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Biological underpinnings of an internalizing pathway to alcohol, cigarette, and marijuana use

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Abstract

There is a limited understanding as to how specific genes impact addiction risk. Applying a developmental framework and research domain criteria (RDoC) to identify etiological pathways from genetic markers to addiction may have utility. Prior research has largely focused on externalizing pathways to substance use. Although internalizing mechanisms have received less attention, there is strong support that addiction is a longer-term consequence of using substances to cope with internalizing as well as externalizing problems. This study tests whether temperament and depression mediate the association between specific genetic variants and substance use. The sample consisted of 426 adolescents from the Michigan Longitudinal Study (70.9% boys, 84.0% White). Four specific genetic variants were examined: *SLC6A4* (*5HTTLPR*), *BDNF* (rs6265), *NPY* (rs3037354), and *CRHBP* (rs7728378). Childhood resiliency and behavioral control were examined as potential mediators, in addition to early adolescent depression, using a multiple-mediator path model. Resiliency and depression were supported as mediators in the association between genetic risk and later substance use. Important differences emerged across substances of abuse. Indirect effects via depression were not significant with the inclusion of aggression. Early difficulties with emotional coping may represent non-specific neurobiological underpinnings for an internalizing pathway to addiction.

Keywords

genetics; adolescence; substance use; mediation; internalizing

Understanding neurobiological mechanisms that contribute to the etiology of substance use disorder (SUD) has been challenging. This is due in part to the nature of SUD as a heterogeneous and complex disorder that develops gradually across ontogeny (Zucker, Hicks, & Heitzeg, 2016). Recently, a research domain criteria (RDoC) approach in which objective intermediate phenotypes across multiple levels of analysis are identified to

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understand the etiology of clinical syndromes has been advocated (Ellingson, Richmond-Rakerd, Statham, Martin, & Slutske, 2016). The RDoC approach specifies five domains/constructs of functioning as an organizing framework: negative valence systems (e.g., loss, fear), positive valence systems (e.g., reward, approach motivation), cognitive systems (e.g., inhibition, attention), systems for social processes (e.g., affiliation), and arousal/regulatory systems (e.g., sleep-wake; Cuthbert, 2014). This approach emphasizes the delineation of “building blocks” such as genes, temperament, and maladaptive experiences to elucidate the mechanisms through which biological factors impact psychopathology in adulthood.

The RDoC approach may have particular utility for understanding factors linking genetic risk to SUD given its complexity. Yet, few studies have adopted this approach to understanding heterogeneous pathways from genetic risk to SUD. One exception is a study by Trucco and colleagues (2016), which investigated the role of temperament and externalizing behavior as potential mediators between genetic risk factors (*5-HTTLPR*, *DRD4*, *SLC6A2*, *GABRA2*, *GABRA6*) and substance use (SU). Although that work contributes to the field by demonstrating the utility of incorporating multiple levels of analysis to understand how addiction develops, only one pathway was tested. The aim of the current study is to adopt a similar approach to identify an internalizing pathway to SU.

Developmental Pathways to SUD

Prior research demonstrates that risk for SUD is evident during childhood, with risk factors unfolding well before SU initiation (Zucker et al., 2016). Cascade models posit that genetic influences place individuals on a trajectory for adult psychopathology that is characterized by a progression from early risk factors in childhood, to sequentially riskier behaviors across development (Dodge et al., 2009). The externalizing pathway to SUD is an empirically supported cascade model reflecting the gradual progression of risk (e.g., Zucker et al., 2016). In this model, early risk is expressed as childhood temperament characterized by behavioral disinhibition and impulsivity. Impulsive temperament then paves the way for the sequential progression to adolescent externalizing problems, such as aggression, and the eventual onset of SU.

Consistent support for externalizing pathways has contributed to the limited exploration into alternative etiological pathways to addiction. One notable alternative is the internalizing pathway to SUD. Consistent with cascade models, the internalizing pathway is also grounded in developmental theory and characterizes a sequential progression to increasingly problematic behavior across development. Yet in this model, the key temperamental factors that set SUD into motion are behavioral inhibition, emotional dysregulation, and negative emotionality. These aspects of temperament lead to increased risk for internalizing symptoms, such as depression, during adolescence. In turn, internalizing symptoms increase risk for the onset of SU (Hussong, Jones, Stein, Baucom, & Boeding, 2011). Although the role of depression and anxiety on SU has been articulated in several seminal studies (e.g., Type I and Type II Alcoholism; Cloninger, Sigvardsson, & Bohman, 1996), the internalizing pathway to SU provides the contextualization of these mechanisms within a developmental framework whereby risk for later SU can be identified as operational in childhood.

Anxiety and depressive symptoms have both been supported as risk factors for SU. The self-medication theory posits that individuals are likely to consume substances to offset negative affect contributing to a positive correlation between anxiety and depression on SU (Khantzian, 1997). The long-term reliance on substances to regulate emotions is believed to contribute to the onset of SUD (Hussong et al., 2011). Although these associations are fairly consistent for adults, these associations are varied in adolescence. Depressive symptoms are more consistent predictors of adolescent SU than anxiety symptoms, which show few associations with SU (Hussong, Ennett, Cox, & Haroon, 2017). During adolescence, SU is less normative and perceived as risky. For example, younger adolescents perceive that the likelihood of negative outcomes from SU (e.g., getting sick) is greater than the likelihood of positive outcomes (e.g., I'll feel more relaxed; Colder et al., 2017). As SU becomes more normative, there is a shift towards greater balance between negative and positive expectancies. Thus, some adolescents may view SU as not a viable option to cope with anxiety as SU may exacerbate worries. This is consistent with risk avoidance theory (Wills, Windle, & Cleary, 1998), which supports a negative correlation between anxiety and SU, as anxious adolescents tend to avoid taking risks or engage in social situations where substances are often used.

In contrast, adolescent depression has been demonstrated to increase the odds of SU onset, even after controlling for externalizing behavior (e.g., Hussong et al., 2017). Moreover, twin studies indicate that nicotine and alcohol dependence may share a common genetic liability with depression (Edwards, Maes, Pedersen, & Kendler, 2011). Testing an internalizing pathway to SUD could provide a greater understanding of alternative mechanisms that characterize the equifinality of addiction. Moreover, examining neurobiological mechanisms underlying the development of SU may facilitate the development of novel treatment and prevention efforts aimed to address early biological risk factors of SUD.

Relevant Temperamental Traits

Temperament is defined as heritable and early emerging differences in emotional, motor, attentional, and self-regulation processes that modulate reactivity (Eisenberg et al., 2001). According to cascade models, temperament is critical in understanding early risk for SUD. The temperament model originally articulated by Block and Block (1980) and later re-conceptualized by Eisenberg and colleagues (2001) reflects two types of control: reactive (involuntary) and effortful (voluntary) control. Resiliency reflects the ability to modulate negative emotions and control in response to environmental stressors. It has been linked to effortful control (Rothbart & Bates, 1998), emotion regulation (Eisenberg et al., 2001) and low negative emotionality (Martel & Nigg, 2006; Trucco et al., 2016). In contrast, behavioral control is closely related to reactive control, reflecting a less voluntary control process, which includes both positive incentive approach and negative withdrawal (Martel & Nigg, 2006). Behavioral control has been associated to delayed gratification (Funder & Block, 1989) and low impulsive approach tendencies (Eisenberg, Ma, Chang, Zhou, West, & Aiken, 2007). Resiliency and behavioral control reflect two of the domains highlighted in the RDoC proposal: the negative valence system and the cognitive system (Ellingson et al., 2016).

Low behavioral control and low resiliency in childhood predict adolescents' social functioning, including internalizing problems (Ellingson et al., 2016). In turn, internalizing problems, especially depression, predict SU onset (Hussong et al., 2011). Although internalizing symptoms have an overall weaker effect on SU compared to externalizing symptoms, there is evidence that depression uniquely predicts SU beyond externalizing problems. For example, a review by Hussong and colleagues (2017) indicated that depression predicted subsequent alcohol, cigarette, marijuana, and drug use after controlling for co-occurring externalizing symptoms. Thus, depression may represent a viable alternative pathway to SUD.

Associations between Depression and SU

Work examining prospective associations between depression and SU has been mixed. There is evidence to support depression predicting SU as well as SU predicting depression (Fergusson, Goodwin, & Horwood, 2003). One factor contributing to these discrepancies is age and stage of SU. One study demonstrated that when examining later waves of a longitudinal dataset (ages 18–25) the prospective model where alcohol use disorder (AUD) predicted depression was a better fit to the data than alternative models (Fergusson, Boden, & Horwood, 2009). When examining earlier waves of the same dataset (16–21 years), the prospective model where depression predicted nicotine dependence was a better fit to the data (Fergusson et al., 2003). This is consistent with a meta-analysis concluding that depression predicting smoking during adolescence was the stronger effect compared to competing models (Chaiton, Cohen, O'Loughlin, & Rehm, 2009).

It is possible that the link between SU to adult depression is a result of regular SU rather than initiation. For example, social, economic, and legal problems resulting from high levels of SU predict depression (Boden & Fergusson, 2011). Similarly, changes in circadian rhythms, metabolism, and neurochemical pathways resulting from high levels of SU also predict adult depression (e.g., Koob & Le Moal, 2001). These findings suggest that the association between depression and SU is bidirectional and influenced by developmental age and severity of SU.

Relevant Genetic Factors

There are likely several genetic factors involved in the cascading pathway to SUD through depression. Namely, genetic factors impacting neurotransmitter systems involved in emotionality and adaptability to stress likely play a role in the development of SU (Zhu et al., 2003). We selected four genetic factors based on empirical support for their association with neurological correlates, endophenotypes, and behavioral outcomes of interest.

Serotonin Transporter Gene

The linked polymorphic region of the serotonin transporter gene, *SLC6A4* (or *5-HTTLPR*), has two common repeat alleles: long (L; 16-repeat) and short (S; 14-repeat). The S allele has decreased *SLC6A4* transcriptional efficiency, thus eliciting less serotonin reuptake compared to the long variant. The S allele has been associated with increased aversive responses to stressful events (Karg, Burmeister, Shedden, & Sen, 2011), lower emotional resiliency

(Stein, Campbell-Sills, & Gelernter, 2009) as well as AUD (Rubens et al., 2016) and cigarette smoking (Dick et al., 2007) compared to L allele homozygotes.

Neuropeptide Y (NPY)

NPY is a 36-amino acid peptide and a key modulator of stress-related behaviors (Zhu et al., 2003). Low NPY plasma concentrations are associated with mood disorders (Mickey et al., 2011) whereas high concentrations are associated with resilience to stress (Feder, Nestler, & Charney, 2009). Variation in the expression of NPY is driven partly by variation in the *NPY* gene. Haplotypes of six polymorphisms predicted levels of NPY messenger and plasma levels (Zhou et al., 2008). Individuals carrying the TG insertion of the *NPY* promoter variant (present in the low-expression haplotype group) rs3037354, demonstrated higher processing of NPY (Mickey et al., 2011). Not surprisingly, since low levels of NPY are observed in individuals with major depression, those without the TG insertion (Deleted allele) had reduced risk to depression-related disorders (Mickey et al., 2011). Findings indicate that the association between low-expression *NPY* genetic variation and risk for depression may be due to individual differences in stress sensitivity (Mickey et al., 2011). Yet findings on the association between *NPY* and depression are not consistent, with some demonstrating a protective effect of *NPY* variants that increase the plasma concentration of NPY (e.g., the Pro7 allele; Bosker et al., 2011), and others a risk effect (Koefoed et al., 2012). Other findings suggest that *NPY* variants that increase the plasma concentration of NPY are associated with increased alcohol consumption (Lappalainen et al., 2002).

Brain-derived Neurotrophic Factor (BDNF)

BDNF is important in the regulation of neural circuits that are involved in depression (Martinowich & Lu, 2008). An amino acid substitution (Val to Met) at codon 66 in the 5' pro domain of the *BDNF* gene (Val66Met, rs6265) has consistent support in relation to stress-related psychopathology. The Met allele is associated with a decrease in activity-dependent secretion of BDNF compared to the Val allele (Colzato, Van der Does, Willem, Kouwenhoven, Elzinga, & Hommel, 2011). Decreased expression of BDNF is related to increased susceptibility to depression (Haerian, 2013). Similarly, the Met allele has been associated with negative emotionality, depression, pronounced negative-affect to stress, as well as greater alcohol consumption (Colzato et al., 2011) and risk of being a current smoker (Lang et al., 2007) compared to Val/Val homozygotes.

Corticotropin-Releasing Hormone-Binding Protein (CRHBP)

The corticotropin-releasing hormone system is central to both the neuroendocrine hypothesis of depression and the anti-reward mechanism that is integral in addiction (DeRijk & de Kloet, 2005). Accordingly, it is logical to consider the role of the *CRHBP* gene in stress-related pathology, such as depression, and SUDs. Single nucleotide polymorphisms (SNPs; rs6159, rs1870392, rs1870393) of the *CRHBP* gene are associated with behavioral inhibition (Smoller et al., 2003) and depression in individuals with AUD (SNPs rs10474485 and rs1715747; Kertes et al., 2011).

Current Study

This study utilizes developmental theory and an RDoC approach to examine internalizing pathways from genetic risk factors to SU. This work expands on prior work demonstrating the utility of applying a similar approach to examine externalizing pathways to SU (Trucco et al., 2016). Prospective mechanisms by which multiple gene systems impact SU indirectly via childhood temperament and early adolescent depressive symptomatology as mediators using a multi-informant design will be tested. We also test whether internalizing pathways remain significant above and beyond externalizing behavior. We hypothesized that prospective multiple mediator models would provide a more comprehensive understanding of pathways critical for the unfolding of genetic risk than has been possible with cross-sectional direct effect models.

Method

Participants

Participants were 426 adolescents from 276 families enrolled in a prospective study. The Michigan Longitudinal Study (MLS) follows a high-risk community sample of families of men charged with drunk driving who met criteria for AUD, their son, and their son's biological mother whose AUD status could vary. A community contrast sample of families drawn from the same neighborhoods was also recruited. These families comprised both low-risk (i.e., no family history of AUD) and moderate-risk (i.e., men making a AUD diagnosis when assessed by research staff, but not having a prior history of out of control social problems with SU). Biological siblings were also included if they were within eight years of the male target child. Due to original recruitment strategies, the sample was predominantly male (302, 70.9%) and of European American ancestry (358, 84.0%; African American = 31, 7.4%; bi-racial = 17, 4.0%; Hispanic/Latino = 20, 4.7%). See Zucker and colleagues (1996) for additional description of the MLS.

Procedure

Parents, teachers, and children completed assessments following initial recruitment (Wave 1, ages 3 to 5) with assessments occurring every three years (e.g., Wave 2, ages 6 to 8). The current study focused on childhood (Wave 3, ages 9 to 11) to late adolescence (Wave 5, ages 15 to 17). Reports from multiple sources were included to minimize shared method variance. This study focuses on the subset of families that provided blood or saliva for genotyping (61.6%). Adolescents without genetic data did not differ from those included in this study on temperament, depression, aggression, and SU. Informed consent and assent were obtained from participants. The University of Michigan's Institutional Review Board approved this study (Family Study of Risk for Alcoholism over the Life Course, HUM00039806).

Measures

Temperament—Temperament was assessed at ages 9–11 using the California Child Qsort (Block & Block, 1980) consisting of 100 cards reflecting various characteristics and descriptors of the child's behavior. Cards are sorted into nine categories ranging from “least descriptive” to “most descriptive” by a research interviewer who spent a full day of data

collection with the participant. Resiliency and behavioral control subscales were derived from prior work (Eisenberg et al., 2001). The resiliency subscale (Cronbach's $\alpha = 0.86$) reflects adaptability to environmental stressors and modulation of negative emotions. It is based on 23 items such as: "open to new experiences," "responds to reason," "resourceful," and "tends to go to pieces under stress" (reverse-coded). The behavioral control subscale (Cronbach's $\alpha = 0.77$) reflects the ability to control impulses and behaviors. It is based on 12 items such as: "is inhibited and constricted," "is physically cautious," "is reflective; deliberates before speaking or acting," and "reacts and moves quickly" (reverse-coded).

Depression—Child report of depression at ages 12–14 was assessed using the Youth Self Report form (Achenbach, 1991). A subscale was created following the work of Lengua and colleagues (2001). This represents an alternative scoring system that closely corresponds to current conceptualizations of depressive symptomatology. It is based on 12 items such as: "unhappy, depressed" and "feels worthless" (Cronbach's $\alpha = 0.77$). Items are rated on a 3-point Likert scale (0 = *not true* to 2 = *very true or often true*).

Aggression—Parent report of aggressive, disruptive, and noncompliant behavior at ages 12–14 was assessed using the Aggression subscale of the Child Behavior Checklist (Achenbach, 1991). It is based on 20 items such as: "argues" and "bullying" (Cronbach's $\alpha = 0.88$). Items are rated on a 3-point Likert scale (0 = *not true* to 2 = *very true or often true*).

SU—It is possible that internalizing pathways to SU may vary across substances. Prior work demonstrates a stronger association between depression and alcohol use compared to cigarette smoking (Costello, Erkanli, Federman, & Angold, 1999). Accordingly, we tested alcohol, cigarette, and marijuana use models, as these are the most commonly used substances during adolescence (Johnston, O'Malley, Miech, Bachman, & Schulenberg, 2017). Problematic alcohol use at ages 15–17 was assessed with an item reflecting past year maximum alcoholic beverages consumed in 24 hours, using the Drinking and Drug History form (Zucker, Fitzgerald, & Noll, 1990). We examined the frequency of past year cigarette and marijuana use using the same form. In addition to utilization of extent of use as an indicator of problem use, it is important to note, that prior research demonstrates a strong negative association between age of first SU and lifetime prevalence of SUD (prevalence of lifetime AUD among those who started drinking at ages 15, 16, and 17 was 38.7%, 30.6%, and 24.5%, respectively; Grant & Dawson, 1997). Thus, even though we focused on misuse that often precedes SUD, SU during this age is of itself indicative of problematic current use as well as being a prodromal to later disorder.

Covariates—Prior research indicates different rates of depression, aggression, and SU across biological sex and race (e.g., Chen & Jacobson, 2012). Accordingly, biological sex (0 = girls, 1 = boys) and race (0 = non-European Ancestry, 1 = European Ancestry) were included as covariates. Given that this was a high-risk sample, family risk group status was also a covariate. We chose to combine "moderate" and "high-risk" families using a dummy code (i.e., low [0] vs. moderate or high risk [1]) consistent with prior work (Trucco et al., 2016) and research indicating that youth of parents with SUD are more at risk for difficult

temperament, problem behavior, and SU (e.g., Barnow, Schukit, Lucht, John, & Freyberger, 2002).

Genotyping

Polymorphisms from four genes were genotyped. SNPs rs6265 (*BDNF*; 67.2% [287] = CC (Val/Val), 30.0% [128] = CT (Val/Met), 2.8% [12] = TT (Met/Met)) and rs3037354 (*NPY*; 54.1% [231] = TG/TG, 39.3% [168] = TG/del, 6.6% [28] = del/del) were genotyped using the Sequenom iPLEX SNP Genotyping method (Gabriel, Ziaugra, & Tabbaa, 2009), and rs7728378 (*CRHBP*, TT [31.4%], TC [47.3%], and CC [21.3%]) was genotyped using TaqMan® SNP Genotyping Assays (Applied Biosystems, Carlsbad, CA). The promoter polymorphism (*5HTTLPR*) of *SLC6A4* was genotyped by PCR following recommended methods (Sen et al., 2004). The S allele (14.5% [62] = SS, 51.3% [219] = LS) corresponds to a product of 14-repeat, and the long allele (34.2% [146] = LL) reflects the 16-repeat. No deviation from HardyWeinberg Equilibrium was detected and no discrepancies or duplications were observed.

Data Analysis

First, patterns of missing data were examined. Data were determined to be missing completely at random (Little's MCAR test = 13.28[12], $p = 0.35$). Given that the sample included siblings, multilevel linear modeling analyses were used to assess family clustering. A significant clustering effect of families on behavioral control, resiliency, and cigarette use (Intraclass Correlations range = 0.08 to 0.57) was found. Accordingly, multilevel analyses accounting for family clustering were estimated.

Multiple mediator path models were estimated in Mplus 7.4 (Muthén & Muthén, 1998-2015). Separate models were conducted for each substance (see Figure 1 for a conceptual model). A second set of models were tested, which included early adolescent aggression, to determine whether pure depression mediated the effect of genetic factors on SU.¹ Models were just identified and hence model statistics are not provided.

Several options exist to assess mediated effects in Mplus. The first is the product-of-coefficients approach using the IND command. The second involves calculating bias-corrected bootstrap confidence intervals (BBCIs), which is more robust (Preacher & Hayes, 2008). In Mplus, it is not possible to account for clustering while using resampling approaches. Therefore, indirect effects when controlling for clustering effects were compared to BBCIs, a procedure used in prior work (Trucco, Villafuerte, Heitzeg, Burmeister, & Zucker, 2014; Trucco et al., 2016). Although results were largely similar across procedures, we present path estimates for clustering effects and report BBCIs for indirect effects. Study variables were all normally distributed except for aggression (skewness = 2.08, kurtosis = 5.85) and cigarette smoking (skewness = 1.99, kurtosis = 4.07). Accordingly, a log transformation of aggression (skewness = 1.65, kurtosis = 2.98) and a square root transformation of cigarette use (skewness = 1.29, kurtosis = 0.08) were performed.

¹A two-way interaction between depression and aggression was also tested across models, but was not significant.

Results

Descriptive information for study variables are presented in Table 1. Girls reported more depressive symptoms. Adolescents with European ancestry were rated as having lower resiliency and reported more alcohol and cigarette use. Family AUD risk was associated with higher rates of depression and SU. Those carrying the *BDNF*T allele had lower resiliency, while those carrying the *NPY*TG allele reported more depression. Resiliency was associated with lower behavioral control, aggression, and cigarette use. Behavioral control was associated with lower depression, aggression, problematic alcohol use, and marijuana use. Given the low percentage of individuals carrying the TT genotype, *BDNF* was also examined as CC versus T-carriers. In addition, given the low percentage of individuals carrying the Del/Del genotype, *NPY* was also examined as TG/TG versus Del-carriers. Findings were comparable to those examining each genotype separately.

Pathways to Problematic Alcohol Use

This model accounted for approximately 12% of the variance in problematic alcohol use (see Figure 2, Panel A). *BDNF*rs6265 was associated with resiliency, whereby those with the T allele had lower resiliency. *NPY*rs3037354 was associated with depression, whereby those with the TG allele reported greater depression. Low resiliency predicted increased depression, however, this effect was marginal ($p = 0.07$). Low behavioral control predicted increased depression and problematic alcohol use; however, this last pathway was marginal ($p = .06$). Lastly, greater depression predicted increased problematic alcohol use. Accordingly, we tested the significance of several indirect pathways. First, the indirect effect from *BDNF* to depression through resiliency was significant (estimate = 0.073, BCCI = 0.005 – 0.216), despite the marginal association between resiliency and depression. Approximately 16% of the total effect of *BDNF*rs6265 on depression operated through resiliency. Second, the indirect effect from resiliency to problematic alcohol use through depression was significant (estimate = -0.090, BCCI = -0.299 – -0.001). Approximately 23% of the total effect of resiliency on problematic alcohol use operated through depression. Third, the indirect effect from *NPY* to problematic alcohol use through depression was significant (estimate = -0.128, BCCI = -0.373 – -0.011). Approximately 16% of the total effect of *NPY*rs3037354 on problematic alcohol use operated through depression. Lastly, the indirect effect from behavioral control to problematic alcohol use through depressive symptomatology was significant (estimate = -0.134, BCCI = -0.358 – -0.009). Approximately 14% of the total effect of behavioral control on problematic alcohol use operated through depression. Thus, there was evidence for two mediated pathways.

When including aggression in the model (see Figure 2, Panel B), there was a direct association between aggression and problematic alcohol use. The direct effect between depression and problematic alcohol use was reduced to marginal significance ($p = .09$). Adding aggression also resulted in reducing prior indirect effects to non-significance. However, evidence for new mediated pathways emerged. First, the indirect effect from *BDNF* to aggression through resiliency was significant (estimate = 0.006, BCCI = 0.001 – 0.014). Approximately 13% of the total effect of *BDNF* on aggression operated through resiliency. Second, the indirect effect from resiliency to problematic alcohol use through

aggression was significant (estimate = -0.307 , $\text{BBCI} = -0.847 - -0.058$). Approximately 3% of the total effect of resiliency to alcohol use operated through aggression. Thus, there was evidence for one mediated pathway.

Pathways to Cigarette Use

This model accounted for approximately 17% of the variance in frequency of cigarette use (see Figure 3 Panel A). In contrast to the alcohol model, there was a direct association between low resiliency and cigarette use. Although the indirect effect between resiliency to cigarette use via depression was not significant, there was evidence for another mediated pathway. Namely, the indirect effect from *BDNF* to cigarette use through resiliency was significant (estimate = 0.030 , $\text{BBCI} = 0.004 - 0.079$). Approximately 13% of the total effect of *BDNF*rs6265 on cigarette use operated through resiliency. The indirect effect from *NPY* to cigarette use through depression was not significant. Lastly, the indirect effect from behavioral control to cigarette use through depression was not significant. Thus, there was evidence for one mediated pathway.

Adding aggression to the model (see Figure 3 Panel B) resulted in reducing the prior indirect effect to non-significance. However, evidence for new mediated pathways emerged. First, the indirect effect from *BDNF* to aggression through resiliency was significant (estimate = 0.006 , $\text{BBCI} = 0.001 - 0.014$). Approximately 13% of the total effect of *BDNF*rs6265 on aggression operated through resiliency. Second, the indirect effect from resiliency to cigarette use through aggression was significant (estimate = -0.057 , $\text{BBCI} = -0.116 - -0.023$). Approximately 3% of the total effect of resiliency on cigarette use operated through aggression. Thus, there was evidence for one mediated pathway.

Pathways to Marijuana Use

This model accounted for approximately 14% of the variance in frequency of marijuana use (see Figure 4 Panel A). In contrast to the alcohol model, there was a direct association between low resiliency and marijuana use. Although the indirect effect between resiliency to marijuana use via depression was not significant, there was evidence for another mediated pathway. Namely, the indirect effect from *BDNF* to marijuana use through resiliency was significant (estimate = 0.074 , $\text{BBCI} = 0.004 - 0.218$). Approximately 11% of the total effect of *BDNF*rs6265 on marijuana use operated through resiliency. The indirect effect from *NPY* to marijuana use through depression was not significant. Lastly, the indirect effect from behavioral control to marijuana use through depression was not significant. Thus, there was evidence for one mediated pathway. When including aggression in the model (see Figure 4 Panel B), there was not a direct association between aggression and marijuana use and indirect effects were nonsignificant.

A post-hoc model which included a latent variable comprised of problematic alcohol, cigarette, and marijuana use as indicators was also tested given prior evidence demonstrating that depression may have a stronger impact on overall SU (Hussong et al., 2017). Findings were consistent with the alcohol model. That is, in the first model there was support for two mediated pathways from *BDNF*rs6265 and *NPY*rs3037354 via resiliency and depression.

When including aggression, these two mediated pathways were reduced to non-significance, and a new pathway to SU emerged from *BDNF*rs6265 via resiliency and aggression.

Discussion

Understanding neurobiological mechanisms contributing to SU onset has been challenging. This is partly due to the nature of SUD as a gradually developing heterogeneous syndrome. Although there is strong support for externalizing pathways to SUD, current work supports the role of internalizing mechanisms. The current study demonstrates the utility of an RDoC approach to examine internalizing pathways from genetic risk factors to SU. Childhood temperament and early adolescent depression were examined as mediators in the association between four specific genetic factors on late adolescent SU. Findings support resiliency and depression as mediators by which polymorphisms of *BDNF* and *NPY* increase risk for later SU. In contrast to prior work examining externalizing pathways to SU (Trucco et al., 2016), this pathway operates differently across substances. Moreover, findings indicate that aggression may play a more prominent role in the etiology of SU.

Consistent with prior research demonstrating weak genetic influences on SUD during adolescence (e.g., Rose, Dick, Viken, Pullkkinen, & Kaprio, 2001), there was no evidence for direct effects of genetic factors on SU. Yet, there was evidence for the role of temperament and depression as precursors to the onset of SU, consistent with prior work (e.g., Hussong et al., 2011). Namely, there was evidence for the role of resiliency and depression as mediators in the association between *BDNF*rs6265 and SU. Those carrying the T (Met) allele had lower resiliency. *BDNF* is critical for the regulation of the formation, plasticity, and integrity of neurons in brain circuits that regulate emotion (Colzato et al., 2011). Prior work demonstrates that Met carriers have heightened reactivity to psychological stress in the hypothalamic-pituitary-adrenal (HPA) axis. Namely, Met carriers demonstrated higher anticipatory cortisol response before engaging in a stressful task and were more anxious during the task itself (Colzato et al., 2011). Similarly, children with at least one Met allele exhibited high negative emotionality when exposed to in-home stressors compared to Val homozygotes (Hayden et al., 2010). This suggests that Met carriers may have greater difficulties managing novel experiences and stressors.

In turn, lower resiliency was associated with depression, which in turn predicted later problematic alcohol use. Prior work suggests that although *BDNF* has weak direct effects on depression, Met carriers are at greater risk of affective disorders given their enhanced stress response (Vinberg et al., 2009). Brain regions that regulate the HPA axis such as the hippocampus, also have connections with the amygdala and prefrontal cortex, which are involved in emotional and cognitive processes contributing to depression (Duman & Monteggia, 2006). It is likely that *BDNF* polymorphisms have a stronger impact on the emotional processes associated with depression compared to the cognitive processes, as we found a direct effect of *BDNF*rs6265 on resiliency but not behavioral control. Accordingly, difficulties with emotion regulation may act as a potential mechanism through which carrying a susceptible *BDNF* polymorphism may increase risk for depression.

Our findings are also consistent with work indicating that *BDNF*Met carriers are more likely to use substances to manage negative emotions associated with stress (Colzato et al., 2011). Yet, these pathways were somewhat different depending on the substance. Both resiliency and depression acted as key mediators in the association between *BDNF* and problematic alcohol use; however, depression was not a key factor in the pathway from *BDNF* to cigarette and marijuana use. Resiliency had a direct effect on later cigarette and marijuana use, supporting the role of resiliency as a mediator in the association between *BDNF*rs6265 and SU. Despite these differences, there is evidence indicating that adolescents may use a variety of substances to cope with depression, consistent with the self-medication hypothesis (Khantzian, 1997).

A second etiological pathway involving depression as a mediator in the association between *NPY*rs3037354 also emerged. Those carrying the *NPY*rs3037354 TG allele reported greater depression, which in turn was associated with increased SU. However, the indirect effect was only significant for problematic alcohol use. NPY is expressed primarily in the central nervous system including certain affective regions such as the amygdala (Domschke et al., 2010). Impaired NPY function is involved in the pathophysiology of depression. Research indicates that levels of NPY are decreased in plasma (Mickey et al., 2011) of individuals with depression. Research demonstrates that polymorphisms in the *NPY* gene may impact the variation in NPY expression within key stress-regulatory neural circuits, thus increasing risk for affective disorders (Zhou et al., 2008). For example, one study found that C allele carriers of *NPY*rs16147 (a low expression *NPY* genotype) are at increased risk for depression because they have increased bilateral amygdala activation when processing angry faces (Mickey et al., 2011).

Other research examining *NPY* has focused on the direct effects on SU. For example, the Pro7 allele (rs16139) has been associated with alcohol consumption (Kauhanen et al., 2000) and AUD (Lappalainen et al., 2002). Yet, researchers state that the precise nature of this association remains elusive (Zhu et al., 2003). Although, prior research examining the role of temperament as a potential mediator in the association between *NPY* polymorphisms and SU are currently lacking, several researchers propose that individuals carrying susceptible *NPY* polymorphisms are at increased risk for addiction as their primary motivation to use is stress relief (Bhaskar et al., 2013). That is, problematic SU may develop to offset the relative lack of tension-reducing NPY among carriers of low expression *NPY* genotypes (Kauhanen et al., 2000).

Additional models were conducted to examine whether internalizing pathways remained after controlling for aggression. Overall, findings demonstrated that significant indirect effects via depression were reduced to non-significance. However, there was evidence for resiliency as a mediator in the association between *BDNF*rs6265 and aggression. In turn, aggression mediated the association between resiliency and problematic alcohol and cigarette use. Accordingly, even though the role of depression on SU was suppressed when including aggression, there was still evidence that early difficulties regulating stressors may represent one pathway through which genetic risk factors impact SU.

Although some studies demonstrate a unique role for internalizing symptoms as a pathway to SU when controlling for externalizing behavior (e.g., King et al., 2004), findings supporting the stronger impact of externalizing symptoms on SU are well replicated (Hussong et al., 2011; 2017). It is important to note, that the current sample is at elevated risk for AUD and therefore adolescents have high rates of externalizing behavior. Moreover, our sample is comprised primarily of boys. Research suggests that girls tend to have higher rates of depression compared to boys (Nolen-Hoeksema, 2001). Moreover, other studies indicate that negative affect among boys may be expressed as aggression, whereas negative affect among girls is expressed by crying and sadness (Zeman & Garber, 1996). Together the demographic characteristics of our sample may have limited our ability to support the role of depression as a mediator across all models and internalizing pathways to SU while controlling for aggression.

Adolescents with depression are likely to have co-occurring aggressive symptomatology (Lewinsohn, Roberts, Seeley, Rohde, Gotlib, & Hops, 1994). Given that most research on the prevalence of child psychopathology has been cross-sectional, it has been difficult to determine how comorbidity between internalizing and externalizing symptoms develop. Some posit that the course of comorbidity fits one of three models: common vulnerability, accumulation, or differentiation over time (Willner, Gatzke-Kopp, & Bray, 2016). The common vulnerability model presumes that a shared trait increases risk for symptoms across both domains; comorbidity across depression and aggression is likely to manifest early and be stable across development. The accumulation model posits that comorbidity occurs when symptoms of one clinical syndrome increase the risk for another clinical syndrome (Willner et al., 2016). For example, prior work indicates that youth with internalizing problems are more likely to engage in aggression compared to healthy participants (Lewinsohn et al., 1994). In turn, these adolescents may resort to SU as a coping mechanism when aggressive behavior leads to social rejection, consistent with the Capaldi's (1992) failure model. Finally, the differentiation model theorizes that during childhood, symptoms of distress are undifferentiated (temper tantrums, aggression), and may be manifested as symptoms of internalizing and externalizing problems (Willner et al., 2016). These undifferentiated symptoms of distress are then shaped by the environment (e.g., parents and peers) over time, which leads to more specific clinical presentations in adulthood. Given high rates of comorbidity, it will be important that future work examine how aggression and depression unfolds over time and how alternative developmental sequences may impact the association between genetic risk factors and SU.

Limitations and Future Directions

Although the current study has a number of strengths, including the prospective study design, the inclusion of multiple genetic factors, and the use of multiple reporters, it also has limitations. First, some researchers argue that since the association between depression and SU is less robust, methodological decisions have a strong impact on results (McCarty et al., 2012). Although we chose a well-validated measure, it will be important to test whether findings replicate with other assessments (e.g., clinical interviews). Second, there are likely other important etiological pathways not tested in these models. The relation between temperament, depression and SU is more complex than the model tested. Although the

current study was informed by prior research and developmental theory, future work should examine alternative models that include reciprocal effects and heterogeneous pathways to SU. Including social environments could inform alternative pathways as well as the interaction between genetic risk factors and environmental contexts (GxE) and interactions between multiple genetic risk factors (GxG). For example, some studies support the synergistic effect of *5-HTTLPR* and *BDNF* on depression (Comasco, Aslund, Orelund, & Nilsson, 2013). The current study was underpowered to detect these effects. Third, our study cannot be generalized to samples with different demographic characteristics. This sample was comprised mostly of boys. It is likely that internalizing pathways to SU may be stronger in samples evenly split on biological sex. Moreover, this was a high-risk sample. Findings may not generalize to population-based samples. Previous findings also suggest that during adolescence, depression is likely to predict early stages of SU (Fergusson et al., 2003), whereas regular SU may predict depression in adulthood (Fergusson et al., 2009). Thus, findings may not generalize to older samples or later stages of SU. Finally, although the MLS represents an ideal dataset for this type of research, it limits the identification of comparable independent samples that are suitable to replicate findings. As comprehensive longitudinal studies become available it will be important to test whether these findings replicate. Until then, caution is warranted when drawing inferences.

Clinical Implications

Understanding how specific genes contribute to risk for addiction remains unclear. Work in this area will help inform current interventions and the development of tailored treatments. Although internalizing pathways to SU has received less attention, there is strong support that addiction may be a longer-term consequence of using substances to cope with depression. Our findings demonstrate that for some youth, genetic risk may be expressed as early difficulties with effective coping and that this may represent non-specific neurobiological underpinnings for an internalizing pathway to addiction. It follows that these youths may benefit from preventive interventions that focus on emotion regulation and stress management. Still, given the current state of knowledge regarding genetic effects, more work is necessary before individualized treatment decisions based on an individual's genome can be made. More work focused on interactions (GxG, GxE), replication studies, and samples that are more diverse are necessary before firm conclusions can be made to inform preventive interventions.

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General Scientific Summary

Prior work demonstrates that genetic risk factors may influence the likelihood of problematic substance use through intermediate pathways marked by adolescent impulsivity and aggression. However, these factors only represent one pathway to substance use. This study suggests that early difficulties in coping effectively with stressors, along with later depression, may represent another pathway through which genetic risk factors impact substance use.

Figure 1.
Conceptual Model

A.

B.

Figure 2. Models for problematic alcohol use

Note. Values represent standardized path coefficients accounting for family clustering. Only significant and marginal paths ($* = p < .05$, $*** = p < .001$, $^\dagger =$ marginal effect) are depicted. Covariates are not depicted. Bold lines represent significant mediated pathways. Panel A only includes self-report of depression as a mediator between temperament and alcohol use. Panel B also includes parent-report of aggression. Models are just identified.

A.

B.

Figure 3. Models for cigarette use

Note. Cig = Cigarette. Values represent standardized path coefficients accounting for family clustering. Only significant and marginal paths (* = $p < .05$, ** = $p < .01$, *** = $p < .001$, † = marginal effect). Covariates are not depicted. Bold lines represent significant mediated pathways. Panel A only includes self-report of depression as a mediator between temperament and alcohol use. Panel B also includes parent-report of aggression. Models are just identified.

A.

B.

Figure 4. Models for marijuana use

Note. Mar = marijuana. Values represent standardized path coefficients accounting for family clustering. Only significant and marginal paths ($^* = p < .05$, $^{**} = p < .01$, $^{***} = p < .001$, † = marginal effect). Covariates are not depicted. Bold lines represent significant mediated pathways. Panel A only includes self-report of depression as a mediator between temperament and alcohol use. Panel B also includes parent-report of aggression. Models are just identified.

Table 1

Means, standard deviations, and correlations for study variables.

	Range	Mean	SD	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Biological sex		--	--	--												
2. Race		--	--	0.16	--											
3. Family Risk		--	--	-0.06	-0.14	--										
4. <i>SLC6A4</i> 45-HTTLPR		--	--	0.08	0.16	0.05	--									
5. <i>BDNF</i> rs6265		--	--	-0.02	0.06	0.05	-0.04	--								
6. <i>NPY</i> rs3037354		--	--	-0.04	0.02	-0.07	0.01	0.04	--							
7. <i>CRHBP</i> rs7728378		--	--	-0.02	-0.32	0.09	-0.02	0.02	0.16	--						
8. Resiliency	3–8	5.86	0.87	-0.02	-0.12	0.01	0.06	-0.14	-0.05	0.03	--					
9. Behavioral Control	2–8	4.80	1.04	-0.06	0.07	-0.06	0.07	-0.00	0.01	0.02	-0.20	--				
10. Depression	0–19	2.89	3.05	-0.11	0.07	0.11	-0.04	0.01	-0.10	-0.04	-0.07	-0.17	--			
11. Aggression ^a	50–95	54.32	6.39	-0.04	-0.06	0.09	-0.03	-0.06	-0.06	-0.09	-0.15	-0.22	0.23	--		
12. Problem Alcohol Use	0–30	4.22	6.30	0.03	0.18	0.20	0.07	-0.02	-0.03	-0.05	-0.01	-0.14	0.18	0.24	--	
13. Cigarette Use ^a	0–9	0.72	1.37	0.03	0.12	0.21	0.03	-0.03	-0.07	-0.07	-0.12	-0.07	0.15	0.37	0.49	--
14. Marijuana Use	0–9	1.26	2.32	0.02	0.08	0.23	0.04	-0.03	-0.06	0.02	-0.08	-0.21	0.17	0.27	0.64	0.57

^aUntransformed variable. Bold font represents significant associations.