Development and Psychopathology **27** (2015), 1489–1502

doi:10.1017/S0954579415000899

Developmental pathways from child maltreatment to adolescent marijuana dependence: Examining moderation by FK506 binding protein 5 gene (*FKBP5*)

ELIZABETH D. HANDLEY,[a] FRED A. ROGOSCH,[a] AND DANTE CICCHETTI[a,b]
[a]*University of Rochester Mt. Hope Family Center; and* [b]*University of Minnesota Institute of Child Development*

Abstract

The current study examined the prospective association between child maltreatment and the development of substance use disorder in adolescence with the aim of investigating pathways underlying this relation, as well as genetic moderation of these developmental mechanisms. Specifically, we tested whether youth who experienced maltreatment prior to age 8 were at risk for the development of marijuana dependence in adolescence by way of a childhood externalizing pathway and a childhood internalizing pathway. Moreover, we tested whether variation in FK506 binding protein 5 gene (*FKBP5*) CATT haplotype moderated these pathways. The participants were 326 children (n =179 maltreated; n = 147 nonmaltreated) assessed across two waves of data collection (childhood: ages 7–9 and adolescence: ages 15–18). Results indicated that higher levels of child externalizing symptoms significantly mediated the effect of child maltreatment on adolescent marijuana dependence symptoms for individuals with one or two copies of the *FKBP5* CATT haplotype only. We did not find support for an internalizing pathway from child maltreatment to adolescent marijuana dependence, nor did we find evidence of moderation of the internalizing pathway by *FKBP5* haplotype variation. Findings extend previous research by demonstrating that whether a maltreated child will traverse an externalizing pathway toward substance use disorder in adolescence is dependent on *FKBP5* genetic variation.

According to recent Monitoring the Future data (Johnston et al., 2014), 50.4% of 12th graders in the United States reported some illicit drug use in their lifetimes, with marijuana use being the most commonly used illicit drug. Moreover, 22.7% of 12th graders reported using marijuana within the past 30 days. Among this age group, 9.7%–16.3% met DSM-IV criteria for drug abuse or dependence (Merikangas et al., 2010). Given the associations between heavy and persistent marijuana use during adolescence and serious consequences throughout adolescence and adulthood such as academic underachievement, unemployment, crime, and neuropsychological decline (Green, Doherty, Reisinger, Chilcoat, & Ensminger, 2010; Green & Ensminger, 2006; Lynskey & Hall, 2000; Meier et al., 2012), adolescent marijuana use and disorder represent significant public health concerns.

Children who have experienced maltreatment represent a high-risk group for whom the risk for developing substance use disorder (SUD) is heightened. More specifically, research clearly shows that maltreated children are vulnerable to developing problematic substance use and disorder (e.g., Buckingham & Daniolos, 2013; Cicchetti & Toth, in press; Moran, Vuchinich, & Hall, 2004; Shin 2012; Shin, Hong, & Hazen, 2010; Vilhena-Churchill & Goldstein, 2014). For example, Huang et al. (2011) found that young adults with a history of childhood physical abuse were 37% more likely to use illicit drugs compared to those without abuse histories. Research also indicates that with each additional adverse childhood experience, such as physical and sexual abuse and neglect, the likelihood of early initiation of illicit drug use increases two- to fourfold (Dube et al., 2003). Understanding the underlying mechanisms by which maltreated children are at risk for developing SUD and also identifying which maltreated children may be at most risk for SUD will be critical to informing effective preventive interventions for this population, as well as advancing our understanding of the etiology of SUD more broadly.

Pathways to Adolescent SUD

A developmental pathway to adolescent SUD that has received much attention and robust empirical support is the externalizing pathway, also commonly referred to as the behavioral undercontrol–disinhibition pathway, antisocial pathway, and/or deviance–proneness pathway (Chassin, Hussong, & Beltran, 2009; Chassin, Sher, Hussong, & Curran, 2013; Costello, 2007; Sher, 1991; Zucker, Heitzeg, & Nigg, 2011). This pathway is often characterized by bidirectional processes and dynamic interactions throughout development among a difficult early temperament style, behavioral undercontrol, suboptimal parental monitoring and support,

We are grateful to the Jacobs Foundation, the National Institute of Mental Health (Grant R01-MH83979), and the National Institute on Drug Abuse (Grants R01-DA017741 and R01-DA12903) for their support of this work.

Address correspondence and reprint requests to: Elizabeth Handley, Mt. Hope Family Center, 187 Edinburgh Street, Rochester, NY 14608. E-mail: elizabeth_handley@urmc.rochester.edu.

childhood externalizing behavior (i.e., aggression and delinquency), affiliation with deviant substance using peers, and an early initiation of substance use. An underlying genetic lability toward disinhibition in the context of a risky peer or family environment appears central to this pathway (Dick, 2011; Zucker et al., 2011). Evidence supporting an externalizing pathway to SUD is clear (for reviews, see Chassin et al., 2009, 2013; and Zucker et al., 2011), with results suggesting that the externalizing pathway to marijuana use may be relatively stronger than the externalizing pathway to other substances (i.e., alcohol and nicotine; King, Iacono, & McGue, 2004).

One mechanism by which maltreated children may go on to develop SUDs is by way of this externalizing pathway. Specifically, children who have experienced maltreatment are at risk for behavioral undercontrol and impulsivity (e.g., Brodsky et al., 2001; Kim, Cicchetti, Rogosch, & Manly, 2009; Oshri, Rogosch, & Cicchetti, 2013) and are more likely to live in homes with less parental monitoring (Rogosch, Cicchetti, Shields, & Toth, 1995). Moreover, there is clear evidence that maltreated children are at risk for various externalizing symptoms throughout development (see Cicchetti & Toth, in press, for review). For example, Manly, Kim, Rogosch, and Cicchetti (2001) showed that the severity of emotional maltreatment and/or neglect experienced during infancy/toddlerhood predicted higher levels of aggression and externalizing behavior in middle childhood. Using a longitudinal cascade model, Rogosch, Oshiri, and Cicchetti (2010) showed that child maltreatment before age 8 predicted higher levels of externalizing symptoms at that time, which predicted continuity in externalizing symptoms across childhood and early adolescence, ultimately resulting in heightened risk for marijuana abuse and dependence symptoms in late adolescence. Thus, there is support for an externalizing pathway from child maltreatment to the development of adolescent SUD.

A not mutually exclusive pathway to adolescent substance use and disorder is the internalizing pathway that involves stress and negative affect regulation. Central to this pathway is the notion that individuals experiencing stress, negative affect, and internalizing symptoms may engage in substance use as a means to reduce negative affect (i.e., self-medication). Hussong, Jones, Stein, Baucom, and Boeding (2011) proposed that, similar to the externalizing pathway, the internalizing pathway has early developmental roots and is marked by complex bidirectional and cumulative influences over time. Specifically, they argue that individuals at risk for developing SUDs via an internalizing pathway may exhibit a behaviorally inhibited temperament in infancy; symptoms of depression and anxiety in early childhood; and social withdrawal, peer rejection, and interpersonal skill deficits throughout childhood and adolescence. In addition, they may espouse substance-using motives such as peer acceptance of substance use and/or self-medication. Unlike the externalizing pathway, which has been widely researched and empirically supported, the internalizing pathway has received less attention, and support has been inconsistent (e.g., Dahne, Banducci, Kurdziel, & MacPherson, 2014; Edwards et al., 2014; McCarty et al., 2012).

That maltreated children may traverse an internalizing pathway to SUD is also plausible. Maltreated children are at well-documented risk for internalizing symptoms, including depression and anxiety (e.g., Cicchetti & Toth, in press; Maniglio, 2010; Scott, Smith, & Ellis, 2010), and have been shown to experience difficulties with peers, including peer bullying, rejection, and victimization (Banny, Cicchetti, Rogosch, Oshri, & Crick, 2013; Bolger & Patterson, 2001; Kim & Cicchetti, 2010; Shields & Cicchetti, 2001) and social withdrawal (Lansford et al., 2002), all etiological factors theorized to be included on the internalizing pathway to SUD. Rogosch et al. (2010) found that although child maltreatment was related to higher levels of early adolescent internalizing symptoms, higher internalizing symptoms in early adolescence were predictive of lower marijuana abuse/dependence symptoms in later adolescence, thus calling into question whether child maltreatment leads to adolescent substance problems via an internalizing pathway.

FK506 Binding Protein 5 Gene (*FKBP5*)

Although child maltreatment is associated with a host of negative developmental outcomes, including SUD, the risk is not deterministic. Gene × Environment (G × E) interactions take place when the effect of an environmental risk, such as maltreatment, on an individual's adaptation/maladaptation is dependent on the individual's genotype (Moffitt, Caspi, & Rutter, 2005). Enoch (2012) espoused the role of stress and anxiety-related genes, such as *FKBP5*, as likely candidates for G × E interactive effects on the development of SUDs; however, to date, *FKBP5* × Child Maltreatment effects on SUD have yet to be examined.

The *FKBP5* gene is involved in modulating the stress response. Specifically, stressors lead to secretion of the catecholamines and glucocorticoids, the primary effectors of the stress system. Circulating glucocorticoids trigger the glucocorticoid receptor (GR) that results in the rapid transcriptional regulation of genes such as molecular activators of the hypothalamic–pituitary–adrenal axis. *FKBP5* is an important regulator of GR activity. *FKBP5* is induced by cortisol and acts within a negative feedback loop to promote the transcription of stress-response target genes, leading to downstream release of adrenocorticotropic hormone and plasma cortisol. *FKBP5* is located on chromosome 6 (chromosome 6p21.31) and contains a number of single nucleotide polymorphisms (SNPs) that are associated with differential ability for *FKBP5* to be induced by cortisol and bind to the GR (see Zannas & Binder, 2014, for review of *FKBP5*).

SNPs rs3800373, rs9296158, rs1360870, and rs9470080, which span the 103 kb of the *FKBP5* gene, contain variants that are associated with functional differences at the GR. Genotypes containing the minor "high-induction" alleles are associated with greater expression of *FKBP5* and increased

https://doi.org/10.1017/S0954579415000899 Published online by Cambridge University Press

binding at the GR, whereas genotypes containing the complement major "low-induction" alleles are associated with weaker induction by cortisol and GR receptor binding (Binder et al., 2004; Dackis, Rogosch, Oshiri, & Cicchetti, 2012).

Prior research indicates that *FKBP5* interacts with childhood adversity in the development of both internalizing and externalizing symptomatology (see Zannas & Binder, 2014, for review). For instance, Dackis et al. (2012) found *FKBP5* moderation of child maltreatment effects on adult women's depressive symptoms, such that women with maltreatment histories and the *FKBP5* CATT haplotype were at risk for limbic system irritability, which in turn predicted higher levels of depressive symptoms. Studies have also shown that variation in *FKBP5* moderates childhood trauma effects on adult suicide attempts (Roy, Gorodetsky, Yuan, Goldman, & Enoch, 2010; Roy, Hodgkinson, DeLuca, Goldman, & Enoch, 2012) and posttraumatic stress disorder symptoms (Binder et al., 2008; Xie et al., 2010). In addition to internalizing outcomes, Bevilacqua et al. (2012) found that adults with a history of child maltreatment evidenced greater aggressive and violent behavior depending on *FKBP5* variation. Although the majority of this prior work has identified *FKBP5* minor alleles as conferring risk in interaction with childhood adversity (e.g., Bevilacqua et al., 2012; Binder et al., 2008; Dackis et al., 2012; Xie et al., 2010), there are a few studies that have demonstrated interactive risk associated with child adversity and major alleles (e.g., Roy et al., 2010, 2012). Whether *FKBP5* moderates the association between child maltreatment and adolescent SUD has yet to be examined. Moreover, whether *FKBP5* moderates the underlying pathways between child maltreatment and adolescent SUD remains unknown.

A recent review examining *FKBP5*×Environment interactions concluded that interactions between trauma and *FKBP5* are robust when the trauma occurs during childhood specifically, rather than adulthood, suggesting a developmentally sensitive period (Zannas & Binder, 2014). This conclusion is supported by recent epigenetic research that identified DNA demethylation as a mechanism underlying the interactive effects of *FKBP5* polymorphism and trauma in the development of stress-related psychopathology that was restricted to trauma occurring during childhood.

Current Study

Using longitudinal data from a sample of maltreated and nonmaltreated youth, we investigated whether child maltreatment impacts the development of adolescent marijuana dependence by way of child externalizing and child internalizing developmental pathways. Moreover, we tested whether *FKBP5* genetic variation moderates these pathways as well as moderates the direct effect of child maltreatment on adolescent marijuana dependence. In doing so, we extend prior research by being the first to examine the interactive effects of *FKBP5* and child maltreatment in the development of adolescent SUD and theorized developmental pathways. It was hypothesized that *FKBP5* would moderate the direct effect of child maltreatment on adolescent marijuana dependence, as well as moderate the internalizing and externalizing pathways, such that youth with one or two copies of the *FKBP5* CATT haplotype would be more likely to traverse these pathways of accumulating risk.

Method

Participants

The participants for this longitudinal investigation included 326 children who were assessed across two waves of data collection (childhood ages 7–9 and adolescence ages 15–18). During the first wave of data collection children attended a summer camp research program designed for school-aged low-income children (Cicchetti & Manly, 1990). These children were followed in adolescence and invited to take part in a series of individual interviews and research assessments examining the developmental sequelae of child maltreatment. The sample included both maltreated ($n = 179$) and nonmaltreated ($n = 147$) children. The maltreatment groups were comparable in terms of gender, χ^2 (1) = 0.73, *ns*, and 55.8% of the sample were male. The groups also did not differ in child's parent-reported race/ethnicity, χ^2 (3) = 2.74, *ns*. The majority of participants were African American (58.5%), 23.7% were Caucasian, 11.1% were Hispanic, and 6.8% identified as another race/ethnicity. Moreover, both maltreatment groups were impoverished and did not differ in markers of socioeconomic status. Specifically, 96.4% of the families reported having received public assistance, χ^2 (1) = 0.84, *ns*, and 73.1% of the mothers were nonmarried, χ^2 (1) = 2.15, *ns*.

Children in the maltreated group had been identified by the county Department of Human Services (DHS) as having experienced child abuse and/or neglect. A recruitment liaison from the DHS contacted eligible maltreating families, explained the study, and if parents were interested, then their names were released to the project team for recruitment. Families were free to choose whether to participate. Comprehensive searches of DHS records were completed, and maltreatment information was coded utilizing operational criteria from maltreatment nosology specified in the Maltreatment Classification System (MCS; Barnett, Manly, & Cicchetti, 1993). The MCS utilizes DHS records detailing investigations and findings involving maltreatment in identified families over time. Rather than relying on official designations and case dispositions, the MCS codes all available information from DHS records, making independent determinations of maltreatment experiences. Based on operational criteria, the MCS designates all of the subtypes of maltreatment children have experienced (i.e., neglect, emotional maltreatment, physical abuse, or sexual abuse). Coding of the DHS records was conducted by trained research assistants, doctoral students, and clinical psychologists. Adequate reliability has been obtained (weighted κs = 0.86–0.98; Manly, 2005;

https://doi.org/10.1017/S0954579415000899 Published online by Cambridge University Press

Manly et al., 2001). Other investigators have demonstrated that the MCS is reliable and valid in classifying maltreatment (Bolger & Patterson, 2001; Manly et al., 2001; Stouthamer-Loeber, Loeber, Homish, & Wei, 2001). Among the maltreated children, 69.3% had experienced neglect, 53.6% had experienced emotional maltreatment, 38.0% had experienced physical abuse, and 14.5% had experienced sexual abuse. As is typical in maltreated populations (Bolger & Patterson, 2001; Manly et al., 1994, 2001), the majority of maltreated participants had experienced multiple subtypes of maltreatment. Specifically, 65.8% of the maltreated children had experienced two or more maltreatment subtypes. Prior research demonstrates cumulative risk associated with multiple maltreatment subtypes (e.g., Kim & Cicchetti, 2010; Kim et al., 2009).

Because maltreated children are predominantly from low socioeconomic status families (Fourth National Incidence Study of Child Abuse and Neglect; Sedlak et al., 2010), demographically comparable nonmaltreated children were recruited from families receiving Temporary Assistance for Needy Families. A DHS recruitment liaison contacted eligible nonmaltreating families, described the project, and if interested, parents signed a release for their names to be given to the project team for recruitment. DHS record searches were completed for these families to verify the absence of any record of child maltreatment. Trained research assistants also interviewed mothers of children recruited for the nonmaltreatment group to confirm a lack of DHS involvement and prior maltreatment experiences. Only children from families without any history of documented abuse or neglect were retained in the nonmaltreatment group. In addition, families who had received preventive services through the DHS due to concerns over risk for maltreatment were excluded from the sample to reduce the potential for unidentified maltreatment existing within this group.

Procedure

At Wave 1, children attended a weeklong day camp program and participated in research assessments (see Cicchetti & Manly, 1990, for detailed descriptions of camp procedures). At the camp, children were assigned to groups of eight (four maltreated, four nonmaltreated) same-age and same-sex peers. Each group was led by three trained camp counselors, who were unaware of the maltreatment status of children and the hypotheses of the study. The camp lasted 7 hr per day for 5 days, providing 35 hr of interaction between children and counselors. In addition to the recreational activities, after providing assent, children participated in various research assessments and peer evaluations. Trained research assistants, who also were unaware of research hypotheses and maltreatment status, conducted individual research sessions with children, in which questionnaires and other research measures were administered. Clinical consultation and intervention occurred if any concerns over danger to self or others emerged during research sessions. The counselors, who had been trained extensively for 2 weeks prior to the camp, also completed assessment measures on individual children, based on their 35 hr of observations and interactions with children in their respective groups.

At Wave 2, adolescent participants were individually interviewed in private interview rooms by trained research assistants who were unaware of the participant's maltreatment group status and the research hypotheses. The participants completed a range of assessments, including self-report measures and interviews regarding their substance use and disorder.

Measures

Dimensions of child symptomatology. The camp context and associated measurement battery provided a multiple-informant, multiple-perspective view of child behavioral functioning. Measures include peer evaluations, counselor observations, and counselor-report assessments of individual children. After children interacted with each other during the week of summer camp, children evaluated the characteristics of their peers in their respective camp groups using a peer-nomination method (PNM) on the last day of camp (cf. Coie & Dodge, 1983). Counselors conducted the peer-nomination assessment with individual children. For each peer in the camp group, children were given brief behavioral descriptors characterizing different types of social behavior and asked to select one peer from the group who best fit the behavioral description for a child who was disruptive and a child who was a fighter. The total number of nominations that each individual child received from peers in each category was determined. These totals were converted to proportions of the possible nominations in each category, and these scores in each category were standardized. Thus, these scores were used to measure peer nominations of child fighting and disruptive behavior.

The Pupil Evaluation Inventory (PEI; Pekarik, Prinz, Liebert, Weintraub, & Neale, 1976) was completed by camp counselors for children in their respective groups at the end of each camp week. The PEI consists of 35 items assessing social behavior, yielding three factors, including aggression and withdrawal. Similar to peer-nomination procedures, counselors were asked to select no more than two children who were best characterized by each individual item. Aggregate scores for each of the scales were generated on the basis of the number of nominations each child received on the respective scale items. Scores were averaged across counselors to obtain subscale scores for individual children.

Counselor report of child behavioral symptomatology was evaluated at the end of each week by counselors' completion of the Teacher Report Form (TRF; Achenbach, 1991). The TRF is a widely used and validated instrument to assess behavioral disturbance from the perspective of teachers. This measure was used in the present study because camp counselors are able to observe similar behaviors to that of teachers. The TRF, containing 118 items rated for frequency, assesses two broadband dimensions of child symptomatology,

https://doi.org/10.1017/S0954579415000899 Published online by Cambridge University Press

externalizing and internalizing, as well as total behavior problems. Subscales scores are also computed for the following factors: withdrawn, somatic problems, anxiety/depression, delinquent behavior, and aggressive behavior. Across the years, reliabilities ranged from 0.56 to 0.84 ($M = 0.68$) for internalizing and from 0.78 to 0.88 ($M = 0.83$) for externalizing. The counselors' scores for each child were averaged to obtain individual child scores for delinquency, aggression, withdrawn, somatic problems, and anxiety/depression.

Adolescent marijuana dependence. Current (i.e., past 12 months) marijuana dependence symptoms were assessed using the Diagnostic Interview Schedule for Children (Shaffer et al., 1993). The Diagnostic Interview Schedule for Children is a well-validated structured interview for children and adolescents (Fisher et al., 1993; Piacentini et al., 1993) and provides diagnostic scoring based on the DSM. Given the relative young age of participants ($M = 16.2$), only 7.1% of the sample met criteria for marijuana dependence. Therefore, the total count of marijuana dependence symptoms was used as the dependent variable in subsequent models. See Table 1 for descriptive statistics.

DNA collection, extraction, and genotyping. Using an established protocol, trained research assistants obtained DNA samples from children by collecting buccal cells with the Epicentre Catch-All Collection Swabs. Subsequently, using the conventional method, DNA was extracted with the Epicentre BuccalAmp DNA Extraction Kit, in order to prepare DNA for polymerase chain reaction amplification. Genotyping was conducted following previously published protocols. DNA was whole-genome amplified using the Repli-g kit (Qiagen, Chatsworth, CA, Catalog No. 150043) per the kit instructions to ensure the availability of data over the long term for this valuable sample. Amplified samples were then diluted to a working concentration.

All DNA samples were genotyped in duplicate for quality control. In addition, human DNA from cell lines was purchased from Coriell Cell Repositories for all representative genotypes in duplicate and genotypes confirmed by sequencing using dye terminator cycle sequencing chemistry on an ABI 3130x1. These and a no-template control were run alongside study samples representing 9% of the total data output. Any samples that were not able to be genotyped to a 95% or greater confidence level were repeated under the same conditions.

FKBP5 was genotyped using assays for SNPs rs3800373, rs9296158, rs1360780, and rs9470080 purchased from Applied Biosystems, Inc. (ABI, Bedford, MA) as C27489960_10, C1256775_10, C8852038_10, and C92160_10, respectively. Individual allele discriminations were made using Taq Man Genotyping Master Mix (ABI Catalog No. 4371357) with amplification in an ABI 9700 thermal cycler and analyzing the endpoint fluorescence using a Tecan M200. If a genotype for either gene or SNP could not be determined after the first run, then it was repeated up to four times. The call rates for the four *FKBP5* SNPs ranged from 99.8% to 100%. Genotype distributions were in Hardy–Weinberg equilibrium (all $p > .05$). Haplotypes for the four *FKBP5* SNPs were determined using Arlequin v3.5.1.3, which employs a pseudo-Bayesian approach to estimate phase (Excoffier & Lischer, 2010). Arlequin was able to estimate haplotypes for every participant with a posterior probability higher than 0.97, which allowed us to assign a score of zero, one, or two copies of the CATT haplotype to participants with a high degree of certainty (see Table 1 for overall distributions of SNPs and CATT haplotype). The

Table 1. *Description of FKBP5 SNPs and CATT haplotype by maltreatment groups*

SNP	Overall Sample	Maltreated ($n = 179$)	Nonmaltreated ($n = 147$)	
rs3800373				χ^2 (2) = 1.23, *ns*
AA	45.1%	47.2%	42.5%	
AC	43.2%	40.4%	46.6%	
CC	11.7%	12.4%	11.0%	
rs9296158				χ^2 (2) = 1.13, *ns*
GG	38.9%	41.3%	35.4%	
AG	46.0%	43.6%	49.0%	
AA	15.1%	15.1%	15.2%	
rs1360780				χ^2 (2) = 1.67, *ns*
CC	42.8%	45.8%	39.0%	
CT	44.9%	41.9%	48.6%	
TT	12.3%	12.3%	12.3%	
rs9470080				χ^2 (2) = 0.73, *ns*
CC	36.0%	38.0%	33.6%	
CT	48.6%	47.5%	50.0%	
TT	15.4%	14.5%	16.4%	
CATT haplotype				χ^2 (1) = 0.44, *ns*
No copies	47.2%	48.9%	45.1%	
1–2 copies	52.8%	51.1%	54.9%	

https://doi.org/10.1017/S0954579415000899 Published online by Cambridge University Press

CATT haplotype accounted for 32.7% of all haplotypes in the sample, with its complement, AGCC accounting for 57.5%. Approximately 47.2% of participants had zero copies of the CATT haplotype, 42.2% had one copy, and 10.6% had two copies of the haplotype. Because of the small number of participants with two copies, individuals with one or two copies of the haplotype were combined.

African ancestry. To address potential population stratification, ancestral proportion testing was conducted. DNA from study participants was subjected to SNP genotyping of the Burchard et al. panel of 106 SNPs (Lai et al., 2009; Yaeger et al., 2008), known to be informative for ancestry from Africa, Europe, and Native America. The SNPs were genotyped using the iPLEX platform from Sequenom Bioscience, Inc., which uses the Sequenom MassArray. Samples are subjected to single base primer extension (SBE) with fluorophore labeled nucleotides from primers designed for SNPs of interest. The samples including the SBE products were placed on the iPLEX platform, and matrix assisted laser desorption/ionization time of flight was used to identify the allele based on the fluorophore passing the detector at the expected time associated with the mass of the SBE primer. The SNP genotyping results were then recoded and uploaded into STRUCTURE v2.3.4, which uses algorithms developed by Falush Stephens and Pritchard (2003, 2007) and Hubisz, Falush, Stephens, and Pritchard (2009). Three SNP tests were excluded based on high allele call rates of the non-DNA containing wells. The data from the remaining 103 loci were uploaded into the software and set to analyze with an admixture model of ancestry and initialization of the simulation on the Genetics of Asthma in Latino Americans cohort (initialize of POPINFO). The simulation was set to run with a burn-in of 10,000, 1000 Markov chain Monte Carlo repetitions, and assuming three populations within the group. The results of the simulations were subsequently identified as percent association to each ancestry group based on the known ancestry of the Genetics of Asthma in Latino Americans cohort.

To facilitate Gene × Race interaction tests, a grouping variable using ancestral proportion continuous scores was created using multinomial logistic regression to classify cases. Parent-reported race/ethnicity (coded 1 = African American, 2 = Caucasian, 3 = Hispanic, and 4 = other race/ethnicity) was predicted from proportion African ancestry and proportion Native American ancestry. Given the large proportion of African American children in our sample, we then created a binary variable to classify those with predominately African ancestry (58.6%) versus others (41.4%).

Data analytic plan

Analyses were performed using Mplus Version 7.0 (Muthén & Muthén, 1998–2012) with maximum likelihood estimator with robust standard errors, which computes parameter estimates for continuous outcomes with standard errors that are robust to nonnormality. Measurement modeling was conducted to determine the appropriate factor structure of the five hypothesized indicators of child externalizing (i.e., TRF counselor-reported delinquency, TRF counselor-reported aggression, PNM peer-nominated fighting, PNM peer-nominated disruptive behavior, and PEI counselor-reported aggression). Next, measurement invariance testing was conducted to examine factor loading invariance across *FKBP5* CATT haplotype variation. Results of measurement modeling informed model building in subsequent structural equation models (SEMs).

Missing data for endogenous variables were handled using full information maximum likelihood. Model fit for confirmatory factor analyses (CFAs) and SEMs was evaluated using the comparative fit index (CFI), root mean square error of approximation (RMSEA), and standardized root mean square residual (SRMR). CFI values greater than 0.95, RMSEA values less than 0.06, SRMR values less than 0.06, and a nonsignificant χ^2 statistic were considered evidence of good model fit (Hu & Bentler, 1999; Yu & Muthen, 2002). Multiple-group SEMs were conducted to examine whether child externalizing symptoms mediate the effect of child maltreatment on adolescent marijuana dependence symptoms differently depended on adolescents' *FKBP5* CATT haplotype variation. Figure 1 provides a conceptual depiction of the moderated mediation model tested.

These same steps and analytic procedures were repeated to examine child internalizing symptoms as a mechanism of the effect of child maltreatment on adolescent marijuana dependence symptoms. Measurement modeling and invariance testing was conducted. Hypothesized indicators of child internalizing were TRF counselor-reported withdrawal, TRF counselor-reported anxiety/depression, TRF counselor-reported somatic complaints, and PEI peer-reported withdrawal. Next, multiple-group SEMs were examined to determine whether child internalizing symptoms mediate the effect of child maltreatment on adolescent marijuana dependence symptoms differently depending on adolescents' *FKBP5* CATT haplotype variation.

Results

Table 1 presents SNP and haplotype frequencies for the overall sample, and comparing maltreated and nonmaltreated children. The maltreatment groups did not differ on distributions of any of the four FKBP5 SNPs, rs3800373: χ^2 (2) = 1.23, ns; rs9296158: χ^2 (2) = 1.13, *ns*; rs1360780: χ^2 (2) = 1.67, *ns*; rs9470080: χ^2 (2) = 0.73, *ns*, and groups did not differ on CATT haplotype frequency, χ^2 (1) = 0.44, *ns*. These results indicate the absence of gene–environment correlation such that FKBP5 genotype did not affect the likelihood that children would be maltreated.

Externalizing mechanism

Measurement modeling. CFA was conducted to determine the appropriate factor structure of the five child externalizing behavior indicators (TRF delinquency, TRF aggression,

https://doi.org/10.1017/S0954579415000899 Published online by Cambridge University Press

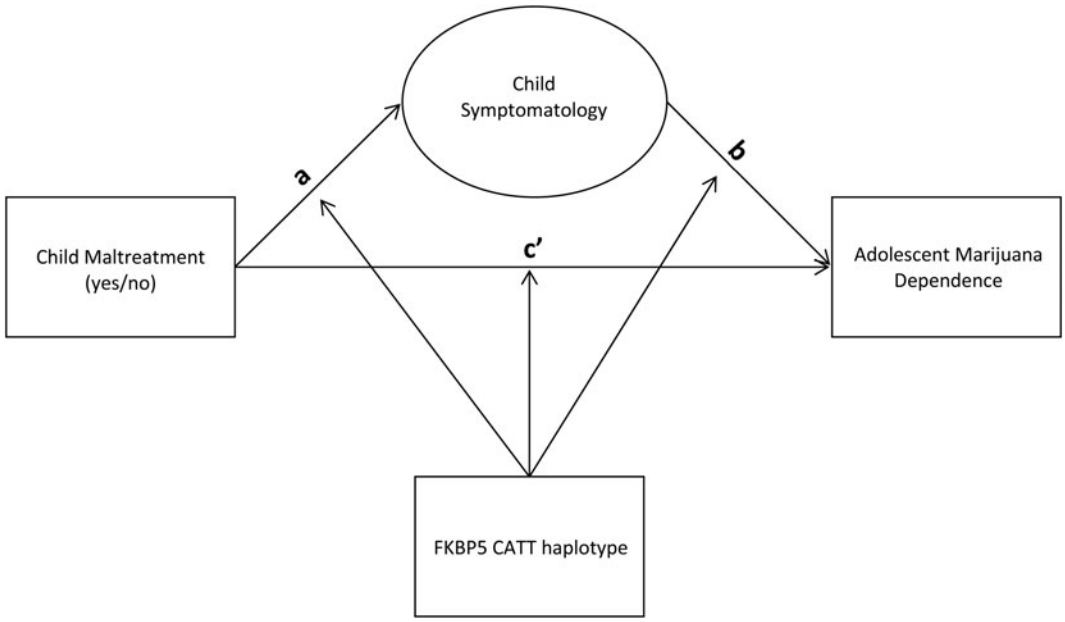

Figure 1. Conceptual *FKBP5* moderated mediation model.

PNM fights scale, PNM disruptive scale, and PEI aggression). Results of the CFA indicated that a one-factor model was a good fit to the data, χ^2 (3) = 0.91, p = .82, CFI = 1.00, RMSEA < 0.001, SRMR = 0.004, with factor loadings all significant at $p < .001$ and ranging from 0.60 to 0.94. Residual covariances were modeled between TRF delinquency and TRF aggression and between PNM fighting and PNM disruptive behavior. Measurement invariance testing was then conducted to examine invariance across *FKBP5* haplotype variation for the latent variable "child externalizing." A model that constrained factor loadings and residual covariances to be equal across groups was tested and demonstrated fair fit to the data, χ^2 (16) = 30.20, p = .02, CFI = 0.97, RMSEA = 0.09, SRMR = 0.26. A model that relaxed all constraints across groups was tested next and also evidenced good fit to the data, χ^2 (10) = 10.80, p = .37, CFI = 0.99, RMSEA = 0.03, SRMR = 0.03. The fully unconstrained model was a significantly better fit to the data than the constrained model, Satorra–Bentler $\Delta\chi^2$ (6) = 16.88, p = .009, evidencing measurement variance across *FKBP5* haplotype group. See Table 2 for standardized parameter estimates for each genotype group. These results informed subsequent model specification as described below.

Multiple-group SEM. Multiple-group SEMs were conducted to examine whether child externalizing symptoms mediated the effect of child maltreatment on adolescent marijuana dependence symptoms differently depended on adolescents' *FKBP5* CATT haplotype variation. Child maltreatment, ancestral-informed race, and adolescent age were entered as exogenous variables with latent variable child externalizing modeled as the mediator and the count of adolescent marijuana dependence symptoms modeled as the endogenous variable. In preliminary models, adolescent gender did not significantly uniquely predict marijuana dependence symptoms. Given the lack of theorized gender effects, and to attain a more parsimonious model, gender was trimmed from the final models.

In keeping with the suggestion proffered by Keller (2014) regarding the importance of including covariate main and interactive effects in G × E models, we not only included child race as a covariate main effect predicting child externalizing and adolescent marijuana dependence symptoms but also allowed paths from child race to child externalizing and adolescent marijuana dependence to vary by *FKBP5* CATT haplotype, thus testing for the Gene × Race interaction effects. A model that constrained all structural paths was tested and evidenced fair fit to the data, χ^2 (50) = 80.54, p = .004, CFI = 0.96, RMSEA = 0.06, SRMR = 0.09. A model that relaxed constraints across groups for paths from child race

Table 2. *Child externalizing factor loadings by FKBP5 CATT haplotype group*

	Factor Loadings by CATT Haplotype	
Indicator	0 Copies	1–2 Copies
TRF: delinquency	.70	.58
TRF: aggression	.81	.75
PNM: fights	.86	.98
PNM: disruptive	.73	.73
PEI: aggression	.85	.82

Note: All factor loadings are significant at $p < .001$. TRF, Teacher Report Form; PNM, peer-nomination method; PEI, Pupil Evaluation Inventory.

https://doi.org/10.1017/S0954579415000899 Published online by Cambridge University Press

to child externalizing and adolescent marijuana dependence was tested next and also demonstrated fair fit to the data, χ^2 (48) = 76.38, p = .006, CFI = 0.96, RMSEA = 0.06, SRMR = 0.09. The racial unconstrained model was not a significantly better fit to the data than the constrained model, Satorra–Bentler $\Delta\chi^2$ (2) = 4.10, *ns*, suggesting the lack of gene moderation of race effects on child externalizing and adolescent marijuana dependence symptoms. Although we did not find evidence for moderation by gene, we left the paths from race to child externalizing and adolescent marijuana dependence symptoms unconstrained across genotype group in the subsequent models given Keller's (2014) recommendation that interactions be included in models when testing G × E.

We then tested a series of SEMs relaxing constraints across genotype groups for various paths in the mediation model of interest (see Figure 1). Specifically, we tested whether the "a" path (i.e., child maltreatment → child externalizing) varied by *FKBP5* CATT haplotype group by relaxing this parameter constraint. This model evidenced fair model fit, χ^2 (47) = 76.594, p = .004, CFI = 0.96, RMSEA = 0.06, SRMR = 0.08. Comparing the fit of this model to the fit of the model described above, in which paths from child race to externalizing and marijuana dependence were relaxed, revealed a nonsignificant improvement in model fit, Satorra–Bentler $\Delta\chi^2$ (1) = 0.12, *ns*. This indicates that the effect of child maltreatment on child externalizing behavior does not vary by genotype group. Next, we tested whether the "b" path (i.e., child externalizing → adolescent marijuana dependence) varied by haplotype group by relaxing this parameter constraint. This model demonstrated good model fit, χ^2 (47) = 71.88, p = .01, CFI = 0.97, RMSEA = 0.06, SRMR = 0.08, that was significantly better than the model with this path constrained, Satorra–Bentler $\Delta\chi^2$ (1) = 5.76, p = .02, suggesting that the effect of child externalizing on adolescent marijuana dependence is moderated by *FKBP5* CATT haplotype. Finally, whether the "c" path (i.e., child maltreatment → adolescent marijuana dependence) was moderated by *FKBP5* CATT haplotype was also tested. A model that relaxed the constraint across groups for this path fit the data well, χ^2 (47) = 73.62, p = .01, CFI = 0.96, RMSEA = 0.06, SRMR = 0.08, was a marginally significantly better fit than the model with this path constrained, Satorra–Bentler $\Delta\chi^2$ (1) = 2.91, p = .09, suggesting that the effect of child maltreatment on adolescent marijuana dependence is moderated by *FKBP5* CATT haplotype. Because of evidence for gene moderation of the "b" and "c" paths, a final model was tested that allowed both paths to vary by genotype group. This model fit the data well, χ^2 (46) = 70.88, p = .01, CFI = 0.97, RMSEA = 0.06, SRMR = 0.07, and was a significantly better fit than the model with these two path constrained, Satorra–Bentler $\Delta\chi^2$ (2) = 6.05, p = .04. Standardized parameter estimates for this final model are presented in Figure 2.

The results indicated that child maltreatment was related to higher levels of child externalizing symptoms (b = 0.31, SE = 0.08, p < .001) regardless of *FKBP5* haplotype variation. However, higher levels of child externalizing predicted more symptoms of adolescence marijuana dependence among adolescents with at least one copy of the *FKBP5* CATT haplotype only (b = 0.18, SE = 0.08, p = .03). Child externalizing behavior was unrelated to adolescent marijuana dependence for adolescents without a copy of the *FKBP5* CATT haplotype (b = –0.04, SE = 0.09, *ns*). Moreover, child maltreatment predicted more marijuana dependence symptoms among adolescents with one or two copies of the CATT haplotype only (b = 0.14, SE = 0.07, p = .04). As anticipated, older adolescents reported more marijuana dependence symptoms (b = 0.12, SE = 0.06, p = .04). Child race was unrelated to marijuana dependence symptoms for either genotype group (zero copies: b = 0.07, SE = 0.08, *ns*; one or two copies: b = –0.013, SE = 0.08, *ns*). African American children without copies of the CATT haplotype were viewed as demonstrating higher levels of externalizing behavior (b = 0.23, SE = 0.10, p = .02). Child race was unrelated to externalizing behavior for children with one or two copies of the CATT haplotype (b = 0.08, SE = 0.10, *ns*).

To determine whether child externalizing behavior mediated the effect of child maltreatment on adolescent marijuana dependence symptoms differently for individuals with and without copies of the *FKBP5* CATT haplotype, 95% asymmetrical confidence intervals were used (Tofighi & MacKinnon, 2011). Confidence intervals that do not include the value zero indicate significant mediation. Results indicated that higher levels of child externalizing significantly mediated the effect of child maltreatment on adolescent marijuana dependence symptoms for individuals with one or two copies of the CATT haplotype only (95% confidence interval = 0.006, 0.109).

Internalizing mechanism

Measurement modeling. CFA was conducted to determine the appropriate factor structure of the four child internalizing behavior indicators (TRF withdrawal, TRF anxiety/depression, TRF somatic complaints, and PEI withdrawal). The results of the CFA indicated that a one-factor model was a good fit to the data, χ^2 (1) = 2.93, p = .09, CFI = 0.99, RMSEA = 0.08, SRMR = 0.02, with factor loadings all significant at p < .001 and ranging from 0.37 to 0.79. Residual covariances were modeled between TRF withdrawal and PEI withdrawal. Measurement invariance testing was then conducted to examine invariance across *FKBP5* haplotype variation. A model that constrained factor loadings and residual covariance to be equal across groups was tested and demonstrated fair fit to the data, χ^2 (9) = 8.24, p = .51, CFI = 1.00, RMSEA < 0.001, SRMR = 0.10. A model that relaxed all constraints across groups was tested next and also evidenced fair fit to the data, χ^2 (5) = 8.17, p = .15, CFI = 0.98, RMSEA = 0.07, SRMR = 0.05. The fully unconstrained model was not a significantly better fit to the data than the constrained model, Satorra–Bentler $\Delta\chi^2$ (4) = 1.49, *ns*. Therefore, there

https://doi.org/10.1017/S0954579415000899 Published online by Cambridge University Press

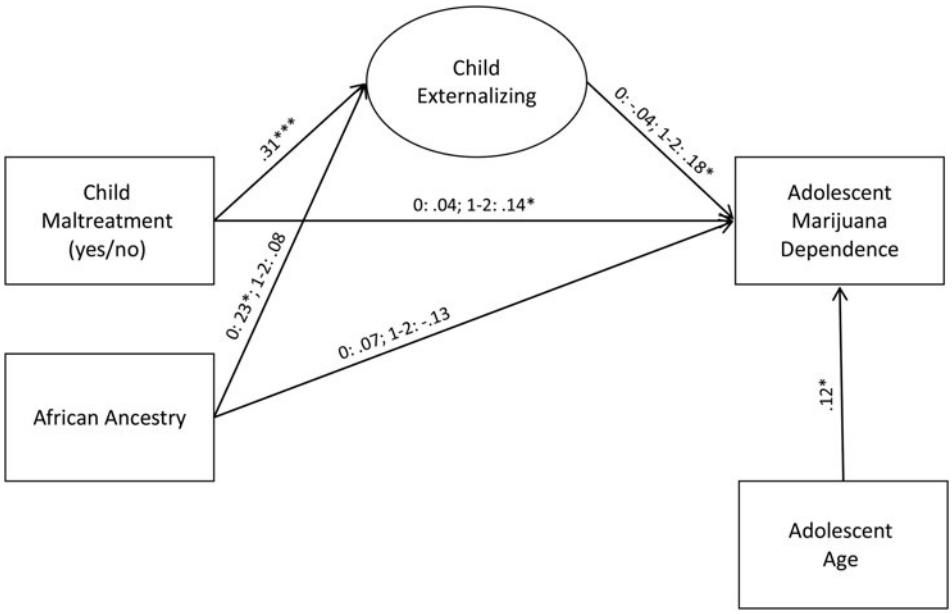

Figure 2. Genetic moderated mediation of child maltreatment effects on adolescent marijuana dependence. 0 = no copies of *FKBP5* CATT haplotype; 1–2 = one or two copies of haplotype. Child race is coded 1 = African American; 0 = other. Only statistically significant ($p < .05$) standardized parameter estimates are presented. *$p < .05$, ***$p < .001$.

was evidence for measurement invariance across *FKBP5* haplotype group.

Multiple-group SEM. The same procedures described above were employed for the internalizing models. A model that constrained paths from child race to internalizing and marijuana dependence was tested and evidenced poor fit to the data, χ^2 (41) = 60.84, $p = .02$, CFI = 0.89, RMSEA = 0.06, SRMR = 0.10. A model that relaxed constraints across groups for these two paths was tested next and also demonstrated poor fit to the data, χ^2 (39) = 57.09, $p = .03$, CFI = 0.90, RMSEA = 0.05, SRMR = 0.10. The racial unconstrained model was not a significantly better fit to the data than the constrained model, Satorra–Bentler $\Delta\chi^2$ (2) = 3.63, *ns*. Although we did not find evidence for moderation by race, we left the paths unconstrained across racial groups in the subsequent models given Keller's (2014) recommendation that interactions be included in models when testing G × E.

We then tested a series of SEMs relaxing constraints across haplotype groups for various paths in the mediation model of interest (see Figure 1). Specifically, we tested whether the "a" path (i.e., child maltreatment → child internalizing) varied by *FKBP5* CATT haplotype group by relaxing this parameter constraint. This model evidenced fair model fit, χ^2 (38) = 56.39, $p = .03$, CFI = 0.90, RMSEA = 0.06, SRMR = 0.10. Comparing the fit of this model to the fit of the model described above in which paths from child race to internalizing and marijuana dependence were relaxed revealed a nonsignificant improvement in model fit, Satorra–Bentler $\Delta\chi^2$ (1) = 0.68, *ns*. Thus, we did not find evidence that the effect of child maltreatment on child internalizing symptoms is moderated by *FKBP5* CATT haplotype. Next, we tested whether the "b" path (i.e., child internalizing → adolescent marijuana dependence) varied by haplotype group by relaxing this parameter constraint. This model demonstrated good model fit, χ^2 (38) = 56.48, $p = .03$, CFI = 0.90, RMSEA = 0.06, SRMR = 0.10, that was not significantly better than the model with this path constrained, Satorra–Bentler $\Delta\chi^2$ (1) = 0.53, *ns*, suggesting the lack of evidence for gene moderation of the effect of child internalizing on adolescent marijuana dependence symptoms. Finally, whether the "c" path (i.e., child maltreatment → adolescent marijuana dependence) was moderated by *FKBP5* CATT haplotype was also tested. A model that relaxed the constraint across groups for this path fit the data well, χ^2 (38) = 56.48, $p = .04$, CFI = 0.91, RMSEA = 0.05, SRMR = 0.10, and was not significantly better than the model with this path constrained, Satorra–Bentler $\Delta\chi^2$ (1) = 2.65, $p = .10$.

Given the lack of evidence for moderation of any path within the mediation model by *FKBP5* CATT haplotype, a single group SEM was then conducted. We trimmed child race from this model given the lack of significant main effects of child race on child internalizing and adolescent marijuana symptoms, the lack of interactive effects of race with *FKBP5* CATT haplotype in predicting both child internalizing and adolescent marijuana symptoms, and that this model no longer included a test of G × E. This final internalizing model fit the data well, χ^2 (11) = 16.81, $p = .11$, CFI = 0.97, RMSEA = 0.04, SRMR = 0.03. Results indicated that child maltreatment predicted higher levels of child internalizing symptoms regardless of *FKBP5* haplotype variation ($b = 0.23$, $SE = 0.06$, $p < .001$). Higher levels of child internalizing symptoms did not predict

https://doi.org/10.1017/S0954579415000899 Published online by Cambridge University Press

more adolescent marijuana dependence symptoms ($b = –0.01$, $SE = 0.10$, *ns*). Finally, child maltreatment significantly predicted higher levels of adolescent marijuana dependence symptoms ($b = 0.13$, $SE = 0.06$, $p = .02$). See Figure 3 for standardized parameter estimates.

Discussion

The current study examined the prospective association between child maltreatment and the development of SUD in adolescence with the aim of investigating pathways underlying this relation, as well as genetic moderation of these developmental mechanisms. Specifically, we tested whether youth who experienced maltreatment prior to age 8 were at risk for the development of marijuana dependence in adolescence by way of a childhood externalizing pathway and a childhood internalizing pathway. Moreover, we tested whether variation in *FKBP5* CATT haplotype moderated these pathways, thus aiming to partially explain why some maltreated children go on to develop problems with substance use later in development while others do not.

Interaction of child maltreatment and FKBP5 genetic variation

Our data replicated the well-documented finding that children who experience maltreatment are at risk for developing SUD (e.g., Buckingham & Daniolos, 2013; Cicchetti & Toth, in press; Moran et al., 2004; Oshri, Rogosch, Burnette, & Cicchetti, 2011; Shin 2012; Shin et al., 2010; Vilhena-Churchill & Goldstein, 2014). Moreover, consistent with Rogosch et al. (2010), our results support an externalizing pathway from child maltreatment to adolescent marijuana dependence. In addition, we extend previous research by demonstrating that whether a maltreated child will traverse an externalizing pathway toward SUD in adolescence is dependent on *FKBP5* genetic variation. More specifically, we found evidence for moderated mediation, such that child externalizing symptoms mediated the effect of child maltreatment on adolescent marijuana dependence only for adolescents with one or two copies of the *FKBP5* CATT haplotype. Although child maltreatment conferred risk for child externalizing symptoms regardless of *FKBP5* polymorphism, whether externalizing symptoms in childhood progressed into marijuana dependence in adolescence depended on the presence of the *FKBP5* CATT haplotype. Given that *FKBP5* is involved in altering the stress response, our findings indicate that the snowballing of childhood externalizing symptoms into adolescent SUD may be dependent on gene variants that affect stress sensitivity.

In addition, we found preliminary support for *FKBP5* genetic variation as a moderator of the direct effect of child maltreatment on adolescent marijuana dependence. Our externalizing models demonstrated that adolescents with maltreatment

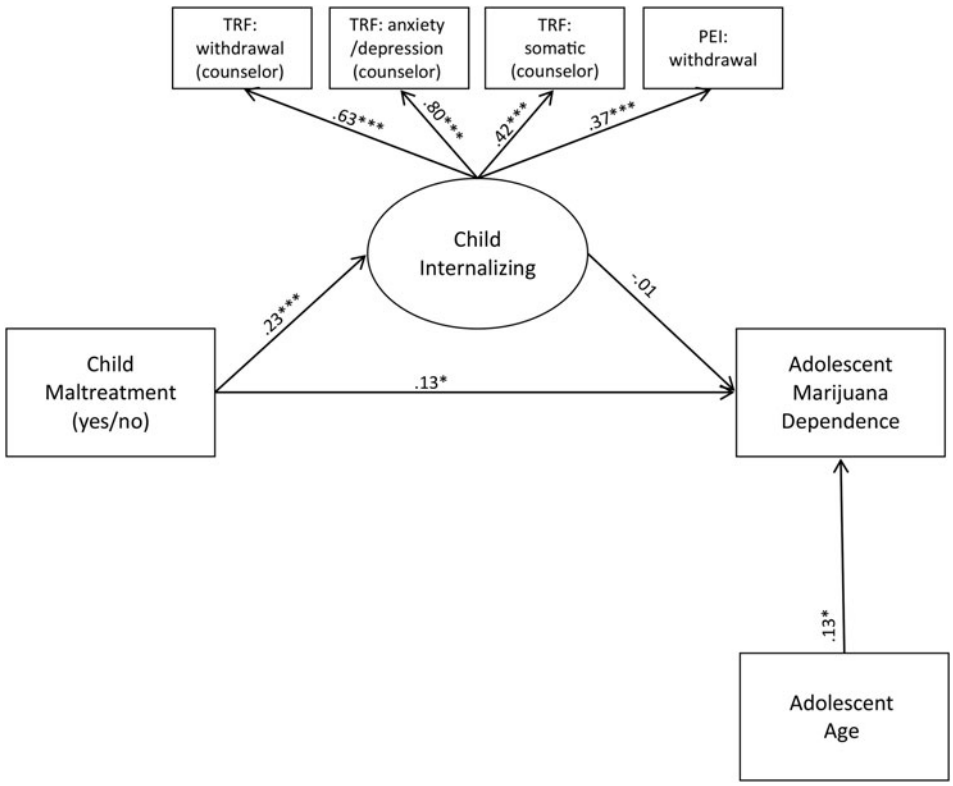

Figure 3. Standardized parameter estimates. Only statistically significant ($p < .05$) standardized parameter estimates are presented. *$p < .05$, ***$p < .001$.

https://doi.org/10.1017/S0954579415000899 Published online by Cambridge University Press

experiences prior to age 8 reported more marijuana dependence symptoms in adolescence only if they carried one or two copies of the *FKBP5* CATT haplotype. For adolescents without copies of the *FKBP5* CATT haplotype, child maltreatment was unrelated to marijuana dependence in adolescence. It is worth noting that moderation of this direct effect did not reach statistical significance in our internalizing models. Therefore, we regard these findings as tentative and, as is the case with all G × E findings, in need of replication.

Together, these results contribute to a growing literature demonstrating that *FKBP5* moderates the effect of childhood adversity on the development of later psychopathology (see Zannas & Binder, 2014, for review). To our knowledge, our findings represent the first evidence that *FKBP5* interacts with childhood maltreatment to affect substance-related outcomes, an interactive effect posited by Enoch (2012) but not yet tested. This also represents the first evidence for *FKBP5* moderation of the developmental pathway from child maltreatment to adolescent SUD.

Although much of the prior research has focused on the interaction of childhood trauma and *FKBP5* in the prediction of internalizing symptoms such as posttraumatic stress disorder (Binder et al., 2008; Xie et al., 2010), depression (Appel et al., 2011; Dackis et al., 2012), and suicide (Roy et al., 2010), one other study has examined *FKBP5* × Child Adversity interactive effects on the externalizing spectrum. Specifically, Bevilacqua et al. (2012) found child physical abuse significantly predicted adult aggressive and violent behavior only for individuals with the *FKBP5* CATT haplotype. We advance the extant literature by being the first to show that *FKBP5* moderates the externalizing mechanism by which youth exposed to maltreatment are at risk for marijuana dependence in adolescence. Moreover, we provide preliminary evidence that *FKBP5* also moderates the direct effect of child maltreatment on adolescent marijuana dependence. In doing so, our data support Zannas and Binder's (2014) assertion that *FKBP5*, a genetic variant that alters stress sensitivity, may confer a general vulnerability in the context of childhood trauma that may result in a number of varied phenotypes.

Regarding internalizing symptoms, our results are consistent with the well-documented finding that child maltreatment confers risk for internalizing symptoms (e.g., Cicchetti & Toth, in press). Contrary to our hypotheses, we did not find support for *FKBP5* variation as a moderator of the effect of child maltreatment on child internalizing symptoms or as a moderator of the internalizing pathway from child maltreatment to adolescent SUD. Although, as described above, there is emerging support for the interaction of *FKBP5* and child adversity in the prediction of internalizing symptoms, prior studies have examined internalizing symptoms in *adulthood*, rather than childhood. Our focus on childhood internalizing symptoms, which are more proximal to the maltreatment experience, may provide a possible explanation for the difference in findings. Moreover, the present study assessed maltreatment via the MCS (Barnett et al., 1993). The MCS utilizes DHS records detailing investigations and findings involving maltreatment in identified families over time and codes all available information, making independent determinations of maltreatment experiences. Thus, the MCS does not rely on retrospective reporting of participants or participants' memory. Prior studies that have identified a *FKBP5* × Child Adversity interaction in the prediction of adult internalizing symptoms have utilized the Childhood Trauma Questionnaire (Bernstein et al., 2003), an adult retrospective self-report measure. Differences in G × E findings with *FKBP5* and child adversity have been documented within the same study across various measures of childhood adversity. Specifically, Buchman et al. (2014) found a significant *FKBP5* × Child Adversity interactive effect on cortisol stress response when adversity was measured by the Childhood Trauma Questionnaire and not when measured by a parent-reported prospective measure of family adversity. Therefore, our lack of support for *FKBP5* moderation in the relation between child maltreatment and internalizing symptoms may be a consequence of two methodological differences between our study and prior research, namely, the developmental timing of our assessment of internalizing symptoms and our measurement of child maltreatment. More research investigating the way in which *FKBP5* polymorphisms may or may not enhance vulnerability to childhood internalizing symptoms in the context of child trauma is necessary.

Pathways to SUD

Our data support the widely documented externalizing pathway to SUD. However, our test of Gene × Environment interaction within the externalizing pathway highlights the importance of examining individual differences within this risk pathway and, more broadly, the criticality of a multiple levels of analysis approach to understanding the etiology of SUD. Although externalizing symptoms in childhood are robustly associated with substance use and disorder later in development (see Chassin et al., 2009, 2013; Zucker et al., 2011, for reviews), consistent with the notion of multifinality (Cicchetti & Rogosch, 1996), not all children who exhibit externalizing symptoms follow a pathway to substance problems in adolescence. Our data provide a possible explanation for this heterogeneity by demonstrating that individuals with at least one copy of the *FKBP5* CATT haplotype are more likely to progress from childhood externalizing symptoms to adolescent substance use problems, possibly as a result of alterations in the stress response due to *FKBP5* polymorphisms.

As described previously, the internalizing pathway to SUD has received less attention than the externalizing pathway, and results have yielded equivocal support for the internalizing mechanism (e.g., Chassin et al., 2009, 2013). We did not find evidence that child internalizing symptoms prospectively predicted adolescent marijuana dependence, nor did we find evidence for an internalizing pathway to SUD from child maltreatment. For instance, our lack of support for the internalizing mechanism to SUD is consistent with prior research showing that child externalizing, but not internalizing,

https://doi.org/10.1017/S0954579415000899 Published online by Cambridge University Press

symptoms prospectively predict adolescent or young adult marijuana use and disorder (Englund & Siebenbruner, 2012; Tarter, Kirisci, Ridenour, & Vanyukov, 2008). However, many others have found associations between internalizing symptoms and subsequent substance involvement (see Chassin et al., 2013, for review), including McCarty et al. (2012), who showed that early adolescent depressive symptoms were predictive of greater alcohol use a year later, over and above adolescent conduct problems.

Why might child maltreatment confer risk for the development of adolescent SUD by way of an externalizing pathway, rather than an internalizing pathway? First, it is clear from the broader literature on the etiology of SUD that externalizing symptoms are more robust predictors of SUD than are internalizing symptoms (Hussong et al., 2011). Second, the time lag between our measurement of childhood symptomatology (ages 7–9) and our measurement of adolescent marijuana dependence (ages 15–18) may not be optimal for uncovering an internalizing mechanism. For instance, self-medication is one proposed aspect of the internalizing pathway, which is likely a more immediate process that is more appropriately captured by a much shorter time lag between assessments. Third, our measurement of child internalizing symptoms included diverse measures of internalizing symptoms such as withdrawal, anxiety, depression, and somatic complaints. There is evidence that different components of the internalizing spectrum may be differentially related to SUD (Edwards et al., 2014; Hussong et al., 2011), although whether depression or anxiety symptoms are more robustly linked to SUD is not yet clear. Using these components of the internalizing spectrum as indicators of one latent factor may have obscured relations. Fourth, we relied on counselor-reported internalizing symptoms, rather than child self-report. Prior research has indicated weaker relations between internalizing symptoms and substance use for observer versus self-reported internalizing measures (McCarty et al., 2012). Fifth, and perhaps of the most importance, the internalizing and externalizing pathways are not mutually exclusive, and comorbidity across the internalizing and externalizing spectrums is widely reported (Hussong et al., 2011). Recent theorized models of the etiology of SUD suggest that externalizing symptoms may mediate and/or moderate the internalizing pathway to SUD (Hussong et al., 2011). Therefore, it is likely that complex dynamic models of these developmental pathways that incorporate genetic variations will be critical for understanding why maltreated children are at risk for developing SUD and for understanding the etiology of SUD more broadly.

Limitations and conclusions

The present study contributes to the literature in a number of key ways. In addition to identifying an externalizing developmental pathway by which maltreated children may progress to substance disorder in adolescence, the current study demonstrated that whether a maltreated child will traverse this externalizing pathway and develop marijuana dependence in adolescence is dependent on *FKBP5* genetic variation. Strengths of this investigation include our examination of multiple longitudinal mechanisms of child maltreatment risk spanning childhood through adolescence, our use of prospective assessment of maltreatment, and the use of the *FKBP5* CATT haplotype, as opposed to individual *FKBP5* SNPs.

The present study has important implications not only for understanding the developmental progression from child maltreatment to adolescent SUD, and the etiology of SUD more broadly, but also for preventive intervention design. Clearly, intervening with families prior to the occurrence of maltreatment is critical to curbing the cascading effects of maltreatment on child externalizing symptoms and future substance problems. However, intervening with families during childhood when early externalizing symptoms are present may function to thwart the progression into SUS in adolescence.

In spite of these contributions, there are limitations worth noting. We relied on a binary measure of child maltreatment experience prior to age 8. Much research supports the importance of the developmental timing of maltreatment (for a review, see Cicchetti & Toth, in press). A more nuanced understanding of the role of developmental timing of maltreatment may be critical to further explicating the complex pathways from child maltreatment to subsequent SUD. Similarly, Gene × Environment × Development studies may be particularly useful for demonstrating how *FKBP5* may interact with child maltreatment differently at different developmental periods. In addition, we focused on psychosocial and molecular genetic levels of analysis in this investigation. There is evidence that *FKBP5* polymorphism interacts with childhood adversity to affect cortisol recovery after acute stress (Buchman et al., 2014). Future multiple levels of analysis studies of pathways from child maltreatment to adolescent SUD, which can incorporate not only molecular genetics but also other levels such as cortisol regulation and reactivity, will be important to further uncovering the underlying mechanisms of risk associated with childhood maltreatment.

In summary, results of the current study support an externalizing pathway by which certain vulnerable children who experience maltreatment may develop into adolescents with a substance disorder. Specifically, we found that maltreated youth with one or two copies of the *FKBP5* CATT haplotype were at risk for developing marijuana dependence in adolescence partially because of an underlying externalizing mechanism. Our findings contribute to the understanding of how risk associated with child maltreatment unfolds throughout childhood and adolescence and may result in SUD for certain vulnerable youth. The consideration of the molecular genetic level within these mediational pathways was critical to identifying a subgroup of maltreated children most at risk for traversing an externalizing pathway and is consistent with the multiple levels of analysis approach espoused within developmental psychopathology (Cicchetti & Dawson, 2002; Cicchetti & Toth, 2009).

https://doi.org/10.1017/S0954579415000899 Published online by Cambridge University Press

References

Achenbach, T. (1991). *Manual for the Teacher Report Form and 1991 profile*. Burlington, VT: University of Vermont, Department of Psychiatry.

Appel, K., Schwahn, C., Mahler, J., Schulz, A., Spitzer, C., Fenske, K., et al. (2011). Moderation of adult depression by a polymorphism in the FKBP5 gene and childhood physical abuse in the general population. *Neuropsychopharmacology*, *36*, 1982–1991.

Banny, A. M., Cicchetti, D., Rogosch, F. A., Oshri, A., & Crick, N. R. (2013). Vulnerability to depression: A moderated mediation model of the roles of child maltreatment, peer victimization, and serotonin transporter linked polymorphic region genetic variation among children from low socioeconomic status backgrounds. *Development and Psychopathology*, *25*, 599–614.

Barnett, D., Manly, J. T., & Cicchetti, D. (1993). Defining child maltreatment: The interface between policy and research. In D. Cicchetti & S. L. Toth (Eds.), *Child abuse, child development, and social policy* (pp. 7–74). Norwood, NJ: Ablex.

Bernstein, D. P., Stein, J. A., Newcomb, M. D., Walker, E., Pogge, D., Ahluvalia, T., et al. (2003). Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse & Neglect*, *27*, 169–190.

Bevilacqua, L., Carli, V., Sarchiapone, M., George, D. K., Goldman, D., Roy, A., et al. (2012). *Interaction between FKBP5 and childhood trauma and risk of aggressive behavior*. *Archives of General Psychiatry*, *69*, 62–70.

Binder, E. B., Bradley, R. G., Liu, W., Epstein, M. P., Deveau, T. C., Mercer, K. B., et al. (2008). Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *Journal of the American Medical Association*, *299*, 1291–1305.

Binder, E. B., Salyakina, D., Lichtner, P., Wochnik, G. M., Ising, M., Pütz, B., et al. (2004). Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nature Genetics*, *36*, 1319–1325.

Bolger, K. E., & Patterson, C. J. (2001). Developmental pathways from child maltreatment to peer rejection. *Child Development*, *72*, 549–568.

Brodsky, B. S., Oquendo, M., Ellis, S. P., Haas, G. L., Malone, K. M., & Mann, J. J. (2001). The relationship of childhood abuse to impulsivity and suicidal behavior in adults with major depression. *American Journal of Psychiatry*, *158*, 1871–1877.

Buchmann, A. F., Holz, N., Boecker, R., Blomeyer, D., Rietschel, M., Witt, S. H., et al. (2014). Moderating role of FKBP5 genotype in the impact of childhood adversity on cortisol stress response during adulthood. *European Neuropsychopharmacology*, *24*, 837–845.

Buckingham, E. T., & Daniolos, P. (2013). Longitudinal outcomes for victims of child abuse. *Current Psychiatry Reports*, *15*, 342.

Chassin, L., Hussong, A., & Beltran, I. (2009). Adolescent substance use. In R. M. Lerner & L. Steinberg (Eds.), *Handbook of adolescent psychology: Vol. 1. Individual bases of adolescent development* (3rd ed.). Hoboken, NJ: Wiley.

Chassin, L., Sher, K. J., Hussong, A., & Curran, P. (2013). The developmental psychopathology of alcohol use and alcohol disorders: Research achievements and future directions. *Development and Psychopathology*, *25*, 1567–1584.

Cicchetti, D., & Dawson, G. (2002). Multiple levels of analysis. *Development and Psychopathology*, *14*, 417–420.

Cicchetti, D., & Manly, J. T. (1990). A personal perspective on conducting research with maltreating families: Problems and solutions. In G. Brody & I. Sigel (Eds.), *Methods of family research: Families at risk* (Vol. 2, pp. 87–133). Hillsdale, NJ: Erlbaum.

Cicchetti, D., & Rogosch, F. A. (1996). Equifinality and multifinality in developmental psychopathology. *Development and Psychopathology*, *8*, 597–600.

Cicchetti, D., & Toth, S. L. (2009). The past achievements and future promises of developmental psychopathology: The coming of age of a discipline. *Journal of Child Psychology and Psychiatry*, *50*, 16–25.

Cicchetti, D., & Toth, S. L. (in press). A multilevel perspective on child maltreatment. In M. Lamb & C. Garcia Coll (Eds.), *Handbook of child psychology and developmental science: Vol. 3. Socioemotional process* (7th ed.). New York: Wiley.

Coie, J. D., & Dodge, K. A. (1983). Continuities and changes in children's social status: A five-year longitudinal study. *Merrill–Palmer Quarterly*, *29*, 261–282.

Costello, E. J. (2007). Psychiatric predictors of adolescent and young adult drug use and abuse: What have we learned? *Drug and Alcohol Dependence*, *88S*, S97–S99.

Dackis, M. N., Rogosch, F. A., Oshri, A., & Cicchetti, D. (2012). The role of limbic system irritability in linking history of childhood maltreatment and psychiatric outcomes in low-income, high-risk women: Moderation by FK506 binding protein 5 haplotype. *Development and Psychopathology*, *24*, 1237–1252.

Dahne, J., Banducci, A. N., Kurdziel, G., & MacPherson, L. (2014). Early adolescent symptoms of social phobia prospectively predict alcohol use. *Journal of Studies on Alcohol and Drugs*, *75*, 929.

Dick, D. (2011). Developmental changes in genetic influences on alcohol use and dependence. *Child Development Perspectives*, *5*, 223–230.

Dube, S. R., Felitti, V. J., Dong, M., Chapman, D. P., Giles, W. H., & Anda, R. F. (2003). Childhood abuse, neglect, and household dysfunction and the risk for illicit drug use: The adverse childhood experiences study. *Pediatrics*, *111*, 564–572.

Edwards, A. C., Latendresse, S. J., Heron, J., Cho, S. B., Hickman, M., Lewis, G., et al. (2014). Childhood internalizing symptoms are negatively associated with early adolescent alcohol use. *Alcoholism: Clinical and Experimental Research*, *38*, 1680–1688.

Englund, M. M., & Siebenbruner, J. (2012). Developmental pathways linking externalizing symptoms, internalizing symptoms, and academic competence to adolescent substance use. *Journal of Adolescence*, *35*, 1123–1140.

Enoch, M. (2012). The influence of gene-environment interactions on the development of alcoholism and drug dependence. *Current Psychiatry Reports*, *14*, 150–158.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567.

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, *164*, 1567–1587.

Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molecular Ecology Notes*, *7*, 574–578.

Fisher, P., Shaffer, D., Piacentini, J., Lapkin, J., Kafantaris, V., Leonard, H., et al. (1993). Sensitivity of the Diagnostic Interview Schedule for Children (2nd ed., DISC-2.1) for specific diagnoses of children and adolescents. *Journal of the American Academy of Child & Adolescent Psychiatry*, *32*, 666–673.

Green, K. M., Doherty, E. E., Reisinger, H. S., Chilcoat, H. D., & Ensminger, M. (2010). Social integration in young adulthood and the subsequent onset of substance use and disorders among a community population of urban African Americans. *Addiction*, *105*, 484–493.

Green, K. M., & Ensminger, M. E. (2006). Adult social behavioral effects of heavy adolescent marijuana use among African Americans. *Developmental Psychology*, *42*, 1168–1178.

Hu, L., & Bentler, P. (1999). Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Structural Equation Modeling*, *6*, 1–55.

Huang, S., Trapido, E., Fleming, L., Arheart, K., Crandall, L., French, M., et al. (2011). The long-term effects of childhood maltreatment experiences on subsequent illicit drug use and drug-related problems in young adulthood. *Addictive Behaviors*, *36*, 95–102.

Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, *9*, 1322–1332.

Hussong, A. M., Jones, D. J., Stein, G. L., Baucom, D. H., & Boeding, S. (2011). An internalizing pathway to alcohol use and disorder. *Psychology of Addictive Behaviors*, *25*, 390–404.

Johnston, L. D., O'Malley, P. M., Miech, R. A., Bachman, J. G., & Schulenberg, J. E. (2014). *Monitoring the Future national results on drug use, 1975–2013: Overview, key findings on adolescent drug use*. Ann Arbor, MI: University of Michigan, Institute for Social Research.

Keller, M. C. (2014). Gene × environment interaction studies have not properly controlled for potential confounders: The problem and the (simple) solution. *Biological Psychiatry*, *75*, 18–24.

Kim, J., & Cicchetti, D. (2010). Longitudinal pathways linking child maltreatment, emotion regulation, peer relations, and psychopathology. *Journal of Child Psychology and Psychiatry*, *51*, 706–716.

Kim, J., Cicchetti, D., Rogosch, F. A., & Manly, J. T. (2009). Child maltreatment and trajectories of personality and behavioral functioning: Implications for the development of personality disorder. *Development and Psychopathology*, *21*, 889–912.

King, S. M., Iacono, W. G., & McGue, M. (2004). Childhood externalizing and internalizing psychopathology in the prediction of early substance use. *Addiction*, *99*, 1548–1559.

Lai, C. Q., Tucker, K. L., Choudhry, S., Parnell, L. D., Mattei, J., García-Bailo, B., et al. (2009). Population admixture associated with disease prevalence in the Boston Puerto Rican health study. *Human Genetics*, *125*, 199–209.

Lansford, J. E., Dodge, K. A., Pettit, G. S., Bates, J. E., Crozier, J., & Kaplow, J. (2002). A 12-year prospective study of the long-term effects of early child physical maltreatment on psychological, behavioral, and academic problems in adolescence. *Archives of Pediatrics & Adolescent Medicine*, *156*, 824–830.

Lynskey, M., & Hall, W. (2000). The effects of adolescent cannabis use on educational attainment: A review. *Addiction*, *95*, 1621–1630.

Maniglio, R. (2010). Child sexual abuse in the etiology of depression: A systematic review of reviews. *Depression and Anxiety*, *27*, 631–642.

Manly, J. T. (2005). Advances in research definitions of child maltreatment. *Child Abuse & Neglect*, *29*, 425–439.

Manly, J. T., Cicchetti, D., & Barnett, D. (1994). The impact of subtype, frequency, chronicity, and severity of child maltreatment on social competence and behavior problems. *Development and Psychopathology*, *6*, 121–143.

Manly, J. T., Kim, J. E., Rogosch, F. A., & Cicchetti, D. (2001). Dimensions of child maltreatment and children's adjustment: Contributions of developmental timing and subtype. *Development and Psychopathology*, *13*, 759–782.

McCarty, C. A., Wymbs, B. T., King, K. M., Mason, W. A., Stoep, A. V., McCauley, E., et al. (2012). Developmental consistency in associations between depressive symptoms and alcohol use in early adolescence. *Journal of Studies on Alcohol and Drugs*, *73*, 444.

Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S., et al. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences*, *109*, E2657–E2664.

Merikangas, K. R., He, J., Burstein, M., Swanson, S. A., Avenevoli, S., Cui, L., et al. (2010). Lifetime prevalence of mental disorders in U.S. adolescents: Results from the National Comorbidity Survey Replication—Adolescent Supplement (NCS-A). *Journal of the American Academy of Child & Adolescent Psychiatry*, *49*, 980–989.

Moffitt, T. E., Caspi, A., & Rutter, M. (2005). Strategy for investigating interactions between measured genes and measured environments. *Archives of General Psychiatry*, *62*, 473–481.

Moran, P. B., Vuchinich, S., & Hall, N. K. (2004). Associations between types of maltreatment and substance use during adolescence. *Child Abuse & Neglect*, *28*, 565–574.

Muthén, L. K., & Muthén, B. O. (1998–2012). *Mplus user's guide* (7th ed.). Los Angeles: Author.

Oshri, A., Rogosch, F. A., Burnette, M. L., & Cicchetti, D. (2011). Developmental pathways to adolescent cannabis abuse and dependence: Child maltreatment, emerging personality, and internalizing versus externalizing psychopathology. *Psychology of Addictive Behaviors*, *25*, 634.

Oshri, A., Rogosch, F. A., & Cicchetti, D. (2013). Child maltreatment and mediating influences of childhood personality types on the development of adolescent psychopathology. *Journal of Clinical Child & Adolescent Psychology*, *42*, 287–301.

Pekarkik, E., Prinz, R., Liebert, D., Weintraub, S., & Neale, J. (1976). The Pupil Evaluation Inventory: A sociometric technique for assessing children's social behavior. *Journal of Abnormal Child Psychology*, *4*, 83–97.

Piacentini, J., Shaffer, D., Fisher, P., Schwab-Stone, M., Davies, M., & Goia, P. (1993). The Diagnostic Interview Schedule for Children—Revised (DISC-R): III. Concurrent criterion validity. *Journal of the American Academy of Child & Adolescent Psychiatry*, *32*, 658–665.

Rogosch, F. A., Cicchetti, D., Shields, A., & Toth, S. L. (1995). Parenting dysfunction in child maltreatment. In M. H. Bornstein (Ed.), *Handbook of parenting* (Vol. 4, pp. 127–159). Hillsdale, NJ: Erlbaum.

Rogosch, F. A., Oshri, A., & Cicchetti, D. (2010). From child maltreatment to adolescent cannabis abuse and dependence: A developmental cascade model. *Development and Psychopathology*, *22*, 883–897.

Roy, A., Gorodetsky, E., Yuan, Q., Goldman, D., & Enoch, M. A. (2010). Interaction of FKBP5, a stress-related gene, with childhood trauma increases the risk for attempting suicide. *Neuropsychopharmacology*, *35*, 1674–1683.

Roy, A., Hodgkinson, C. A., DeLuca, V., Goldman, D., & Enoch, M. A. (2012). Two HPA axis genes, CRHBP and FKBP5, interact with childhood trauma to increase the risk for suicidal behavior. *Journal of Psychiatric Research*, *46*, 72–79.

Scott, K. M., Smith, D. R., & Ellis, P. M. (2010). Prospectively ascertained child maltreatment and its association with DSM-IV mental disorders in young adults. *Archives of General Psychiatry*, *67*, 712–719.

Sedlak, A. J., Mettenburg, J., Basena, M., Petta, I., McPherson, K., & Greene, A. (2010). *Fourth National Incidence Study of Child Abuse and Neglect (NIS-4): Report to Congress*. Washington, DC: US Department of Health and Human Services, Administration for Children, Youth and Families.

Shaffer, D., Schwab-Stone, M., Fisher, P., Cohen, P., Piacentini, J., Davies, M., et al. (1993). The Diagnostic Interview Schedule for Children—Revised (DISC-R): I. Preparation, field testing, and interrater reliability. *Journal of the American Academy of Child & Adolescent Psychiatry*, *32*, 643–650.

Sher, K. J. (1991). *Children of alcoholics: A critical appraisal of theory and research*. Chicago, IL: University of Chicago Press.

Shields, A., & Cicchetti, D. (2001). Parental maltreatment and emotion dysregulation as risk factors for bullying and victimization in middle childhood. *Journal of Clinical Child Psychology*, *30*, 349–363.

Shin, S. H. (2012). A longitudinal examination of the relationships between childhood maltreatment and patterns of adolescent substance use among high-risk adolescents. *American Journal on Addictions*, *21*, 453–461.

Shin, S. H., Hong, H. G., & Hazen, A. L. (2010). Childhood sexual abuse and adolescent substance use: A latent class analysis. *Drug and Alcohol Dependence*, *109*, 226–235.

Stouthamer-Loeber, M., Loeber, R., Homish, D. L, & Wei, E. (2001). Maltreatment of boys and the development of disruptive and delinquent behavior. *Development and Psychopathology*, *13*, 941–955.

Tarter, R. E., Kirisci, L., Ridenour, T., & Vanyukov, M. (2008). Prediction of cannabis use disorder between childhood and young adulthood using the child behavior checklist. *Journal of Psychopathology and Behavioral Assessment*, *30*, 272–278.

Tofighi, D., & MacKinnon, D. P. (2011). RMediation: An R package for mediation analysis confidence intervals. *Behavior Research Methods*, *43*, 692–700.

Vilhena-Churchill, N., & Goldstein, A. L. (2014). Child maltreatment and marijuana problems in young adults: Examining the role of motives and emotion dysregulation. *Child Abuse and Neglect*, *38*, 962–972.

Xie, P., Kranzler, H. R., Poling, J., Stein, M. B., Anton, R. F., Farrer, L. A., et al. (2010). Interaction of FKBP5 with childhood adversity on risk for post-traumatic stress disorder. *Neuropsychopharmacology*, *35*, 1684–1692.

Yaeger, R., Avila-Bront, A., Abdul, K., Nolan, P. C., Grann, V. R., Birchette, M. G., et al. (2008). Comparing genetic ancestry and self-described race in African Americans born in the United States and in Africa. *Cancer Epidemiology Biomarkers & Prevention*, *17*, 1329–1338.

Yu, C. Y., & Muthén, B. O. (2002). *Evaluation of model fit indices for latent variable models with categorical and continuous outcomes* (Technical Report). Los Angeles: University of California at Los Angeles, Graduate School of Education and Information Studies.

Zannas, A. S., & Binder, E. B. (2014). Gene–environment interactions at the FKBP5 locus: Sensitive periods, mechanisms and pleiotropism. *Genes, Brain, and Behavior*, *13*, 25–37.

Zucker, R. A., Heitzeg, M. M., & Nigg, J. T. (2011). Parsing the undercontrol-disinhibition pathway to substance use disorders: A multilevel developmental problem. *Child Development Perspectives*, *5*, 248–255.