difference in treatment effect between these two groups was large. For 'A' carriers, 18 percent of treated children manifested externalizing psychopathology at adult follow-up, as compared to 75 percent of control children. In contrast, for children who did not carry an 'A' allele, Fast Track had no effect; 56 percent of treated children and 57 percent of control children manifested externalizing psychopathology at follow-up. Sensitivity analyses indicated that moderation of the Fast Track intervention effect on externalizing psychopathology at age 25 years by rs10482672 genotype was primarily due to increased effectiveness of the intervention in preventing alcohol abuse and ADHD in rs10482672 'A' allele carriers. Genetic moderation of intervention effects was observable already during adolescence. Across assessments spanning grade 7 through two years post high school, Fast Track intervention was associated with lower levels of self-reported delinquency, alcohol use, and cannabis in children who carried an rs10482672 'A' allele, but not in their peers who did not carry an 'A' allele. None of the *NR3C1* SNPs genotyped in our study showed evidence of moderating Fast Track intervention response in African-American children.

The absence of replication of findings for rs10482672 in the African-American sample may be an artifact of differences in the genomes of African-American and European-American

children in the Fast Track sample. We used a tagging SNP approach to measure variation in *NR3C1*. (Instead of identifying variants in the gene that were likely to cause differences in intervention effects, we instead identified variants that provided a parsimonious summary of all variation in *NR3C1*.) African-descent populations are more genetically diverse than European-descent populations and there is less linkage in African-descent as compared to European-descent genomes. These differences could have the result that our SNP set was a less comprehensive measure of *NR3C1* variation for the African-American children as compared to the European-American children. Therefore, the possible causal variant that was measured with rs10482672 in the European-American sample might have gone unmeasured in the African-American sample.

We acknowledge limitations. First, our analysis was based on a small sample. Only 242 European-American and 248 African-American Fast Track participants met inclusion criteria. Replication is needed. Nevertheless, use of a randomized controlled trial increases power and bolsters confidence that results are not confounded by unmeasured correlations between genotype and environment (Fletcher & Conley, 2013; McClelland & Judd, 1993). A specific feature of the Fast Track design that may increase power is the focus of the trial on very high-risk children among whom rates of adult externalizing psychopathology absent treatment were expected to be high. This expectation proved correct. By age 25 years, the majority of children in the control condition manifested externalizing psychopathology. A second limitation of our data is that we did not observe the complete sequence of the *NR3C1* gene. Instead, we observed a set of tagging SNPs identified from reference data. Further analyses in additional samples are needed to identify the causal sequence that is the source of the signal detected at rs10482672. Third, it is uncertain whether results generalize to non-white populations. To the extent that the

tagging SNPs we observed captured less variation in the surrounding sequence in African-American as compared to European-American children, it could lead to spurious null findings in the African American sample. Moreover, we were unable to account for the likely substantial genetic diversity within the African American sample. The lack of genome-wide data in our study precluded the use of a comprehensive strategy to address potential confounding by racial admixture. It is also the case that race differences could exist in base rates and/or reporting behaviors related to the outcomes we studied (Costanzo et al., 2007). However, previous Fast Track evaluations have found no evidence for race differences in program impacts on similar outcome measures (CPPRG, 2007; 2011; in press). Fourth, analyses focused on a single gene, the glucocorticoid receptor *NR3C1*. We expect there are dozens, if not hundreds of genes and intergenic loci involved in regulating response to Fast Track and other complex behavioral interventions. Ultimately, genome-wide investigations of variants moderating intervention effects will be needed to uncover genetic signatures of intervention response that can be used to select individuals into interventions. Our study provides proof of concept to motivate the development of databases capable of supporting genome-wide inquiry.

Developing databases to conduct large-scale genome-wide gene-by-intervention interaction research in the field of human capital development depends on two key ingredients. First, randomized trials of interventions must collect genetic data from participants and obtain consent for the use of these data in post hoc analyses. Second, investigators running randomized trials must participate in collaborative networks to share data. Consortia are necessary because genetic influences on the outcomes targeted by human capital interventions are complex and magnitudes of individual genetic effects are likely to be very small (Benjamin et al., 2012; Chabris et al., 2012). Consortia are possible, as illustrated by a recent genome-wide association

study of educational attainment that integrated dozens of datasets to achieve a sample of hundreds of thousands of individuals (Rietveld et al., 2013). Consortia are worth the effort as evidenced by their track record in the biomedical science where, despite high complexity of genetic influences on the outcomes studied, thousands of discoveries have been made (www.genome.gov/gwasstudies) and clinical applications translating these discoveries are beginning to emerge (Manolio, 2013).

Moving beyond implications for a general research agenda, findings from this specific study can provide an opportunity to begin a discussion about the implications of genetic research for the prevention of externalizing psychopathology in particular and, more generally, for program development and policy in the genomic era. With respect to efforts to prevent externalizing psychopathology, our findings indicate that (1) common genetic variation may predispose some children to developing externalizing psychopathology; but that (2) this predisposition is susceptible to preventative intervention. In other words, there is a group of children that is both highly likely to develop externalizing psychopathology and also highly likely to benefit from preventative intervention—and it may be possible to identify them. These observations lend support to intervention strategies that target high-risk children (in contrast to those that are delivered to the entire population), but they raise questions about how that targeting should be conducted. Specifically, they raise the possibility that genetic information could help to identify those children most likely to benefit from intervention. This is of particular interest in the case of long running, costly interventions with high risk populations, such as Fast Track.

With respect to program development and policy, we highlight three issues. (1) Genetic differences between individuals may influence risk for adverse outcomes, e.g. externalizing

psychopathology. But a genetic predisposition does not represent an immutable risk. In fact, genetically mediated risks may be as treatable or more so than risks arising from environmental exposures. (2) The way the genome relates to outcomes is complicated and may depend on features of the environment that are amenable to policy intervention; in the Fast Track treatment group, the same allele predicting increased risk among control children predicted a two-thirds reduction in risk among treated children. Modifiable features of the environment that mitigate or amplify genetic risks are appealing intervention targets because changes to these environments benefit the population and provide added benefit to individuals carrying the genetic risk modified by the environmental change. (3) Policy makers and program developers should begin grappling with the ethical challenges that come with knowledge of how genetic differences between individuals may affect response to publicly financed interventions. Medical science is involved in ongoing debate over ethical means of incorporating genetic information into screening and treatment decisions (Berg, Khoury, & Evans, 2011; Dancey et al., 2012; Evans, Skrzynia, & Burke, 2001; Goldenberg & Sharp, 2012). Our tentative evidence that common genetic variation may affect response to an intervention like Fast Track suggest that this same debate should be going on in the social and behavioral sciences as well. To be clear, findings from our study in particular and from behavioral genetics in general are not ready for "prime time." But that may not always be the case. If we are eventually able to predict which children will respond to an intervention on the basis of their DNA (and this is not guaranteed), there are reasons we may not wish to use this information to restrict access to the intervention. It would be helpful to begin a conversation about such decisions now, in order that social policy may evolve with the technology rather than in response to it.

Figure 1. Fast Track Randomized Controlled Trial Design.

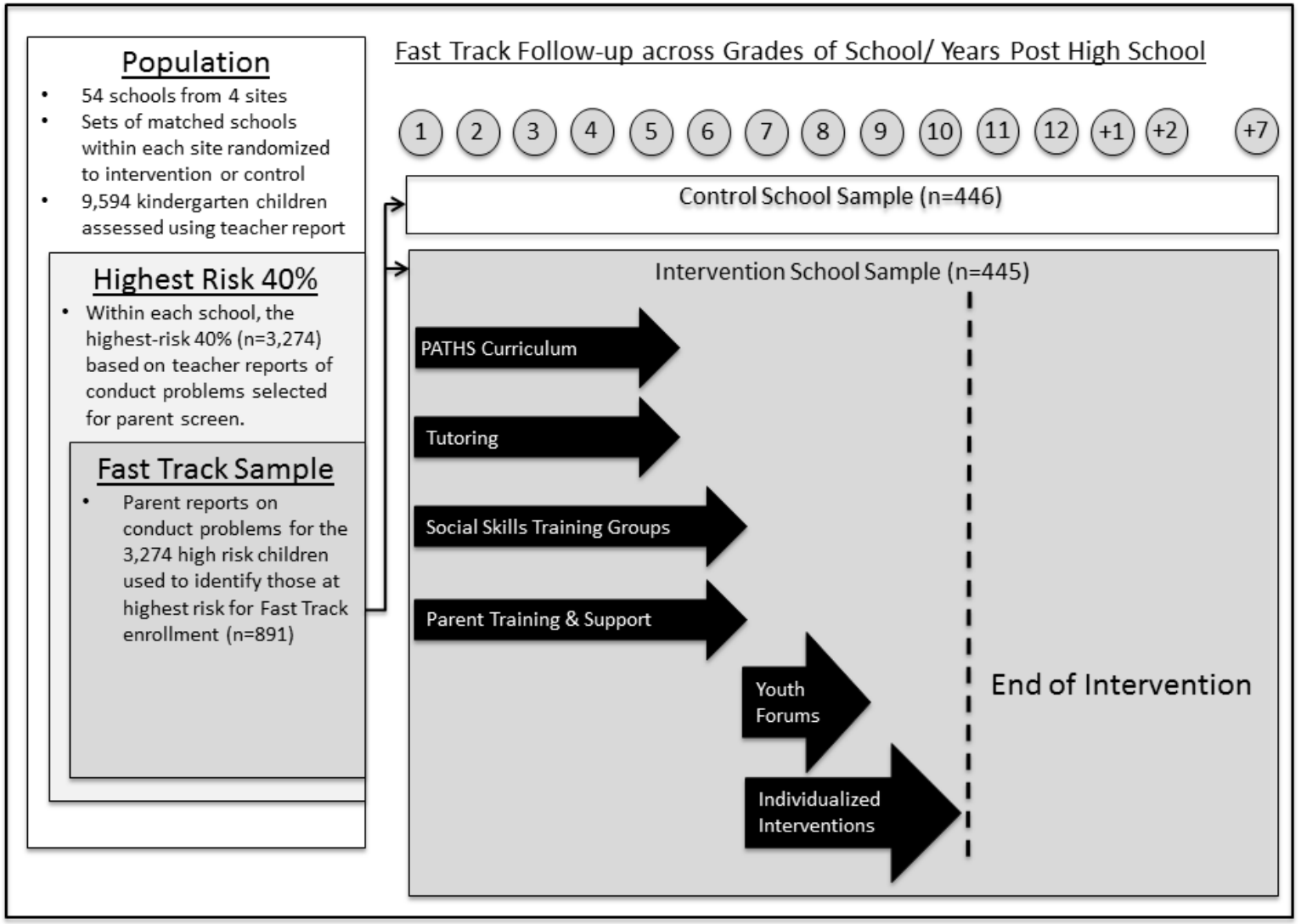


Figure 2. Prevalence of Any Externalizing Psychopathology in European-American Fast Track Intervention and Control Children by Carriage of the rs10482672 'A' Allele. The G/G group carried no copies of the A allele. The A Carrier group carried one or two copies of the A allele.

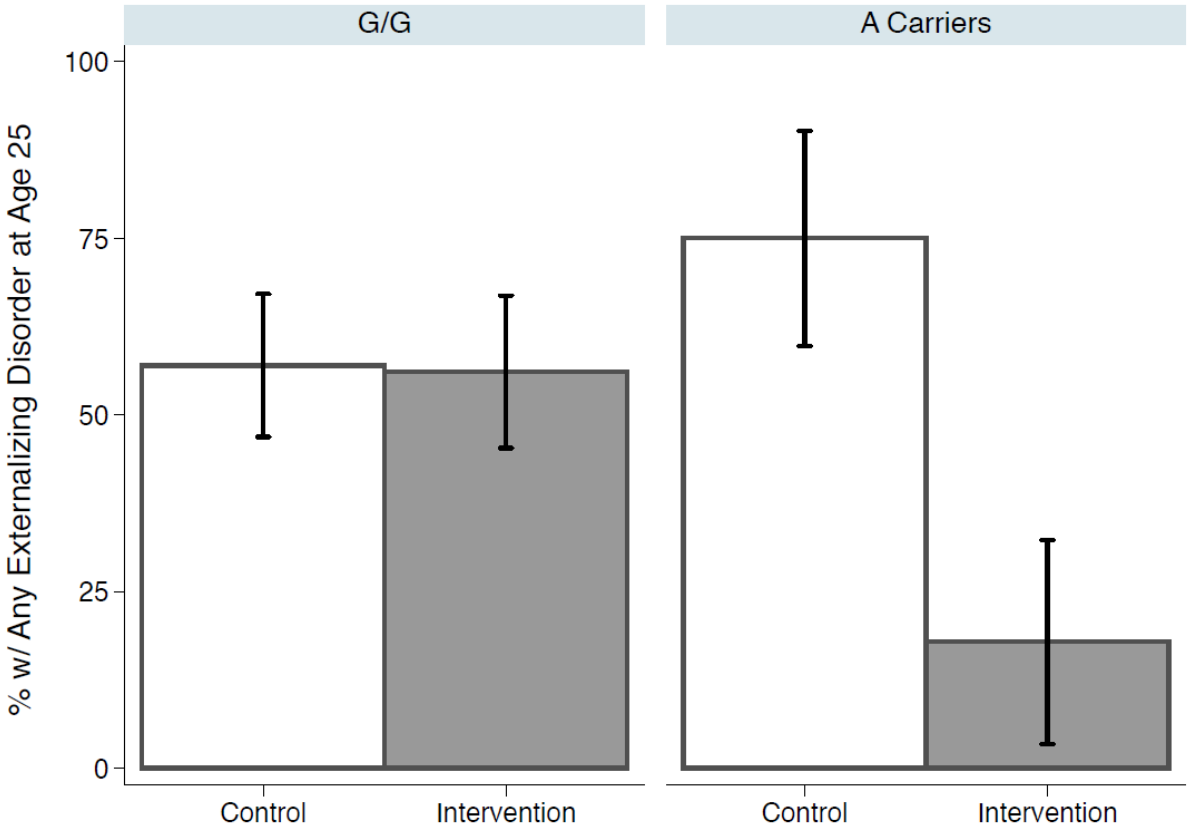


Figure 3. Prevalence of Alcohol Abuse, Cannabis Abuse, Hard Drug Use, Attention Deficit/Hyperactivity Disorder, and Antisocial Personality Disorder in European-American Fast Track Intervention and Control Children by Carriage of the rs10482672 'A' Allele. The G/G group carried no copies of the A allele. The A Carrier group carried one or two copies of the A allele.

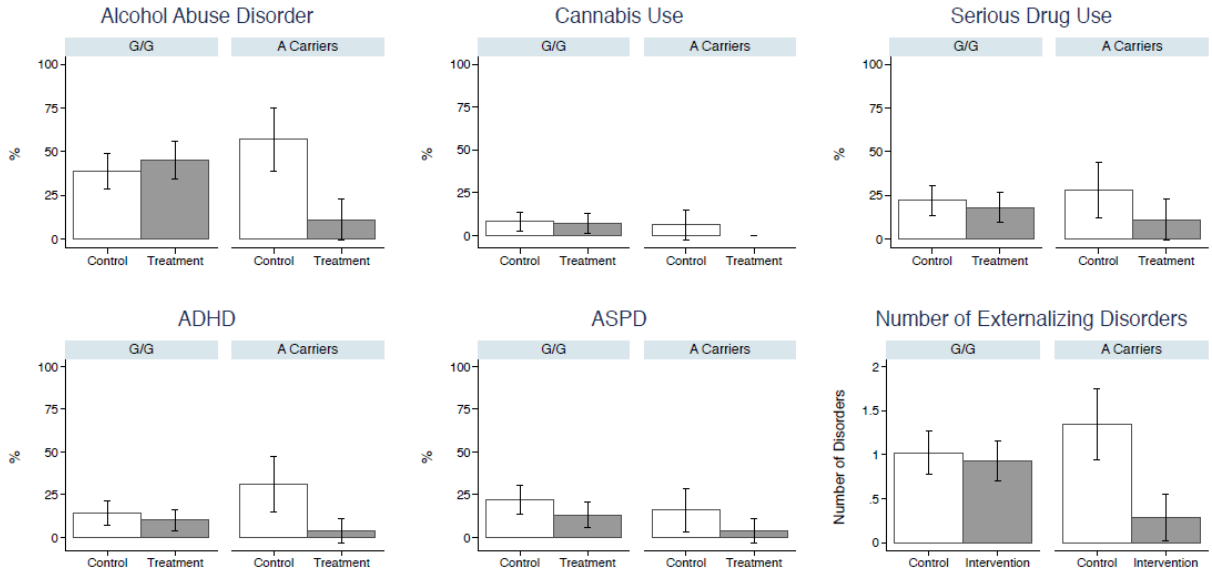
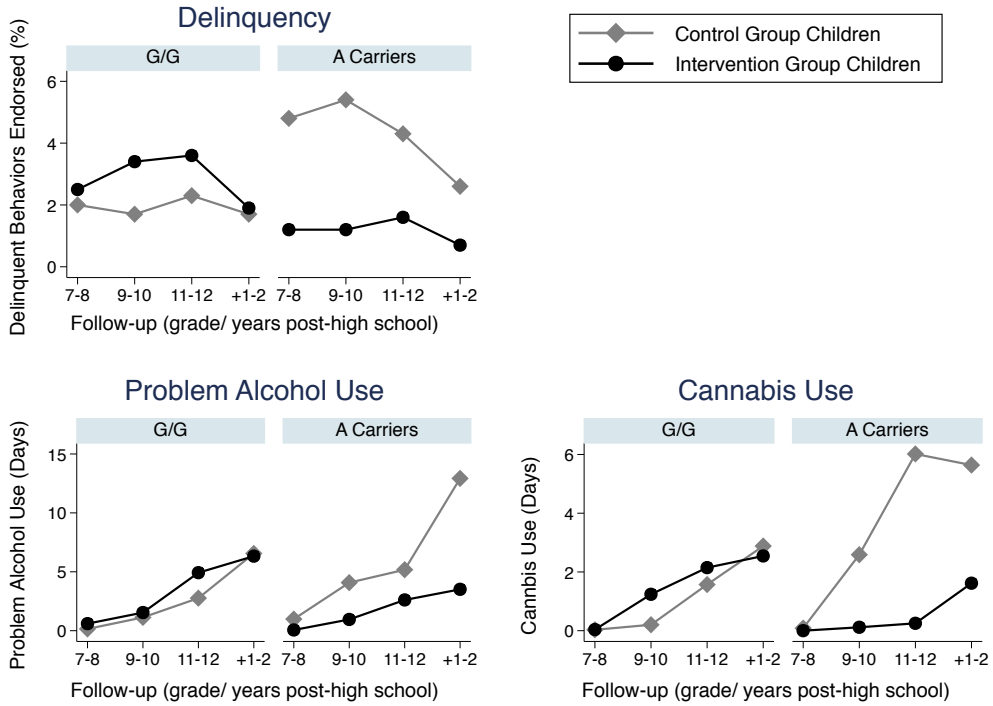


Figure 4. Delinquent Behavior, Problem Alcohol Use, and Cannabis Use in European-American Fast Track Control and Intervention Children by Carriage of the rs10482672 'A' Allele. The G/G group carried no copies of the A allele. The A Carrier group carried one or two copies of the A allele. Gene-by-intervention interaction effects were statistically significant ($p<0.05$) for mean levels of delinquency, alcohol use, and cannabis use and for change over time in alcohol and cannabis use. Details are reported in Appendix Table A5.



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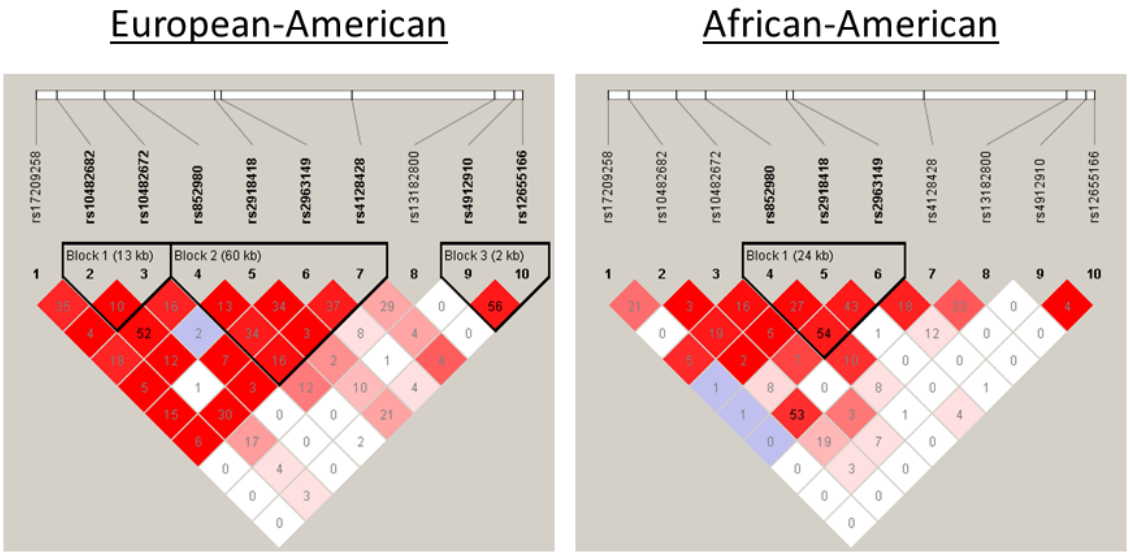
APPENDIX

Appendix Figure A1. Pairwise Linkage Disequilibrium (R^2) Between Candidate SNPs in NR3C1. Linkage disequilibrium plots were obtained from Haploview (Barrett et al., 2005). Values displayed in each box indicate the pairwise correlation (R^2) between markers. Shading indicates the degree of correlation as measured by D', following the standard Haploview color scheme: high-confidence associations (LOD ≥ 2) are shaded from light pink to bright red based on the absolute value of D'; low-confidence associations (LOD < 2) are shaded white (D'<1) or blue (D'=1). Each subset of SNPs grouped within a black triangle is considered a "block" based on proximity and inter-correlation criteria defined by Gabriel et al. (2002). LOD is the log of the likelihood odds ratio, a measure of confidence in the value of D'.



Appendix Table A1. Prevalence of any externalizing psychopathology by intervention group and genotype and test of gene-by-intervention interaction for ten tag SNPs in *NR3C1*. Analyses were conducted separately for European-American (Panel A) and African-American (Panel B) samples. P-values for difference in differences were calculated from linear probability models regressing externalizing psychopathology status on genotype, intervention group, and a product term testing the GxI interaction. Models were adjusted for sex. P-values are based on the test statistic for the product term. P-values are reported for the additive model and the dominance model. Prevalences in the table reflect the dominance model. Bolded p-values are statistically significant at the alpha=0.05 level following Nyholt correction for multiple testing.

Appendix Table A1 - Panel A: European-American Sample

					Prevalence of Any Externalizing Psychopathology at Age 25						
			p-value (Gene x Intervention Interaction)		Test Allele Carriers			Non-Carriers			Difference in Differences
rsID	Alleles (Ref/Test)	Test Allele Frequency	Additive Model	Dominance Model	Control	Intervention	Difference	Control	Intervention	Difference	
rs852980	G/C	0.44	8.40E-02	1.80E-02	64%	39%	-25%	55%	66%	11%	*-36%*
rs10482682	C/T	0.41	1.20E-01	3.41E-01	54%	45%	-10%	72%	49%	-24%	*14%*
rs4912910	G/A	0.35	7.28E-01	3.15E-01	66%	47%	-20%	54%	48%	-6%	*-14%*
rs2963149	A/T	0.34	5.17E-01	7.40E-01	61%	51%	-10%	59%	42%	-17%	*7%*
rs12655166	T/C	0.25	4.63E-01	3.32E-01	71%	48%	-23%	54%	44%	-11%	*-13%*
rs13182800	G/T	0.23	3.61E-01	3.36E-01	61%	40%	-22%	61%	51%	-10%	*-12%*
rs17209258	A/G	0.22	2.00E-03	1.00E-02	51%	59%	8%	66%	37%	-29%	*37%*
rs4128428	T/C	0.17	5.98E-01	6.53E-01	55%	36%	-19%	64%	51%	-13%	*-6%*
rs2918418	G/C	0.15	3.27E-01	1.82E-01	67%	68%	1%	58%	38%	-20%	*22%*
rs10482672	G/A	0.13	**6.20E-05**	**1.83E-04**	75%	18%	-57%	57%	56%	-1%	*-56%*

Appendix Table A1 - Panel B: African-American Sample

					Prevalence of Any Externalizing Psychopathology at Age 25						
			p-value (Gene x Intervention Interaction)		Test Allele Carriers			Non-Carriers			Difference in Differences
rsID	Alleles (Ref/Test)	Test Allele Frequency	Additive Model	Dominance Model	Control	Intervention	Difference	Control	Intervention	Difference	
rs852980	G/C	0.60	9.45E-01	7.55E-01	58%	33%	-25%	75%	44%	-31%	*6%*
rs10482682	C/T	0.12	8.07E-01	7.27E-01	64%	46%	-18%	56%	33%	-23%	*5%*
rs4912910	G/A	0.62	3.83E-01	7.60E-02	60%	32%	-28%	50%	53%	3%	*-31%*
rs2963149	A/T	0.28	9.33E-01	8.63E-01	67%	41%	-26%	50%	28%	-22%	*-3%*
rs12655166	T/C	0.06	3.12E-01	3.33E-01	91%	50%	-41%	55%	34%	-21%	*-20%*
rs13182800	G/T	0.20	7.79E-01	9.73E-01	63%	41%	-21%	55%	33%	-22%	*0%*
rs17209258	A/G	0.05	8.89E-01	8.89E-01	38%	20%	-18%	59%	37%	-22%	*4%*
rs4128428	T/C	0.08	7.32E-01	7.32E-01	68%	41%	-27%	54%	33%	-21%	*-6%*
rs2918418	G/C	0.17	3.64E-01	6.28E-01	68%	52%	-16%	53%	30%	-23%	*7%*
rs10482672	G/A	0.20	5.69E-01	5.99E-01	55%	36%	-19%	59%	34%	-25%	*6%*

Appendix Table A2. Regression model results for age-25 externalizing psychopathology. Analyses were conducted separately for European-American and African-American samples. Unstandardized beta coefficients and coefficient standard errors were calculated from linear probability models regressing externalizing psychopathology status on genotype, intervention group, and a product term testing the GxI interaction. Genotype was coded additively, as the number of test alleles (i.e., 0-1-2). Models were adjusted for sex.

		Main Effect of Genotype		Main Effect of Intervention		GxI Effect	
Sample	SNP	β	SE	β	SE	β	SE
European-American	rs852980	-0.157	0.065	-0.031	0.061	0.155	0.089
	rs10482682	-0.121	0.068	-0.100	0.062	0.147	0.095
	rs4912910	-0.153	0.072	0.062	0.066	-0.033	0.095
	rs2963149	-0.112	0.074	0.006	0.063	0.065	0.101
	rs12655166	-0.207	0.082	0.130	0.073	-0.075	0.102
	rs13182800	-0.209	0.089	-0.019	0.077	-0.101	0.111
	rs17209258	0.041	0.093	-0.129	0.072	0.344	0.111
	rs4128428	-0.198	0.111	-0.054	0.088	-0.070	0.132
	rs2918418	-0.071	0.106	0.108	0.082	0.119	0.121
	rs10482672	-0.534	0.111	0.163	0.080	-0.520	0.127
African-American	rs852980	-0.240	0.067	0.115	0.071	-0.006	0.094
	rs10482682	-0.191	0.127	0.045	0.104	0.035	0.142
	rs4912910	-0.218	0.067	0.006	0.064	-0.083	0.095
	rs2963149	-0.235	0.078	0.136	0.072	-0.009	0.102
	rs12655166	-0.387	0.164	0.308	0.137	-0.175	0.173
	rs13182800	-0.230	0.095	0.070	0.077	-0.032	0.113
	rs17209258	-0.177	0.232	-0.207	0.179	0.034	0.241
	rs4128428	-0.264	0.163	0.138	0.123	-0.061	0.177
	rs2918418	-0.142	0.097	0.079	0.079	0.102	0.112
	rs10482672	-0.181	0.090	-0.081	0.080	0.064	0.113

Appendix Table A3. Mean levels of delinquency, problem alcohol use, and cannabis use by rs10482672 genotype and intervention group and test of gene-by-intervention interaction. Children reported on their delinquent behavior, problem alcohol use, and cannabis each year from grade 7 through two years post high school. Group means are based on average scores across all 8 waves of assessment. Delinquency is the proportion of 25 different delinquent behaviors the child endorsed engaging in in the past year; Alcohol Use is the average of (a) the number of past-year days the child reported consuming 5 or more drinks in one sitting, and (b) the number of past-year days the child reported being drunk. Cannabis Use is the number of days the child reported consuming cannabis in the past month. Gene-by-intervention interaction p-values were calculated from ordinary least squares models regressing average externalizing behavior on on genotype, intervention group, and a product term testing the GxI interaction. For additive models, genotype was coded as the number of rs10482672 'A' alleles (i.e., 0-1-2). For dominance models, genotype was coded as the absence or presence of one or more 'A' alleles (i.e., 0-1). Models were adjusted for sex. Analyses were limited to the European-American subsample of participants. Unstandardized betas and coefficients are reported in Appendix Table A4. Effect size (d) = Cohen's d.

	p-value (GxI Interaction)		**A-Carriers**					**Non-Carriers**					
			Control (n=29)		Intervention (n=26)		Effect Size	Control (n=85)		Intervention (n=82)		Effect Size	Effect Size Difference
	Additive Model	Dominance Model	Mean	SD	Mean	SD	d	Mean	SD	Mean	SD	d	$d_{A\text{-}Carriers} - d_{Non\text{-}Car}$
Delinquency	0.00159	0.00296	0.042	0.064	0.012	0.015	*0.64*	0.019	0.027	0.028	0.048	*-0.23*	*0.87*
Alcohol Use	0.00185	0.00386	5.79	7.27	1.78	3.59	*0.69*	2.65	4.00	3.34	5.07	*-0.15*	*0.84*
Cannabis Use	0.00714	0.00126	3.58	5.57	0.50	1.39	*0.74*	1.17	2.44	1.49	3.17	*-0.11*	*0.86*

Appendix Table A4. Regression model results for adolescent delinquency, problem alcohol use, and cannabis use. Children reported on their delinquent behavior, problem alcohol use, and cannabis each year from grade 7 through two years post high school. Group means are based on average scores across all 8 waves of assessment. Unstandardized beta coefficients and coefficient standard errors were calculated from ordinary least squares models regressing average externalizing behavior on on genotype, intervention group, and a product term testing the GxI interaction. Genotype was coded additively, as the number of rs10482672 'A' alleles (i.e., 0-1-2). Models were adjusted for sex. Analyses were limited to the European-American subsample of participants.

	Main Effect of Genotype		**Main Effect of Intervention**		**GxI Effect**	
	β	**SE**	**β**	**SE**	**β**	**SE**
Delinquency	0.019	0.007	0.008	0.006	-0.034	0.011
Alcohol Use	2.772	0.851	0.589	0.733	-4.165	1.321
Cannabis Use	1.528	0.567	0.071	0.488	-2.390	0.880

Appendix Table A5. Repeated measures ANCOVA results for adolescent delinquency, problem alcohol use, and cannabis use. Children reported on their delinquent behavior, problem alcohol use, and cannabis each year from grade 7 through two years post high school. For each outcome, the repeated measures factor (i.e., "Time") was evaluated from four scores (average scores for grades 7+8, 9+10, 11+12, and post-HS 1+2). Genotype (rs10482672 'A' carriers vs. non-carriers) and treatment group were entered as between-subjects factors. Between-subjects effects correspond to group differences in average behavior over the 8-year span. Within-subjects effects correspond to group differences in magnitude of behavior change over time. Analyses are limited to the European-American sample. Models were adjusted for sex. MS = Mean Square. η_p^2 = Partial Eta Squared.
**p<.05; **p<.01; ***p<.001.*

	Delinquency				Problem Alcohol Use				Cannabis Use			
	df	MS	F	ηp^2	df	MS	F	ηp^2	df	MS	F	ηp^2
Between-Subjects Effects												
Genotype	1	0.002	0.27	0.001	1	102.9	1.07	0.005	1	82.5	2.01	0.009
Intervention	1	0.020	2.90	0.013	1	451.7	4.70 *	0.021	1	315.6	7.70 **	0.034
Genotype by Intervention	1	0.065	9.62 **	0.042	1	912.3	9.49 **	0.042	1	478.3	11.66 ***	0.051
Error	218	0.007			217				217	41.0		
Within-Subjects Effects												
Time	3	0.005	3.30 *	0.015	3	1458.7	35.31 ***	0.140	3	329.5	18.73 ***	0.079
Genotype by Time	3	0.001	0.61	0.003	3	28.8	0.70	0.003	3	11.8	0.67	0.003
Intervention by Time	3	0.001	0.36	0.002	3	195.3	4.73 **	0.021	3	59.8	3.40 *	0.015
Genotype by Intervention by Time	3	0.003	1.64	0.007	3	111.8	2.71 *	0.012	3	67.6	3.84 **	0.017
Error	654	0.002			651	41.3			651	17.6		