Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Sarah Zisser April 2, 2016

Eating and Substance Use Disorders: The Role of Adverse Mother-Daughter Relationships and the Mu-Opioid Receptor Gene

By

Sarah Zisser

Dr. Patricia Brennan
Adviser

Department of Psychology

Dr. Patricia Brennan

Adviser

Dr. Jessica Barber

Committee Member

Dr. Phillip Wolff

Committee Member

Dr. Edward Queen

Committee Member

2016

Eating and Substance Use Disorders: The Role of Adverse Mother-Daughter Relationships and the Mu-Opioid Receptor Gene

By

Sarah Zisser

Dr. Patricia Brennan

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Arts with Honors

Department of Psychology

2016

Abstract

Eating and Substance Use Disorders: The Role of Adverse Mother-Daughter Relationships and the Mu-Opioid Receptor Gene
By Sarah Zisser

Adverse mother-daughter relationships and genetic variation in the mu-opioid receptor gene (*OPRM1)* have demonstrated associations with both eating disorders and substance use disorders. The present longitudinal study examines the independent and gene-environment interaction effects of an adverse mother-daughter relationship and the presence of the Single Nucleotide Polymorphism (SNP) A118G (rs1799971) in the *OPRM1* gene on lifetime severity of substance use and eating disorders in 262 female adolescents. The quality of the mother-daughter relationship was measured using the Five-Minute Speech Sample, the UCLA Life Stress Interview, the Children's Report of Parental Behavior Inventory, and a child-report questionnaire of perceived maternal hostility. The lifetime severities for both disorders were measured using the maximum severity score across age 15 and 20 from the Kiddie-Schedule of Affective Disorders and Schizophrenia for School-Age Children at age 15 and the Structured Clinical Interview for DSM-IV at age 20. Results revealed that the *OPRM1* G-allele and child-report measures of mother-daughter relationship quality independently associated with increased lifetime severity of eating disorders. Mother-report measures and a latent variable constructed from both mother-report and child-report measures of mother-daughter relationship, but not the gene, associated with increased lifetime severity of substance use disorders. No gene-environment interaction for either outcome severity was found. Although the findings of the current study are preliminary, they support the role of *OPRM1* genetic variation in eating disorder outcomes, and point to the potential for future research to explore differences between child and mother-report of mother-daughter relationship quality that may uniquely associate with the development of eating disorder and substance use disorder outcomes.

Eating and Substance Use Disorders: The Role of Adverse Mother-Daughter Relationships and the Mu-Opioid Receptor Gene

By

Sarah Zisser

Dr. Patricia Brennan

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Arts with Honors

Department of Psychology

2016

Acknowledgements

I would like to thank Dr. Patricia Brennan for her support throughout this process. I could not have asked for a better adviser and role model. I would also like to thank Dr. Erica Smearman for her unending guidance and encouragement. Finally, I would like to thank the BUILD Lab for inspiring my love of research and cheering me on every step of the way.

Table of Contents

Introduction....1

Adverse Environments and Development of Eating Disorders....2

Adverse Environments and Development of Substance Use Disorders....4

The Reward Pathway....7

The A118G Polymorphism....10

Aims and Hypotheses....13

Methods....14

Participants....14

Procedure....15

Measures....15

Genotyping....19

Statistical Analyses....20

Results....22

Mother-Daughter Relationship and Child Disorder Outcomes....22

Genotype and Child Disorder Outcomes....23

Mother-Daughter Relationship, Genotype, and Child Disorder Outcomes....24

Supplemental Analyses of Independent Effects....25

Discussion....26

Mother-Daughter Relationship and Child Disorder Outcomes....26

Genotype and Child Disorder Outcomes....28

Mother-Daughter Relationship, Genotype, and Child Disorder Outcomes....30

Limitations and Future Directions....31

References....35

List of Tables

Table 1. Bivariate correlations for mother-daughter relationship and lifetime severity of eating and substance use disorders.....44

List of Figures

Figure 1. Conceptual model predicting lifetime severity of eating disorders and substance use disorders from a latent variable for adverse mother-daughter relationship.....45

Figure 2. Structural equation modeling predicting lifetime severity of eating disorder and substance use disorders from a latent variable for adverse mother-daughter relationship.....46

Figure 3. Path analysis independently predicting lifetime severity of eating disorders and substance use disorders from a composite child-report and a composite mother-report variable.....47

Figure 4. Path analysis predicting lifetime severity of eating disorders and substance use disorders from the presence of the G-allele.....48

Figure 5. Structural equation modeling independently predicting lifetime severity of eating disorders and substance use disorders from a latent variable of adverse mother-daughter relationship and the presence of the G-allele.....49

Figure 6. Path analysis independently predicting lifetime severity of eating disorders and substance use disorders from a composite child report variable, a composite mother-report variable, and the presence of the G-allele.....50

Eating and Substance Use Disorders: The Role of Adverse Mother-Daughter Relationships and the Mu-Opioid Receptor Gene

The extant body of literature consistently demonstrates that an adverse mother-daughter relationship is a risk factor for both substance use disorders and eating disorders (Attie & Brooks-Gunn, 1989; Brody & Forehand, 1993; Humphrey, 1986; Stice & Barrera, 1995). Significantly, substance use disorders and eating disorders have also been found to be highly comorbid, to be characterized by similar behavioral characteristics, and to involve similar brain systems, specifically the mesoaccumbens reward pathway (Davis & Claridge, 1998; Hadad & Knackstedt, 2014; Krahn, 1991; Marrazzi, Luby, Kinzie, Munja, & Spector, 1997; Wolfe & Maisto, 2000). Despite the vast amount of research documenting common risk factors and overlapping characteristics, no research has attempted to characterize how substance use and eating disorders may operate within a single model, and further, why an individual at risk for these outcomes might evidence one type of disorder over another.

Recently, psychological and genetic researchers have turned to gene-environment interactions to explain differential risks for an array of psychopathologies. The A118G (rs1799971) Single Nucleotide Polymorphism (SNP) is an allele variant in the mu-opioid receptor gene (*OPRM1*), and has been associated with differential responsiveness of the brain's reward pathway to opioids and to eating disorder behaviors (Bond et al., 1998). While researchers suspect a gene-environment interaction between this gene, family factors, and the severity of eating disorders based on each variable's individual association with the outcome of interest (Davis & Loxton, 2014; Slavich, Tartter, Brennan, & Hammen, 2014), it is unclear the degree to which this allele interacts with the environmental variables to predict eating disorder severity. In addition, although the A118G allele variant is associated with the brain's

responsiveness to opioids (Mague et al., 2009), it is also unclear how it might impact the severity of substance use disorders when combined with an environmental stressor such as an adverse mother-daughter relationship. The current study seeks to clarify the potential gene-environment interactions involving the presence of an adverse mother-daughter relationship and the A118G gene allele, while also elucidating how this specific genetic variant may impact the type of psychological disorder evidenced by an individual at risk.

Adverse Environments and Development of Eating Disorders

According to a study investigating the burden of different diseases on Australian society, eating disorders are a leading cause of disability for female adolescents (Mathers, Vos, Stevenson, & Begg, 2000). Due to their clinical significance, efforts to pin down risk factors for the development of eating disorders are especially relevant and important. Based on past studies, many researchers conclude that the most important risk factor for the development of an eating disorder is being female (Striegel-Moore, Silberstein, & Rodin, 1986). While it is clear that not all females develop eating disorders, research has focused attention almost exclusively on the co-occurring risk factors that drive female adolescents to partake in disordered eating behaviors. Since the coining of the term "anorexia nervosa" in 1873 by Sir William Gull, parental relationships have been suspect in the etiology of eating disorders (Lock & Le Grange, 2001). Today, the association between family characteristics and eating disorders is well documented in the extant body of literature (Attie & Brooks-Gunn, 1989; Kichler & Crowther, 2001; Polivy & Herman, 2002). Of particular interest to this study is which specific family characteristics have the greatest association with the development and severity of an eating disorder, and, further, how these family characteristics overlap those that are associated with substance use disorders.

A longitudinal study looking at a non-clinical sample of 193 adolescent girls and their mothers demonstrated that maternal psychological control, lack of boundaries, and lack of support had the greatest impact on daughters' self-esteem and disordered eating patterns (Attie & Brooks-Gunn, 1989). They suggested that these family features significantly impacted the adolescent's conception of her effectiveness and competencies when it came to regulating and controlling her own behavior. Although the study was particularly interested in the adolescent's perception of the family environment, maternal ratings were more predictive of eating problems than the adolescent's ratings. Based on the mothers' reports, most eating problems were found in families characterized by less cohesive and less expressive communication styles, implicating these characteristics as additional risk factors for the development of eating disorders.

In a separate study, 148 college-aged women completed questionnaires pertaining to general family dysfunction, family communication, and eating disorder symptomology (Kichler & Crowther, 2001). Findings suggested that when compared to maternal modeling of negative eating attitudes and behaviors, negative family communication, but not general family dysfunction, had a greater direct effect on eating attitudes in daughters. General family dysfunction is involved in a number of different psychopathologies and, as such, may not have as much predictive power as negative parent-child communication when predicting to eating disorders specifically (Benninghoven et al., 2007).

Indeed, a study looking explicitly at family influences on adolescent body image within a clinical sample demonstrated that particular family relationships, specifically relationships characterized by parental control and negative communication styles, were risk factors for the development of body-image problems and subsequent eating problems (Benninghoven et al., 2007). In another study focusing on family factors involved in the etiology of eating disorders,

patients with eating disorders most frequently described critical family environments and coercive parental control (Haworth-Hoeppner, 2000). Finally, Ogden and Steward (2000) and Smolak and colleagues (1999) concluded that, when compared to parental modeling of weight concerns, both general and body or weight related critical comments, as perceived by the adolescent, demonstrated more predictive power. These findings suggest that, although a myriad of family factors have important implications for the development of disordered eating, characteristics specific to the quality of parent-child interactions seem to be those most strongly related to eating disorder severity.

Adverse Environments and Development of Substance Use Disorders

Just as an adverse family environment has been implicated in the development of eating disorders, it has also been implicated in the emergence of substance use disorders. While most studies investigating eating disorders focus on female participants, the literature on substance use is far more varied. As a result, it is important to note how the characteristics discussed above may be uniquely relevant to the development and severity of substance use disorders in women. A second discrepancy between eating disorder literature and substance use disorder literature involves the point of focus within the family environment. In contrast to the literature on eating disorders, which focuses mainly on family relationships, studies of psychosocial family factors relating to adolescent substance use have historically focused on family structure, the marital status of the parents, and parental rule enforcement (Brody & Forehand, 1993; Stice & Barrera, 1995). In a study following 80 adolescents over the course of one year, Brody and Forehand (1993) sought to disentangle the skein of family factors that are purported to influence substance use. The results of this study suggested that, according to the mothers' report, the prospective relationship between interparental conflict and the development of substance use disorders in

adolescents is nonsignificant. Rather, mother-child relationship quality seemed to play a greater role in adolescent problem behaviors for both female and male participants such that mother-adolescent conflict and maternal rejection uniquely predicted substance use disorders in adolescence (Brody & Forehand, 1993).

In a study investigating the pathways leading to marijuana use among adolescents in Columbia, 1,687 participants and their mothers indicated that a high level of mother-child conflict and a lack of maternal affection and identification are related to an increase in adolescent marijuana use, particularly for female participants (Brook et al., 1998). This study also demonstrated that a gender difference exists such that the parent-child relationship relates more strongly to unconventional behavior in females than in males, and peer delinquency relates more strongly to unconventional behavior in males than in females. Similarly, a longitudinal study following 444 adolescents over the course of one year examined the prospective relationship between parental support and control, as reported by the adolescent, and substance use disorders among participants (Stice & Barrera, 1995). The results of this study suggested that deficits in both of these family factors predicted a higher level of substance use one year later, and that this finding was marginally stronger in female participants than in male participants. This study further suggested that an adolescent's disassociation from their parents as a result of this low parental support and affection may drive the relationship between the aforementioned family factors and the level of adolescent substance use (Stice & Barrera, 1995).

In addition, Schwartz and colleagues (1990) investigated the relationship between maternal expressed emotion, including critical attitudes, hostile attitudes, and emotional overinvolvement, and substance use in children. The results of the study demonstrated a three-fold increase in risk for substance use when mothers had high critical expressed emotion.

Importantly, the results of this study remained significant even after controlling for maternal psychopathology. This finding is particularly interesting given the widely accepted assumption that parental mental illness mediates the relationship between adverse parent-child relationship factors and adolescent substance use disorders (Stice & Barrera, 1995). Although Schwartz and colleagues (1990) looked at both female and male adolescents, they did not investigate differences between the two groups. Though expressed emotion seems to be a viable risk factor on its own, a meta-analyses of the existing studies assessing family correlates of substance use also identified rejection and over-domination as precursors to adolescent addiction (Braucht et al., 1973).

While family factors have demonstrated associations with substance use in both female and male adolescents, the family interaction theory was developed to offer a framework through which the psychosocial forces that predict female adolescent substance use can best be understood (Brook et al., 1998). According to this theory, mother-daughter relationships characterized by warmth and a nurturing dynamic protect against the adolescent's use of alcohol and drugs. Conversely, mother-daughter relationships characterized by a lack of support and affection are correlated with an increase in drug and alcohol use (Schinke, Fang, & Cole, 2008). Based on the extant body of literature, and consistent with this theory the impact of family relationships on substance use problems are consistently stronger among female than male adolescents (Brook et al., 1998; Schinke et al., 2008; Stice & Barrera, 1995).

The current study builds on the previous research by focusing on a high-risk cohort of females and examining the mother-daughter relationship as it relates to both eating disorder and substance use disorder severity. The presence of negative communication styles, including criticism and hostility, and psychological control in the mother-daughter relationship are the

most commonly discussed and widely cited family factors examined in the prospective prediction of both eating disorders and substance abuse. Therefore, an adverse mother-daughter relationship, operationally defined by the presence of psychological control, hostility, criticism, or overall stress in the relationship, seems to provide the relevant environmental risk focus for the current gene-environment interaction study.

The Reward Pathway

In addition to having shared family risk factors, the development of and differentiation between eating disorders and substance use disorders is further muddled by shared behavioral symptomology. Both disorders display the progression of an addiction, including loss of control over the behavior, preoccupation with the behavior, consequences on health, and utilization of the behavior to escape from a negative affect (Krahn, 1991; Lesieur & Blume, 1993; Wolfe & Maisto, 2000). Further, individuals with either substance use or eating disorders are often characterized by an ambivalence to treatment, suggesting a co-occurring denial of the addictive behavior (Krahn, 1991). Finally, both are often characterized by repeated use or consumption, obsession with using or completing the behavior, failed efforts to preclude the behavior or usage, and withdrawal from other areas of life for the sake of use (Hadad & Knackstedt, 2014). Born from these clinical similarities, many of the treatment options for eating disorders unsurprisingly mirror addiction models (Hadad & Knackstedt, 2014). It is also unsurprising that the same neural pathway, the brain's reward pathway, has been implicated in the development and severity of both substance use disorders and eating disorders. Specifically, the mu-opioid receptor system, uniquely linked to behaviors related to addiction (Davis & Claridge, 1998), operates by eliciting the experience of reward through the firing of different neurons along the mesoaccumbens dopamine pathway in the brain (Contet, Kieffer, & Befort, 2004). When this pathway is

repeatedly stimulated, the produced reward can lead to the formation of an addiction (Contet et al., 2004).

Different classes of drugs utilize this pathway by plugging either directly or indirectly into the mu-opioid receptors to incite the experience of euphoria (Hadad & Knackstedt, 2014). Most notably, morphine and other related opiates, bind directly to the mu-opioid receptors and increase the activation of the mesoaccumbens dopamine pathway, specifically by decreasing the efficacy of gamma-aminobutyric-acid (GABA), an inhibitory neurotransmitter released on this pathway (Contet et al., 2004). Although different substances stimulate the mesoaccumbens dopamine pathway through separate mechanisms, the mu-opioid receptor system is implicated in the experience of reward elicited across both opioid and non-opioid drug classes (Contet et al., 2004).

Similarly, behaviors associated with eating disorders are also influenced by the mu-opioid receptor system (Davis & Claridge, 1998). Theorists argue that eating disorders result from the rewarding properties associated with the body's endogenous opioids, or Beta-endorphins, which are released in the brain following eating disorder related behaviors, including self-starvation, binging, and purging (Davis & Claridge, 1998). In a study seeking to verify the release of Beta-endorphins in conjunction with the aforementioned behaviors, Marrazzi and colleagues (1997) demonstrated that both patients with anorexia nervosa and bulimia exhibited higher levels of endogenous opioids in the brain than individuals without eating disorders. As a result of these findings, a number of researchers have argued that eating disorders qualify as auto-addictions, or addictions to the body's endogenous opioids (Davis & Claridge, 1998; Marrazzi et al., 1997). In other words, these behaviors may be addictive because they increase

endogenous opioids, which then bind to the mu-opioid receptor, attenuate GABA inhibition, and result in increased activation of the reward pathway, resulting in addiction-like behaviors.

The auto-addiction opioid model suggests that this addiction pattern both mirrors that of substance use and engages the same mechanistic neurotransmitter system that facilitates the formation of addiction to exogenous substances (Davis & Claridge, 1998). In contrast to addiction models for exogenous substances, the auto-addiction model stipulates that the addicting aspect need not be an experience of euphoria. Rather, the result of mu-opioid receptor stimulation may simply be the reduction of anxiety and depression, resulting from the induced reward. In this sense, what is addicting to individuals with eating disorders is not so much the pleasure associated with binging or purging, but rather the degree that anxiety and negative affect are offset by the implicit rewarding properties of beta-endorphins (Davis & Claridge, 1998).

The evidence supporting the common brain mechanism through which eating disorders and substance use disorders operate is further bolstered by the finding that Naltrexone, an opioid receptor antagonist, has been effective in reducing the behaviors associated with both eating disorders (bulimia), and substance use disorders (Hadad & Knackstedt, 2014; Ziauddeen et al., 2013). This finding suggests that, not only is the mu-opioid receptor system implicated in both types of disorders, but it may also be a crucial driving factor associated with the continuation of related behaviors. Without the mu-opioid receptor system as a driving force, it seems likely that many of the rewarding and addictive properties of both eating disorders and substance use disorders would be attenuated.

The A118G Polymorphism

To date, the A118G polymorphism is both one of the most studied and one of the most consistently befuddling polymorphisms in research concerning the brain's reward system. Although it has been widely documented that the A118G polymorphism has a three-times greater binding affinity for Beta-endorphins than the more common A118A variant (Bond et al., 1998), it is still widely contested whether or not the polymorphism results in a gain or loss of function (Mague et al., 2009). According to Bond and colleagues (1998), a three-fold increase in binding affinity implies a tighter bond between the opioid and the receptor site, thus resulting in increased activation of the mu-opioid receptor system and an increase in the hedonic experience. In contrast, other studies suggest that the A118G polymorphism actually results in less opioid receptor efficacy and thus an inhibited experience of reward (Olsen et al., 2012; Slavich et al., 2014). Despite the controversies surrounding the specific properties and effects of the polymorphism, the association between the A118G polymorphism and eating disorders seems to be far less divisive.

Several studies demonstrated that individuals with binge eating disorder (BED) were more likely than controls to carry the G-allele of the A118G polymorphism (Davis et al., 2009; Davis, 2015). This documented association suggests that the polymorphism may lead to an increased experience of reward and reactivity to palatable foods (Davis, 2015). These results also corroborate previous findings indicating that the intake of palatable foods releases Beta-endorphins in the brain, which in turn leads to the experience of reward via the mu-opioid receptor system and the mesoaccumbens dopamine pathway (Davis & Claridge, 1998). The study by Davis and Claridge (1998) further demonstrates that the presence of the G-allele amplifies the experience of reward due to the increased binding affinity of Beta-endorphins with

the mu-opioid receptors. Although the current body of literature is limited such that it is unclear how this polymorphism impacts the development of other eating disorders, the auto-addiction model supports the existence of an analogous relationship between the polymorphism and both anorexia nervosa and bulimia, which are similarly characterized by the release of Beta-endorphins (Marrazzi et al., 1997).

Based on the similarities between eating disorders and substance use disorders, it is unsurprising that researchers have been motivated to demonstrate a parallel association between the A118G polymorphism and substance use disorders. A myriad of studies have sought to demonstrate this relationship; however, while some studies were able to demonstrate a significant relationship between the polymorphism and substance dependence (Deb, Chakraborty, Gangopadhyay, Choudhury, & Das, 2010; Miranda et al., 2010), the vast majority of studies were either unable to demonstrate any relationship or proffered a significant relationship in the opposite direction. One such study analyzed the genetic data of 398 heroin or alcohol-addicted individuals and found, contrary to the study's hypothesis, that the A118G polymorphism was not a risk factors for substance dependence (Franke et al., 2001). Further, a similar study suggested that the polymorphism may be a protective factor against substance dependence, given the finding that more non-substance dependent subjects carried the polymorphism than subjects in the substance-dependent group (Bond et al., 1998). Despite researchers' best efforts to corroborate the existence of a relationship, a recent meta-analysis demonstrated a lack of association between dependence on substances, including opioids, alcohol, nicotine, and cocaine, and the A118G polymorphism (Coller et al., 2009).

At a more proximate level of analysis, several studies investigated how the A118G polymorphism impacts the experience of reward and overall effect derived from exogenous

substances. In a study assessing morphine dosage needs of patients in the first year following a lumbar disc herniation, researchers found that patients with the A118G polymorphism required more morphine than patients with the A118A polymorphism to achieve the same analgesic effect (Olsen et al., 2012). Significantly, a preclinical study investigating differential morphine preference in mice with an analogous polymorphism noted similar findings (Mague et al., 2009). Namely, mice with the G-allele displayed reduced morphine related effects, had a lower preference for morphine-paired environments, and achieved a lower analgesic reaction following morphine administration than mice with the A-allele (Mague et al., 2009). Interestingly, Mague and colleagues (2009) also indicated that there might be a sex difference in G-allele responsiveness to morphine. In their study, only female mice with the G-allele failed to show any conditioned place preference for the morphine-paired environments. These findings suggest that, while morphine and related drugs are able to bind to the same receptor sites as endogenous opioids, the polymorphism may operate in such a way that the OPRM1 binding affinity to exogenous opioids is reduced, most pronouncedly in females, in contrast to the greater binding suggested for endogenous opioids. In this respect, a G-allele carrier would experience less reward following exposure to substances of abuse. This supports the claim made in the initial work of Bond and colleagues (1998), which again suggested that the A118G polymorphism might serve as a protective factor against the development of substance use disorders.

The proposed differential relationship of the A118G polymorphism with endogenous opioids and exogenous opioids provides a new lens through which researchers can examine the conflicting conclusions drawn from the extant literature. It is clear that, while past studies selectively investigate the polymorphism in relation to either endogenous or exogenous opioids, they largely fail to actually disentangle the conflicting behavior of the polymorphism across

studies. Mague and colleagues (2009) revealed that researchers who argue that the A118G polymorphism implies a gain of function cite the elevated Beta-endorphin binding, while researchers who claim that the polymorphism indicates a loss of function cite the well-documented decreases in morphine sensitivity associated with the G-allele. In other words, proponents of the gain in function hypothesis draw on receptor activation by endogenous opioids, while proponents of the loss in function hypothesis draw on reduced activation by exogenous opioids.

The current study seeks to examine the unique associations between the A118G polymorphism and the development of eating disorders and substance use disorders in young adults with varying levels of mother-daughter relationship quality. Given the existence of a shared environmental risk factor in the development of both eating disorders and substance use disorders, the current research attempts to broaden our current understanding of how multifinality is achieved over the course of child and adolescent development, within the context a gene-environment interaction paradigm. Three aims were investigated in this study. The first aim of the study was to investigate the association between mother-daughter relationship quality and the lifetime severity of both eating disorders and substance use disorders. On the basis of the existing empirical literature, we hypothesized that an adverse mother-daughter relationship during adolescence will associate with the presence and lifetime severity of both eating disorders and substance use disorders. The second aim was to investigate the association between the A118G polymorphism and the lifetime severity of both disorders of interest. Based on the extant research, we hypothesized that the polymorphism will associate with both outcomes of interest such that the presence of the G-allele will be positively associated with the lifetime severity of eating disorders and negatively associated with the lifetime severity of substance use disorders.

The final aim of this research was to investigate the interaction of an adverse mother-daughter relationship and the A118G polymorphism in predicting the lifetime severity of eating disorders and substance use disorders. To this end, we hypothesized that a gene-environment interaction will be evident, such that, among participants who have experienced an adverse mother-daughter relationship during adolescence, those that have the A118G polymorphism will evidence elevated risk for lifetime eating disorder severity and lessened risk for lifetime substance use disorder severity, relative to participants without this polymorphism.

Methods

Participants

A total of 262 female participants and their mothers were recruited from a larger birth cohort of 7,223 mother-child dyads in Brisbane, Australia. The original cohort was part of the Mater-University Study of Pregnancy in which mothers and children born between 1981 and 1984 were followed from birth through age 25 (Keeping et al., 1989). The participants of the current study were females who continued to participate in the study at ages 15 (N=402) and 20 (N=363), and who gave DNA samples between the ages 22 and 25 (N=262). Male participants from the original cohort and those who did not complete assessments and testing at each of the three noted age benchmarks were not included in this study.

Participants of the current study were 91.6% Caucasian, 4.6% Asian, and 3.8% Pacific Islander or Aboriginal. Participants were largely from middle to lower class incomes, and parents had a median education of 10^{th} grade (equivalent to high school graduates in the U.S.). Compared to females who participated in assessment at age 20 (N=363), participants in the current study did not differ significantly on substance use severity, $t(401)=-1.01$, $p=0.31$, or eating disorder severity, $t(401)=-1.46$, $p=0.14$. In addition, the current group of female

participants did not differ significantly from the original cohort of female participants on ethnicity (p=0.81) or on family income (p=0.48).

Procedure

As part of the Mater-University Study of Pregnancy, each mother-daughter dyad completed large-scale assessments at several time points. The current study focused on adverse mother-daughter relationship characteristics at age 15, which were derived from an expert-rated linguistic speech sample and self-report measures completed by the mother and the target child, and lifetime substance use and eating disorder severity, which were based on assessments rated by a trained diagnostic interviewer at age 15 and 20. This study also focused on genotyping data, which was gathered from blood samples drawn between the ages of 22 and 25. All participants provided written informed assent and consent prior to assessment at each study visit. The institutional review boards of the University of Queensland, University of California, Los Angeles, Emory University, and the Queensland Institute of Medical Research Genetic Epidemiology Laboratory approved all procedures.

Measures

Indicators of mother-daughter relationship quality at age 15.

Mother-report measures.

Five-Minute Speech Sample (FMSS). At adolescent age 15, mothers' perception of the relationship with the target child was captured using a Five-Minute Speech Sample (Magana et al., 1986). The FMSS indicates parents' expressed emotion, encompassing both criticism and emotional overinvolvement, toward their child. In the FMSS protocol, parents are asked to speak freely about their child and their relationship for five minutes without interruption or prompting. The FMSS was coded based on a coding scheme developed by Magana and colleagues (1986). A

speech sample is considered high in expressed emotion (2 points) if parents meet criteria for a high score on the criticism dimension or a high score on the emotional overinvolvement dimension. A high score on the criticism dimension is given if there is a negative initial statement, parents express a negative relationship with the child, or there is more than one explicitly critical statement, as defined by the original coding scheme (Magana et al., 1986). A high score on the emotional overinvolvement dimension is given if the parent displays self-sacrificing/overprotective behavior, excessive emotional display, or five or more statements of affection. Similarly, a speech sample is considered borderline-high in expressed emotion (1 point) if parents exhibit either borderline-high critical behavior, which is characterized by expressed dissatisfaction, or borderline-high emotional overinvolvement, which is characterized by moderate-levels of overprotective behavior. If none of the above criteria are satisfied, the speech sample is categorized as low in expressed emotion (0 points). For the purpose of this study, only scores of expressed emotion related to criticism were included in our analyses. FMSS raters used in this study were trained by the research group that developed the measure, and interrater reliability of the raters with an expert from the original research group was sufficient. Kappa values for expressed emotion on the dimension of criticism ranged from 0.63 to 0.82 (Brennan, Le Brocque, & Hammen, 2003). In the current sample, the mean score for maternal criticism was 0.41 (SD=0.71).

UCLA Life Stress Interview (LSI). Mothers' report of parent-child relationship stress was evaluated using the "Relationship with Target Child" domain of the LSI. This subpart of the LSI assessed aspects of the parent-child relationship, such as conflict, closeness, and rule-compliance, that result in maternal stress. For this domain, each mother was given a score between 1 and 5 based on behavioral scoring from a trained interviewer. A score of 5 signifies a

parent-child relationship marked by severe stress. The LSI demonstrates sufficient validity (Hammen, Brennan, & Keenan-Miller, 2008), and has a kappa value of 0.82 (Humphreys et al., 2013). In the current study, the mean score was 2.18 (SD=0.49).

Child-report measures.

Children's Report of Parental Behavior Inventory (CRPBI). Youth report of parental behaviors was evaluated using the CRPBI, originally developed by Schaefer (1965). This measure was used to assess adolescents' feelings about the relationship with both their mother and father by rating parental behaviors along three dimensions: positive involvement (acceptance versus rejection), negative control (psychological autonomy versus psychological control), and lax discipline (firm control versus lax control; Schludermann & Schludermann,1988). Measures of the CRPBI have demonstrated reliability, validity, and internal consistency (α=0.79- 0.91; Safford, Alloy, & Pieracci, 2007). For the purpose of this study, only subscales relating to maternal positive involvement and negative control were included in the analyses. Each subscale consisted of 10 items rated on a 3-point Likert scale. The highest score possible for both maternal positive involvement and negative control was 30, with higher scores signifying more maternal acceptance and more psychological control. For consistency, the maternal acceptance score was reverse coded by subtracting the maximum score (30) from each score of maternal acceptance and taking the absolute value to create a score for maternal rejection. The mean score for maternal rejection was 6.12 (SD=4.93), and the mean score for maternal psychological control was 16.20 (SD=3.99). Subscales of maternal positive involvement and maternal negative control from the current sample had Cronbach's α values of 0.90 and 0.81 respectively.

Maternal Hostility. Youth perception of maternal hostility was assed using a 24-item questionnaire with each item rated on a 7-point Likert scale. The maximum possible score on this

measure was 168, indicating extremely high levels of maternal warmth. For the purpose of this study, the maximum possible score was subtracted from each participant's score, and the absolute value was taken to create a score for maternal hostility. Higher scores reflected higher levels of maternal hostility, as perceived by the adolescent. The measure was originally developed by the Iowa Youth and Families Project and exhibits sufficient internal reliability (Ge, Best, Conger, & Simons, 1996). In the present study, the Cronbach's α value was 0.93 and the mean score was 35.77 (SD=21.50).

Indicator of lifetime substance use and eating disorder severity.

Kiddie-Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS-E). At age 15, substance use severity and eating disorder severity were measured using the K-SADS-E (Orvaschel, 1995). The K-SADS-E is a semistructured interview with documented reliability and concurrent validity that is used to assign diagnoses to children based on interviews from the mother and child (Kaufman et al., 1997). Diagnoses were assigned to the child if either the child or the mother indicated the necessary criteria for a diagnosis. Though the K-SADS-E is equipped to diagnose current and past Axis I psychiatric disorders, only diagnoses relevant to substance use and eating disorders were analyzed in the current study. Diagnoses were converted to a 4-point severity scale, with a score of 0 indicating the absence of symptoms, and a score of 4 indicating severe symptoms. Weighted kappa values were greater than 0.75 for both diagnoses (Conway, Hammen, & Brennan, 2014). The mean score for substance use disorder severity at age 15 was 0.07 (SD=0.40), and the mean score for eating disorder severity at age 15 was 0.15 (SD=0.61).

Structured Clinical Interview for DSM-IV (SCID). At age 20, adolescent substance use severity and eating disorder severity were measured using the SCID for DSM-IV (First, Spitzer,

Gibbon, & Williams, 1995**)**. Again, although the SCID is designed to diagnose all current and past Axis 1 psychiatric disorders, only diagnoses relating to substance use and eating disorders were analyzed in the current study. Participants were again rated on a scale from 0 to 4, based on the severity of current symptoms. The mean score for substance use disorder severity at age 20 was 1.26 (SD=1.51), and the mean score for eating disorder severity at age 20 was 0.18 (SD=0.67).

A lifetime severity score for each disorder was calculated by taking the maximum severity score across the age 15 and age 20 visits. The mean score for lifetime substance use severity was 0.99 (SD=1.42), and the mean score for lifetime eating disorder severity was 0.29 (SD=0.83).

Genotyping

Adolescents who participated in either the age 15 or age 20 follow up were asked to participate in genotyping data collection between the ages of 22 and 25 years. Blood was drawn at participants' local pathology lab using a blood collection kit. After blood samples were transported to the Genetic Epidemiological Laboratory at the Queensland Institute of Medical Research for storage, DNA aliquots were sent to the UCLA Inflammatory Biology Core Laboratory for Processing. The A118G polymorphism was genotyped multiple times using a series of real-time polymerase chain reactions (PCR). Formal protocol was followed during this procedure. The test-retest reliability of the samples had an error rate <1% (Slavich et al., 2014). Results of the genotyping revealed that 75.6% (N=198) of participants had the AA genotype, 23.7% (N=62) of participants had the AG genotype, and 0.8% (N=2) of participants had the GG genotype. The allele frequencies satisfied the Hardy-Weinberg Equilibrium, $\chi^2(2)=1.30$, $p=0.52$. Based on the research documenting the influence of the G polymorphism (e.g., Mague et al.,

2009), participants were coded as either G-allele carriers (N=64) or not G-allele carriers (N=198).

Statistical Analyses

Structural equation modeling (SEM) was used to test all models evaluating the three primary aims in this study. All correlation and regression analyses were performed using SPSS (Version 23.0), and all SEM analyses were performed using the AMOS (Version 23.0) software package (Arbuckle, 2015). The alpha level was set at $p<0.05$.

In all SEM analyses, model fit was evaluated using the χ^2 index, the comparative fit index (CFI), and the root mean square of approximation (RMSEA) with its 90% confidence interval. The χ^2 test statistic is used to evaluate whether the population covariance matrix is equal to the covariance matrix implied by the model when distribution assumptions are satisfied. A significant χ^2 test indicates that the population covariance matrix does not equal the model's covariance matrix, and the model should be rejected, although exceptions are made to this rule in the case of very large sample sizes or non-normal distributions of outcomes (Schermelleh-Engel, Moosbrugger, & Müller, 2003). The CFI measures model fit by comparing the current model to a baseline model. Scores range from 0 to 1 with scores above 0.90 indicating adequate fit and scores above 0.95 indicating good fit (Hu & Bentler, 1999). The RMSEA measures approximate fit in the population where the 90% CI interval indicates the precision of the estimate. RMSEA values less than 0.08 indicate adequate fit, and scores less than 0.05 indicate good fit. The lower bound of the CI should be no greater than 0.05, and a lower bound of 0.00 indicates perfect fit (Hu & Bentler, 1999; Schermelleh-Engel et al., 2003).

To evaluate the first study aim, a latent variable was constructed using mother and child ratings of the mother-daughter relationship. Table 1 displays the bivariate correlations among

measures for mother-daughter relationship and lifetime severity of both eating disorders and substance use disorders. Contrary to expectation, child-rated maternal rejection was not significantly correlated with either eating disorder severity or substance use disorder severity. As our gene-environment interaction test presupposes an overall significant effect of the environmental risk factor on its own, child-rated maternal rejection was excluded from the remaining analyses. The four remaining measures of mother-daughter relationship quality (i.e., child-rated maternal psychological control, mother-rated maternal criticism, mother-rated parent-child relationship stress, and child-rated maternal hostility) were used to construct a latent variable for use in hypothesis testing. Based on previous literature suggesting a significant relationship between maternal depression and adverse mother-daughter relationships (Brennan et al., 2003; Stoneman, Brody, & Burke, 1989), maternal depression was tested as a possible predictor of the latent variable. Maternal depression was significantly associated with the latent factor for mother-daughter relationship (β=0.28, p<0.01). Therefore, it was controlled for in all models.

In the confirmatory factor analyses, the standardized residual error covariance between maternal psychological control and maternal hostility was significant at p<0.01, indicating that the error terms were significantly correlated. As a result, this relationship was controlled for in the subsequent SEM analyses. In the final latent model, the standardized factor loadings ranged from 0.35 to 0.82 and the model indicated an adequate fit (χ^2(df=1, N=262)=3.12, p=0.07, CFI=0.99, RMSEA=0.08 with at 90% CI 0.00-0.21). This latent variable was then used in a path model to test the hypothesis that an adverse mother-daughter relationship associates with both lifetime eating and substance use disorder severity (for conceptual model, see Figure 1).

To evaluate the second aim concerning *OPRM1* genotype and eating and substance use disorders, a SEM model was created to test the association between the *OPRM1* genotype (GG/AG vs AA) and the lifetime severity of both disorders. The genotype distribution differed by ethnicity in this sample such that the allele distribution within the Asian ethnicity differed significantly from the distribution evidenced in the rest of the sample, $t(260)=2.06$, $p<0.01$. The model was first run with all ethnic groups included in the sample. Fitness indices signified that the model had moderate fit based on a CFI of 0.87 (Hu & Bentler, 1999). Asian ethnicity was subsequently controlled for in the model, and it was found that fit statistic improved. Therefore, it was included in the final genotype model.

Finally, to evaluate the third aim, we exported the latent mother-daughter relationship variable to SPSS and created an interaction term using the latent scores and the genotype. Linear regressions were performed with the interaction term predicting to study outcomes.

Results

Mother-Daughter Relationship and Child Disorder Outcomes

To test the hypothesis that an adverse mother-daughter relationship will be significantly related to an increase in both eating disorder and substance use disorder severity, SEM with maximum likelihood procedures were used to assess a model linking the latent variable for mother-daughter relationship to the lifetime severity of both disorders (see Figure 2). Fit indices suggested adequate model fit: χ^2 (df=13, N=262)=27.68, $p<0.01$; CFI= 0.94; RMSEA= 0.07 with a 90% CI of 0.03-0.10. Although the χ^2 test statistic was significant, it was likely inflated due to non-normal distributions in both substance use and eating disorder severity. The other fit statistics, which are less influenced by nonnormality, suggested an adequate fit (Schermelleh-Engel et al., 2003). While the latent variable was significantly associated with lifetime substance

use severity (β=0.36, p<0.01), it was not significantly associated with lifetime eating disorder severity (p=0.35).

Since the latent mother-daughter relationship model did not significantly associate with both outcomes, and the correlations between mother and child ratings appeared to differentially associate with eating disorders and substance use disorders (see Table 1), we created an alternative environmental risk factor prediction model. Specifically, separate composite variables were created for the child-rated measures (maternal psychological control and maternal hostility) and for the mother-rated measures (parent-child relationship stress and maternal criticism). SEM was then used to evaluate the independent relationship between each of the composite variables and lifetime severity of both disorders (see Figure 3). The standardized residual error covariance between the child-rated composite variable and mother-rated composite variable was significant at p<0.01, indicating that the error terms were significantly correlated. As a result, this relationship was controlled for in the model. This model was an excellent fit to the data (χ^2 (df=1, N=262)=0.90, p=0.34; CFI= 1.00; RMSEA= 0.00 with a 90% CI of 0.00-0.16) and indicated that child-rated measures of mother-daughter relationship quality significantly associated with lifetime eating disorder severity (β=0.18, p<0.01) whereas mother-rated measures of mother-daughter relationship quality associated with lifetime substance use disorder severity (β=0.27, p<0.01). No other associations were significant.

Genotype and Child Disorder Outcomes

To test the second hypothesis that the A118G polymorphism will be positively associated with lifetime severity of eating disorders and negatively associated with the lifetime severity of substance use disorders, a SEM model was created with the G-allele predicting to both of these

outcomes. Based on fit indices, this model was a good fit to the data: χ^2 (*df*=3, N=262)=3.40, *p*=0.33; CFI= 0.98; RMSEA= 0.02 with a 90% CI of 0.00-0.11.

This model demonstrated that the presence of the G-allele was significantly associated with eating disorder severity (β=0.14, *p*=0.03), as hypothesized, but was not associated with substance use severity (β=-0.07, *p*=0.26).

Mother-Daughter Relationship, Genotype, and Child Disorder Outcomes

The third study aim sought to explore the interplay between the mother-daughter relationship variables, genotype, and lifetime severity of eating and substance use disorders. As the latent variable containing both mother and child ratings of mother-daughter relationship quality did not significantly associate with eating disorder severity, the gene-latent variable environment interaction was only tested for substance use disorder severity by exporting the latent scores and creating an interaction term with the genotype. Linear regressions revealed that there was no gene-environment interaction effect on substance use disorder severity (*p*=0.21).

We also tested for gene-environment effects using the separate mother-report and child-report measures. Specifically, we examined child-reported measures of relationship quality in interaction with the A118G polymorphism in the prediction of eating disorder severity and mother-reported measures of relationship quality in interaction with the A118G polymorphism in the prediction of substance abuse disorder severity. Linear regression analyses revealed that G-allele did not interact with the child-report composite variable to predict eating disorder severity (*p*=0.42). Similarly, the mother-report composite variable did not interact with the G-allele to predict substance use disorder severity (*p*=0.47). Therefore, these findings do not support a gene-by-environment association.

Supplemental Analyses of Independent Effects of Genetic and Environmental Predictors

Subsequently, we sought to test whether the A118G polymorphism and the mother-daughter relationship quality latent variable served as independent predictors of the outcomes of interest (see Figure 5). Statistical fitness indices were again mixed with regard to the model fit: χ^2 (*df*=18, N=262)=30.82, *p*=0.03; CFI= 0.95; RMSEA= 0.05 with a 90% CI of 0.02-0.08. Given the non-normal distribution of the disorder outcomes, more emphasis was given to the goodness-of-fit indices not dependent on assumptions of normality, which suggest that the model had acceptable fit.

Taking both predictors into account, the G-allele continued to significantly associate with eating disorder severity (β=0.14, *p*=0.02) but not to substance use disorder severity (*p*=0.368), and the latent variable continued to relate to substance use disorder severity (β=0.36, *p*<0.01) but not to eating disorder severity (*p*=0.28). Therefore, all relationships held when controlling for the other predictors of interest.

We also tested independent effects of genetic and environmental factors in our alternative model that separated mother and child-reports of mother-daughter relationship quality (see Figure 6). The model was an excellent fit to the data: χ^2 (*df*=3, N=262)=2.68, *p*=0.44; CFI= 1.00; RMSEA= 0.00 with a 90% CI of 0.00-0.10. The results of this model indicated that the G-allele continued to significantly relate to eating disorder severity (β=0.13, *p*=0.03) but not to substance use disorder severity (β=-.06, *p*=0.35). The composite variable of the child-report measures continued to relate to lifetime eating disorder severity (β=0.18, *p*<0.01), and the composite variable of the mother-report measures continued to relate to lifetime substance use severity (β=0.26, *p*<0.01). Again, all relationships held when controlling for the other predictors of interest.

Discussion

In this study, the relationship between a polymorphism of the *OPRM1* receptor gene, mother-daughter relationship quality, and adolescent eating disorder and substance use disorder severity was evaluated. Our study is one of the first of its kind to evaluate substance use and eating disorders within the same gene-environment theoretical model and to directly compare the effects of possible differential activation associated with the *OPRM1* receptor gene in a human sample. The study findings provide insight into a potential mechanism through which adolescents may be genetically at risk for developing specific mental health disorders.

Mother-Daughter Relationship and Child Disorder Outcomes

A large body of literature demonstrates that an adverse parent-child relationship is a risk factor for both substance abuse and eating disorders (Attie & Brooks-Gunn, 1989; Benninghoven et al., 2007; Brody & Forehand, 1993; Stice & Barrera, 1995). The current study utilized data from a variety of mother and child-report measures on the mother-daughter relationship. Contrary to expectation, none of our mother-daughter relationship variables were significantly associated with both substance use and eating disorder outcomes. However, we did find that the child's perception of the mother-daughter relationship was particularly important in predicting lifetime eating disorder severity, while the mother's perception was especially important in predicting lifetime substance use disorder severity. In addition, our findings demonstrated that a latent measure of mother-daughter relationship quality, which represented the convergence of mother-report and child-report measures, does not predict to eating disorder severity. Thus, not only is the child's perception particularly important, but it is specifically the components of the child's perceptual experience that do *not* overlap with those experienced by the mother that appeared to uniquely predict to eating disorder severity.

This finding can be interpreted in two ways. First, it is possible that the areas at which the child-report and mother-report measures converge represent the more objective and apparent instances of an adverse mother-daughter relationship. In this sense, the adolescent may be having some perceptual experience, which relates specifically to eating disorder severity, that is unique to their way of viewing the world and is specifically captured in the child-report measure. This interpretation suggests that there is some unobservable component of the mother-daughter relationship that is felt by the adolescent and not experienced externally by the mother, though it might also suggest that the youth's perception is biased or incorrect in some way. Alternatively, it is possible that the cases in which only the adolescent's report relates to eating disorder severity are the cases in which the mother is unaware of the state of the mother-daughter relationship. In this sense, maybe it is specifically the mother's lack of awareness surrounding the relationship that predicts to eating disorder severity.

Overall, the findings suggest that more attention should be given to the daughter's report of mother-daughter relationship quality when evaluating risk for eating disorders. For example, clinicians might want to focus on the child's perception of the mother daughter relationship, particularly in cases where maternal and child perceptions differ. In addition, if a plan of action in treating an adolescent's eating disorder involves creating a more supportive family environment, it will be important to assess how the adolescent herself perceives any changes that occur in the family environment.

Both maternal perceptions of maternal-child relationship quality, and the convergence of maternal and child perceptions of their relationship predict to substance use disorder severity in adolescence and young adulthood. This finding is consistent with the literature linking mother-child relationship quality and the outcome of substance use problems. It may be that substance

use problems and their precursors are less "hidden" than eating disorder pathology and therefore put an earlier strain on the mother-daughter relationship, which is then captured in ratings during the adolescent phase of development.

The findings of the current study somewhat contradict the existing literature on maternal versus child perception of the mother-child relationship and child mental disorders. For example, Attie and Brooks-Gunn (1989) remarked that, when compared to the adolescent's report of family cohesion and communication styles, the mother's report had a greater relationship with the adolescent's eating disorder behavior. It is possible that there was something specific to the measures used in the current study that differed from past studies. This interpretation seems likely given that there was no overlap between the measures used to assess mother-daughter relationship in the current study and those used in previous studies investigating either eating disorders or substance use disorders. Further, past studies used alternative methods for evaluating the severity of the outcomes. For example, Benninghoven and colleagues (2007) assessed eating disorder behavior among adolescents with a self-report questionnaire measuring body image and desired body size, and Stice and Barrera (1995) assessed substance use disorder behavior with a self-report questionnaire measuring quantity and frequency of substance use in the past three months. Our diagnostic measure of eating and substance use disorders may have reflected more severe psychopathology than these self-report measures.

Genotype and Child Disorder Outcomes

As supported by the extant body of literature, the presence of the *OPRM1* gene G-allele significantly associated with increased lifetime severity of eating disorders; however, the G-allele did not significantly associate with substance use disorder severity. Past studies are mixed regarding the behavior of the G-allele with exogenous opioids such that some studies report a

negative association (Bond et al., 1998; Mague et al., 2009), and others report a lack of significance in either direction (Coller et al., 2009; Franke et al., 2001). Therefore, our findings concerning the G-allele relationship with substance use outcomes align with some of the previous studies in terms of the noted lack of association. Given the inconsistency in the field, it may be that other genetic or environmental variables not assessed in the current study moderate the relationship between this polymorphism and substance use disorders. Future studies should investigate other environmental variables, such as peer relations or past trauma, that might be relevant for these outcomes.

Interestingly, the G-allele polymorphism of the *OPRM1* gene remains a significant predictor of eating disorder severity even when accounting for the impact of an adverse mother-daughter relationship. This suggests that biological influences may have a substantial role in the severity of eating disorders. Specifically, when evaluating associations with eating disorder severity, the effect size of the gene (β=0.14) was comparable to the effect size of an adverse mother-daughter relationship (β=0.18), implying that the gene may have a similar degree of impact on eating disorder severity as the environmental risk factor. This finding aligns with the current literature in supporting the contribution of biological risk factors in the development and severity of eating disorders. The significant associations found linking both the Brain-derived neurotropic factor Val66Met and disturbances in serotonin activity with eating disorder symptomology provide evidence for this contribution (Gratacòs et al., 2007; Kaye, Gendall, & Strober, 1998).

The findings of this study are preliminary and in need of replication. Only a small subsample of our study participants was characterized by the G-allele (n=64), and an even smaller subsample exhibited a lifetime eating disorder severity greater than zero (n=32). While

this study was able to demonstrate a moderate relationship between the gene and lifetime eating disorder severity, future studies should work to see if the effect size continues to be seen at this level in larger samples of adolescents and young adults.

Mother-Daughter Relationship, Genotype, and Child Disorder Outcomes

The results of the current study demonstrated that the A118G polymorphism and adverse mother-daughter relationship quality were independent predictors of eating disorder severity. Contrary to what was hypothesized, there was no gene-environment interaction between this gene and the environmental risk factor. While more studies are needed to clarify the impact of our limited sample size and evaluate alternative risk factors, it is possible that this environmental risk factor and the polymorphism of the *OPRM1* gene are actually independent in their effects.

Given this interpretation, there are likely many clinical implications associated with this finding. The finding that the presence of the gene alone predicts to elevated severity of eating disorders allows us to better understand the complexity and development of the brain and associated behavior while also inspiring greater research into the genetic antecedents of mental illness and patterns of heritability within families. While an adverse mother-daughter relationship did not strengthen the relationship between the gene and the severity of eating disorders in this study, there may be other variables that either strengthen or weaken the observed relationship. Thus, future studies should investigate potential moderating environmental risk and protective factors that might attenuate the effects observed in this study. In addition, given the apparent importance of the mesoaccumbens dopamine pathway in the severity of this outcome, eating disorder treatments should continue to explore how this reward pathway can be targeted for both prevention and intervention.

Limitations and Future Directions

The current study did not differentiate between different types of eating disorders or different types of substance use disorders. Although the existing literature cites the impact of an adverse mother-daughter relationship in the development of all subtypes of eating disorders (Benninghoven et al., 2007), some studies suggest that this effect may be particularly pertinent in individuals with bulimia nervosa compared to those with anorexia nervosa or other eating disorders (Humphrey, 1986; Kim, 1998). Similarly, while behaviors related to both anorexia nervosa and bulimia nervosa result in a release of endogenous opioids (Marrazzi et al., 1997), this release may be especially important in the severity and continuation of bulimia nervosa (Hadad & Knackstedt, 2014). In the current study, the sample size of individuals who displayed bulimia nervosa symptomology (n=6) was too small to investigate independently as an exploratory hypothesis; however, future studies would benefit from using a clinical sample from which eating disorder specific effects can be determined.

Correspondingly, the lumping together of all substance use disorders into one category is of additional concern. As with eating disorders, all substance use disorders interact to some degree with the mesoaccumbens dopamine pathway, which is influenced by the mu-opioid receptor system. What is different, however, is the extent to which each substance interacts directly with mu-opioid receptors to incite the reward pathway. While other substances activate the mu-opioid receptors indirectly, only exogenous opioids, such as heroin and other opiates, plug directly into these receptors (Contet et al., 2004). Further, Gianoulakis (2004) suggests that other substances might activate the mu-opioid receptors by releasing endogenous opioid peptides. Taken together with the current findings, the inclusion of other drug classes may have washed out any association between exogenous opioids and the gene, thus explaining the lack of

association found in the current study. Interestingly, the past studies cited in this paper that found nonsignificant effects were those that included other drug classes in their analyses (Coller et al., 2009; Franke et al., 2001). In contrast, studies that found a significant relationship between substance use and the gene were those that only evaluated opioid use (Bond et al., 1998; Mague et al., 2009). The inclusion of only opiate users and individuals with bulimia nervosa in a model assessing the respective associations with the A118G polymorphism would clarify the extent to which our own findings were attenuated by the addition of other forms of the relevant disorders.

The current study had several other important methodological limitations. First, ratings of eating disorder and substance use disorder severity relied predominantly on self-reports by the youth during structured clinical interviews. As with all self-report measures, there is a chance that participants did not respond honestly, thus leading to either the overreporting or underreporting of a particular phenomenon based on what the participant views as the socially desirable response (Hebert, Clemow, Pbert, Ockene, & Ockene, 1995). Underreporting is of particular concern in the present study given the commonly experienced emotions of shame associated with eating disorders and the illegality concerns associated with substance use disorders (Shillington & Clapp, 2000; Swan & Andrews, 2003). In a clinical study seeking to understand the impact of self-report bias, Swan and Andrews (2003) found that 42% of individuals with eating disorder symptomology reported non-disclosure during clinical interviews or treatment. Similarly, Shillington and Clapp (2000) revealed that older adolescents were significantly less likely to report illegal substance use when compared to cigarette use and alcohol use. While current methods of clinical assessment are largely restricted to self-report measures, future studies should gather data on eating disorder and substance use disorder symptomology from multiple sources to avoid potentially biased reporting.

Attrition may have also impacted our results. In the Mater-University Study of Pregnancy, the study from which our sample was derived, genotype data was gathered as an add-on to the original paradigm. Due to attrition, DNA data were unavailable for 101 female participants who had provided parenting and diagnostic data. Although our sample size (N=262) is considered adequate for SEM analyses (Iacobucci, 2010), a larger sample size may have allowed for additional analyses that were not feasible in the current study. Specifically, multiple-group analyses, which were not possible with our sample size, would have allowed us to more efficiently evaluate a gene-environment interaction between an adverse mother-daughter relationship and the G-allele.

Given the finding that the A118G polymorphism plays a significant role in the lifetime severity of eating disorders, future studies should investigate what other types of behaviors are impacted by the endogenous reward system and how this system influences the development, progression, or prognosis of other mental illnesses. Extending beyond eating disorders, studies should examine whether other behaviors that release endogenous opioids are affected by the G-allele in a similar way. Although the impact observed in the current study was deleterious, it is also possible that the presence of the G-allele may be beneficial to an individual by helping to motivate positive or productive behaviors that also engage the endogenous reward pathway.

In addition, future studies should investigate how the endogenous reward system influences other brain mechanisms involved in mental illnesses. For example, past studies suggest that variants in the *OPRM1* gene might be related to the function of the HPA axis in that individuals with the A118G variant exhibit a lower cortisol response to stress (Chong et al., 2006; Pratt & Davidson, 2009). Overall, future studies should investigate how the endogenous reward pathway influences other areas of the brain also relevant to mental disorder outcomes.

Although the findings of the current study are in need of replication, they suggest that future research may benefit by continuing to uncover the mechanisms involved in differential susceptibility to eating disorders versus substance use disorders. Studies that incorporate both gene and environmental influences may be particularly informative in understanding the development of these mental disorders, and will allow us to continue to investigate which variables set an individual on a path to one outcome over another.

References

Attie, I., & Brooks-Gunn, J. (1989). Development of eating problems in adolescent girls: A longitudinal study. *Developmental Psychology*, *25*(1), 70–79. http://doi.org/10.1037/0012-1649.25.1.70

Benninghoven, D., Tetsch, N., Kunzendorf, S., & Jantschek, G. (2007). Body image in patients with eating disorders and their mothers, and the role of family functioning. *Comprehensive Psychiatry*, *48*(2), 118–23. http://doi.org/10.1016/j.comppsych.2006.08.003

Bond, C., LaForge, K. S., Tian, M., Melia, D., Zhang, S., Borg, L., Yu, L. (1998). Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(16), 9608–9613. http://doi.org/10.1073/pnas.95.16.9608

Braucht, G. N., Brakarsh, D., Follingstad, D., & Berry, K. L. (1973). Deviant drug use in adolescence: A review of psychosocial correlates. *Psychological Bulletin*, *79*(2), 92–106.

Brennan, P. A., Le Brocque, R., & Hammen, C. (2003). Maternal depression, parent-child relationships, and resilient outcomes in adolescence. *Journal of the American Academy of Child and Adolescent Psychiatry*, *42*(12), 1469–77. http://doi.org/10.1097/00004583-200312000-00014

Brody, G. H., & Forehand, R. (1993). Prospective associations among family form, family processes, and adolescents' alcohol and drug use. *Behaviour Research and Therapy*, *31*(6), 587–593. http://doi.org/10.1016/0005-7967(93)90110-G

Brook, J. S., Brook, D. W., De La Rosa, M., Duque, L. F., Edgar, R., Montoya, I., & Whiteman, M. (1998). Pathways to Marijuana Use Among Adolescents: Cultural/Ecological, Family,

Peer, and Personality Influences. *Journal of the American Academy of Child and Adolescent Psychiatry*, *37*(7), 759 – 766. http://doi.org/10.1097/00004583-199807000-00016

Chong, R. Y., Oswald, L., Yang, X., Uhart, M., Lin, P.-I., & Wand, G. S. (2006). The Mu-Opioid Receptor Polymorphism A118G Predicts Cortisol Responses to Naloxone and Stress. *Neuropsychopharmacology, 31,* 204-211.

Clark, S., & Coker, S. (2009). Perfectionism, self-criticism and maternal criticism: A study of mothers and their children. *Personality and Individual Differences*, *47*(4), 321–325. http://doi.org/10.1016/j.paid.2009.03.020

Coller, J. K., Beardsley, J., Bignold, J., Li, Y., Merg, F., Sullivan, T., Somogyi, A. A. (2009). Lack of association between the A118G polymorphism of the mu opioid receptor gene (OPRM1) and opioid dependence: A meta-analysis. *Pharmacogenomics and Personalized Medicine*, *2*, 9–19.

Contet, C., Kieffer, B. L., & Befort, K. (2004). Mu opioid receptor: a gateway to drug addiction. *Current Opinion in Neurobiology*, *14*(3), 370–8. http://doi.org/10.1016/j.conb.2004.05.005

Conway, C. C., Hammen, C., & Brennan, P. a. (2014). Adolescent Precursors of Adult Borderline Personality Pathology in a High-Risk Community Sample. *J Pers Disord*, *29*(3), 1–18. http://doi.org/10.1521/pedi_2014_28_158

Davis, C. (2015). The epidemiology and genetics of binge eating disorder (BED). *CNS Spectrums*, (SEPTEMBER), 1–8. http://doi.org/10.1017/S1092852915000462

Davis, C. a., Levitan, R. D., Reid, C., Carter, J. C., Kaplan, A. S., Patte, K. a., Kennedy, J. L. (2009). Dopamine for "Wanting " and Opioids for "Liking": A Comparison of Obese Adults With and Without Binge Eating. *Obesity*, *17*(6), 1220–1225. http://doi.org/10.1038/oby.2009.52

Davis, C., & Claridge, G. (1998). The eating disorders as addiction. *Addictive Behaviors*, *23*(4), 463–475. http://doi.org/10.1016/S0306-4603(98)00009-4

Davis, C., & Loxton, N. (2014). A Psycho-Genetic Study of Hedonic Responsiveness in Relation to "Food Addiction." *Nutrients*, *6*(10), 4338–4353. http://doi.org/10.3390/nu6104338

Deb, I., Chakraborty, J., Gangopadhyay, P. K., Choudhury, S. R., & Das, S. (2010). Single-nucleotide polymorphism (A118G) in exon 1 of OPRM1 gene causes alteration in downstream signaling by mu-opioid receptor and may contribute to the genetic risk for addiction. *Journal of Neurochemistry*, *112*(2), 486–96. http://doi.org/10.1111/j.1471-4159.2009.06472.x

Franke, P., Wang, T., Nöthen, M. M., Knapp, M., Neidt, H., Albrecht, S., Maier, W. (2001). Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. *American Journal of Medical Genetics*, *105*(1), 114–119. http://doi.org/10.1002/1096-8628(20010108)105:1<114::AID-AJMG1074>3.0.CO;2-L [pii]

Gratacòs, M., González, J. R., Mercader, J. M., de Cid, R., Urretavizcaya, M., & Estivill, X. (2007). Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. *Biological Psychiatry*, *61*(7), 911–22. http://doi.org/10.1016/j.biopsych.2006.08.025

Hadad, N. a., & Knackstedt, L. a. (2014). Addicted to palatable foods: comparing the neurobiology of Bulimia Nervosa to that of drug addiction. *Psychopharmacology*, *231*(9), 1897–1912. http://doi.org/10.1007/s00213-014-3461-1

Hammen, C., Brennan, P. a., & Keenan-Miller, D. (2008). Patterns of Adolescent Depression to Age 20: The Role of Maternal Depression and Youth Interpersonal Dysfunction. *Journal of Abnormal Child Psychology*, *36*(8), 1189–1198. http://doi.org/10.1007/s10802-008-9241-9

Haworth-Hoeppner, S. (2000). The critical shapes of body image: The role of culture and family in the production of eating disorders. *Journal of Marriage and the Family*, *62*(1), 212–227. http://doi.org/10.1111/j.1741-3737.2000.00212.x

Hebert, J. R., Clemow, L., Pbert, L., Ockene, I. S., & Ockene, J. K. (1995). Social Desirability Bias in Dietary Self-Report May Compromise the Validity of Dietary Intake Measures. *International Journal of Epidemiology*, *24*(2), 389–398. http://doi.org/10.1093/ije/24.2.389

Hu, L., & Bentler, P. M. (1999). Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Structural Equation Modeling: A Multidisciplinary Journal*, *6*(1), 1–55. http://doi.org/10.1080/10705519909540118

Humphrey, L. L. (1986). Structural analysis of parent-child relationships in eating disorders. *Journal of Abnormal Psychology*, *95*(4), 395–402.

Humphreys, K. L., Katz, S. J., Lee, S. S., Hammen, C., Brennan, P. a., & Najman, J. M. (2013). The association of ADHD and depression: Mediation by peer problems and parent–child difficulties in two complementary samples. *Journal of Abnormal Psychology*, *122*(3), 854–867. http://doi.org/10.1037/a0033895

Iacobucci, D. (2010). Structural equations modeling: Fit Indices, sample size, and advanced topics. *Journal of Consumer Psychology*, *20*(1), 90–98. http://doi.org/10.1016/j.jcps.2009.09.003

Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P., Ryan, N. (1997). Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime

Version (K-SADS-PL): initial reliability and validity data. *Journal of the American Academy of Child and Adolescent Psychiatry*, *36*(7), 980–8. http://doi.org/10.1097/00004583-199707000-00021

Kaye, W., Gendall, K., & Strober, M. (1998). Serotonin neuronal function and selective serotonin reuptake inhibitor treatment in anorexia and bulimia nervosa. *Biological Psychiatry*, *44*(9), 825–838. http://doi.org/10.1016/S0006-3223(98)00195-4

Kichler, J. C., & Crowther, J. H. (2001). The effects of maternal modeling and negative familial communication on women's eating attitudes and body image. *Behavior Therapy*, *32*(3), 443–457. http://doi.org/10.1016/S0005-7894(01)80030-7

Kim, S. (1998). Opioid antagonists in the treatment of impulse-control disorders. *The Journal of Clinical Psychiatry*, *59*(4), 159–164. http://doi.org/10.4088/JCP.v59n0403

Krahn, D. D. (1991). The relationship of eating disorders and substance abuse. *Journal of Substance Abuse*, *3*(2), 239–253. http://doi.org/10.1016/S0899-3289(05)80039-2

Lesieur, H. R., & Blume, S. B. (1993). Pathological gambling, eating disorders, and the psychoactive substance use disorders. *Journal of Addictive Diseases*, *12*(3), 89–102. http://doi.org/10.1300/J069v12n03_08

Lock, J., & Le Grange, D. (2001). Can family-based treatment of anorexia nervosa be manualized? *The Journal of Psychotherapy Practice and Research*, *10*(4), 253–261.

Mague, S. D., Isiegas, C., Huang, P., Liu-Chen, L.-Y., Lerman, C., & Blendy, J. A. (2009). Mouse model of OPRM1 (A118G) polymorphism has sex-specific effects on drug-mediated behavior. *Proceedings of the National Academy of Sciences*, *106*(26), 10847–10852. http://doi.org/10.1073/pnas.0901800106

Marrazzi, M. A., Luby, E. D., Kinzie, J., Munja, I. D., & Spector, S. (1997). Endogenous Codeine and Morphine in Anorexia and Bulimia Nervosa, *60*(20), 1741–1747.

Mathers, C. D., Vos, E. T., Stevenson, C. E., & Begg, S. J. (2000). The Australian Burden of Disease Study: measuring the loss of health from diseases, injuries and risk factors. *The Medical Journal of Australia*, *172*(12), 592–6.

Miranda, R., Ray, L., Justus, A., Meyerson, L. A., Knopik, V. S., McGeary, J., & Monti, P. M. (2010). Initial evidence of an association between OPRM1 and adolescent alcohol misuse. *Alcoholism, Clinical and Experimental Research*, *34*(1), 112–22. http://doi.org/10.1111/j.1530-0277.2009.01073.x

Olsen, M. B., Jacobsen, L. M., Schistad, E. I., Pedersen, L. M., Rygh, L. J., Roe, C., & Gjerstad, J. (2012). Pain Intensity the First Year after Lumbar Disc Herniation Is Associated with the A118G Polymorphism in the Opioid Receptor Mu 1 Gene: Evidence of a Sex and Genotype Interaction. *Journal of Neuroscience*, *32*(29), 9831–9834. http://doi.org/10.1523/JNEUROSCI.1742-12.2012

Polivy, J., & Herman, C. P. (2002). Causes of Eating Disorders. *Young*, 187–213.

Pratt, W. M., & Davidson, D. (2009). Role of the HPA axis and the A118G polymorphism of the mu-opioid receptor in stress-induced drinking behavior. *Alcohol and Alcoholism (Oxford, Oxfordshire)*, *44*(4), 358–65. http://doi.org/10.1093/alcalc/agp007

Raskin, A., Boothe, H., Reatig, N., Schulterbrandt, J., & Odle, D. (1971). Factor Analyses of Normal and Depressed Patients' Memories of Parental Behavior. *Psychological Reports*, *29*, 871–879.

Safford, S. M., Alloy, L. B., & Pieracci, A. (2007). A comparison of two measures of parental behavior. *Journal of Child and Family Studies*, *16*(3), 375–384. http://doi.org/10.1007/s10826-006-9092-3

Schermelleh-Engel, K., Moosbrugger, H., & Müller, H. (2003). Evaluating the Fit of Structural Equation Models : Tests of Significance and Descriptive Goodness-of-Fit Measures. *Methods of Psychological Research Online*, *8*(2), 23–74. http://doi.org/10.1002/0470010940

Schinke, S. P., Fang, L., & Cole, K. C. A. (2008). Substance use among early adolescent girls: risk and protective factors. *The Journal of Adolescent Health : Official Publication of the Society for Adolescent Medicine*, *43*(2), 191–4. http://doi.org/10.1016/j.jadohealth.2007.12.014

Schwartz, C. E., Dorer, D. J., Beardslee, W. R., Lavori, P. W., & Keller, M. B. (1990). Maternal expressed emotion and parental affective disorder: Risk for childhood depressive disorder, substance abuse, or conduct disorder. *Journal of Psychiatric Research*, *24*, 231–250. http://doi.org/10.1016/0022-3956(90)90013-G

Shillington, A. M., & Clapp, J. D. (2000). Self-report stability of adolescent substance use: are there differences for gender, ethnicity and age? *Drug and Alcohol Dependence*, *60*(1), 19–27. http://doi.org/10.1016/S0376-8716(00)80004-6

Simons, R. L., & Robertson, J. F. (1989). The impact of parenting factors, deviant peers, and coping style upon adolescent drug use. *Family Relations*, *38*(3), 272–281. http://doi.org/10.2307/585052

Slavich, G. M., Tartter, M. a., Brennan, P. a., & Hammen, C. (2014). Endogenous opioid system influences depressive reactions to socially painful targeted rejection life events. *Psychoneuroendocrinology*, *49*, 141–149. http://doi.org/10.1016/j.psyneuen.2014.07.009

Stice, E., & Barrera, M. (1995). A longitudinal examination of the reciprocal relations between perceived parenting and adolescents' substance use and externalizing behaviors. *Developmental Psychology*, *31*(2), 322–334. http://doi.org/10.1037/0012-1649.31.2.322

Stoneman, Z., Brody, G. H., & Burke, M. (1989). Marital quality, depression, and inconsistent parenting: Relationship with observed mother-child conflict. *American Journal of Orthopsychiatry*, *59*(1), 105–117.

Striegel-Moore, R. H., Silberstein, L. R., & Rodin, J. (1986). Toward an understanding of risk factors for bulimia. *American psychologist, 41*(3), 246.

Swan, S., & Andrews, B. (2003). The relationship between shame, eating disorders and disclosure in treatment. *The British Journal of Clinical Psychology / the British Psychological Society*, *42*(Pt 4), 367–78. http://doi.org/10.1348/014466503322528919

Thompson, R., & Zuroff, D. C. (1999). Development of Self-Criticism in Adolescent Girls: Roles of Maternal Dissatisfaction, Maternal Coldness, and Insecure Attachment. *Journal of Youth and Adolescence*, *28*(2), 197–210. http://doi.org/10.1023/A:10216014312

Wolfe, W. L., & Maisto, S. A. (2000). The relationship between eating disorders and substance use. *Clinical Psychology Review*, *20*(5), 617–631. http://doi.org/10.1016/S0272-7358(99)00009-4

Ziauddeen, H., Chamberlain, S. R., Nathan, P. J., Koch, a, Maltby, K., Bush, M., Bullmore, E. T. (2013). Effects of the mu-opioid receptor antagonist GSK1521498 on hedonic and

consummatory eating behaviour: a proof of mechanism study in binge-eating obese subjects. *Molecular Psychiatry*, *18*(12), 1287–1293. http://doi.org/10.1038/mp.2012.154

Table 1

Bivariate Correlations for mother-daughter relationship and lifetime severity of eating and substance use disorders.

	1	2	3	4	5	6
1. Mother-reported maternal criticism						
2. Mother-reported parent-child relationship stress	.38**					
3. Child-reported maternal psychological control	.23**	.26**				
4. Child-reported maternal hostility	.25**	.45**	.57**			
5. Child-reported maternal rejection	.26**	.41**	.39**	.79**		
6. Lifetime eating disorder severity	.07	.02	.17**	.13*	.07	
7. Lifetime substance use disorder severity	.16*	.31**	.11	.10	.02	.07

Note. *$p < .05$; **$p < .01$

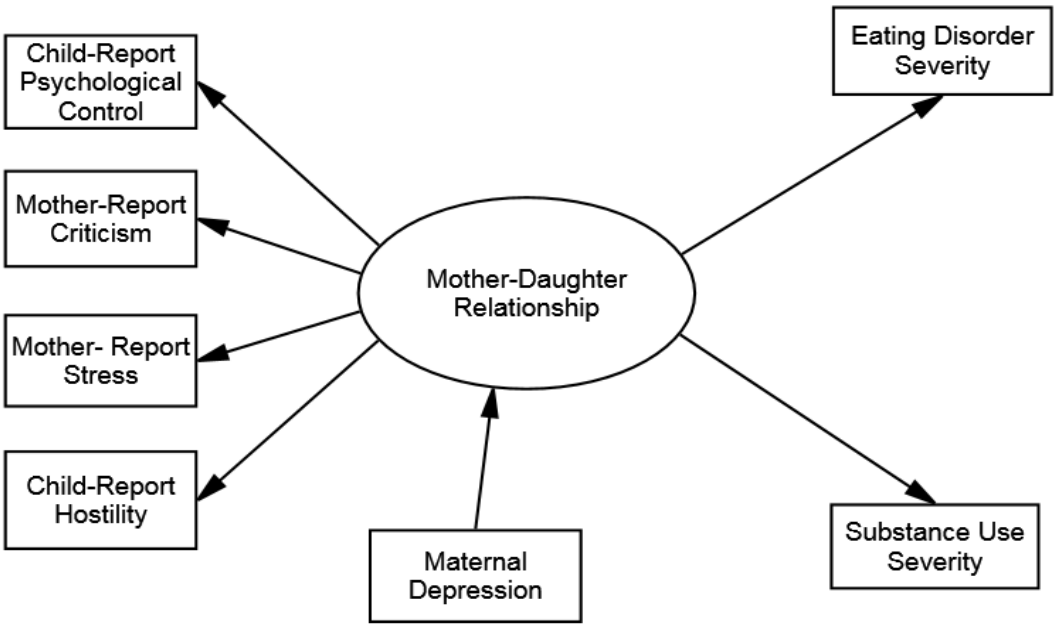

Figure 1. Conceptual model predicting lifetime severity of eating disorders and substance use disorders from a latent variable for adverse mother-daughter relationship.

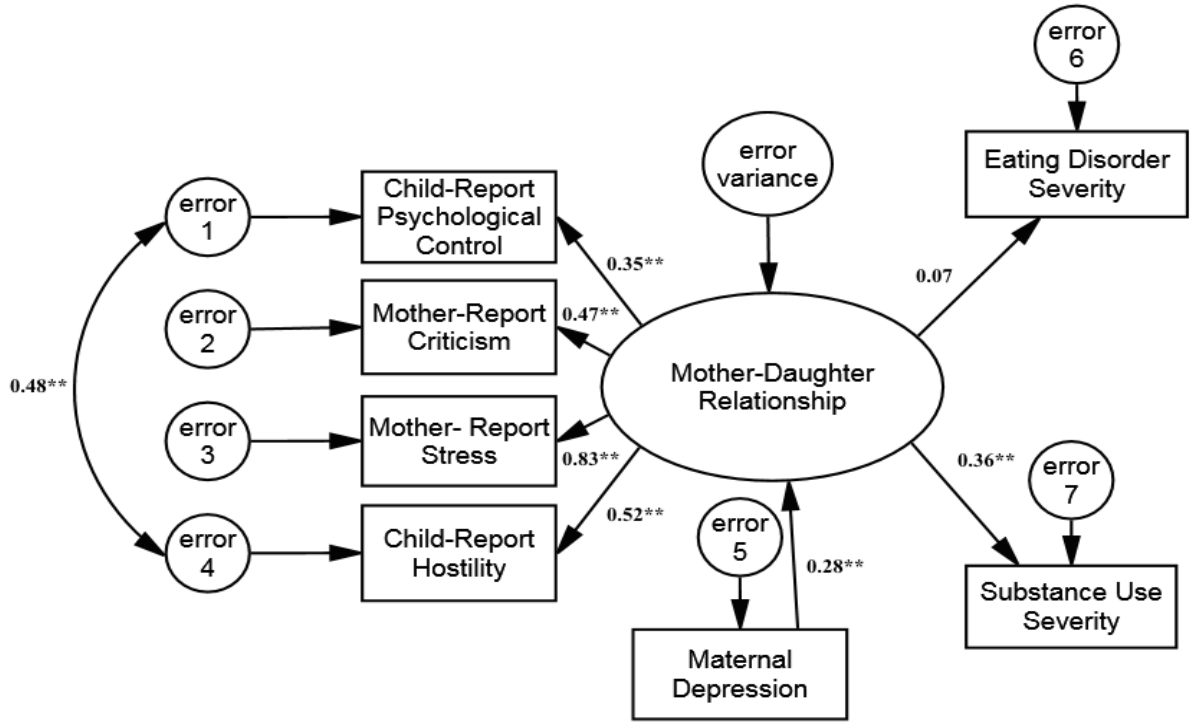

Figure 2. Structural equation modeling predicting lifetime severity of eating disorders and substance use disorders from a latent variable for adverse mother-daughter relationship. *p<0.05; **p<0.01.

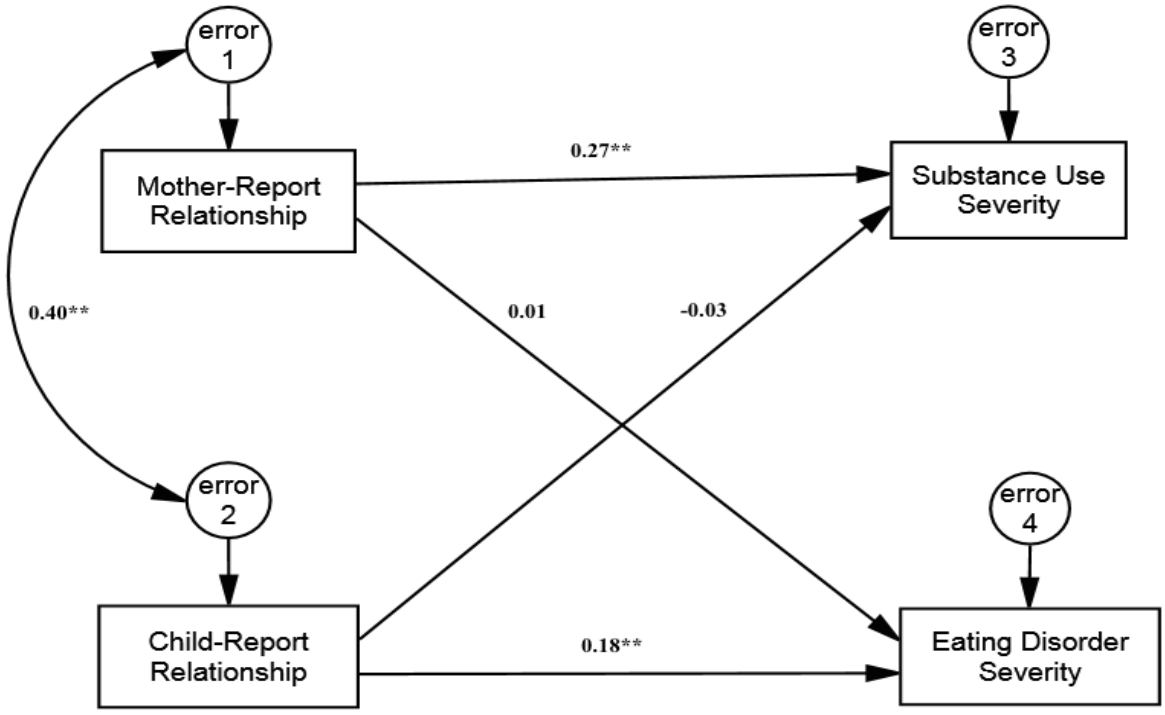

Figure 3. Path analysis independently predicting lifetime severity of eating disorders and substance use disorders from a composite child-report variable and a composite mother-report variable. *p<0.05; **p<0.01.

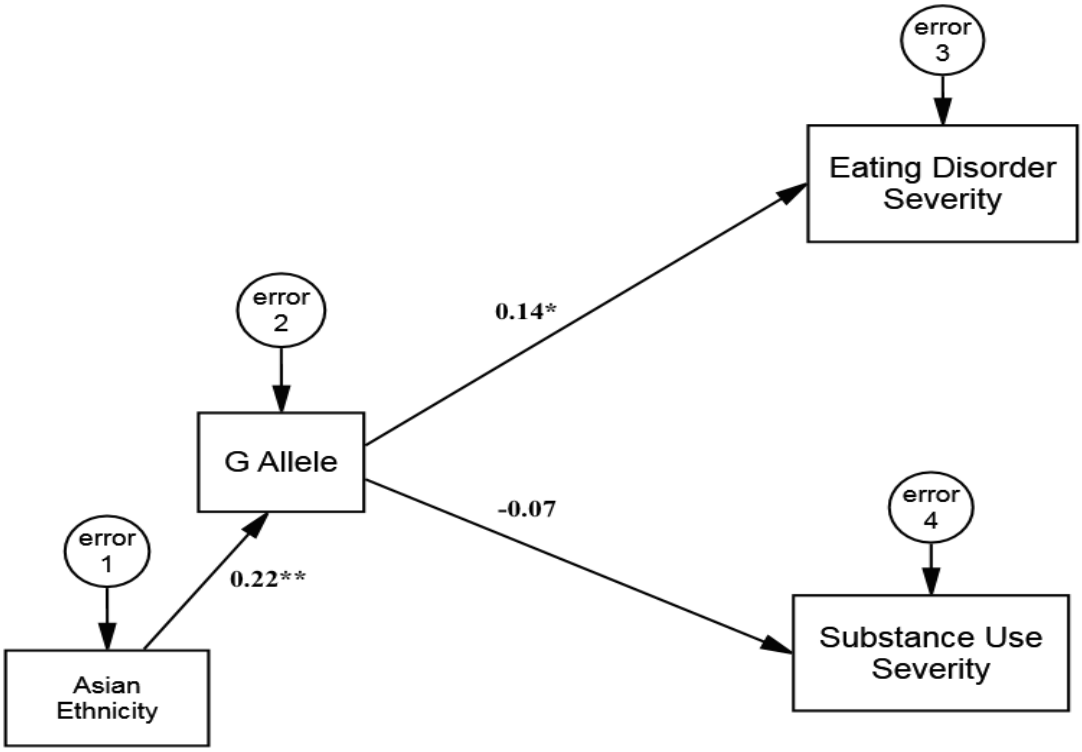

Figure 4. Path analysis predicting lifetime severity of eating disorders and substance use disorders from the presence of the G-allele. *p<0.05; **p<0.01.

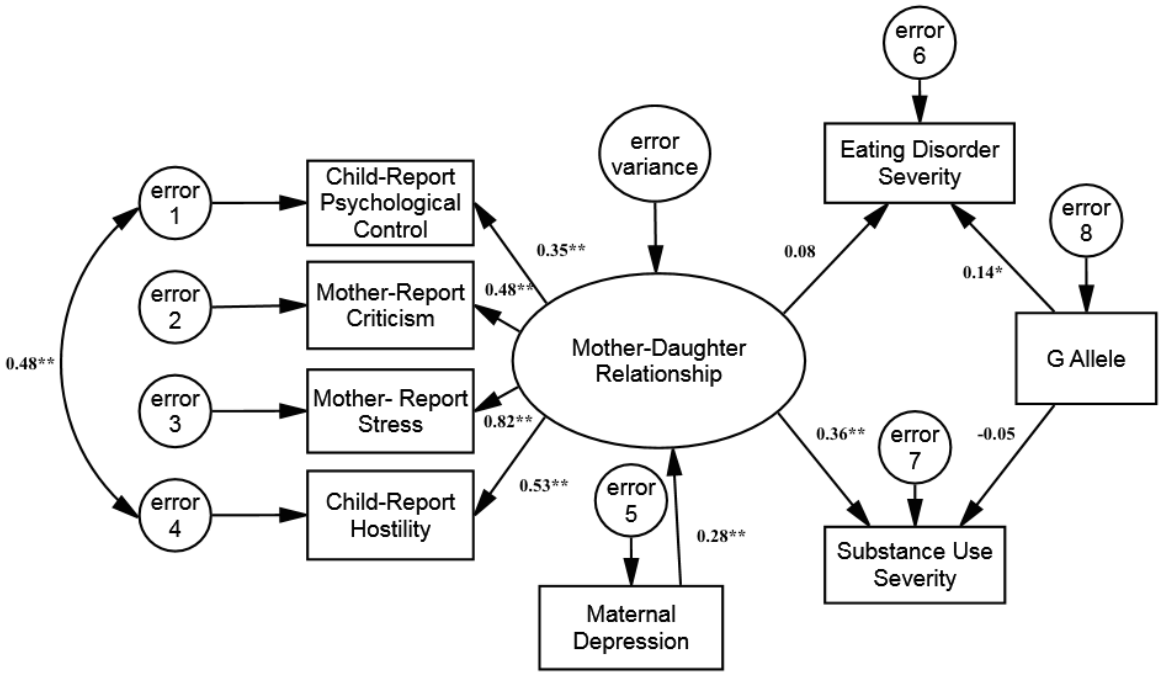

Figure 5. Structural equation modeling independently predicting lifetime severity of eating disorders and substance use disorders from a latent variable for adverse mother-daughter relationship and the presence of the G-allele. **p*<0.05; ***p*<0.01.

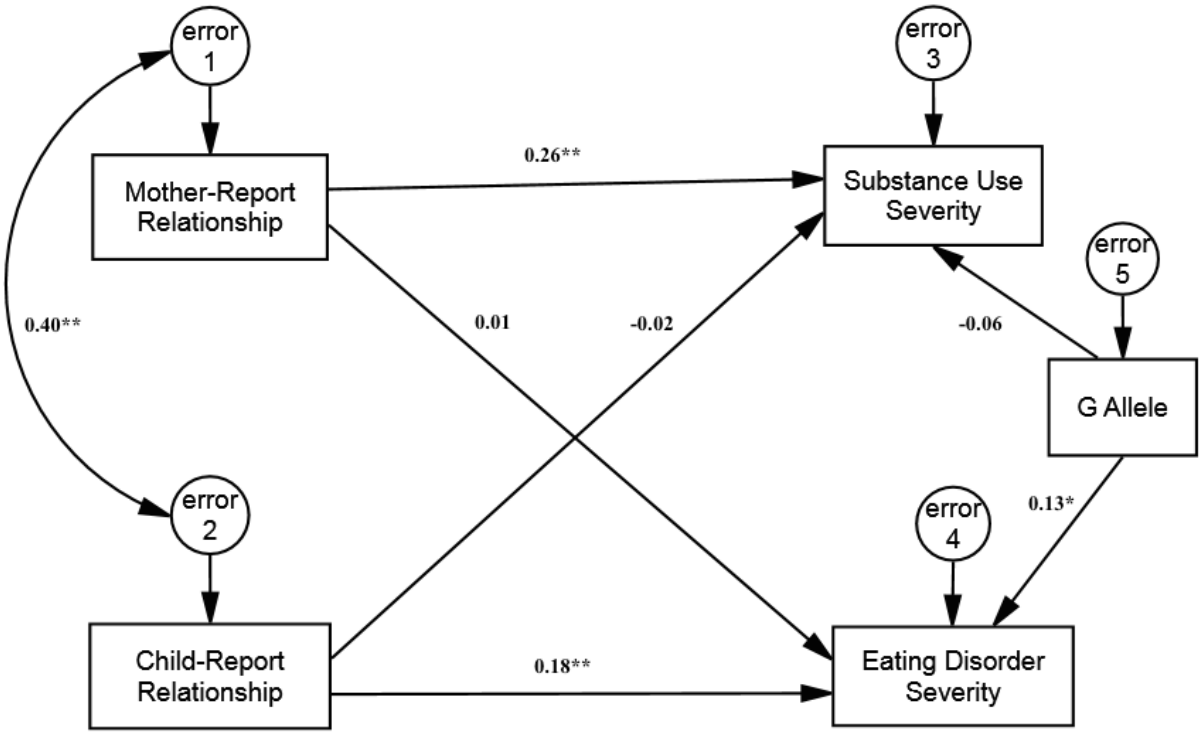

Figure 6. Path analysis independently predicting lifetime severity of eating disorders and substance use disorders from a composite child-report variable, a composite mother-report variable, and the presence of the G-allele. *p<0.05; **p<0.01.