Re-Conceptualizing Genetic Influence in GxE Studies:

Does Inherited Sensitivity to Environmental Influence Moderate the Indirect Effect of Parent Knowledge on Future Drinking?

by

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ABSTRACT

Excessive drinking in adolescence is a public health issue with major consequences on both an individual and societal level. Elucidating genetic and environmental influences could be particularly informative for prevention efforts. One potential source of genetic influence is sensitivity to environmental influences. It was hypothesized that parent knowledge would interact with genetic sensitivity to the environment to indirectly reduce risk for alcohol problems through less adolescent rule breaking behavior. Participants (N=316) provided genetic data and reported their rule breaking behavior and past year frequency of heavy drinking, and participants' custodial parents reported their perceived knowledge of their child's activities. A novel index of genetic sensitivity to environmental influence was created using published methylation quantitative trait locus data from the frontal lobe. Study hypotheses were mostly not supported. The study results likely reflect the poor distribution of study variables and the limitations of the current study's sensitivity gene score. The current study underscored the importance of adhering to methodological rigor and explored alternate conceptualizations and methods that future research could use to elucidate the role of inherited to sensitivity to environmental influences in adolescent drinking.

DEDICATION

This dissertation is the product of support from the ASU Psychology Department, faculty, friends and my family. I am grateful for the time my committee members spent in talking, reading drafts, writing letters, providing guidance and being so generous with opportunities to collaborate. Thank you Kit for all your mentorship, both on papers and on overall professional development and life in general. This dissertation is also dedicated to Dr. Laurie Chassin—I would not be where I am as a scientist, a professional, and overall as a person without her consistent guidance and her insistent belief that I am capable of producing high-caliber work. Thank you for helping me grow in ways I could not have anticipated and just for everything.

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Finally, this dissertation is for my family—my mom, my dad, Ate Melissa and Alexa. This dissertation is to honor all the sacrifices my parents and our family had to make over the years. It's been a hard, long, unpredictable ride—thank you for being so supportive of me throughout all of this. We did it!!

And most important of all, I dedicate this dissertation to my daughter Mutiara Soul Smith. I love you so much, this is for you.

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CHAPTER 1

INTRODUCTION

Excessive drinking in adolescence is a public health issue with major consequences on both an individual and societal level. Animal models suggest that binge drinking alcohol as a youth may have impacts on brain development that are detectable even in adulthood in brain regions important for executive functioning (Coleman, Liu, Oguz, Styner, & Crews, 2014). Adolescence is also a developmental period characterized by greater risk-taking behavior, with alcohol use exacerbating this tendency (Lane, Cherek, Pietras, & Tcheremissine, 2004; Steinberg, 2008). Due to this potent combination, youth drinking and driving continues to be a major public safety concern (McCambridge, McAlaney, & Rowe, 2011). Given the high societal and personal costs, continued research on the development of youth drinking is warranted. Moreover, greater alcohol consumption in adolescence is related to future alcohol problems and alcohol disorder diagnosis in adulthood (McAmbridge et al., 2011). Thus, understanding adolescent drinking is an opportunity to better understand an important aspect of alcohol disorder development. Elucidating genetic and environmental influences could be particularly informative for prevention efforts by identifying protective environmental contexts, and individuals who may optimally benefit from such interventions

Although there is consistent support for the heritability of youth drinking and alcohol problems, identifying specific genetic and gene-by-environment (GxE) influences has proven challenging because there are multiple distinct and correlated etiological pathways that may lead to elevated drinking and alcohol problems (McGue, Iacono, Krueger, 2006; Vernulst, Neale, & Kendler, 2015; Sher, 1991). Much genetic research

does not account for this phenotypic complexity, and simply tests for relations between genetic or GxE factors and a positive alcohol disorder diagnosis. Genetic research that uses more nuanced alcohol phenotypes and tests theoretically-informed models may yield more consistent results and insight into adolescent drinking. Heavy drinking in adolescence is one alcohol phenotype that is associated with future alcohol problems and alcohol disorder in adulthood (McAmbridge et al., 2011) and is also associated with the deviance proneness pathway to alcohol disorder (Sher, 1991). The present study tested for GxE effects on the development of heavy drinking in adolescents.

A challenge for behavior genetic research is that genetic influence is indexed based on risk for a particular behavior or psychopathology. Evolutionary psychology research suggests that there are other sources of individual differences that may be important in the development of behavior. The differential susceptibility model integrates evolutionary theory with developmental psychology. According to the differential susceptibility model, individual differences in sensitivity or plasticity to environmental influences interact with environmental contexts to shape behavior. Thus, particularly plastic individuals are sensitive to both harmful effects of a risky environment and the benefits of a secure environment (Belsky & Pluess, 2013). Variation in plasticity to the environment may be an important, understudied influence on risky behaviors, such as heavy adolescent drinking. Indexing genetic sensitivity based on plasticity to environmental influence may be a novel strategy for improving detection of genetic effects in GxE studies of drinking.

An additional challenge is effectively characterizing genetic influence so that it captures both the genetic architecture of excessive adolescent drinking as it relates to

future alcohol disorder, *and* the biological mechanisms of the genetic variants and psychological phenotypes being tested. Gene scores are one way to capture the likely polygenic genetic architecture of the etiological processes that contribute to alcohol disorder through heavy adolescent drinking and was used to index genetic risk in the present study (Wray et al., 2014). To integrate biological context into genetic studies, researchers are currently selecting variants related to neural or other physiological systems important to alcohol disorder development (e.g. Wang & Chassin, 2018). However, few studies have used polygenic and functionally-informed indices of genetic influence for GxE studies. DNA methylation (hereafter referred to as just methylation) is a type of epigenetic modification that is directly influenced by the environment and alters the *expression* of a DNA sequence or gene without altering the alleles that comprise the sequence. Methylation is a biological mechanism that accounts for environmental modification of genetic influence and could be integrated into a GxE study. Although a few genetic studies have looked at variants associated with the methylation system (e.g. Pishva et al., 2014), no GxE study to date has indexed multiple genetic variants related to methylation either in general or in the context of adolescent drinking.

DNA Methylation as a Plasticity Phenotype to Index Inherited Sensitivity

Differential Susceptibility theory is an evolutionary-based framework for understanding and exploring individual differences in human behavior and the underlying mechanism driving these differences. Recognizing that an organism's sensitivity, or plasticity, to environmental conditions is a driving element in natural selection, the differential susceptibility model posits that heritable individual differences in this plasticity account for differences in "normal" human behavior as well as

psychopathology. Thus, individuals inherit varying levels of sensitivity to environmental cues indicating which type of behavioral strategies will be most effective in promoting reproductive fitness in the given environment (Boyce, 2016). Testing the assumptions of the differential susceptibility model is beyond the scope of the present study. However, plasticity to the environment may be an important phenotype for understanding how genes interact with environmental influences on adolescent drinking. The present study indexed genetic influence based on the differential susceptibility theory conceptualization of plasticity to the environment.

Plasticity refers to the biological and physiological processes that facilitate an individual's perception, processing, and response to environmental cues. Thus, plasticity lends itself to elucidating the underlying processes of psychopathology because of its definitively mechanistic nature. Additionally, differences in plasticity are *biologically* driven. Although plasticity may manifest at different levels of biological influence—ranging from physiological, to neural, to genetic—it is broadly this biological moderation of environmental influences on a behavior that is fundamental to differential susceptibility. Thus, differences in environmental sensitivity are a function of biological differences (Boyce, 2016).

Candidate genes have often been used as "plasticity factors," particularly in studies focusing on drinking and alcohol disorder. However, most of these polymorphisms were selected due to their functional relation to a specific disorder rather than their functional role in environmental sensitivity. For example, genetic variants in the dopamine or GABA system are often used as plasticity factors (e.g. Laucht et al., 2012; Brody, Yu, & Beach, 2015) in studies due the role of both neurotransmitter

systems in the development and maintenance of alcohol disorder. Less clear is the role of these neurotransmitter systems in creating individual differences in plasticity to the environment. Despite the emphasis placed on plasticity as an individual difference factor, few studies have selected genetic variants related to a plasticity phenotype. Indeed, on contemplating genetic variants used as plasticity factors, Belsky & Pluess (2013) suggested that investigators "[…] expand the list of genetic 'suspects' beyond those thought to be related to disturbances in functioning by thinking biologically about genes that could be related to physiological processes instantiating plasticity."

Empirical research on differential susceptibility has been mixed on how plasticity phenotypes are characterized. However, the theoretical literature implicates three qualities as constituting an ideal plasticity phenotype:

1) biological in nature
2) part of a process or mechanism that facilitates sensitivity the environment and could influence downstream responsivity
3) has significant, heritable variation

The epigenetic process of DNA Methylation is a physiological mechanism that conforms to these plasticity phenotype criteria and could therefore expand the list of genetic 'suspects' used in studies of sensitivity to the environment.

Epigenetics and DNA Methylation

Epigenetic modification refers to the various biological processes that substantially modify gene expression, without altering the actual genetic sequence, in response to an environmental stimulus (Polderman et al., 2015; Isles, 2015). It is worth emphasizing that epigenetics refers to processes that change *gene expression*—the

specific sequence of alleles that comprise an individual's DNA remains intact. These modifications are physical in nature—for instance, histone modifications are a type of epigenetic change that modify gene expression by changing which sequences of DNA are physically exposed to transcription, translation, and ultimately expression (Egger, Liang, Aparicio, & Jones, 2004). The environmental stimuli that affect these changes can be either internal (e.g. ingestion of a high fat diet, elevated stress hormones) or external (e.g. toxins in the environment, physical maltreatment). Epigenetic modification is a process by which environmental factors become integrated into genetic influence (Isles, 2015).

Epigenetic modifications were initially identified as central to cell specialization, or the process by which developing cells become specialized to their specific role in the body. For example, epigenetic processes modify the genes that are and are not expressed in skin cells, which may be distinct from the genes expressed in liver cells (Isles, 2015). Later research found that epigenetics was not limited to early development, and indeed continues to occur throughout an organism's life in response to environmental influences (Gertz et al., 2011). These findings imply that epigenetic modifications are tissue-specific. That is, epigenetic modifications differ depending on the tissue from which the epigenetic modifications are evaluated (Barker, Walton, & Cecil, 2018). Indeed, they are even significantly different across different brain regions (Davies et al., 2012). Thus, environmental influences on gene expression may differentially affect different body tissues, which may manifest in different downstream consequences—including differences in complex phenotypes, such as behavior.

The present study focused on DNA methylation as a measure of epigenetic modification. DNA methylation is a type of epigenetic modification consisting of methyl

attaching to cytosine-guanine di-nucleotides in the DNA sequence. Consequently, transcription of the sequences is effectively stopped due to the methyl physically blocking transcription factors from accessing the now methylated DNA sequence. Occasionally, the methylated sequence is part of a gene or a promoter region of the gene, ultimately silencing the expression of the gene (Egger et al., 2004; Barker, et al., 2018). Thus, greater DNA methylation is related to less gene expression (Meaney, 2010). Although long thought to be the most stable epigenetic modification, emerging research on methylation at non-CpG sites and on demethylation indicate that methylation may be quite dynamic, particularly in neural tissue (Isles, 2015; Qureshi & Mehler, 2014). The fluidity and enrichment of methylation in neural tissue make it an ideal measure of epigenetic modification in the context of behavioral research. Moreover, it is possible to capitalize on the tissue specificity of DNA methylation. That is, for psychological phenotypes with fairly well-characterized neural undeprinnings, researchers can test whether DNA methylation in functionally-significant tissue indeed relates to behavior differences. Finally, DNA methylation is an ideal epigenetic modification to study from a practical stand-point due to its popularity and the availability of methylation literature and bioinformatic resources.

DNA Methylation as a Plasticity Phenotype

DNA methylation fulfills all the criteria for a plasticity phenotype. First, it is a biological process. Second, researchers posit that DNA Methylation is a central mechanism underlying GxE effects in that it drives the integration of environmental with genetic influence on behavior (Meaney, 2010) Thus, it promotes environmental sensitivity by embedding environmental stimuli into a person's biology as changes in

gene expression. Moreover, methylation mediates the influence of the environment on stable changes to downstream phenotypes, including physiology, cognition, and behavior (Ijzendoorn, Bakermans-Kranenburg, & Ebstein, 2011). Human and animal research supports this mediating role of methylation. In animal research, environmental enrichment and maternal care significantly relate to methylation (Weaver et al., 2004; Kuzumaki et al., 2011). In humans, a range of psychosocial environments relate to greater methylation, including being raised in institutional care since birth (Naumova et al., 2012) and sexual and physical child abuse (McGowan et al., 2009; Romens, McDonal, Svaren & Pollack, 2015). Indeed, a systematic literature review on the effects of social environmental stressors on methylation at the glucocorticoid receptor gene found that various parental and early life adversity stressors had significant effects on methylation (Turecki & Meaney, 2016).

In turn, differential methylation is related to differences in social behavior. Entomological research has found that the enzyme important for DNA methylation is exclusively present in social insects and absent in non-social insects, suggesting that DNA Methylation may be particularly important in shaping social behaviors (Yan et al., 2014; Isles, 2015). This is evident in human research, where significant relations have been found between methylation and physical aggression, callous-unemotional interactions, and externalizing psychopathology (e.g. ADHD, conduct disorder; Barker et al., 2018). Importantly, there are studies that have formally tested and support the mediating effect of methylation. Specifically, researchers have found significant indirect effects of prenatal environmental stressors on ADHD symptoms, adolescent substance use, and early onset drinking through methylation at the candidate gene and epigenome

level (Rijlaarsdam et al., 2017; Cecil et al,. 2016). Altogether, research points to methylation as an important biological mechanism in environmental sensitivity and subsequent behavior—a definitive quality of a plasticity factor.

Third and finally, methylation is an ideal plasticity phenotype because there is growing evidence that it has significant, heritable variation. Across the entire epigenome an estimated 80% of the variance in methylation is attributable to genetic influence (Gertz et al., 2011). Although heritability estimates for methylation range widely at specific candidate genes (20-97% variance attributable to genes; Heijmans, Kremer, Tobi, Boomsma, & Slagboom, 2007), overall differences in methylation are significantly heritable. Moreover, this heritability appears to remain stable over time and is driven by allelic variation, making it ideal to use as an index for genetic sensitivity (Heijmans et al., 2007; Gaunt et al., 2016). In summary, empirical research demonstrates that methylation fulfills the parameters of an ideal plasticity phenotype as outlined in seminal differential susceptibility literature.

Although theoretically ideal, few empirical studies have tested methylation as a moderator in a GxE framework. Indeed, most studies have focused on methylation as a *mediator* of environmental effects on subsequent gene expression and/or changes in behavior (e.g. Rijlaarsdam et al., 2017; Cecil et al,. 2016). However, evidence of significant individual differences or variability in methylation patterns suggest that it may also be a *moderator* that can distinguish who is differentially affected by environmental conditions. The present study addressed this gap in the literature by characterizing genetic influence in a GxE model using DNA Methylation as the plasticity phenotype.

Inherited Sensitivity to DNA Methylation

The present study created a gene score using secondary data relating the entire genome to differences in DNA methylation (i.e. methylation quantitative trait locus data; meQTL) in post-mortem brain tissue from the frontal cortex. These data are available through previously published meQTL data from the North American Brain Expression Consortium (NABEC; Gibbs et al., 2010). This score does not represent actual levels of DNA methylation present in an individual's tissue. Rather, it represents the number of single nucleotide polymorphisms (SNPs) an individual has that have previously been related to greater DNA methylation in the brain. More details on creating the polygenic score are presented below in the Methods section. A gene score is well suited for exploring methylation as a plasticity phenotype.

Genetic influence on methylation appears to remain stable over time. For example, heritability estimates of methylation at candidate genes were consistent between an adolescent twin-pair sample and an independent middle-aged adult twin-pair sample (Heijmans et al., 2007). One study comparing methylation patterns across the life span (i.e. birth, childhood, adolescence, middle age) did detect a slight decline in heritability estimates from childhood into adulthood—from 0.24 to 0.21 (Gaunt et al., 2016). However, this change likely reflects the increased influence of environment on individual variation over time (as opposed to a decrease in genetic influence), particularly given that methylation increases with age. A twin-study on this phenomenon found that methylation significantly diverged between monozygotic twins as a function of age and also lifestyle differences. Researchers speculate that increased methylation with age reflects the accumulation of different environmental exposures, and also a "breakdown" in the cellular machinery that regulates methylation (Fraga et al., 2005). Although this

conclusion may seem in conflict with findings that additive genetic influence on psychological phenotypes increases with age, recall that methylation is a distinct phenotype. Indeed, methylation is a biological process *driven* by environmental influence—as people get older, they will have more exposure to various environments which may result in environmental factors accounting for a greater proportion of variability in methylation. Thus, methylation itself may not be stable over time, however genetic influence on methylation *is* consistent (Gaunt et al., 2016). An environmental sensitivity gene score captures this more stable aspect of methylation influence on behavior. Interestingly, research suggests that the heritability of methylation is largely attributable to allelelic variation, or the effects of SNPs, as opposed to gametic imprinting or other sources of genetic influence (Gertz et al., 2011). Common SNPs account for 20% of the variance in methylation, with effects likely being polygenic (Gaunt et al., 2016). Altogether, these studies highlight that a polygenic score may be the most effective method of indexing genetic influence on methylation.

Using a gene score of environmental sensitivity also addresses several challenges that accompany working with methylation data. For instance, collecting, processing and analyzing methylation data is costly. Moreover, there are no safe, minimally invasive ways to access brain tissue samples, and thus evaluate tissue-specific methylation, in living research participants. A gene score indexing sensitivity to methylation circumvents these challenges by relying on secondary meQTL data—minimizing cost and participant burden while also maintaining tissue specificity of methylation effects. Additionally, with a sensitivity gene score we are not measuring actual levels of methylation. This is not a limitation. Indeed, it is a strength of the study because methylation levels are inherently

conflated by environmental influence and change over the course of development. Indexing the sensitivity or *potential* for methylation may capture a more pure form of plasticity to the environment.

Parent Knowledge as Environmental Context

Alcohol etiology literature establishes several psychosocial environments as important to the development of alcohol disorder through adolescent drinking. Specifically, the deviance proneness model points to parenting behaviors as particularly influential context in the development of risky adolescent drinking through adolescent externalizing behaviors (Sher, 1991). For individuals who are more sensitive to their environment, these contextual influences should be more impactful than for less sensitive individuals. The present study capitalized on the well-supported effects of parent knowledge on adolescent problem behaviors to test the moderating effect of inherited sensitivity to environmental influences.

Parent knowledge refers to how aware parents are of where their children are, who they are with, and what activities they're engaged in. Greater parental knowledge has consistently been associated with significantly less problem behavior in adolescence (Dishion & McMahon, 1998; Keijsers, 2016). As typically measured in the literature and in the present study, parent knowledge refers to parent's awareness of various aspects of their child's life and is not limited to their child's drinking or engagement in specific problem behavior. The effects of parent knowledge are nuanced because there are several ways that parents could gain knowledge, and there are different implications for how knowledge ultimately influences adolescent behavior. Like most measures in a non-experimental design, parent knowledge is likely not a purely environmental influence.

However, it may broadly reflect how parents structure their child's environment. Indeed, greater parent knowledge is related to greater rules and restrictions that limit/control their child's activities (Stattin & Kerr, 2000). Thus, parent knowledge is a useful to measure for the present GxE study because it is a developmentally appropriate parenting influence that may broadly, albeit indirectly, capture the extent to which parents use different strategies to control their children's physical and social environments. The present study tested how genetic sensitivity to the environment moderates the effect of parent knowledge on adolescent problem behaviors.

Previous studies that have measured parent knowledge characterized the construct as "parental monitoring" (e.g. Dishion & McMahon, 1998). However, the theoretical conceptualization of parental monitoring (i.e. as active behaviors aimed at imposing rules and surveilling children) is distinct from the construct that was commonly measured as "monitoring" in earlier studies (i.e. parental knowledge; Stattin & Kerr, 2000). That is, previous research conflated their measures of greater parental knowledge--the hypothesized *outcome* of parental monitoring—with parental monitoring, despite their being several other ways that parents could gain knowledge of their children's activities. In addition to monitoring (e.g. actively imposing rules and surveillance), parents can acquire knowledge by directly soliciting information, and through their children spontaneously disclosing information. Indeed, research suggests that child-disclosure may be a particularly important source of parent knowledge and predictor of problem behavior in adolescence (Stattin & Kerr, 2000; Keijsers, 2016). The present study focused on the effects of parent knowledge directly on adolescent problem behavior and indirectly on drinking rather than on the specific mechanisms of gaining knowledge.

However, these mechanisms are important to consider when interpreting the effects of parent knowledge on adolescent drinking.

Parent Knowledge and Adolescent Rule Breaking Behavior

Externalizing behavior is problem behavior characterized by poor inhibition and is often overtly disruptive and socially inappropriate (i.e. physical aggression, property damage). These behaviors manifest in two related but ultimately distinct ways: aggression and rule breaking behaviors. Aggression is characterized by physical acts, such as hitting or fighting, and interpersonal tendencies, such as arguing and having a hot temper. Rule breaking behaviors are characterized by breaking rules, often with peers and include, theft, vandalism and truancy (Eley, Lichtenstein, & Moffitt, 2003). Research suggests that there may be a greater heritable component in the etiology of aggressive behavior, whereas shared environmental influence may play a greater role in the etiology of rule breaking behaviors. Indeed, a twin-study of externalizing behavior development from late childhood to early adolescence found that genes accounted for greater variability in the development of aggressive behaviors, whereas shared environmental influences also accounted for variability in the development of rule breaking behaviors (Eley et al., 2003). Consistent with these findings, Vries and colleagues (2016) found that cognitive distortions were associated with aggressive behavior in adolescents, whereas environmental factors—including parent knowledge—were significantly associated with rule breaking behavior. Moreover, a twin-study using an externalizing composite heavily weighted to capture rule breaking behavior found that shared environmental influence, and little to no genetic influence, accounted for the relation between parent knowledge and rule breaking behaviors (Marceau et al., 2015). These findings suggest that, relative

to aggression, rule breaking behavior is an externalizing outcome significantly shaped by environmental factors. The goal of the present study is to test how differences in genetic sensitivity to the environment ultimately moderates *environmental* influence on adolescent externalizing behavior and subsequent drinking. To these ends, and to optimize detection of the environmental effects of parent knowledge, the present study focused on adolescent rule breaking behavior as an externalizing outcome.

Empirical research consistently supports a relation between parent knowledge and adolescent rule breaking behavior, although the nature of this relation is complex. Early research using middle- and high school-aged youth found significant relations between self-reported parent knowledge and rule breaking behavior, such that greater perceived parent knowledge related to less rule breaking behavior (Stattin & Kerr, 2000; Fletcher, Steinberg, & Williams-Wheeler, 2004). However, these relations were cross-sectional and thus could not support a direction of effect. To clarify this ambiguity, recent research has used longitudinal data to test for the direction of effect and potential bi-directional relation between parent knowledge and youth rule breaking behavior. Findings have been mixed. For instance, a longitudinal study of high-risk youth found that greater parent knowledge predicted less rule breaking behavior, and the bi-directional relation was not significant (Bendezu, Pinderhughes, Hurley, McMahon, & Racz, 2016). Similarly, a bi-directional relation was not significant in a in a community sample of twin-pairs, however only the prospective relation between youth externalizing behavior and future parent knowledge was significant (Wertz et al., 2016). The discrepancies in these studies may reflect methodological differences, such as the nature of the sample (community versus high-risk) and the measure used for problem behaviors. For instance, Wertz and

colleagues (2016) operationalized problem behavior using both aggression and rule breaking behaviors, which may have tapped into more individual, genetically driven effects versus environmental effects. Indeed, the relation between externalizing behavior and future parent knowledge was genetically-mediated (Wertz et al., 2016).

Additionally, the discrepant outcomes may be partially due to the number and distribution of the time points used in each study. One study that modeled both latent trajectories of growth and short-term cross-lagged models over four annual data points found evidence of bi-directional relations between parent knowledge and adolescent rule breaking behavior (Abar, Jackson, & Wood, 2014). Specifically, initially higher levels of parent knowledge predicted less growth in adolescent rule breaking, and less declines in parent knowledge over time was related to greater declines in adolescent rule breaking behavior. Conversely, consistently elevated levels of delinquency related to declines in parent knowledge over time. These findings suggest that the relation between parent knowledge and adolescent delinquency may reflect characteristics of both the familial environment *and* the child. Parent knowledge may be a familial environment that predicts less rule breaking behavior, and youth who engage in rule breaking behavior may be less likely to disclose knowledge to their parents. Interestingly, bi-directional relations were also found in cross-lag models controlling for long-term growth, although the effects were in an unanticipated direction (Abar et al., 2014). Greater parent knowledge predicted more rule breaking behavior and greater rule breaking behavior predicted greater parent knowledge. These findings are consistent with evocative effects, such that parents attempt to increase their knowledge in response to their child's rule breaking

behavior. Altogether, the literature strongly supports a relation between parent knowledge and adolescent rule breaking behavior and suggests that this relation may be reciprocal.

Implications of the Relation between Parent Knowledge and Adolescent Delinquency

Like many other environmental influences, parent knowledge may reflect a confluence of contextual and individual difference effects on adolescent drinking. For instance, parent knowledge is highly correlated with parent-child relationship quality (Dishion, Li, Spracklen, Brown, & Haas, 1998). Greater parental knowledge may reflect a closer parent-child relationship that may facilitate communication or encourage behaviors that ultimately buffer against risky alcohol use. Alternately, more parental knowledge may occur through greater control over a youth's activities, thereby limiting opportunities to drink.

The relation between parent knowledge and problem behavior may also reflect characteristics of the *child*. Researchers have found that parents seek more knowledge with children who are warm and open, and seek less knowledge with children who are closed off (Racz & McMahon, 2011). Relative to youth with little to no antisocial behavior, greater antisocial behavior prospectively predicts greater declines in parent knowledge (Laird et al., 2003). A longitudinal twin-study found that such effects may be partially genetically mediated, such that heritable externalizing behaviors prospectively predicted later levels of parent knowledge (Wertz et al., 2016). Indeed, a study from our research group found that a greater genetic predisposition for behavioral under control indirectly predicted less parent knowledge through greater levels of child impulsivity—a pattern consistent with evocative gene-environment correlation (rGE; Elam, et al., 2017). Thus, parent knowledge may be *evoked* by heritable child characteristics. Youth prone to

problem behaviors may be less likely to spontaneously disclose to their parents and actively discourage parents from seeking more knowledge through solicitation and monitoring, ultimately contributing to low parent knowledge. The present study is aimed at understanding the environmental effects of parent knowledge on adolescent rule breaking behaviors. To limit confounding by child effects, earlier levels of child rule breaking behavior was a covariate in the present study.

The effects of parent knowledge also differ as a function of demographic characteristics of the family. For example, parent knowledge changes over the course of development as the child's interpersonal abilities, social contexts, and relationship with their parents change (Dishion & McMahon, 1998). Longitudinal research indicates that overall levels of parent knowledge decline over time (Laird, Marrero, & Sherwood, 2010). Thus, parents of younger children may have greater levels of knowledge relative to parents of older children. Additionally, there are significant sex differences in parent knowledge with parents having more knowledge about female children relative male children (Stattin & Ker, 2000; Crouter & Head, 2002; Racz & McMahon, 2011). The present study evaluated child-report of parent knowledge as it is moderated by genetic sensitivity to environmental influence to predict teen drinking. Adolescent age and sex will additionally be included as covariates.

Rule Breaking Behavior as a Mediator of Parent Knowledge Effects on Drinking

Aberrations in top-down cognitive control are proposed to underlie externalizing behavior, including rule breaking behavior (Lee, Derefinko, Milich, Lynam, & DeWall, 2017; White et al., 2014). Research suggests that brain regions and neural circuits important to top-down cognitive control include the frontal lobe (Nigg 2017; Hwang,

Velanova, & Luna, 2010). Thus, methylation in the frontal lobe could be important to the development of rule breaking behaviors. The present study proposed that rule breaking behavior mediates the effects of environmental context and heritable sensitivity to frontal lobe methylation on adolescent drinking. Cognitive and neuropsychological research support the importance of frontal lobe methylation in rule breaking behaviors. Moreover, both alcohol and behavior genetic research support the mediating effect of rule breaking behavior, demonstrating that it has a significant etiological role in adolescent drinking.

Methylation in the Frontal Lobe

Methylation in the frontal lobe may be particularly important to rule breaking behavior due to the role of top-down cognitive control. Top-down cognitive control refers to deliberate, sequential processes that ultimately maintain representation with a current task, and recruit the working memory, attention, and inhibition (Botvinick & Braver, 2015; Nigg, 2017). These cognitive processes are referred to as "top-down" because they are driven by internal mental representations (e.g. a goal) as opposed to external stimuli and are related to neural signaling that travel from the cortical-to-subcortical or anterior-to-posterior regions of the brain. Deficits in top-down cognitive control may manifest as either a non-reflective, immediate response to some cue or relatively greater weighing of immediate versus delayed rewards (Nigg, 2017). Cognitive tasks that capture these manifestations of top-down cognitive control include delay discounting and Stroop tasks.

Researchers hypothesize that deficits in top-down cognitive control may contribute to rule breaking behaviors, with empirical studies supporting this position. For instance, adolescents diagnosed with conduct disorder demonstrated significantly greater delay discounting—poorer top-down cognitive control—as compared to a healthy,

community-based control sample (White et al., 2014). Additionally, greater delay discounting was related to higher levels of rule breaking behavior in an adult community-based sample, and prospectively predicted greater criminal activity in a sample of college students (Mishra & Lalumiere, 2017; Lee et al., 2017). These studies suggest that individual differences in top-down cognitive control may be important in rule breaking.

Although current neuroscientific models of the relation between the brain and cognition suggest that strict localization is inaccurate, regions and neural networks important to top-down cognitive control include the frontal lobe (Egner, 2011; Diamond, 2013; Petersen & Posner, 2012). For example, researchers found that differences in neural activity in the ventrolateral prefrontal cortex accounted for 40% of the variation in performance on the Stroop task, a measure of top-down cognitive control (Egner, 2011). Importantly, research implicates deficits in top-down cognitive functioning *and* rule breaking behavior as related to frontal lobe aberration. As compared to healthy controls, individuals with ADHD, an externalizing disorder, had significantly less frontal lobe surface area and less neural activity when demonstrating greater poorer top-down cognitive control during a delay discounting task (Dirlikov et al., 2015; Ortiz et al., 2015). These studies suggest that the neural processes that contribute to top-down cognitive processing may occur at the frontal lobe and may account for individual differences in rule breaking behaviors. Characterizing inherited sensitivity to methylation in the frontal lobe may be important to testing rule breaking behavior as a mediator in the present study and capitalized on the tissue-specificity of methylation effects.

Although not tested specifically at the frontal lobe, differential methylation is also related to rule breaking outcomes. Recent prospective research linked greater methylation

with increased self-reports of impulsivity (Ruggeri et al., 2015). Differential methylation also distinguished youth with lifetime externalizing disorders from those with a depression diagnosis and healthy controls (Heinrich et al,, 2015), as well as adults with chronic aggression from controls (Provencal et al., 2013). This distinction was detected with methylation at the candidate gene and epigenome level (Wang et al., 2012; Provencal et al., 2013). Thus, inherited sensitivity to DNA methylation may be important in the development of externalizing behaviors, including adolescent rule breaking.

Adolescent Rule Breaking Behavior and Risk for Drinking

Externalizing behaviors broadly and rule breaking behaviors specifically predict adolescent drinking and future alcohol problems. Indeed, the deviance proneness model posits that externalizing behavior is one major etiological mechanism that mediates the influence of familial alcohol disorder on increased risk for pathological drinking—in adolescence, this elevated risk may manifest as greater alcohol consumption (Sher, 1991). Thus, using rule breaking behavior as a mediator in the current study is consistent with etiological theory.

Empirical studies also support a mediating effect. As reviewed earlier, research consistently supports a relation between parent knowledge and rule breaking behavior. The literature also supports a relation between rule breaking behaviors and heavy adolescent drinking. For example, both parent-reported and teacher-reported rule breaking behavior in early and late childhood predicted greater self-reported alcohol consumption in late adolescence (Timmermans, van Lier, & Koot, 2008; Trucco et al., 2011). In addition, interviewer-assessed diagnoses of externalizing psychopathology (i.e. conduct disorder, attention deficit hyperactivity disorder) at age 11 predicted greater odds

of early onset of drinking, as well as greater odds of regularly and more heavily drinking alcohol at age 14 (King, Iacono, & McGue, 2004).

Moreover, recent research indicates that a significant proportion of the effect of rule breaking behavior on future drinking is causal. Kendler and colleagues (2018) used marginal structural and linear probability models to estimate causality in the relations between greater rule breaking behaviors in middle adolescence, and heavy drinking and alcohol problems in late adolescence. In comparing marginal structure and linear probability models, they found a modest attenuation of predictive power for these relations (~19% reduction in beta coefficients). These findings indicate that a significant proportion of the relations between rule breaking behavior and subsequent heavy drinking and problems is causal (Kendler et al., 2018). Additionally, studies aimed at testing indirect effects on adolescent drinking have found that rule breaking behaviors mediate the influence of parent knowledge on drinking. For instance, one study found that low parent knowledge at age 12 predicted greater alcohol problems at 17 through a positive conduct disorder diagnosis at 15 years (Edwards, Gardner, Hickman, & Kendler, 2016). Altogether, theoretical and empirical research support rule breaking behavior as a mediator of parent knowledge effects on adolescent drinking. The present study is the first to test for the moderating effect of inherited sensitivity to methylation on the relation between the parent knowledge and adolescent drinking using rule breaking behavior as a mediating psychological phenotype.

In summary, rule breaking behavior is the ideal mediating psychological phenotype for the present study. Cognitive psychology, and structural and functional neuroimaging research support focusing on DNA Methylation in the frontal lobe given

the importance of top-down cognitive control in rule breaking behaviors. Thus, the present study capitalized on the tissue-specificity of methylation effects and cognitive research on rule breaking behavior to optimize detection of GxE effects using methylation in the frontal lobe as a plasticity phenotype. Moreover, rule breaking behavior is theoretically consistent with the deviance prone-ness model of alcohol etiology and is empirically related to parent knowledge and drinking in ways consistent with a mediating effect.

The Present Study

Innovations in behavior genetic and developmental psychology research have added nuance to studies on the development of adolescent drinking, an important public health issue. Specifically, this research has yielded novel conceptualizations of how individual difference factors and the environment influence problem behaviors, including youth drinking. These innovations underscore gaps in the literature where continued work is needed. The differential susceptibility model posits that heritable variation in sensitivity to environmental influences—or plasticity—is an understudied individual difference factor important in shaping behaviors. A plasticity phenotype ideally suited for genetic research is biological in nature, involved in mechanisms that facilitate environmental sensitivity, and demonstrates significant heritable variation. However there remains a significant discrepancy between plasticity phenotypes used in behavior genetic studies versus the phenotypes conceptualized in theoretical literature. The present study addressed this gap in the literature.

The present study used an ethnically-diverse sample of youth participating in a longitudinal study of familial alcohol disorder. It adds to our understanding of adolescent

drinking etiology by using a novel index of inherited sensitivity to environmental influences and an empirically and theoretically well-supported pathway to adolescent drinking to test for GxE effects on heavy adolescent drinking. Specifically, the present study developed a novel, polygenic index of inherited sensitivity to environmental influence by using methylation in the frontal lobe as a plasticity phenotype. The genetic score represents an individual's sensitivity to greater methylation in frontal lobe tissue, with a higher score indicating greater sensitivity to higher levels of methylation. The present study tested whether this sensitivity gene score moderated the influence of parental knowledge as it indirectly influenced heavy adolescent drinking through rule breaking behavior.

Because the sensitivity gene score indexes heritable, individual differences in plasticity to the environment, we hypothesized that higher gene scores would exacerbate or moderate environmental effects. We hypothesized that the sensitivity gene score would significantly moderate the indirect effect of parental knowledge on adolescent heavy drinking through rule breaking behavior. Specifically, we hypothesized that:

1. Parent knowledge will significantly relate to rule breaking behavior such that greater parent knowledge will predict less rule breaking behavior. Rule breaking will significantly predict heavy drinking such that less rule breaking behavior will relate to less heavy drinking.
2. Inherited environmental sensitivity will significantly moderate the effect of parent knowledge on rule breaking such that greater parent knowledge will predict the least levels of rule breaking behavior for adolescents with higher

sensitivity gene scores as compared to adolescents with moderate to low scores

3. Parent knowledge will have a significant indirect effect on heavy drinking as mediated by rule breaking behavior, such that greater parent knowledge will predict less rule breaking behavior which will relate to less heavy drinking.
4. Inherited environmental sensitivity will significantly moderate the indirect effect of parent knowledge on heavy drinking. Compared to adolescents with moderate to low sensitivity gene scores, greater parent monitoring will predict the least levels of heavy drinking through the least levels of rule breaking behavior for adolescents with higher sensitivity gene scores.

CHAPTER 2

METHODS

The Original Study

Participants

Participants were a subset from a larger, longitudinal study of familial alcohol disorder called the Adult and Family Development Project (AFDP; PI: Dr. Laurie Chassin). The study started with 454 adolescents (i.e. generation 2s, or G2s) and their parents (G1s). Of the G2s recruited, 54% had one biological custodial parent with an AUD (COAs), and both G1s and G2s were interviewed regularly over the course of 13 years. At the sixth wave of assessment, 745 children of G2s (i.e. "G3s") were interviewed and genetic data were collected. Three more follow-up assessments were completed with only G3 participants at 18 months, three years, four years, and 7 years after wave 6.

Recruitment

Families with one or more parents with an alcohol disorder parent were recruited by identifying potential G1s through court records, health maintenance organization (HMO) wellness questionnaires, and telephone surveys. Inclusion criteria included: having a child 11-15 years old, Hispanic or non-Hispanic Caucasian ethnicity, birth dates between 1927 and 1960, and Arizona residency. In addition, one biological parent must have met lifetime DSM-III criteria for an alcohol disorder based on structured, face-to-face interviews using the DIS-III (Robins, Helzer, Croughan, & Ratcliff, 1981). Control families were recruited via reverse directories that identified families residing in the same neighborhood as families with an alcoholic parent. Families that met sub-threshold levels of a lifetime alcohol disorder (n=17) were not included in the study to reduce chances of

a control family later transitioning into a family with an parent who had an alcohol disorder. Recruitment methods are further detailed in Chassin and colleagues (1992).

Recruitment Bias

There are two potential sources of recruitment bias in the original study. First, there may be a significant difference between families with alcoholic parents who were successfully contacted versus families who were not contacted. The court and HMO records of successfully contacted versus not contacted individuals were compared using t-test and chi-square analyses. Self-identification as an alcoholic, Michigan Alcohol Screening Test scores, blood alcohol level at time of arrest, and number of prior alcohol-related arrests did not differ as a function of successful contact versus no contact. However, relative to those not contacted, successfully contacted participants were more likely to have a lower SES, be younger, unmarried, and of Hispanic descent. Second, there may be a significant difference between contacted individuals who agreed versus declined participation in the original study. A comparison among families who had a parent with an alcohol disorder parent indicated that SES, sex, age, and measures of alcohol disorder did not significantly differ as a function of agreeing versus declining to participate. Relative to those who agreed, individuals who declined were significantly more likely to be married and of Hispanic descent. A comparison among control families indicated that family composition and SES did not significantly differ as a function of agreeing versus declining to participate. However, relative to those who agreed, control individuals who declined were more likely to be of Hispanic descent. Potential recruitment biases are further detailed in Chassin and colleagues (1992).

Procedure

Families who agreed to participate were required to provide consent and assent for minor children prior to enrollment. Data were collected through in-person interview or phone if needed, and confidentiality was reinforced with a DHHS Certificate of Confidentiality. In-person interviews were conducted by trained research staff either at participants' residence or at Arizona State University. Research staff read interview items from a laptop to participants, who had the option of verbally responding or manually inputting their response into the laptop. If multiple family members were being interviewed at once, staff conducted interviews in separate, private rooms to ensure response confidentiality. The follow-up interviews 18 months and 4 years after Wave 6 data collection were completed by telephone. Out-of-state participants completed the survey via mail or the phone. The follow-up interviews 3 and 7 years after Wave 6 were collected via web survey.

Genotyping

AFDP gene data were collected and processed with NIAAA funding to the Midwest Alcohol Research Center (MARC). The Washington University Genome Sequencing Center

processed the genetic data through a revised Illumina Golden Gate array using a panel of well supported markers of AUD and SUD (Hodgkinson et al., 2008). To ensure high quality data, the genetic data were evaluated for Mendelian inconsistencies, sample swaps, incorrect gender assignments, cryptic relatedness, deviations from Hardy-Weinberg equilibrium (HWE), ambiguous genotype calls, and SNPs with low call rates (<95%). SNPs that deviated from HWE, had a call rate <95%, or had a minor allele

frequency (MAF) <2% were omitted. Relatedly, participants with poor quality genetic data were not included in the study (n=5).

The Current Study

Participants

The study used genetic and environmental data on G3 participants at Wave 6 (hereafter referred to as Time 1; T1), rule breaking behavior data from the 18-month follow-up (hereafter referred to as T2), and drinking data when participants were age 18-20 years (hereafter referred to as T3). Participants (N=316) were included in the study if they were of Hispanic or Non-Hispanic Caucasian ethnic descent and were between the ages of 11-14 years old at T1. Because the number of participants who reported a history of heavy drinking prior to T3 was low (n=15), we excluded them to reduce the number of covariates included in the analyses. The ethnicity inclusion criterion was intended to reduce effects related to population stratification, which was further controlled for with a genetic ancestry variable (detailed below in the Measures section). The age inclusion criterion was intended to reduce age heterogeneity in the proposed sample and still capture effects of environmental influences.

The participants included in the study did not significantly differ from those excluded (N=533) on measures of: gender, T2 rule breaking, T1 custodial mother and custodial father knowledge, and T3 heavy drinking. However, compared to excluded individuals, participants included the in the study were younger (t=-7.825, p<.001), had lower levels of T1 rule breaking (t==3.30, p=.001) and more likely to be non-Hispanic Caucasian (t=-2.86, p=.004). Additionally, a greater percentage of included participants had a parent with a lifetime alcohol disorder diagnosis compared to excluded participants

($\chi^2 = 4.214$, $p = 0.04$). The sample for the present study being younger than excluded individuals reflects selection criteria aimed at reducing age heterogeneity. Relatedly, the sample having less previous heavy drinking than the excluded participants may be due to the sample being younger in age; older individuals are more likely to have experiences drinking heavily. The sample being more non-Hispanic Caucasian than excluded participants may reflect the finding in the larger AFDP sample that Hispanic participants were less likely to consent to providing genetic data.

Measures

Descriptive data, including means, for all measures in the current study are in Table 1.

Demographics. Adolescents self-reported their gender, which was dummy coded such that male=1 and female=0. Participant age was calculated as a continuous variable based on the date they were interviewed, and their date of birth.

Adolescent Ancestry. The present study controlled for ethnicity and population stratification using a genetic ancestry component score. The ancestry score was created via principal components analysis in the larger G3 sample with 37 ancestry informative markers that distinguish Mexican-American from European-American ancestry. A higher ancestry score reflects greater European-American ancestry. The component score is highly correlated with participant self-reported ethnicity ($r = -0.83$, $p < 0.001$) and has successfully controlled for ethnicity in other behavior genetic studies (Tian, Gregersen, & Seldin, 2008; Wang, Pandika, Chassin, Lee, & King, 2016).

Parent Alcohol Disorder. At T1, the biological parents of G3s self-reported whether they met DSM-IV criteria for lifetime alcohol disorder (abuse or dependence) via the Computerized Diagnostic Interview Schedule (Robins et al., 2000). Absent

biological parents' alcohol disorder statuses were assessed by spousal reports with the Family History Research Diagnostic Criteria (Endicott, Anderson, & Spritzer, 1975). Adolescents were positive for parent alcohol disorder if any of their biological parents met criteria for lifetime alcohol disorder (47.5% positive for parent lifetime alcohol disorder). A dichotomous parent alcohol disorder variable was used as a covariate.

Custodial Parent Knowledge. Custodial mothers and fathers separately reported their knowledge of their G3 child's activities in the past 3 months at T1 using a parent monitoring scale (Lamborn, Mounts, Steinberg, & Dornbusch, 1991). The scale consists of five items with responses on a 5-point Likert scale and ranging from 1 = "Didn't know at all" to 5 = "Knew all the time." See Appendix A for scale items. The summary score of parent knowledge was the mean of all the scale items. A higher score reflects greater parent knowledge. However, the summary scores for both custodial mother and father reported knowledge in the current study were highly skewed, kurtotic, and reflected ceiling effects. Thus, we created dichotomous variables indicating complete knowledge of child's activities (i.e. a mean score of 5) versus less than total knowledge. For custodial mothers, 36.6% reported total knowledge, and for custodial fathers 17.8% reported total knowledge of their children's activities. We used both mother and father reports of their knowledge in separate models.

Inherited Sensitivity to DNA Methylation in the Frontal Lobe. We created a sensitivity gene score from publicly available methylation quantitative trait loci (meQTL) data published by Gibbs and colleagues (2010). These data are part of the North American Brain Expression Consortium. Similar to genome wide association studies (GWAS), meQTL data are collected by testing associations between every SNP in the

genome and methylation levels at CpG sites. In short, meQTL analyses are GWASs with methylation as the phenotype.

In the discovery sample, the meQTL data were collected from post-mortem brain tissue from N=150 individuals who prematurely died due to an accident or disease, or from natural causes. These individuals provided 600 tissue samples from four brain regions: the cerebellum, pons, temporal cortex, and the frontal cortex (Gibbs et al., 2010). Phenotypes for the meQTL analyses were brain region specific. Phenotypes consisted of unique CpG methylation sites that had a significant Illumina detection value ($p \leq .01$) and were detected in 95% of the samples collected from the same brain region. Gibbs and colleagues (2010) used PLINK to estimate linear regressions for each tissue, with methylation at selected CpG sites as the dependent variable and genotype as the independent variable. The p-values for each SNP tested (~1.6 million) were adjusted for multiple testing and were used to calculate an FDR threshold of significance. Biological and methodological covariates were also included in the meQTL analyses.

The present study used meQTL data from frontal cortex tissue, which had 27,532 unique CpG sites that could potentially be methylated and 10,679 SNPs that were significantly related to methylation levels at these sites (Gibbs et al., 2010). We compared SNPs genotyped in the AFDP data with SNPs significantly related to frontal cortex methylation in the discovery sample and with SNPs in high linkage disequilibrium with those in the discovery (i.e. proxy SNPs). We identified 6 SNPs in common (see Table 2 for list of SNPs and details). These SNPs passed quality control procedures. Quality control included checking for rare variants, minor allele frequencies > 0.45, palindromic SNPs (e.g. variants that were C-G or A-T). AFDP genetic data were also evaluated for

strand flips. The sensitivity allele was the reference allele related to methylation in the discovery sample. For proxy SNPs, we used the minor allele as the sensitivity allele. We employed an additive coding approach, where participants received a score between 0 and 2 based on the number of sensitivity alleles they possessed for each SNP. The sensitivity gene score reflected the total number of sensitivity alleles a participant possessed across all 6 SNPs in the score. The sensitivity gene scores could range between 0 and 12, with a higher score reflecting greater sensitivity to DNA methylation in the frontal cortex. Participants in the current study had low sensitivity gene scores. For our analyses, sensitivity scores were standardized via z-score transformations.

Adolescent Rule Breaking Behavior. Participants completed the Youth Self-Report scale (YSR) at T1 and T2 (Achenbach & Rescoria, 2001). The present study used the summary score of the Rule-Breaking Behavior sub-scale as a measure of adolescent rule breaking behavior. The scale has 12 items, with response options on a three-point scale (1 = Not True, 2 = Somewhat true, 3 = Very true/often true). Drinking-related items from the original scale were omitted. See Appendix B for complete list of items. The summary score of rule breaking behavior was the sum of responses to all scale items, with a higher score reflecting greater rule breaking behavior. Internal consistency is adequate ($\alpha = 0.73$). The current sample had low to moderate levels of rule breaking behavior. We used reported rule breaking behavior at T2 as the mediating variable and controlled for rule breaking behavior at T1.

Heavy Adolescent Drinking. Participants self-reported the number of times they had consumed three or more alcoholic beverages per occasion in the past year at every wave of data collection. Frequency of heavy drinking was measured using a trichotomous

variable (0 = Never, 1 = Heavily Drank 1-5x in past year, 2 = Heavily Drank >5x). The present study used reports of heavy drinking when participants were between the ages of 18 and 20 years old. Participants reported low levels of young adult heavy drinking (47% reported Never).

Data Analytic Plan

Primary Analyses

The present study used MPlus (Muthén & Muthén, 1998-2012) to estimate the proposed relations using path analyses. Separate custodial mother and father knowledge models were estimated with maximum likelihood with robust standard errors (MLR) and full information maximum likelihood to account for data that were missing completely at random or at random (Schafer & Graham, 2002). Additionally, we estimated models with a robust sandwich estimator (TYPE = COMPLEX) that adjusts standard errors and chi-square statistics to account for nonnormality and clustering of siblings within families.

Predictor variables were T1 Parent Knowledge, the sensitivity gene score, and their cross-product. The mediator variable was T2 rule breaking behavior, and the outcome variable was T3 heavy drinking. Covariates included adolescent age, sex, ancestry, parent lifetime alcohol disorder status, and T1 rule breaking. We estimated paths from the sensitivity score, T1 parent knowledge, their cross-product term and all relevant covariates (T1 rule breaking, age, sex, ancestry, parent lifetime alcohol disorder) to T2 rule breaking behavior (the mediator) using linear regression. Additionally, we estimated paths from the sensitivity gene, parent knowledge, their cross-product, T2 rule breaking behavior, and the relevant covariates (age, sex, ancestry, parent lifetime alcohol disorder status) to T3 heavy drinking (the outcome) using an ordinal logistic regression

model with Monte Carlo integration (see Figure 1). We first estimated main effects models, then estimated moderation models by including the cross-product term.

We created the cross-product term between the sensitivity gene score and parent knowledge and used it as a predictor. The sensitivity gene score was standardized to a z-score. We probed significant interactions by estimating simple slopes 1 standard deviation above the mean, at the mean, and 1 standard deviation below the mean of the sensitivity score (Aiken & West, 1991).

We used the joint significance test to evaluate the mediating effect of adolescent rule breaking behavior in the proposed model. According to the joint significance test, a significant "a" and "b" path in a mediation model indicates a significant mediating effect. Relative to other tests of mediating influence, the joint significance test demonstrates the best balance between sufficient statistical power and accurate Type I error rates (MacKinnon, Lockwood, Hoffman, West & Sheets, 2002).

CHAPTER 3

RESULTS

Preliminary Analyses

For all study variables, we completed model diagnostics and identified outliers using Mahalonobis distance, and influential cases using Cook's D (Rousseuw & Van Zomeren, 1990; Cook, 1977). We identified two cases that were significant outliers due to unusually high T1 rule breaking scores. However, no cases, including the two outliers, were significantly influential and therefore all participants were kept in the final analyses.

To reduce confounding of the hypothesized sensitivity score x parent knowledge interaction by unaccounted for gene x covariate effects, we tested the relations between all sensitivity score x covariate cross-products and rule breaking behavior. Specifically, we estimated a hierarchical linear regression where Block 1 estimated the covariates, hypothesized main and sensitivity score x parent knowledge effects on T2 rule breaking, and Block 2 additionally tested all the sensitivity score x covariate cross-products effects on T2 rule breaking. We then calculated the squared semi-partial correlation. If Block 2 significantly contributed to predicting rule breaking, it was kept in the final analyses. This approach is a modification of Keller's (2014) suggested best practice of testing all gene x covariate interactions and keeping all gene x covariate interactions in the final model if any one interaction effect is significant. The block 2 sensitivity score x covariate cross products did not significantly contribute to T2 rule breaking variance over and above block 1 for the custodial mother ($F_{gain} = 0.341$ [4,303], $p = .850$) and father models ($F_{gain} = 0.229$, [4, 303], $p = .922$). Additionally, no single gene x covariate interaction term was significantly related to T2 rule breaking. Thus, the final models did not include

any gene x covariate interaction terms. To test for potential confounding due to gene-environment correlations, we estimated a regression between parent knowledge and the sensitivity score. The relation was not significant for custodial mother (beta = 0.014, SE = 0.059, p=.818) or custodial father knowledge (beta = -.008, SE = .074, p=.910), and was therefore not included in the final models.

Correlations

Table 3 displays the zero-order correlations of all study variables. The simple correlations supported the hypothesized mediated effects, with custodial father and mother knowledge related to less G3 rule breaking at T1 (r_{father} = -.145, p=.029; r_{mother} = -.148, *p*=.012), and at T2 for custodial mother knowledge (r = -.169, *p*=.005), and less T2 rule breaking related to less frequent T3 Heavy Drinking (r=.125, p=.05). Although significant, the relation between custodial mother versus father knowledge was small (r=.159, p=.023) supporting our decision to test models separately by reporter.

Consistent with previous literature, older age was related to greater rule breaking at T1 and T2, and more frequent T3 heavy drinking. Also consistent with previous findings, having a parent with a lifetime alcohol disorder diagnosis was related to greater rule breaking and heavier drinking, and less than total custodial mother knowledge of activities. Greater Hispanic ancestry was related to custodial mother and father knowledge, more frequent T3 heavy drinking, and a greater likelihood of being male.

Primary Analyses

Prediction of T2 Rule Breaking

For the custodial mother models, there were main effects. Mother knowledge related to less T2 rule breaking (beta = -.086, SE=.045, p=.057). Additionally, greater

levels of T1 rule breaking (beta = .496, SE = .072, p<.001), older age (beta = .113, SE = .061, p=.063) and having a parent with a lifetime alcohol disorder diagnosis (beta = .091, SE = .055, p=.096) were related to greater T2 rule breaking. However, when the cross-product term was included in the model, the interaction was not significant (see Table 4).

For the custodial father models, there were no significant main effects of either the sensitivity score or father knowledge on T2 rule breaking. Levels of T1 rule breaking (beta = .507, SE = .073, p<.001), older age (beta = .119, SE = .060, p=.048) and having a parent with a lifetime alcohol disorder diagnosis (beta = .105, SE = .054, p=.053) were related to greater T2 rule breaking. When the cross-product term was included in the model, the interaction was not significant. The effects of the sensitivity score and father knowledge on T2 rule breaking remained not significant (see Table 5).

Prediction of T3 Frequency of Heavy Drinking

Greater T2 rule breaking did not significantly relate to frequency of heavy drinking at T3 for the custodial mother or father models (see Tables 4 & 5). Older age and more Mexican-American ancestry related to greater odds of more frequent heavy drinking at T3 for both models.

Indirect Effect

Although greater custodial mother knowledge predicted less T2 rule breaking (the "a-path"), T2 rule breaking did not significantly predict T3 heavy drinking (the "b-path"), Thus, according to the joint significance test, the indirect effect of greater mother knowledge on less T3 heavy drinking through less T2 rule breaking is not significant.

Post-Hoc Analyses

We performed a series of post-hoc analyses to better understand the polygenic score and the results of the present study.

Non-Linear Relations Between Gene Score and Study Variables

Study results may reflect a non-linear relation between the gene score and a study variable. To evaluate potential non-linear effects, bivariate plots between the gene score and continuous study variables and covariates were visually inspected (see Figure 2). There was no evidence of non-linear relations.

Multi-Collinearity between Interaction Term and Gene Score

We considered whether study results were attributable to multi-collinearity between the gene score and interaction terms. Indeed, the simple correlation between the interaction terms for custodial mother knowledge and father knowledge and the gene score were r=0.327, $p < .001$ and r=0.520, $p < .001$. However, these relations are likely structural as opposed to stochastic relations. A stochastic relation between variables reflects a true relation between the broader constructs captured by the variables (e.g. a correlation between two predictors). A structural relation between variables reflect statistical manipulations of the data (e.g. the correlation between a predictor, and another variable which the predictor was used to create). McClelland and colleagues (2017) demonstrated via change-of-origin transformations that relations between predictors and their cross-products in moderated multiple regression models can always be reduced to zero, indicating that such relations are structural as opposed to stochastic, which are invariant to such transformations. The effects of stochastic multi-collinearity are not comparable to the structural relations that emerge in moderated multiple regression

models (Irwin & McClelland, 2001). McClelland and colleagues (2017) suggest that multi-collinearity diagnostics targeting the relation between predictor and interaction terms are not warranted and were therefore not pursued in this study.

Overcontrolling Analyses

Finally, we explored whether estimating models with MLR and controlling for sibling clustering with TYPE = COMPLEX may have "overcontrolled" models and significantly impacted study results. Intraclass correlations coefficients (ICCs) on T2 rule breaking behavior (ICC=0.238) and T3 frequency of heavy drinking (ICC=0.3044) were low. Moreover, estimating study models without controlling for clustering did not significantly change results. Altogether, these findings suggest that over-controlling did not compromise study results.

CHAPTER 4

DISCUSSION

The present study sought to explore alternate sources of genetic influence in the development of alcohol disorders. Specifically, the study developed a novel, polygenic index of inherited sensitivity to environmental influence based on methylation quantitative trait locus data. The study tested whether this gene score moderated the indirect influence of parent knowledge on young adult drinking via early adolescent rule breaking, an empirically well-supported pathway increasing risk for adult drinking problems. The results mostly did not support the study hypotheses. Custodial mother knowledge of adolescent activities did relate to less rule breaking behavior as hypothesized relative to mothers with less than total knowledge. However, the gene score did not significantly moderate the effect of parent knowledge on adolescent rule breaking, and adolescent rule breaking behavior did not relate to frequency of heavy drinking in young adulthood—thereby also failing to support the hypothesized indirect effect of parent monitoring on frequency of heavy drinking. These results highlight several methodological and theoretical considerations.

Custodial Mother Knowledge and Predicted Rule Breaking

Parent knowledge related to less rule breaking behavior in adolescents in the current study. This finding is consistent with study hypotheses and previous research (Stattin & Kerr, 2000; Fletcher et al., 2004; Bendezu et al., 2016) and supports a prospective relation between parent knowledge and adolescent conduct problems.

Interestingly, parent knowledge only related to adolescent rule breaking for custodial mothers and not custodial fathers. This finding is similar to those of previous

studies of parent knowledge and problem behavior, which used less clearly operationalized measures of parent knowledge. For instance, early research used youths' reports of both of their parents' knowledge and did not distinguish between mother's versus father's knowledge of their activities (e.g. Stattin & Kerr, 2000; Fletcher et al., 2004). Given traditional expectations of mothers versus fathers in child-rearing, responses in these studies may have captured youths' perceptions of mostly their mothers' knowledge. More recent studies exclusively used mother report of perceived knowledge of their child's activities, but they referred to the construct as "parent knowledge" (Bendezu et al., 2016; Wertz et al., 2016). The current study findings are consistent with previous research that may have primarily captured the influence of mother knowledge on adolescent problem behavior.

Studies that distinguished between mother versus father knowledge found similar results to the present study. For instance, one study using intact families found that mothers' greater knowledge of their adolescents' activities related to less adolescent delinquent behavior, but this relation was not significant for fathers' knowledge (Waizenhofer, Buchanan, Jackson-Newsom, 2004). These parent gender differences may reflect the relatively greater role mothers have traditionally played in child-rearing. Indeed, compared to fathers, mothers dedicate more time to caretaking activities with their adolescent children (Crouter, McHale, & Bartko, 1993; Craig, 2006). Thus, mothers may not only be more likely to acquire and gain more knowledge of their adolescents' activities relative to fathers, they may also be more likely to intervene and either stop or prevent future problem behavior.

Inherited Sensitivity to the Environment did not Moderate the Effects of Parent Knowledge and Rule Breaking

Study results did not support the hypothesis that inherited sensitivity to the environment moderated the relation between parent knowledge and rule breaking.

These findings most likely reflect the poor distribution of study variables, particularly parent knowledge. There were ceiling effects for custodial parent reports of knowledge. Despite being a continuous measure, nearly 40% of mothers and nearly 20% of fathers reported having total knowledge of their adolescent children's various activities. Parent knowledge was converted into a binary variable to accommodate the low variability. The limited distribution of the measure nevertheless may have interfered with effectively testing study hypotheses. Indeed, the relation between custodial mother knowledge and adolescent externalizing was only marginally significant despite strong support for this relation in the literature. Ceiling effects in one of the interaction variables could lead to non-linear relations that make it difficult to detect moderation.

Beyond its distribution issues, an additional limitation in using parent knowledge is that it may not have been the best-suited construct theoretically to use in the present study. Specifically, parent knowledge may not have been sufficiently "environmental" enough of a context to test for the moderating influence of the study's gene score. Emerging research has characterized parent knowledge as the product of a complex dynamic between adolescents' information management strategies and parents' knowledge gathering strategies (Rote & Smetana, 2018). Parent knowledge may not lend itself to optimally exploring the moderating influence of inherited sensitivity to the environment because adolescent qualities play a large role in eliciting the parenting

environment that contribute to parent knowledge. Although it is questionable whether a purely "environmental" influence exists, future research ought to test the moderating influence of inherited sensitivity to the environment using constructs and designs that attenuate confounding individual-level influences. For instance, by testing the moderating influence of inherited sensitivity to the environment within an intervention or clinical trials framework.

Gene Score Issues

Study findings may also be attributable to the low number of SNPs in the study's gene score. The genetic data available in the current sample did not align with the discovery sample meQTL data resulting in a gene score with a small number of SNPs. The SNPs genotyped for the larger AFDP study were selected due to their location in specific genes and neurobiological systems important in the development of substance use disorders and other externalizing pathologies. Sensitivity to methylation, the plasticity phenotype for which the score in the present study is based, is a broader construct and therefore not limited to variants important in alcohol disorder etiology. Indeed, of the 10,679 SNPs that were significant in the discovery sample meQTL, there were only 6 in common with the AFDP genotyped SNPs. The small number of SNPs in the study's gene score captures only a minute fraction of the variance of sensitivity to the environment, and it is unclear whether the amount captured is meaningful. Moreover, there is no proxy or equivalent construct for levels of methylation to confirm the construct validity of the gene score.

The non-significant moderating influence may also reflect limitations of the discovery sample and resulting meQTL data from which the study's gene score was

based. For instance, although age, sex, and the time between death and autopsy were controlled for in the meQTL analyses, cause of death was not controlled for. This is particularly problematic for meQTL data because some causes of death may have differential impacts on methylation levels. For example, n=14 individuals died due to substance misuse, including several overdoses and accidents while acutely intoxicated. Prolonged and/or heavy substance use influences methylation levels (Cervera-Juanes, Wilhelm, Park, Grant, & Ferguson, 2017; Liu et al., 2018) and is related to structural and functional differences in the frontal lobe (Silveri, Dager, Cohen-Gilbert, & Sneider, 2016) -- thus these individuals may have relatively greater levels of methylation due to their substance use rather than any differences in genetic variation. Similarly, n=3 discovery sample participants had violent causes of death ranging from gunshot wounds to a hanging. These deaths may reflect extended exposure to adverse environmental stressors or serious psychopathology—both of which may have downstream effects on methylation levels. Additionally, n=37 individuals had causes of death described as "multiple injuries"—which may also capture violent and/or substance related causes. Having intensive environmental stressors is not necessarily problematic—indeed, it may even be ideal to best capture an index of possible sensitivity the environment. However, the diversity of intensive environments and failing to control for the cause of death may have confounded the meQTL data and the sensitivity score used in the present study.

Differences in sensitivity to the environment may change over the course of development, which may also account for the current study's failure to find a significant moderating effect of the sensitivity gene score. Some theories conceptualize environmental sensitivity as developmentally limited, having greater influence during

periods characterized by more environmental programming (Pluess, 2015). Thus, the moderating influence of the gene score in this study may not have been apparent because the parenting effects at 12 years of age occurred outside of a critical developmental period. Alternately, as individuals grow older they may learn coping strategies that can offset differences in sensitivity to the environment. In the current study, participants who inherited greater sensitivity to environmental influences may have developed strategies to respond to their parenting environment at levels comparable to their moderately/less sensitive peers. Empirical support for these possible explanations is limited due to a lack of GxE studies on adolescents that use environmental sensitivity gene markers.

Rule Breaking did not Relate to Frequency of Heavy Drinking

Early adolescent rule breaking behavior did not significantly relate to frequency of heavy drinking in young adulthood, controlling for previous rule breaking behavior. This finding is counter to both theory and previous empirical literature and may reflect the poor distribution of both adolescent rule breaking and drinking measures. Analyses were re-estimated without controlling for earlier adolescent rule breaking to explore for potential over-controlling. However, rule breaking and heavy drinking were still not related. Interestingly, estimating analyses with the earlier measure of rule breaking behaviors (T1 Rule Breaking) did yield a significant relation, with adolescents engaging in greater rule breaking having 1.14 greater odds of engaging in more frequent heavy drinking in young adulthood (OR = 1.140, SE = 0.068, $p = 0.038$).

This difference in T1 versus T2 rule breaking as they relate to T3 problem drinking likely reflects distribution issues with participants' reports of rule breaking. Specifically, rule breaking was fairly kurtotic in the current sample, particularly at T1

(kurtosis statistic = 3.78). Preliminary regression diagnostics identified two significant outliers that were due to unusually high T1 rule breaking scores. Although these cases were not significantly influential and thus did not merit removal, the two unusually high rule breaking scores may be driving the kurtosis at T1 and the significant relation between rule breaking behavior and young adult drinking at T1 and not T2.

It is less clear why T2 rule breaking did not predict young adult heavy drinking, even when not controlling for earlier rule breaking behavior. This result may also be attributable to the poor distribution of both T2 rule breaking and T3 heavy drinking in the current study. Participants reported fairly low levels of rule breaking behavior—the max score reported at T2 was 10 out of a total possible max score of 36, with the mean score being 1.65. Similarly, participants reported low frequencies of heavy drinking, with a little over 70% reporting never or only drinking 1-5 times in the past year. Levels of both rule breaking and heavy drinking may have been too low to detect a significant relation.

Limitations, Strengths, and Future Direction

The current study findings most likely reflect the poor distribution of study variables, particularly custodial parent knowledge, and the small number of variants used in the study's gene score. To a lesser extent, the study may have also been theoretically limited by the use of parent knowledge as the "environmental" influence used to test the moderating influence of inherited sensitivity to the environment. The quality of the discovery meQTL data used to create the study gene score is an additional limitation of the current study.

Despite these limitations, this study has notable strengths. First, it indexes genetic influence based on DNA methylation in the frontal lobe, a novel plasticity phenotype that

is consistent with theoretical conceptualizations of a plasticity phenotype. Relatedly, a second strength of the proposed study is that it indexes genetic influence in a GxE study using a phenotype based on differential sensitivity to the environment as opposed to the psychological outcome. Finally, a third strength of the study is its' integration of the tissue-specificity of methylation into a study of human behavior by using secondary bioinformatics data of brain tissue from the frontal lobe.

The strengths and the limitations of the current study inform future research on inherited sensitivity to the environment and its role in the development of substance use. For instance, future research should continue to use gene scores based on theoretically consistent plasticity phenotypes and attempt to couch behavior genetic research within a theoretically and empirically well-supported etiological pathway to substance use problems. The current study highlighted the crucial importance of future studies using well-distributed measures and having ample genetic data available to maximize the number of variants included in a gene score. Additionally, the current study raised important questions on how to best characterize "environmental" influence, particularly given that parenting is not purely environmental—that is, qualities of the participant are likely part of the parenting environment they experience. Future research should test for the moderating influence of heritable sensitivity to the environment within randomized clinical trials or interventions. An experimental design attenuates the influence of such confounds by randomly assigning individuals to an environment. Altogether, the current study underscores the importance of adhering to methodological rigor and explores exciting alternate conceptualization and methods future research could use to elucidate

the role of inherited to sensitivity to environmental influences within the context of substance use disorder development.

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APPENDIX A

PARENT KNOWLEDGE ITEMS

In the past 3 months, how much did your mother/father know:

1. Who your friends were?
2. Where you were at night?
3. How you spent your money?
4. What you did with your free time?
5. Where you were most afternoons after school?

APPENDIX B

YOUTH SELF REPORT RULE BREAKING BEHAVIOR ITEMS

1. Lack guilt
2. Break rules
3. Bad friends
4. Lie/cheat
5. Prefer older kids
6. Run away
7. Set fires
8. Steal at home
9. Steal outside of home
10. Swear
11. Think of sex too much
12. Truant

Table 1

Descriptive Statistics for Study Variables

Continuous/Count Variables	N	Mean	*SD*	Min.	Max.	Skewness	Kurtosis
T1 Age	316	12.08	1.19	11.00	14.95	0.91	-0.54
T2 Age	301	13.34	1.23	12.01	16.98	0.94	-0.32
T3 Age	233	19.04	0.87	18.00	20.94	0.63	-0.88
Ancestry Factor Scores	300	0.03	0.93	-2.87	1.32	-1.09	0.04
T1 Rule Breaking Behavior	316	1.72	2.12	0.00	12.00	1.76	3.78
Sensitivity Gene Score	302	3.83	1.95	1.00	11.00	0.97	0.95
T2 Rule Breaking Behavior	301	1.65	2.20	0.00	10.00	1.54	1.99
T1 Life Stress Events	316	1.13	2.28	0.00	6.00	1.09	0.72
T3 Past Year Frequency of Drinking	233	1.47	1.66	0.00	7.00	0.76	-0.60
Dichotomous Variables							
Custodial Mother Knowledge	287	105 (36.6%) Total Knowledge 182 (63.4%) Less Than Total Knowledge					
Custodial Father Knowledge	225	40 (17.8%) Total Knowledge 185 (82.2%) Less Than Total Knowledge					
T3 Frequency of Heavy Drinking	249	118 (47.4%) Never 60 (24.1%) 1-5x In Past Year 71 (28.5%) >5x in Past Year					
Gender	316	166 (52.5%) Males 150 (47.5%) Females					
Parent Alcohol Disorder	316	166 (52.5%) No parent with AD 150 (47.5%) Parent with AD					

Table 2
Single nucleotide polymorphisms (SNPs) in Sensitivity gene Score

SNP	MAF	Ref Allele	Gene	Gene Function
rs12706832	0.40	A	*LEP*	Regulation of energy/homeostasis
rs1828774	0.37	C	---	non-genic
rs224546	0.42	C	*TRPV1*	Protein transducer of painful thermal stimulus in vivo
rs4332303	0.16	T	*HTR2C*	Serotonin transporter gene
rs6318*	0.12	C	*HTR2C*	Serotonin transporter gene
rs7055144*	0.29	C	*HTR2C*	Serotonin transporter gene

* Proxy SNPs

Table 3

Zero-Order Correlations Among Study Variables

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10
1. Sensitivity Gene Score	1									
2. Custodial Mom Knowledge	0.01	1								
3. Custodial Dad Knowledge	-0.03	0.16*	1							
4. Gender	-0.04	-0.03	0.03	1						
5. Parent Alcohol Disorder	-0.10†	-0.12*	0.06	-0.02	1					
6. Age	0.04	-0.09	-0.05	-0.03	-0.03	1				
7. Ancestry	0.09	-0.12*	-0.20**	-0.12*	-0.10	-0.02	1			
8. T1 Rule Breaking Behavior	0.009	-0.15*	-0.15*	0.15**	0.07	0.27***	0.03	1		
9. T2 Rule breaking Behavior	0.02	-0.17**	-0.05	0.09	0.13*	0.25***	-0.06	0.54***	1	
10. T3 Heavy Drinking	-0.05	0.05	0.06	-0.07	0.14*	0.24***	-0.19**	0.17**	0.13†	1

Note: N = 316. † $p < 0.10$, *$p < 0.05$, **$p < 0.01$, ***$p<.001$. Higher gene score = higher sensitivity to DNA methylation in the frontal cortex. Custodial Mom & Dad Knowledge: 0 = Less Than Total Knowledge of G3 Child's Activities, 1 = Total Knowledge of Activities. Past Year Frequency of Heavy Drinking: 0 = Never, 1 = 1-5x/Past Year, 2 = >5x Past Year. Gender is coded 0 =Females and 1=Males. Parent Alcohol Disorder: 0 = parents without alcohol disorder, 1 =parents with alcohol disorder. Higher ancestry= greater levels of Caucasian ancestry.

Table 4

Sensitivity Score x Custodial Mother Knowledge Predicting T3 Heavy Drinking Through T2 Rule Breaking - Standardized Path Coefficients & Odds Ratios

Predictors	Mediator T2 Rule Breaking β(SE)	Outcome T3 Freq Heavy Drinking OR(SE)
Gender	-0.005 (0.048)	0.724 (0.189)
Age	0.118 (0.059)*	1.475 (0.157)**
Parent Alcohol Disorder	0.090 (0.056)	1.671 (0.531)
Ancestry	-0.058 (0.063)	0.677 (0.104)**
T1 Rule Breaking	0.497 (0.073)***	---
Sensitivity gene Score	-0.008 (0.087)	1.027 (0.181)
Custodial Mother Knowledge	-0.085 (0.045)†	1.402 (0.376)
Gene Score x Cust Mother Knowledge	0.039 (0.067)	0.735 (0.203)
T2 Rule Breaking	---	1.049 (0.062)

Note. † $p < 0.10$, *$p<.05$, **$p<.01$, ***$p<.001$. Gene Score = Sensitivity gene Z-Score

Table 5

Sensitivity Score x Custodial Father Knowledge Predicting T3 Heavy Drinking Through T2 Rule Breaking - Standardized Path Coefficients & Odds Ratios

Predictors	Mediator T2 Rule Breaking β(SE)	Outcome T3 Freq Heavy Drinking OR(SE)
Gender	0.013 (0.045)	0.683 (0.175)†
Age	0.116 (0.060)†	1.504 (0.164)**
Parent Alcohol Disorder	0.102 (0.054)†	1.557 (0.502)
Ancestry	-0.059 (0.068)	0.676 (0.106)**
T1 Rule Breaking	0.507 (0.073)**	---
Sensitivity gene Score	-0.023 (0.055)	0.993 (0.155)
Custodial Father Knowledge	0.033 (0.074)	1.045 (0.410)
Gene Score x Cust Father Knowledge	0.113 (0.116)	0.576 (0.243)
T2 Rule Breaking	---	1.052 (0.060)

Note. † $p < 0.10$, *$p<.05$, **$p<.01$, ***$p<.001$. Gene Score = Sensitivity gene Z-Score

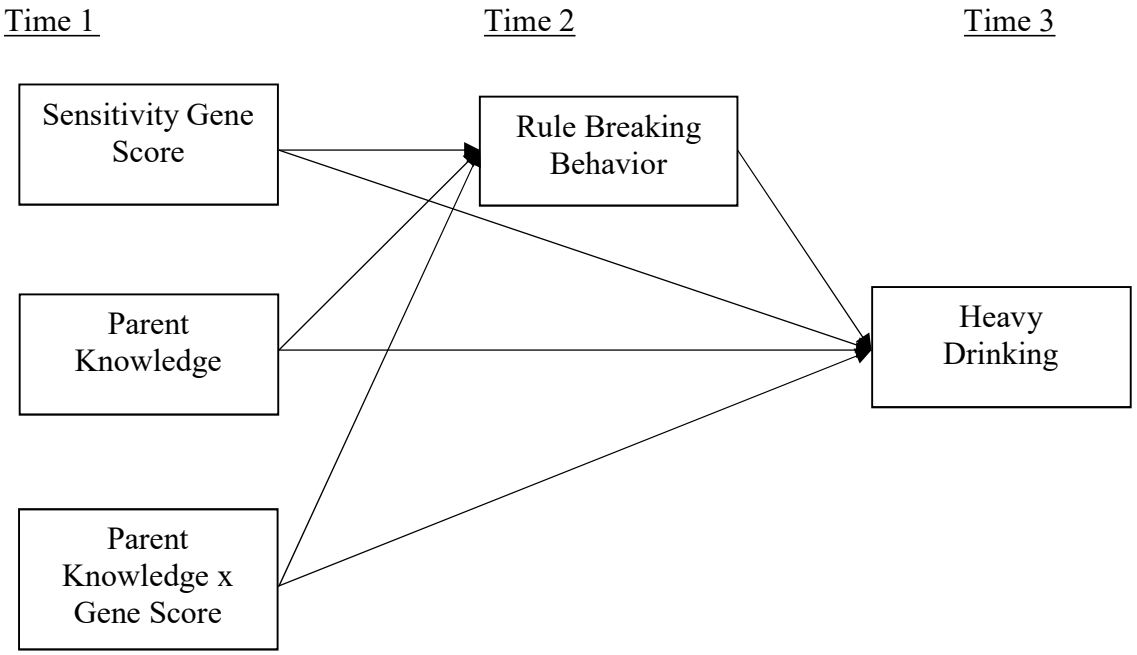

Figure 1. Hypothesized Model. Time 1 =11-14 years old, Time 2=12-16 years old, T3=18-20 years old. For ease of presentation, covariates not shown. Refer to Methods section for additional details.

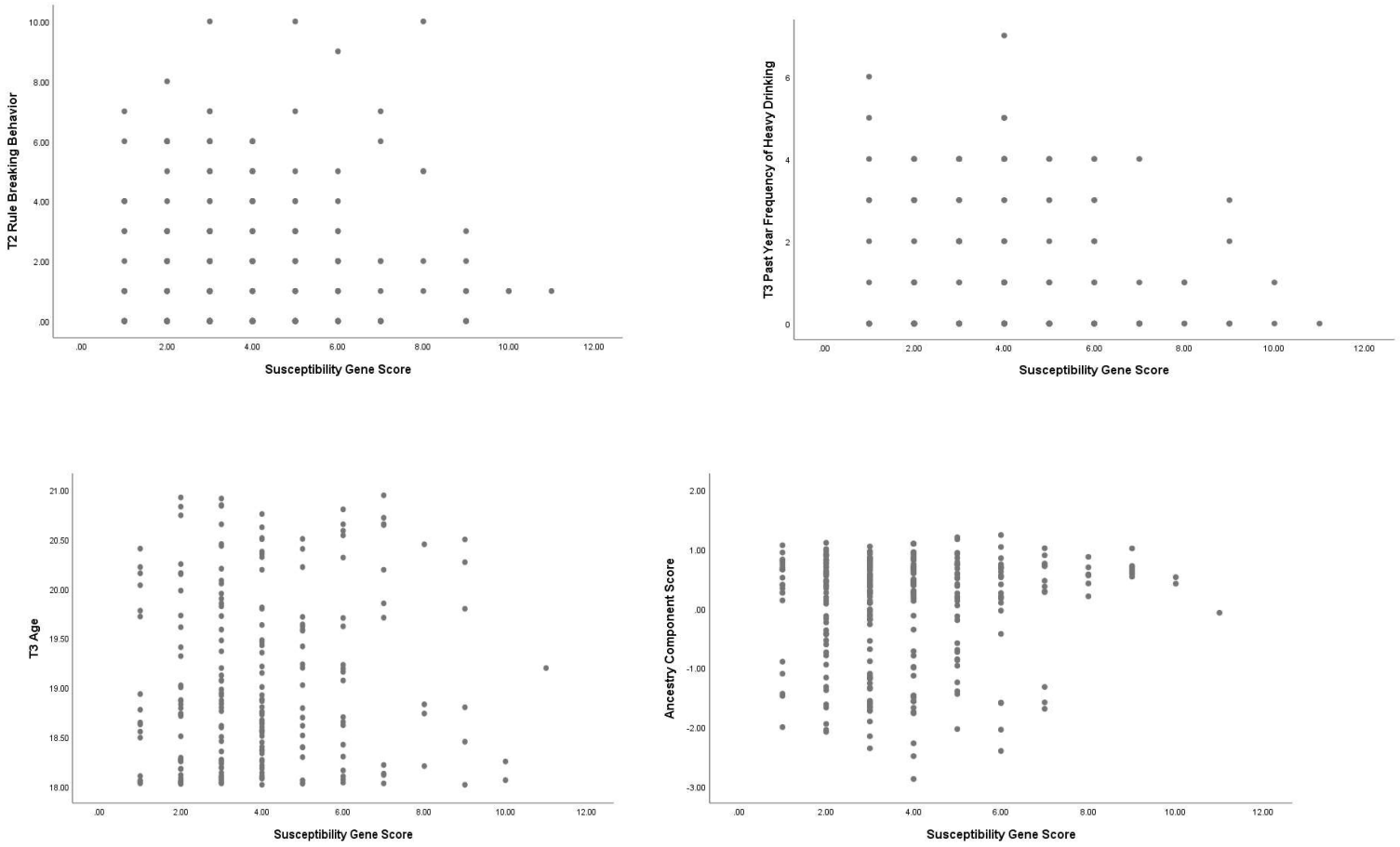

Figure 2. Bivariate scatterplots between the sensitivity gene score and continuous study variables