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THE CONTRIBUTION OF A POLYGENIC RISK SCORE TO INDIVIDUAL
DIFFERENCES IN AGGRESSIVE BEHAVIOR: THE MODERATING AND
MEDIATING ROLES OF STRESSFUL EVENTS

by

Christa C. Christ

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THE CONTRIBUTION OF A POLYGENIC RISK SCORE TO INDIVIDUAL
DIFFERENCES IN AGGRESSIVE BEHAVIOR: THE MODERATING AND
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University of Nebraska, 2016

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Although aggression can be beneficial in certain situations (e.g. playing sports, self-defense), excessive and inappropriate aggression can lead to adverse physical and psychological health outcomes in both perpetrators and victims. Genetic susceptibility to negative environments can increase the likelihood of aggressive behavior in the context of situational risk factors. Low efficiency of serotonin neurotransmission and exposure to stress appear to play a prominent role in the etiology of aggressive behavior. A set of three studies assessed the contribution of polygenic risk (*TPH2* rs4570625, *SLC6A4* 5-HTTLPR+rs25531, *HTR1B* rs13212041, *MAOA* uVNTR) to aggressive behavior, including alcohol-related aggression, in university students at varying reported levels of childhood stress (i.e. exposure to childhood trauma, lack of social support). Additionally, the mediating role of acute stress on the association between the polygenic risk score and aggression was examined using both self-report measures and experimental manipulation. It was expected that increased genetic susceptibility would predict higher aggressive behavior resulting from stress, and that the association would be greater as level of exposure to childhood stress increased. Hypotheses for the study were partially supported. Findings showed that individuals with higher genetic susceptibility (i.e. high polygenic risk score) reported engaging in more aggression if

they reported experiencing higher levels of childhood trauma and reported engaging in less aggression if they reported experiencing lower levels of childhood trauma. In women only, higher genetic susceptibility and higher reported levels of childhood trauma also predicted more aggression indirectly via higher acute perceived stress. However, these results did not generalize to alcohol-related aggression. The pattern of results is consistent with the Differential Susceptibility Model based on a visual inspection and suggests that individuals with genetic risk who have experienced childhood trauma may benefit from intervention and prevention strategies. Because the association in women between the polygenic risk score and aggression was mediated by stress, intervention and prevention strategies that focus on teaching adaptive coping techniques may be particularly useful in reducing aggressive behavior in women that occurs as a result of stress.

DEDICATION

To God who gave me the gift of my husband and to my husband who gives me everything else in life I could ever need. I love you.

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CHAPTER 1: INTRODUCTION

This first chapter introduces the prevalence and consequences of aggressive behavior, in addition to the theoretical framework considered in examining the pathway between genetic variation and aggressive behavior. The three studies that comprise this dissertation address critical concerns within candidate gene-by-environment (cGxE) research while contributing to our current understanding of the etiology of aggressive behavior. A review of related literature important in interpreting the findings that are discussed later in this dissertation is presented in Chapter 2.

Statement of the Problem

Aggression is broadly defined as “behavior intended to inflict harm on another person who is motivated to avoid harm” (Denson, Dewall, & Finkel, 2012). Although this definition may seem simple, aggression is a complex trait; the ways of acting aggressively are numerous. Aggression can be expressed through various behaviors, including but not limited to, bullying, assault, road rage, domestic or workplace violence, and homicide. Aggressive behavior can also be sub-divided based on motives (reactive/impulsive vs. proactive/instrumental) and means (direct vs. indirect, physical vs. verbal).

While aggressive behavior can be beneficial in matters such as self-defense, aggressive behavior can also result in problems for both victims and perpetrators. The World Health Organization (WHO) has identified violence—“the intentional use of physical force or power, threatened or actual, against oneself, or against a group or community that either results in or has a high likelihood of resulting in injury, death, psychological harm, maldevelopment or deprivation”—as a global health problem and recommends strengthening support for comprehensive violence

prevention (World Health Organization, 2014).

Violence is the fourth leading cause of death worldwide for individuals aged 15-44 years (World Health Organization, 2014), and many more are victims to non-fatal violence. Each year in the U.S. alone, 20.1 per 1000 persons aged 12 or older are victims of violent crimes (Truman & Langton, 2015), 22% of students report being bullied (U.S. Department of Education, National Center for Education Statistics, 2015), and 9.4 per 1000 children are victims of child maltreatment (U.S. Department of Health and Human Services, 2015).

Aggressive behaviors are costly to society, with violent crimes alone costing \$124 billion in 2010 (\$137 per American) to cover costs of police, courts and correctional institutions, victim out-of-pocket medical expenses, and loss of earnings by victims and perpetrators (Shapiro & Hassett, 2012). In addition to the economic burden of aggressive behaviors, both victims and perpetrators of aggression are at risk of adverse outcomes such as delinquent behaviors (Wolke, Copeland, Angold, & Costello, 2013), negative health outcomes (Krug, Mercy, Dahlberg, & Zwi, 2002; Ttofi & Farrington, 2008; Wolke et al., 2013), poor social relationships (Ttofi & Farrington, 2008; Wolke et al., 2013), and academic problems (Ttofi & Farrington, 2008; Wolke et al., 2013), which can also create a financial burden (Wolke et al., 2013). In order to reduce aggression that results in these adverse outcomes, it is important to have a better understanding of the etiology of various aggressive behaviors. There are several theories that provide a framework for investigating the etiology of aggressive behaviors.

Theoretical Framework

The General Aggression Model (Figure 1.1) provides an overarching framework for understanding aggression and violence (Anderson & Bushman, 2002; DeWall, Anderson, & Bushman, 2011). The General Aggression Model proposes that there

are biological and environmental factors that interact to contribute to the development of an individual's personality. An individual's personality then contributes to the episodic cycle of aggression. The episodic cycle is a feedback loop consisting of three critical stages that produce aggression: (1) person and situation inputs, (2) present internal states (i.e., cognition, arousal, and affect), and (3) outcomes of appraisal and decision-making processes. Person inputs are characteristics that an individual brings to a situation, which predisposes them to behave aggressively (e.g. sex, beliefs, attitudes, values, scripts). Situation inputs are features of the situation that increase the likelihood of aggression (e.g. provocation, drugs, incentives, discomfort). Person and situation inputs influence aggression by altering the individual's present internal state. Internal state is an interconnection of cognition (e.g. priming aggressive concepts in memory), physiological and psychological arousal, and affect (e.g. mood and emotion). The internal state subsequently influences the appraisal and decision-making processes. There is an initial automatic appraisal that, depending on the circumstances, can result in an impulsive action or a more conscious reappraisal and thoughtful action. Both an impulsive action and thoughtful action can be aggressive or nonaggressive. The action then becomes part of the input for the next cycle creating a feedback loop. The General Aggression Model also encompasses all forms of aggression by noting that knowledge is created through social learning processes upon which all forms of aggression are based.

Previous research has demonstrated an association between proximate factors and processes (i.e. person and situation factors, present internal state, appraisal and decision making process) and aggressive behavior (Anderson & Anderson, 2008; Anderson & Bushman, 2002; Anderson et al., 2010; DeWall et al., 2011; Dewall, Anderson, & Bushman, 2012). The General Aggression Model also proposes that

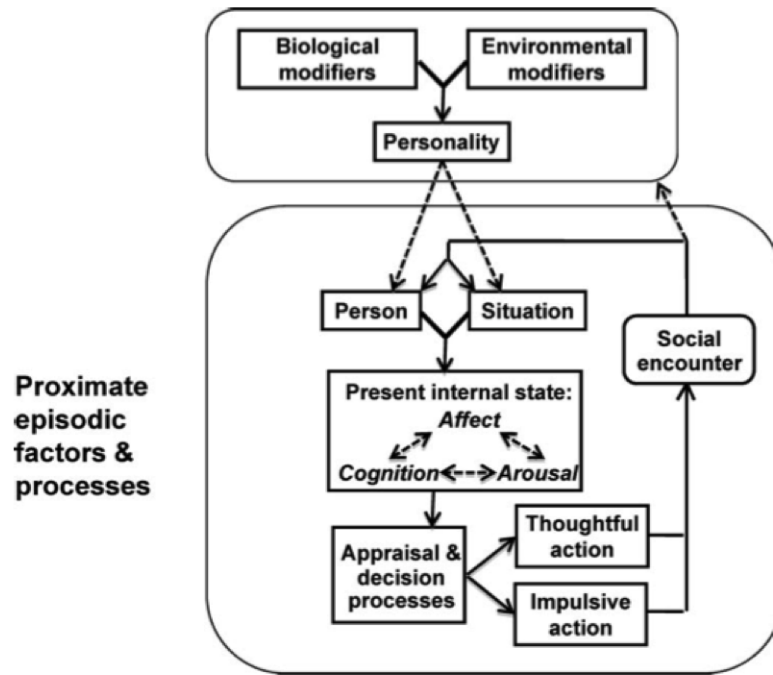


Figure 1.1: General Aggression Model diagram; image taken directly from (DeWall et al., 2011).

the interaction between biological and environmental factors is a distal cause of aggression. Two additional theories regarding the pattern of the interaction between genetic and environmental factors on personality and behavior are (1) Differential Susceptibility and (2) Diathesis Stress. The Differential Susceptibility Model is founded in evolutionary biology and suggests that some individuals will have genotypes more susceptible to both negative and positive experiences or environmental influences (Belsky & Pluess, 2009). The Diathesis Stress Model alternatively proposes that some individuals will have genotypes that are more vulnerable to adverse effects of negative experiences or environments (i.e. stressors) (Monroe & Simons, 1991).

It has been suggested that the Differential Susceptibility Model has greater empirical support than the Diathesis Stress Model (Belsky et al., 2009). However, a

meta-analytic review of previous research to assess which model is a better fit may be inappropriate given that the majority of previous research has focused on a restricted range of environment and behavior (Belsky et al., 2009). Due to the restricted range in measurement of environment and behavior, the ability to detect findings that support differential susceptibility is decreased. Furthermore, the majority of interaction effects that demonstrate a crossover effect are considered to be the result of a Type I error, especially when sample size is small (Boardman et al., 2014). Although statistical methods are available to properly test whether results of cGxE research support the Differential Susceptibility or Diathesis Stress Model (Roisman et al., 2012), researchers must first determine the range of interest for environmental modifiers and have a large enough sample size to properly test for regions of significance.

Although it is important to the field of cGxE research to investigate which model more accurately portrays the relationship of the interaction between genes and environment, both models suggest that individuals with higher genetic plasticity or vulnerability, who have been exposed to adverse environments, are at the highest risk of engaging in aggressive behavior. Indeed, there is considerable evidence that adverse environments moderate the association between genetic variation and aggression (Brendgen et al., 2008; Byrd & Manuck, 2014; Craig & Halton, 2009; Moffitt, 2005). According to the General Aggression Model, biological and environmental factors contribute to person inputs, such as scripts. Scripts are knowledge about how people behave in situations, such as conflict. Therefore, environmental factors that provide knowledge that aggression is an appropriate response to certain situations may be particularly important to examine in cGxE research.

Environmental factors, in which aggression is learned by either directly

experiencing or observing others' aggressive behavior, may contribute to an individual's scripts. Which in turn can predispose an individual to behave aggressively in certain situations. Children who experience or witness violence are more likely to be violent themselves (Bauer et al., 2006; Moretti, Obsuth, Odgers, & Reebye, 2006; Roberts, McLaughlin, Conron, & Koenen, 2011). Children who observed aggressive behavior by adults toward a Bobo Doll were subsequently more aggressive in their behavior toward the doll compared to children who observed nonaggressive behavior (Bandura, Ross, & Ross, 1961). Bandura and colleagues (1961) suggested that aggressive behavior by an adult toward the doll signaled the permissiveness of aggressive behavior, increasing the likelihood of aggressive responding by children in future situations. Additionally, increased exposure to violent video games or media has also been associated with increased aggressive behavior (Anderson et al., 2010; Huesmann, Moise-Titus, Podolski, & Eron, 2003). By learning through their exposure to parental violence or media violence that aggression can be used to solve a problem or gain control, children may be more likely to resolve their own conflicts using aggressive behavior.

Purpose of the Present Studies

The purpose of the present three studies that comprise this dissertation was to better understand the etiology of aggressive behavior. Specifically, the studies examined the environmental context in which genetic variation contributes to individual differences in aggressive behavior. Genetic variation was examined in the environmental context of childhood trauma (e.g. physical, sexual, or emotional abuse or neglect). The environmental context of self-reported exposure to childhood trauma was specifically examined because experiencing or observing aggression may contribute to an individual's scripts, potentially predisposing individuals to behave aggressively. Furthermore, the pathway between genetic variation—as moderated by

childhood trauma—and aggression is examined in regards to proximate factors and processes (i.e. gender, acute stress, alcohol use) as proposed by the General Aggression Model.

Significance of the Present Studies

The General Aggression Model integrates other proposed theories on aggressive behavior forming, to our knowledge, the only current integrative framework for understanding the etiology of aggressive behavior. The current studies were informed by the General Aggression Model, Differential Susceptibility Model, Diathesis Stress Model, and previous knowledge on the etiology of aggressive behavior. The use of theoretical models provides a framework to appropriately design our research questions and interpret the subsequent results.

Within the field of cGxE research there is a high level of concern for false positives with only 27% of replication attempts resulting in significant effects, as opposed to 96% of novel studies (Duncan & Keller, 2011). Many significant replications also have smaller sample sizes compared to studies that did not replicate previous results (Duncan & Keller, 2011). Therefore, the likelihood of the majority of previous cGxE findings being true effects is low. Given the potential that most cGxE research is potentially the result of a type I error, it is difficult to interpret the effect of specific genetic variants on individual differences in behavior. As a result, the second and third studies included in this dissertation attempt to indirectly replicate the study immediately prior to it.

Methodologies suggested to improve cGxE research, in order to combat recent criticism and low rate of reproducibility (Dick et al., 2015; Duncan & Keller, 2011), were integrated into our present studies. Poor measurement of environmental factors can contribute to the inability to replicate a cGxE effect (Monroe & Reid, 2008); therefore, reliable and theoretically plausible self-report measures of

environmental stressors were used in our studies. The Trier Social Stress Test, which is the most commonly used method of stress manipulation and has been shown to be reliable (Dickerson & Kemeny, 2004), was used in our third study to induce acute stress in participants. We also statistically controlled for factors that could produce spurious interactions including all gene-by-covariate interaction terms, environment-by-covariate interactions terms, and a quadratic term if there was a significant correlation between predictors. To date, the majority of cGxE research does not account for quadratic terms or control for factors that could produce spurious interactions (Dick et al., 2015). Failure to include these terms in statistical analyses of regression-type models can result in false-negative findings or reversal of the sign of the effect (Ganzach, 1997).

The field of genetics has advanced to more comprehensive approaches than examining a single polymorphism. Additionally, traits, such as aggression, are complex and likely influenced by many polymorphisms across multiple genes. The use of a polygenic risk score—a composite score of multiple polymorphisms to represent genetic liability—provides a method of investigating the quantitative nature of aggressive behavior (Plomin, Haworth, & Davis, 2009). There have been three other studies that have examined the association between polygenic risk scores and aggression. The first of these studies included *DRD4*, *SLC6A4* 5-HTTLPR, and *MAOA* uVNTR in its polygenic risk score and found that in high hostile/demoralizing environments (i.e. scores in top 41.4% of the sample range), higher polygenic risk scores (i.e. greater number of plasticity alleles) were associated with higher aggression; in low hostile/demoralizing environments (i.e. scores in bottom 16.83% of the sample range), higher polygenic risk scores were associated with lower aggression (Simons et al., 2012). The second study included *SLC6A4* 5-HTTLPR and *MAOA* uVNTR in its polygenic risk score and found that a higher

polygenic risk score (i.e. alleles corresponding to lower transcription rate) was predictive of higher self-reported intimate partner violence (Stuart et al., 2014). Finally, the last of the three studies combined *DRD2* single nucleotide polymorphisms (SNPs) rs1799732 and rs1800497, *COMT* rs4680, and DAT1 for its polygenic risk score and found that there was a positive association between the polygenic risk score (higher polygenic risk corresponds to greater ventral striatum dopamine signaling and reactivity) and self-reported dating violence at time of higher reported use of alcohol (Foshee et al., 2015). However, to our knowledge, there has been no previously published research that has examined how the association between genetic variation—as measured by a polygenic risk score—and aggression is moderated by experiences of childhood trauma and mediated by acute stress.

Summary

The overall goal of the studies presented in this dissertation is to contribute to our current understanding of the etiology of aggressive behavior, while taking into account recent criticisms of cGxE research. It is through a better understanding of the etiology of aggressive behavior that prevention and intervention efforts can be improved. The following chapter provides the context in which the three studies that comprise this dissertation should be considered.

CHAPTER 2: BACKGROUND & RELATED WORKS

The purpose of the studies presented in this dissertation was to investigate the moderating effect of childhood stress (e.g. childhood trauma) and mediating effect of acute stress on the association between genetic variation in serotonin system genes, measured as a polygenic risk score, and aggressive behavior. This chapter provides a review of the literature related to the current understanding of the association between genetic variation that affects serotonin neurotransmission and aggressive behavior. The chapter also includes previous research to support a moderating role of childhood trauma and a mediating role of acute stress on the association between genetic variation and aggressive behavior.

Association between Genetic Polymorphisms and Aggression

Heritability is the amount of variance accounted for by genetic influences. One method to estimate heritability of a trait is to investigate the expression of a trait among monozygotic twins, who share 100% of their genetic material, compared to the expression in dizygotic twins, who share 50% of their genetic material just like any other sibling. The reported heritability of aggression ranges from 26%-59% depending on the type of aggression (Coccaro, Bergeman, Kavoussi, & Seroczynski, 1997; Seroczynski, Bergeman, & Coccaro, 1999), suggesting a genetic basis for the trait. Adoption studies are another method of estimating heritability. In adoption studies, the correlation of a trait between the adoptee and adoptive relatives is compared to the correlation between the adoptee and their biological relatives. A meta-analysis of twin and adoption studies indicated that heritability of aggression was about 50%, but that heritability was higher in adulthood compared to childhood, and males compared to females (Miles & Carey, 1997). The literature discussed in this paragraph suggests that aggression is highly heritable. Therefore,

the identification of specific polymorphisms that contribute to individual differences in aggression may increase the ability to identify individuals most at risk of engaging in aggressive behavior.

Neurotransmitters are chemicals that regulate neuronal activity in response to stimuli, and some genetic polymorphisms affect the ability of neurotransmitter regulation. In particular, the neurotransmitter serotonin has been widely implicated in aggressive behavior. A meta-analysis using over 200 reported effect sizes from animal studies found an overall inhibitory effect; increased levels of serotonin are associated with decreased aggression (Carrillo, Ricci, Coppersmith, & Melloni Jr, 2009). Therefore, genetic variants that alter the efficiency of serotonin neurotransmission are potential biological factors of interest. Due to their functional effect (i.e. observable change in gene transcription or translation) and association with aggression, as indicated by previous literature, there are four specific genetic polymorphisms that are promising for examining the etiology of aggressive behavior, which are presented in the next few paragraphs.

The second isoform of tryptophan hydroxylase is the rate-limiting enzyme in the synthesis of serotonin within the central nervous system (Zhang, Beaulieu, Sotnikova, Gainetdinov, & Caron, 2004) and is expressed in the human brain (Zill, Büttner, Eisenmenger, Bondy, & Ackenheil, 2004). The *TPH2* gene, which encodes the second isoform of tryptophan hydroxylase, is located on chromosome 12 (Walther et al., 2003). Knockout mice can be used to investigate how gene activity contributes to a phenotype. A knockout mouse is a mouse in which a gene has been inactivated by replacing or disrupting the gene with artificial DNA (NIH, 2015). Mice with one copy of the *Tph2* gene (*Tph2* +/-) showed a 50% reduction in mRNA levels compared to mice with two copies of the gene (*Tph2* +/+), but only a 10% reduction in serotonin. Mice lacking two copies of the gene (*Tph2* -/-;

homozygous knockout), which had relatively no serotonin (98% reduction compared to controls), were significantly more aggressive than *Tph2* $+/+$ mice; while *Tph2* $+/-$ mice showed an intermediate level of aggression that was not significantly different from *Tph2* $+/+$ mice (Mosienko et al., 2012).

In the upstream region of the human *TPH2* gene is the single nucleotide polymorphism (SNP) rs4570625. The T-allele of rs4570625 (-703 G/T) has been shown to be associated with down regulation of in vitro gene expression (Chen, Vallender, & Miller, 2008) and reduced neuronal activity (Scheuch et al., 2007). Additionally, the T-allele has been shown to be associated with greater emotional reactivity (Canli, Congdon, Todd Constable, & Lesch, 2008) and increased amygdala activation in response to angry faces (Brown et al., 2005; Furmark et al., 2009). Individuals homozygous for the T-allele of rs4570625 had decreased performance in impulsive and executive control, and showed greater conflict behavior compared to individuals with at least one G-allele (Reuter, Kuepper, & Hennig, 2007). Another *TPH2* SNP, rs6582071, is in complete linkage disequilibrium with rs4570625. The A-allele of rs6582071, which is inherited with the T-allele of rs4570625, was found to be associated with lower rates of brain serotonin synthesis, as indicated by lower normalized blood-to-brain ^{11}C -AMT trapping (Booij et al., 2012). Lower ^{11}C -AMT trapping was also associated with higher self-reported physical aggression in childhood (Booij et al., 2010). The association between rs4570625 and both gene expression and aggressive and conflict behavior makes it an excellent candidate for inclusion in our analyses.

The *SLC6A4* gene has 14 exons (Lesch et al., 1994), is located on chromosome 17, and encodes the serotonin transporter (Ramamoorthy et al., 1993). The serotonin transporter is responsible for the reuptake of serotonin from the synapse back into the presynaptic neuron (Ramamoorthy et al., 1993). Serotonin

transporter protein and mRNA was found to be almost completely absent in serotonin transporter homozygous knockout rats (5-HTT -/-) that also had low serotonin reuptake. Additionally, heterozygous knockout (5-HTT +/-) rats also had reduced serotonin transporter protein, mRNA, and reduced serotonin reuptake compared to wild-type (5-HTT +/+) rats (Homberg et al., 2007). 5-HTT -/- mice had lower serotonin tissue concentrations in the brain stem, frontal cortex, hippocampus, and striatum (Bengel et al., 1998; Fabre et al., 2000; Kim et al., 2005); higher extracellular serotonin levels (Fabre et al., 2000); and were typically less aggressive (Heiming et al., 2013; Holmes, Murphy, & Crawley, 2002; Lewejohann et al., 2010) compared to 5-HTT +/+ mice. Selective serotonin reuptake inhibitors (SSRIs), which inhibit reuptake of serotonin back into the presynaptic neuron, have been associated with decreased aggression in animal models (Delville, Mansour, & Ferris, 1996; Fuller, 1996; Ho, Olsson, Westberg, Melke, & Eriksson, 2001; Pinna, Dong, Matsumoto, Costa, & Guidotti, 2003) and clinical populations (Blader, 2006; Fava et al., 1996; New et al., 2004).

A variable number tandem repeat (VNTR) polymorphism named 5-HTTLPR in the promoter region of *SLC6A4*, in combination with the SNP rs25531, is the binding site for a functional transcription factor (Hu et al., 2006). The L-allele of 5-HTTLPR, in combination with the rs25531 A-allele, results in higher mRNA transcription and more serotonin transporters within the synapse (Hu et al., 2006). The low serotonin expressing 5-HTTLPR+rs25531 genotype group (S'; S/S, L_G/S, or L_G/L_G), as opposed to the high expressing genotype group (L'; L_A/L_A), was found to be more prevalent in clinically aggressive children compared to a control group (Beitchman et al., 2006). Considering 5-HTTLPR alone, the S-allele has been associated with greater aggression (Gonda et al., 2009; Retz, Retz-Junginger, Supprian, Thome, & Rosler, 2004). 5-HTTLPR is one of the most widely studied

polymorphisms for aggressive behavior (Ficks & Waldman, 2014; Vassos, Collier, & Fazel, 2014) which makes it an excellent candidate for inclusion in our analyses.

HTR1B is an intronless gene located on chromosome 6 that encodes the serotonin 1b receptor (5-HT_{1B}), a 390 amino acid polypeptide containing seven hydrophobic regions (Mochizuki, Yuyama, Taujita, Komaki, & Sagai, 1992). The polypeptide structure shows that 5-HT_{1B} is a G protein linked receptor (Mochizuki et al., 1992) that functions as a presynaptic inhibitory autoreceptor and a postsynaptic heteroreceptor (Hartig, 2000). Mice with the *Htr1b* gene knocked-out showed more aggressive behavior (i.e., faster and more intense attacks) when confronted by an intruder compared to wild-type mice (Ramboz et al., 1996; Saudou et al., 1994). Serenics, a class of psychoactive drugs, are 5-HT_{1B} agonists that inhibit aggressive behavior. Multiple 5-HT_{1B} agonists have shown antiaggressive effects in mice with species-typical aggression, social instigation aggression, and extinction-heightened aggression (de Almeida, Nikulina, Faccidomo, Fish, & Miczek, 2001; de Almeida & Miczek, 2002; de Boer & Koolhaas, 2005).

HTR1B gene SNPs are associated with individual differences in aggressive behaviors. One particular SNP, rs13212041, is a binding site for microRNA-96, which binds to the A-allele of rs13212041 and silences mRNA production (Jensen et al., 2009). Individuals who have a homozygous genotype for the A-allele endorsed more conduct disorder behaviors than individuals carrying a G-allele (Jensen et al., 2009). Examination of a haplotype containing 5 *HTR1B* SNPs (rs11568817, rs130058, rs6296, rs6297, rs12312041) revealed that variation in rs12312041 was significantly associated with self-reported anger and hostility in young men (Conner et al., 2010). Haplotypes comprised of these 5 SNPs, which had expected lower mRNA expression, were also associated with higher reported levels of hostility (Conner et al., 2010). The association between rs13212041 and both gene expression

and aggressive behavior makes it an excellent candidate for inclusion in our analyses.

Lastly, monoamine oxidase-A (MAOA) is an enzyme that regulates serotonin through deamination (Weyler, 1990) and is encoded by the *MAOA* gene, located on the short arm of the X chromosome. Mice lacking the *MAOA* gene displayed increased levels of serotonin and enhanced aggressive behavior (Cases et al., 1995). In humans, lower brain MAOA activity, as measured by PET scans, has been associated with higher trait aggression (Alia-Klein et al., 2008).

A VNTR located in the upstream region of the *MAOA* gene (*MAOA* uVNTR) affects transcriptional potential, with certain variants acting as activators of transcription (*MAOA* uVNTR HA-alleles; 3.5R & 4R) and other variants resulting in lower transcriptional activity (*MAOA* uVNTR LA-alleles; 2R, 3R, & 5R) (Sabol, Hu, & Hamer, 1998). Individuals carrying a *MAOA* uVNTR LA-allele have shown higher antisocial personality traits along with altered negative emotion related brain activity (Williams et al., 2009) and more aggressive behavior when either provoked (McDermott, Tingley, Cowden, Frazzetto, & Johnson, 2009) or socially excluded (Gallardo-Pujol, Andrés-Pueyo, & Maydeu-Olivares, 2013). Men in a batterer intervention program who had a higher polygenic risk score (*MAOA* uVNTR LA-allele and 5-HTTLPR S-allele) reported greater intimate partner aggression (Stuart et al., 2014). *MAOA* uVNTR is the most widely studied polymorphism for aggressive behavior (Ficks & Waldman, 2014; Vassos et al., 2014), and therefore, important to include in our analyses. Additionally, the association between *MAOA* uVNTR and both regulation of gene expression and aggressive behavior also makes it an excellent candidate for inclusion.

The literature reviewed above as a whole supports an inverse association between serotonin and aggression. Knockout models of the various genes, typically demonstrating higher aggression, result in lower gene transcription and subsequently

lower levels of serotonin. Furthermore, the allele associated with lower gene transcription for *TPH2* rs4570625, *SLC6A4* 5-HTTLPR+rs25531, *HTR1B* rs13212041, and *MAOA* uVNTR is typically associated with higher aggressive behavior. However, there is also evidence with some of the polymorphisms mentioned above to support that an opposite association exists. Literature that supports an opposite association is presented in the next few paragraphs. The inconsistent results may simply be the result of a Type III error (i.e. correctly rejecting the null hypothesis, but the direction of the effect is false); however, other potential factors that could contribute to the inconsistencies within the literature are discussed below.

Follow-up analyses of the meta-analysis examining the association between serotonin and aggression indicated that the type of aggression measured (e.g. offensive or predatory) and how aggression was induced (e.g. social isolation or stress) contributed to the differences in effect size across studies (Carrillo et al., 2009). Therefore, the type of aggression and how it was induced may also contribute to differences in candidate gene studies. Alternatively, the difference in the study populations may also account for some of the differences. For example, while the *TPH2* rs4570625 T-allele was associated with higher aggression in European populations, the G-allele was associated with higher amygdala activity in response to angry faces (Lee & Ham, 2008) and anger (Yang et al., 2010; Yoon, Lee, Kim, Lee, & Ham, 2012) in Korean populations. There is also a difference in allele frequency between European (T-allele = 0.20) and Korean populations (T-allele = 0.49) for this allele. Overall, these results suggest that inconsistencies within the literature regarding the association between *TPH2* rs4570625 and aggression may partly be due to racial or ethnic differences. Although the reason for such differences is not clear, the difference could be the result of the polymorphism

having a dissimilar association with risk in different populations, or that the polymorphism interacts with additional factors (biological or environmental) that vary across populations. However, a meta-analysis of genetic effects for complex diseases suggests that genetic effects are consistent across racial groups; therefore, racial differences may be the result of spurious findings (Ioannidis, Ntzani, & Trikalinos, 2004).

Previous literature has also typically focused on one polymorphism; however, multiple polymorphisms contribute to a complex trait, such as aggression. Furthermore, there is evidence that there are interactive effects among polymorphisms; therefore, the interaction between genetic polymorphisms should be considered when examining their association with aggression. Although the 5-HTTLPR S-allele is commonly associated with aggression, when examined along with *DRD4* (dopamine receptor 4 gene), the 5-HTTLPR L-allele was associated with higher aggressive behavior (Nobile et al., 2007). Therefore, the discrepancies in previous literature regarding the association between genetic polymorphisms and aggression could also be due to the lack of considering how the association between genetic polymorphisms and aggression is moderated by other genetic polymorphisms.

Similarly, the lack of examining environmental moderators could also contribute to the discrepancies in the literature. Aggressive children from a clinical sample were more likely to carry a *MAOA* uVNTR HA-allele compared to non-clinical adult male controls (Beitchman, Mik, Ehteshami, Douglas, & Kennedy, 2004). Also, self-reported aggression was higher among non-clinical individuals who were homozygous for a *MAOA* HA-allele compared to individuals who were homozygous for a *MAOA* uVNTR LA-allele (Verhoeven et al., 2012). However, environmental moderators were not considered in either of these studies. A meta analysis of the

moderating effect of childhood adversity on the association between *MAOA* uVNTR and aggressive behavior found that when accounting for the modifying effect of childhood adversity, the *MAOA* uVNTR LA-allele was more often associated with higher aggression (Byrd & Manuck, 2014). The Differential Susceptibility Model proposes that certain alleles are more susceptible to the environment, which would suggest that in negative environments, alleles resulting in lower gene transcription would be associated with higher aggression. However, in positive environments the same alleles would be associated with lower aggression. Without properly examining the moderating effect of the environment, examining the association between genetic polymorphisms and aggression may result in misleading findings (i.e. Type II or Type III errors).

Lastly, Foshee and colleagues (2015) found that, among 8th-12th graders, the L-allele of 5-HTTLPR was predictive of higher self-reported levels of dating violence perpetration during times of greater alcohol consumption. The L/L genotype of 5-HTTLPR has also been associated with early onset alcoholism (Laucht et al., 2009). Therefore situational factors (e.g. alcohol use) should also be considered as moderating factors on the association between genetic polymorphisms and aggression.

The studies in this dissertation are partly intended to address some of the inconsistencies in previous literature by accounting for factors (e.g. racial/ethnic differences, biological modifiers, environmental modifiers, situational factors, type of aggression) that potentially contribute to differences in the association between genetic polymorphisms and aggression. Race (White vs. non-White) was statistically controlled for in our analyses in order to address racial differences. Additionally, we (1) account for potential modifying effects of additional polymorphisms by examining the interaction between polymorphisms prior to

building a polygenic risk score, (2) examine the potential moderating effect of childhood trauma exposure (environmental modifier), (3) examine the potential mediating effect of acute stress (situational factor), and (4) include measures of multiple types of aggression.

Benefits of Using a Polygenic Risk Score

Although a better understanding of the genetic contribution to aggressive behaviors is gained through the examination of specific genetic variants, each variant only contributes a small amount [odds ratio of about 1.1 (Dick et al., 2015)] to individual differences in quantitative traits, such as aggression (Plomin et al., 2009). Through the use of a polygenic risk score (PRS) the collective impact of various polymorphisms on aggressive behavior can be examined (Nikolova, Ferrell, Manuck, & Hariri, 2011). Using a PRS provides greater statistical power and a better understanding of the underlying genetic architecture, which makes PRS particularly useful in intensive and expensive research of complex behaviors (Plomin et al., 2009).

Several methods have been used to calculate a PRS. Genetic polymorphisms are selected based on either previous genome-wide association studies (GWAS; the association of polymorphisms across the human genome with common diseases and behavioral traits) (Belsky et al., 2013a; Hamshere et al., 2013; Ising et al., 2009) or biological function that produces an alteration in how the gene encodes the protein (e.g., rate of transcription, structure of protein) (Nikolova et al., 2011; Vrshek-Schallhorn et al., 2015). A PRS can include as few as two polymorphisms (Stuart et al., 2014); however, given that the genetic influence of quantitative traits, such as aggression, is due to many genes contributing a small amount to the variance (Plomin et al., 2009), the more polymorphisms that are included in the PRS, the greater amount of variance we can account for—assuming that each

polymorphisms included in the PRS does account for some of the variance in aggression. Furthermore, the combination of genetic polymorphisms with different functions that are all associated with aggression represents a systems approach [joint association with overlap in downstream function (Plomin et al., 2009)]. For each polymorphism included in a PRS, genotypes are assigned a score based on their expected risk contribution. Scores for each genotype are then summed or averaged for each individual to create a total score. This method assumes all polymorphisms have equal contribution; therefore, the score of each polymorphism to be added to the PRS is sometimes weighted with effect sizes or odds ratios from meta-analysis data, a priori analyses, etc., prior to creating a total score (Derringer et al., 2010; Rodríguez-Rodríguez et al., 2013). Previously, polygenic risk scores have been associated with many outcomes, such as Alzheimer’s disease (Rodríguez-Rodríguez et al., 2013), asthma (Belsky et al., 2013a), depression (Vrshek-Schallhorn et al., 2015), antidepressant treatment outcome (Ising et al., 2009), cancer (Szulkin et al., 2015), cardiovascular disease (Havulinna et al., 2013), obesity (Ahmad et al., 2013), smoking (Belsky et al., 2013b), drug dependence symptoms (Derringer et al., 2012), ADHD in children with comorbid aggression (Hamshere et al., 2013), sensation seeking (Derringer et al., 2010), aggression (Simons et al., 2012) and intimate partner aggression (Foshee et al., 2015; Stuart et al., 2014).

To date there has only been a handful of GWAS on aggressive-related phenotypes. Only one SNP of unknown function—rs11126630—was near significance when examining children’s aggressive behavior (Pappa et al., 2015). GWAS of oppositional defiant behavior in children with ADHD (Aebi et al., 2015), behavioral disinhibition (McGue et al., 2013), children callous-unemotional behavior (Viding et al., 2013), and adult antisocial behavior (Tielbeek et al., 2012) found no significant effects. It was hypothesized that the lack of significant GWAS findings may, in part,

be due to the polygenic nature of the phenotypes investigated (McGue et al., 2013). Without GWAS data available for aggression to build a PRS, polymorphisms that alter gene regulation or function are considered. The PRS in the three previous studies to examine the association between genetic variation and aggression (Foshee et al., 2015; Simons et al., 2012; Stuart et al., 2014) were all comprised of polymorphisms that alter gene regulation or function, and were based on either transcription efficiency or neurotransmitter activity. Given the established association between serotonin and aggression (Carrillo et al., 2009), the polymorphisms associated with variation in serotonin neurotransmission efficiency previously discussed here (*SLC6A4* 5-HTTLPR+rs25531, *MAOA* uVNTR, *TPH2* rs4570625, *HTR1B* rs13212041) are excellent candidates to be included in a PRS.

A meta-analysis on aggression and antisocial behavior found that the *MAOA* uVNTR LA-allele and 5-HTTLPR S-allele were significantly associated with aggression and antisocial behavior (Ficks & Waldman, 2014). However, another recent meta-analysis examining 12 different polymorphisms, including 5-HTTLPR and *MAOA* uVNTR, found no significant association with violence and aggression (Vassos et al., 2014). Although both studies examined the association between genetic variation and aggression, the search terms used for the aggressive phenotype varied between the two studies. As a results, only 30% of the studies used by Ficks and Waldman (2014) were also included by Vassos and colleagues (2014), which may partly account for the difference in their findings. Given the inconsistent results between these two studies and the lack of meta-analysis data for *HTR1B* rs13212041 and *TPH2* rs4570625, a PRS assuming equal contribution of each polymorphism was used for our studies.

Overall the use of a PRS in our analyses was expected to provide greater statistical power than examining polymorphisms individually, which allows for the

investigation of cumulative biological impact of multiple polymorphisms. The modifying effect of other polymorphisms, which may contribute to inconsistencies in previous candidate gene research, was accounted for by first investigating the interaction between *SLC6A4* 5-HTTLPR+rs25531, *MAOA* uVNTR, *TPH2* rs4570625, and *HTR1B* rs13212041 prior to determining which allele should be considered the risk allele. The selected polymorphisms all contribute downstream to the efficiency of serotonin regulation, which allows for a systems approach (PRS directly corresponds to expected efficiency of serotonin regulation) in the interpretation of our results.

Moderation by Experiences of Childhood Trauma

Although certain alleles of genetic polymorphisms may contribute to increased likelihood of aggressive behavior, aggressive outcomes are a result of the interaction between susceptible alleles and environmental factors according to the Diathesis Stress and Differential Susceptibility Models. In particular, environmental factors during childhood that provide an individual with knowledge that suggests aggression is an appropriate behavior for certain social interactions are expected to increase the likelihood of engaging in aggression as an adult (Dewall et al., 2012). Therefore, exposure to childhood trauma (e.g. physical, sexual, or emotional abuse or neglect) is a potential environmental modifier of the association between genetic variation and aggressive behavior. In general, children who experience abuse (Dodge, Bates, & Pettit, 1990; McKinney, Caetano, Ramisetty-Mikler, & Nelson, 2009; Sansone, Leung, & Wiederman, 2012a; Widom, 1989), neglect (Widom, 1989), or bullying (Sansone, Leung, & Wiederman, 2012b) are at an increased risk for engaging in violent behavior.

Additionally, there is evidence of a moderating effect of exposure to childhood trauma on the association between genetic polymorphisms discussed earlier and

aggressive behavior. In males, during adolescence and early adulthood, *MAOA* uVNTR genotype moderated the association between experiences of childhood trauma (i.e. severe physical discipline, child maltreatment) and aggressive-related outcomes (i.e. delinquent behavior, conduct disorder, violent disposition, antisocial personality), such that the effect of childhood trauma on aggressive outcomes was stronger for males with the *MAOA* uVNTR LA-allele than males with the *MAOA* uVNTR HA-allele (Caspi et al., 2002; Edwards et al., 2010). Furthermore, the presence of a significant interaction may be dependent on the amount of childhood trauma experienced. Children homozygous for a *MAOA* uVNTR LA-allele had significantly higher aggression scores if they had experienced moderate levels of trauma compared to those homozygous for a *MAOA* uVNTR HA-allele, while children who had experienced high levels of trauma were aggressive regardless of their genotype (Weder et al., 2009), suggesting that *MAOA* uVNTR genotype may only moderate the association between childhood trauma and aggression up to a certain threshold of childhood trauma. An association between *MAOA* uVNTR genotype, childhood trauma, and aggression related outcomes, is more commonly present in males (Byrd & Manuck, 2014; Frazzetto et al., 2007). The association between *MAOA* uVNTR genotype, childhood trauma, and aggression related outcomes, is also sensitive to other environmental modifiers. Kinnally and colleagues (2009) investigated the effect both parent care and early life trauma (e.g. abuse, death of a family member, divorce) had on and an impulsivity/aggression factor, and found that parental care moderated the interactive effect of early life trauma and genotype, such that individuals with at least one *MAOA* uVNTR LA-allele who experienced early life trauma, reported lower impulsivity/aggression when they also reported high parental care (Kinnally et al., 2009), suggesting that the *MAOA* uVNTR LA-allele is the more susceptible genotype to environmental

factors, and that those with high parental care are more resilient to the effects of early life trauma.

There have also been several studies to examine the association between *SLC6A4* polymorphisms 5-HTTLPR and rs25531, childhood trauma, and aggression related outcomes. For children with at least one 5-HTTLPR+rs25531 S'-allele there was a positive association between childhood trauma and negative emotionality/behavioral regulation, such that when children had experienced high levels of childhood trauma, children with at least one S'-allele had higher negative emotionality/behavioral regulation compared to children with a L'/L' genotype (Bouvette-Turcot et al., 2015). Individuals homozygous for the 5-HTTLPR S-allele were also at higher risk of suicide attempt if they had reported experiencing childhood trauma (Enoch, Hodgkinson, Gorodetsky, Goldman, & Roy, 2013; Roy, Hu, Janal, & Goldman, 2007). *MAOA* uVNTR and *DRD4* genotypes have also been shown to moderate the association between *SLC6A4* polymorphisms 5-HTTLPR and rs25531, childhood trauma, and aggression related outcomes. Male offenders carrying at least one S-allele were more likely to have committed violent crimes if they had reported exposure to an adverse childhood environment, and were even more likely to have committed violent crimes if they also carried the *MAOA* uVNTR LA-allele (Reif et al., 2007). Adolescents with a greater number of risk alleles (*DRD4* L-allele, *MAOA* uVNTR LA-allele, and *SLC6A4* 5-HTTLPR S-allele) had higher predicted early adulthood aggression scores if they reported exposure to a hostile/demoralizing environment (i.e. harsh parenting, caregiver antisocial behavior and substance use, absence of social control, community crime, racial discrimination), and inversely had lower predicted early adulthood aggression scores if they did not have exposure to a hostile/demoralizing environment (Simons et al., 2012).

While the moderating effect of exposure to childhood trauma has not been examined for the association between *TPH2* rs4570625 and aggressive behavior, it has been loosely examined for other adverse outcomes. Infants who had at least one T-allele had greater difficulty disengaging from fearful faces if their mother was stressed or had depressive symptoms over the past 12 months (Forssman et al., 2014). To date, there has been no examination of the moderating effect of childhood trauma on the association between *HTR1B* rs13212041 and aggression.

Overall, exposure to childhood trauma appears to be a strong potential moderator of the expected association between our PRS and aggressive behavior. The literature discussed above suggests that positive environmental factors (i.e. parental care) also moderate the association between childhood trauma, genetic polymorphisms (i.e. *MAOA* uVNTR genotype), and aggression. The use of a PRS in our analyses provides novel evidence to the potential moderating effect of childhood trauma on the association between additive genetic effects and aggression. Additionally, our third study examined the contribution of positive environmental factors (i.e. family social support, positive family relationships) on the association between PRS, childhood trauma, and aggression.

Mediation by Acute Stress

From an evolutionary standpoint, aggression is beneficial for the purpose of maintaining dominance, protection of self and offspring, and competing for resources. Acute stress can subsequently result in aggressive behavior by triggering the “fight” response in the typical “fight or flight” reaction to stress. Therefore, an aggressive reaction to stress may be considered beneficial from an evolutionary standpoint if the aggressive reaction reduces stress by maintaining dominance or eliminating a threat. In support of this viewpoint, intimate partner violence is used to control one’s partner (Antai, 2011), improving one’s own health (Inslicht et al.,

2006) and immune function (Kiecolt-Glaser et al., 2005) by maintaining dominance.

Stress as HPA Axis Reactivity. The hypothalamic-pituitary-adrenal (HPA) axis is an endocrine system that is involved in regulating the physiological response to stress (Pecoraro et al., 2006). Childhood trauma produces long-lasting alterations to the HPA axis, including higher levels of waking cortisol and altered cortisol secretion in response to stress (Frodl & O’Keane, 2013). The dysregulation of the HPA axis, as a result of childhood trauma exposure, alters subsequent responses of the HPA axis to acute stressors (Kuhlman, Geiss, Vargas, & Lopez-Duran, 2015; MacMillan et al., 2009; Peckins, Dockray, Eckenrode, Heaton, & Susman, 2012; Trickett, Gordis, Peckins, & Susman, 2014), suggesting that individuals exposed to childhood trauma are at an increased likelihood of being more reactive to acute stressors later in life.

A reciprocal relationship between aggression and stress response exists, such that activation of the HPA axis increases sensitivity to aggression promoting factors, and activation in the hypothalamus that occurs as a result of aggressive behavior increases cortisol secretion (Kruk, Halász, Meelis, & Haller, 2004). However, individuals who are aggressive have been shown to have hypoactivity in the HPA axis (Pajer, Rabin, & Gardner, 2002; Popma et al., 2007). Perpetrators of intimate partner violence were also shown to have lower cortisol secretion in response to stress (Romero-Martínez, Lila, Sariñana-González, González-Bono, & Moya-Albiol, 2013). The relationship between HPA axis activity and aggression is further complicated by additional factors, such as age, environmental factors, and type of aggression. A meta-analysis of studies from children and adolescents showed no association between cortisol reactivity and externalizing behaviors (i.e. antisocial, aggressive, oppositional, or overactive behaviors), and only a small association between basal cortisol and externalizing behaviors, which was negative in

elementary-aged children, but positive in preschool age children (Alink et al., 2008). Childhood trauma and type of aggression also seem to moderate the association between HPA axis activity and aggression. Heightened response to an acute stressor was associated with decreased anger regulation in adolescents (Cook, Chaplin, Sinha, Tebes, & Mayes, 2012) and increased aggression in young adults (Scarpa & Ollendick, 2003) who reported experiencing high levels of childhood trauma, while in children, cortisol dysregulation was associated with higher aggression only in those who did not report experiencing childhood trauma (Murray-Close, Han, Cicchetti, Crick, & Rogosch, 2008). For physical aggression, a heightened morning cortisol level and steep decline throughout the day was observed in children, while the opposite was observed for relational aggression (Murray-Close et al., 2008).

There is also a reciprocal relationship between the serotonin system and HPA axis function in animal models, such that activation of the HPA axis by stressful conditions and increased corticosterone results in increased synthesis and release of serotonin, and in turn, activation of the serotonin system is associated with increased corticosteroids (Lanfumey, Mongeau, Cohen-Salmon, & Hamon, 2008). Serotonin levels were also shown to decrease in the prefrontal cortex after responding aggressively to the stress of an intruder (van Erp & Miczek, 2000). Genetic polymorphisms that alter transcription of proteins involved in the regulation of serotonin neurotransmission are also associated with HPA axis reactivity. Individuals homozygous for the 5-HTTLPR S-allele had elevated cortisol production in response to stress compared to individuals carrying at least one L-allele (Gotlib, Joormann, Minor, & Hallmayer, 2008; Way, Taylor, Way, & Taylor, 2010). Caregivers with a *MAOA* uVNTR LA-allele compared to caregivers who had a *MAOA* uVNTR HA-allele had lower daily cortisol secretion, which is suggestive of HPA axis dysregulation (Brummett et al., 2008).

Stress as Amygdala Activity. Additional evidence to support the association between genetic variation in serotonin system genes and aggressive tendencies following environmental stress is provided via amygdala activity. Individuals with higher trait aggression have shown greater amygdala activity to angry faces (Coccaro, McCloskey, Fitzgerald, & Phan, 2007). Additionally, genetic polymorphisms such as, the 5-HTTLPR S-allele and *TPH2* rs4570625 T-allele, have been associated with greater amygdala activity following the presentation of fearful or angry faces and negative stimuli (Brown et al., 2005; Furmark et al., 2009; Hariri et al., 2005, 2002). Furthermore, 5-HTTLPR and *TPH2* rs4570625 had an interactive effect, such that the 5-HTTLPR S-allele and *TPH2* rs4570625 T-allele together produced the largest amount of activation in the amygdala following the presentation of fearful faces compared to the other genotype combinations (Canli et al., 2008). Hyperactivation of the amygdala subsequently interacts with the prefrontal cortex resulting in diminished inhibitory feedback resulting in increased stress vulnerability (Hariri & Holmes, 2006), which could subsequently result in greater aggressive behavior.

Stress as Acute Stress Exposure. Mice stressed through social isolation or social instigation were more aggressive compared to mice that were not stressed (de Almeida & Miczek, 2002; Fish, Faccidomo, & Miczek, 1999; Nosjean et al., 2015). Rats allergic to pollen were also more aggressive during a social intruder task if they were exposed to both pollen and stress (i.e. forced swim test) compared to controls or rats that experienced either stress or tree pollen (Tonelli, Hoshino, Katz, & Postolache, 2008). However, mice exposed to unpredictable stressors over a longer period of time (14 stressors over 16 days) were less aggressive compared to non-stressed control mice (Zebrowska-Lupina, Ossowska, & Klenk-Majewska, 1991).

There has also been mixed results regarding maternal aggression, with acute stress exposure in mice and rats being associated with both increased (Kinsley & Svare, 1988; Meek, Dittel, Sheehan, Chan, & Kjolhaug, 2001; Neumann, Kromer, & Bosch, 2005) and decreased aggression (Gammie & Stevenson, 2006; Maestripieri, Badiani, & Puglisi-Allegra, 1991; Pardon, Gerardin, Joubert, Perez-Diaz, & Cohen-Salmon, 2000). Therefore, the association between stress and aggression appears to be context dependent, with factors such as dominance status (Blanchard et al., 1995; Tamashiro et al., 2004; Wommack & Delville, 2003) or duration of stress exposure (Wood, Norris, Waters, Stoldt, & McEwen, 2008; Wood, Young, Reagan, & McEwen, 2003; Yohe, Suzuki, & Lucas, 2012).

Acute stress also contributes to aggression in humans. Perceived role stress in the work environment was associated with higher levels of enacted workplace aggression (Taylor & Kluemper, 2012). The type of stress experienced may also play a role in gender differences in the association between stress and aggressive behavior. Males were more likely to report perpetrating emotional abuse in a dating relationship if they had also reported higher stress from current personal problems, an effect that was not present in females (Gormley & Lopez, 2010). Females who reported experiencing stress from negative life events over the last 12 months were more psychologically and physically abusive toward their partner than females who did not experience recent stress, an effect that was not present in males (Mason & Blankenship, 1987).

In regard to the role of acute stress on the association between genetic variation and aggression, there was a positive association between chronic stress (past 12 months) and aggression in individuals carrying a 5-HTTLPR S-allele, while in individuals homozygous for the L-allele there was no significant association between chronic stress and aggression (Conway et al., 2012). Males homozygous for the

5-HTTLPR S-allele were also more aggressive than males carrying the L-allele when exposed to a laboratory stressor, an effect that was not found in females (Verona, Joiner, Johnson, & Bender, 2006). Male rhesus monkeys exposed to early life trauma (i.e. early maternal separation, non-maternally reared) were more aggressive after they experience stress from a social intrusion if they carried a 5-HTTLPR S-allele compared to being homozygous for the L-allele (Schwandt et al., 2010).

While there is no previous research on the association between the remaining polymorphisms of interest (*TPH2* rs4570625, *MAOA* uVNTR, *HTR1B* rs13212041), acute stress, and aggression, mice stressed through social instigation are less aggressive when injected with a 5-HT1B receptor agonist (da Veiga, Miczek, Lucion, & de Almeida, 2011; de Almeida & Miczek, 2002; Fish, Faccidomo, & Miczek, 1999). Additionally, mice given a monoamine oxidase inhibitor are more aggressive following the stress of shock administration compared to mice given a saline solution (Eichelman & Barchas, 1975). The pharmacological evidence for an association between the *HTR1B* and *MAOA* genes and aggressive behavior, suggests that acute stress may also play a role in the expected association between the polymorphisms selected for our PRS and aggression.

Summary. The evidence presented here suggests that polymorphisms that regulate transcription of serotonin system genes, and subsequently serotonin neurotransmission, are likely to be important in the association between acute stress and aggression. The HPA axis and amygdala activity literature discussed above provides evidence specifically for a mediating effect of acute stress. However, acute stress, as measured by experimental exposure to stress of self-reported experiences of stress, has only been examined as a moderator of the association between genetic variation and aggression. Furthermore, the mediating effect of acute stress on the association between genetic variation and aggression as moderated by childhood

trauma has yet to be examined. The studies of this dissertation address this critical gap within the literature.

Generalization to Alcohol-Related Aggression

Alcohol use is a common factor in aggressive behavior, with alcohol being used in about 38% of all violent crime (Greenfeld, 1998) and 35% of intimate partner aggression events (Reingle, Jennings, Connell, Businelle, & Chartier, 2014). Alcohol use has been associated with more aggressive behavior in both real-life situations and experimental research studies (Boden, Fergusson, & Horwood, 2012; Chermack & Taylor, 1995; Dougherty, Cherek, & Bennett, 1996; Duke, Giancola, Morris, Holt, & Gunn, 2011; Hoaken & Pihl, 2000; Scott, Schafer, & Greenfield, 1999; Wells, Graham, & West, 2000). However, not all individuals who consume alcohol engage in aggressive behavior. The association between aggression and alcohol intoxication has been shown to be stronger for those with higher trait aggressive personalities (Giancola et al., 2012), suggesting that individual differences contribute to alcohol-heightened aggression.

Mediation by Acute Stress. Alcohol reduces negative affective states that can result from experiencing stress (Conger, 1956) and as such, individuals learn through negative reinforcement to consume alcohol as a means of coping with stress (Cooper, Russell, & George, 1988). Life stress is associated with increased alcohol use (Hutchinson, Patock-Peckham, Cheong, & Nagoshi, 1998; Park, Armeli, & Tennen, 2004), supporting the notion that alcohol may be used as a means of coping with stress. Alcohol Stress-Response Dampening Theory proposes that individuals consume alcohol to relieve adverse effects of various stressors (Levenson, Sher, Grossman, Newman, & Newlin, 1980). In fact, life stressors accounted for 35% of the variance in alcohol use for males with positive alcohol expectancy and avoidant coping strategies (Cooper, Russell, Skinner, Frone, & Mudar, 1992). Individuals

who use alcohol excessively as a method of coping with stress may be more likely to engage in alcohol-related violence. The alcohol dampening effect on stress reactivity results in lower inhibition of aggressive responding to threat (Hoaken, Campbell, Stewart, & Pihl, 2003). University students who consumed alcohol for coping purposes were more likely to engage in alcohol-related aggression compared to those who consumed alcohol for social or aesthetic (e.g. enjoy the taste of alcohol) motives (Mihic, Wells, Graham, Tremblay, & Demers, 2009). The literature reviewed earlier in this chapter suggests that acute stress is a strong potential mediator of the association between genetic variation and aggression. The association between alcohol use resulting from stress and aggression suggests that acute stress may also be a mediator of the association between genetic variation and alcohol-related aggression.

Association between Genetic Polymorphisms and Alcohol-Related Aggression. Serotonin transporter availability accounted for 82% of the variance in the amount of alcohol consumed by rhesus monkeys; specifically, higher serotonin transporter expression in the raphe nucleus, which is associated with lower 5-hydroxyindoleacetic acid (5-HIAA), the primary metabolite of serotonin, and increased aggression (Heinz et al., 1998), was associated with greater alcohol use (Heinz et al., 2003). Rhesus monkeys that experienced early life trauma (i.e. parental separation after birth) were more aggressive and less sensitive to alcohol if they had low serotonin turnover rate (Heinz et al., 1998). These findings suggest that factors (e.g. serotonin, childhood trauma) that contribute to an increased likelihood of engaging in aggression behaviors also increase the likelihood of alcohol use.

Pharmacological evidence also supports an association between serotonin and alcohol-related aggression. Alcohol-heightened aggression is typically reduced in

mice as a result of pretreatment with a 5-HT_{1B} agonist (de Almeida, Nikulina, Faccidomo, Fish, & Miczek, 2001; Fish, Faccidomo, & Miczek, 1999; Fish, McKenzie-Quirk, Bannai, & Miczek, 2008; Miczek & de Almeida, 2001). However, the association between pretreatment with a 5-HT_{1B} agonist and alcohol-heightened aggression is dependent on where the agonist is injected into the brain. More specifically, injection of a 5-HT_{1B} agonist in orbitofrontal cortex and medial prefrontal cortex in the mice brain decreases and increases aggression, respectively (Faccidomo, Bannai, & Miczek, 2008). Mice displaying alcohol-heightened aggression have lower mRNA levels of almost all serotonin receptors in the prefrontal cortex, in addition to increased 5-HT_{1B} mRNA levels in the amygdala (Chiavegatto, Quadros, Ambar, & Miczek, 2010), which further suggests that serotonin involvement in alcohol-heightened aggression is specific to certain brain regions. A significant reduction in alcohol-related aggression is also seen in humans treated with a 5-HT_{1B} agonist (Gowin, Swann, Moeller, & Lane, 2010). Additional pharmacological evidence for an association between serotonin and alcohol-heightened aggression in humans has been found using SSRIs. Diagnosed alcoholic perpetrators of intimate partner violence reported a decrease in both emotional and behavioral aspects of intimate partner violence after treatment with a SSRI (George et al., 2011). Men not diagnosed as alcoholic perpetrators of intimate partner violence, who were treated with an SSRI, were also less aggressive during a behavioral paradigm (McCloskey, Berman, Echevarria, & Coccaro, 2009). However, while alcohol intoxication resulted in greater aggression during the behavioral paradigm, it did not moderate the association between SSRI treatment and aggression (McCloskey et al., 2009). Similarly, males with lowered tryptophan levels or intoxicated by alcohol were more aggressive during a behavioral paradigm; however, alcohol intoxication did not moderate the association between tryptophan

levels and aggression (Pihl et al., 1995), suggesting that the association between serotonin and alcohol-heightened aggression may be stronger in individuals with a more aggressive personality.

An association between some of the genetic polymorphisms selected for our studies and, both alcohol use and alcohol-heightened aggression has also been found. The frequency of the 5-HTTLPR S-allele was higher among individuals diagnosed with violent type 2 alcoholics compared to non-violent type 1 alcoholics and healthy controls (Hallikainen et al., 1999). There are also several studies that indicate an association between 5-HTTLPR and alcohol use, and that this association is moderated by environmental stress. Individuals with an S-allele who have experienced negative life events in the past year (Covault et al., 2007) or exposure to trauma as a child (Kaufman et al., 2007) reported heavier and earlier alcohol use. Given that increased alcohol use is associated with 5-HTTLPR S-allele and exposure to environmental stressors, in addition to the increased risk for aggression associated with alcohol use, it is likely that individuals with the 5-HTTLPR S-allele and exposure to environmental stressors are at an increased risk for alcohol-related aggression. However, in males carrying the 5-HTTLPR L-allele there was a stronger association between alcohol use and dating violence perpetration compared to males with the S-allele (Foshee et al., 2015). Foshee and colleagues (2015) also found no association between *MAOA* uVNTR genotype, alcohol use, and dating violence perpetration (Foshee et al., 2015). Similarly, no association was found between *MAOA* uVNTR genotype and self-reported aggression in an alcoholic population (Koller et al., 2003). However, in Finnish alcohol violent offenders, alcohol consumption was positively associated with recidivism among individuals with a *MAOA* HA-allele (Tikkanen et al., 2009). Lastly, no significant association of *TPH2* rs4570625 was found with alcohol-related suicide (Zill et al., 2007) and an

association between *HTR1B* rs13212041 and alcohol-related aggression has not been examined. Although research, particularly with animal models, supports an association between serotonin system genes and alcohol-heightened aggression, less is known about the association between the genetic polymorphisms included in our risk score and alcohol-related aggression. Therefore, this dissertation will contribute to our limited understanding in this area.

Summary. Although alcohol use does not increase aggression in every individual, a large percentage of aggressive behavior co-occurs with alcohol use (Greenfeld, 1998; Reingle et al., 2014). For this dissertation we propose that a higher polygenic risk score (comprised of *TPH2* rs4570625, *SLC6A4* 5-HTTLPR+rs25531, *HTR1B* rs13212041, *MAOA* uVNTR) is associated with increased aggression, and that the association is moderated by childhood trauma and mediated by acute stress. Given the evidence presented in the previous section to suggest an association between serotonin, stress exposure, and alcohol-related aggression, our proposed relationship may also generalize to alcohol-related aggression. Examining whether the association between our PRS and aggression generalizes to alcohol-related aggression may provide valuable insight into the differences between the etiology of non-alcohol-related aggression and the etiology of alcohol-related aggression.

Specific Research Aims

The research reviewed in this chapter suggests an association between genetic risk and aggression is most likely affected by both childhood trauma exposure and mediated by response to an acute stress (e.g. HPA axis reactivity, drinking to cope). However, there is inconsistency in the literature regarding the association between these factors and aggression, and a comprehensive model of this relationship has not been examined. Therefore, the purpose of the present project is to investigate four

specific aims that attempt to better elucidate the pathway between genetic risk and aggression.

To date there have been three studies to examine the association between a polygenic risk score and aggression (Foshee et al., 2015; Simons et al., 2012; Stuart et al., 2014), and only two of these studies included polymorphisms involved in the regulation of serotonin neurotransmission (Simons et al., 2012; Stuart et al., 2014). The PRS used in these two studies are based on transcriptional efficiency, where lower transcriptional efficiency corresponds to higher PRS. However, because the previous literature is inconsistent regarding which allele is associated with greater risk for engaging in aggressive behavior, the **first specific aim** is to identify which alleles predict aggression in order to determine if polygenic risk for aggression corresponds to lower transcriptional efficiency. Study #1 will address the first specific aim. It is hypothesized that, consistent with theory and the majority of previous literature, the alleles resulting in reduced transcriptional efficiency will be associated with greater aggression.

The **second specific aim** is to examine the moderating effect of childhood stressors on the association between PRS and aggression. Study #1 and Study #2 will address the second specific aim. It is hypothesized that at low reported exposure to childhood trauma there will be a positive association between the PRS and aggression, and that the association will be more positive as the level of reported exposure to childhood trauma increases.

The **third specific aim** is to examine the mediating effect of acute stress on the association between PRS, exposure to childhood trauma, and aggression. Study #2 and Study #3 will address the third specific aim. It is hypothesized that acute stress will mediate the association; more specifically individuals with high PRS and a high level of reported childhood trauma exposure are expected to be more

aggressive via acute stress.

The **fourth specific aim** is to examine the association between PRS, exposure to childhood trauma, acute stress, and alcohol-related aggression. Study #3 will address the fourth specific aim. It is hypothesized that individuals with high PRS and a high level of reported childhood trauma experiences are expected to engage in more alcohol-related aggression via acute stress, particularly if they use alcohol to cope with stress.

Figure 2.1 displays how our research questions fit within the General Aggression Model. To our knowledge, this is the first study to examine if there is an association between polygenic risk and aggression as moderated by childhood trauma and mediated by response to an acute stress, and therefore, may contribute to our limited understanding of the etiology of aggressive behavior. Our approach to addressing the four specific aims listed above also addresses the critical concern of a failure to replicate results in cGxE research by providing converging evidence across multiple studies and using different methodologies (both correlational and experimental approaches).

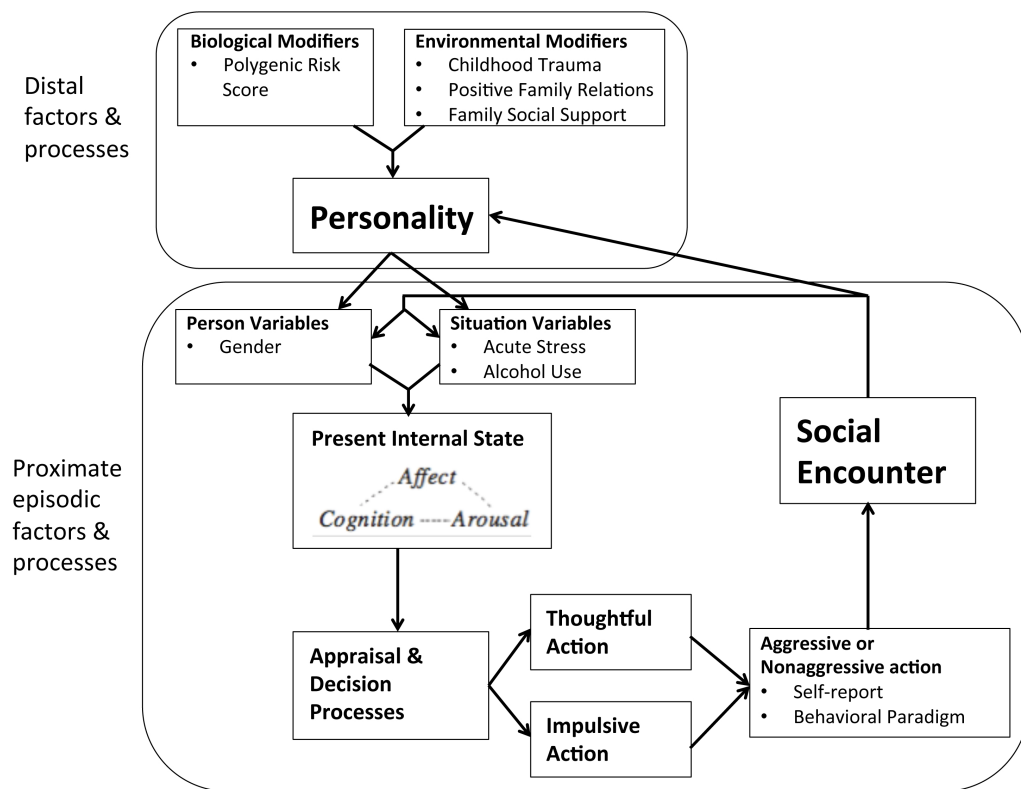


Figure 2.1: An integration of the General Aggression Model and our research questions.

CHAPTER 3: THE ASSOCIATION BETWEEN GENETIC RISK AND AGGRESSION AS MODERATED BY CHILDHOOD TRAUMA EXPOSURE (STUDY #1)

Genetic polymorphisms that alter transcriptional efficiency of serotonin system genes have been previously associated with aggressive behavior (Beitchman et al., 2006; Jensen et al., 2009; Reuter et al., 2007; Stuart et al., 2014). Exposure to childhood trauma has also been shown to moderate the association between these genetic polymorphisms and aggression (Byrd & Manuck, 2014; Caspi et al., 2002; Reif et al., 2007). However, there is inconsistency in the literature regarding which allele contributes to increased risk of engaging in aggressive behaviors (Foshee et al., 2015; Verhoeven et al., 2012; Yoon et al., 2012). There has also yet to be a study to examine the aggregate risk of *TPH2* rs4570625, *SLC6A4* 5-HTTLPR+rs25531, *HTR1B* rs13212041, and *MAOA* uVNTR on aggressive behavior. Therefore, the goal of the first study was to investigate which alleles of *TPH2* rs4570625, *SLC6A4* 5-HTTLPR+rs25531, *HTR1B* rs13212041, and *MAOA* uVNTR are associated with increased aggression, and if a polygenic risk score comprised of these polymorphisms is moderated by previous exposure to childhood trauma. This chapter includes three sections. The first section describes the participants, measures, genotyping, and analytical approaches taken to address the specific aims: to investigate which alleles predict aggression (specific aim #1), and to examine the moderating effect of childhood stressors on the association between PRS and aggression (specific aim #2). The second section reports the quantitative findings. The first set of analyses tested our hypothesis that the alleles corresponding to reduced transcriptional efficiency will be associated with greater self-reported aggression. The second set of analyses tested our hypothesis that at low reported exposure to childhood trauma,

there will be a positive association between PRS and aggression, and that the association will be more positive as the level of reported exposure to childhood trauma increases. The third section summarizes the findings in the context of our research hypotheses and previous literature.

Methods

Participants. Undergraduate students ($N = 825$; 70.5% women; 87.3% White; mean age = 20.43 [$SD = 3.15$; range = 18-55]) from a Midwestern university were recruited from the Psychology Department's subject pool. The study was advertised with the following description to the subject pool:

Why do people engage in risky behavior? In this study we will examine some of the psychological and genetic influences on risky drinking and eating behaviors. Participants will be asked to complete a questionnaire that asks about emotion, alcohol use and eating behaviors. The questionnaire will also ask some questions about specific childhood experiences, your parents, and your political views. Participants will also complete a computer task that investigates decision-making. You will also be asked to provide some cheek cells for genotyping. Participation will take about 1 hour and you will receive 2 research credits. You must be 19 years of age or older to participate in this research study.

Students earned two course credits for an hour of participation in which they completed questionnaires on a desktop computer and provided cheek cells for genotyping purposes. The study was approved by the IRB and all participants gave written informed consent.

Measures. The Buss Perry Aggression Questionnaire (BPAQ) assesses aggression and was developed as an update to the Buss Durkee Hostility Inventory

(Buss & Perry, 1992). This is a 29-item self-report measure comprised of 4 subscales: Physical Aggression (e.g. “If somebody hits me, I hit back”), Verbal Aggression (e.g. “I often find myself disagreeing with people”), Anger (e.g. “I have trouble controlling my temper”), Hostility (e.g. “I am sometimes eaten up with jealousy”). Items are measured on a 7-point scale (adapted from an original 5-point scale; from 1, “Extremely Uncharacteristic of Me”, to 7, “Extremely Characteristic of Me”), where higher scores suggest higher levels of aggression.

Childhood trauma was assessed using the Traumatic Antecedents Questionnaire (TAQ) (Herman & Van der Kolk, 1987). The 42-item self-report questionnaire is comprised of 11 subscales. Items are measured on a 4-point scale (from 0, “Never or not at all”, to 3, “Often or very much”). The 19-items corresponding to the Traumatic experiences factor (Physical Abuse, Sexual Abuse, Witnessing, and Other Traumas subscales) were used for this study and responses of 1 (“Rarely or a little bit”) were converted to 0 prior to averaging item responses to produce an overall mean score. Higher scores correspond to an increased exposure to childhood trauma (Saleptsi et al., 2004).

Genotyping. DNA was extracted from cheek cells using the PURGENE DNA Isolation Kit Protocol (Gentra Systems, Inc., Minneapolis, MN). DNA concentration was determined using the NanoDrop2000 (Fisher Scientific, Inc., Pittsburgh, PA) and all samples were diluted to 20ng/ μ l.

5-HTTLPR and rs25531 were amplified using PCR forward and reverse primers: 5'-TCCTCCGCTTTGGCGCCTCTTCC-3' and 5'-TGGGGGTTGCAGGGGAGATCCTG-3' (Wendland, Martin, Kruse, Lesch, & Murphy, 2006). The PCRs were performed in 25 μ l reactions containing 20ng of DNA, 1X GoTaq Master Mix (Promega, Madison, WI, USA), and 10 μ M of each primer. The PCR amplification conditions were followed as previously described

(Wendland et al., 2006). The rs25531 polymorphism was recognized by digestion with HpaII (New England BioLabs, Ipswich, MA, USA) overnight at 37°C using 15 μ l of the PCR product. Digest product and PCR product were separated by electrophoresis on a 2.5% agarose gel and visualized under UV light with either ethidium bromide or SybrSafe stain. Two trained researchers made genotyping calls independently. The L_G, S_A, and S_G were classified as low activity alleles (S'), while L_A was classified as a high activity allele (L').

MAOA uVNTR was amplified using PCR forward and reverse primers: 5'-TGCTCCAGAAACATGAGCAC-3' and 5'-TAGACTTGGGGATCCGACTG-3'. The PCRs were performed in 25 μ l reactions containing 20ng of DNA, 1X GoTaq Master Mix (Promega, Madison, WI, USA), 10 μ M of each primer, and 10% DMSO. The PCR amplification conditions consisted of 5 minutes initial denaturation at 95°C, followed by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 40 seconds before a final elongation at 72°C for 10 minutes. A secondary set of primers were used to genotype samples unable to be called by the first set of primers and for the discrepancy test. The secondary set of primers (10 μ M of each primer), 5'-ACAGCCTGACCGTGGAGAAG-3' and 5'-AGGCTTACCTCGCAGGCAAG-3', were combined with 20ng of DNA and 1X GoTaq Master Mix (Promega, Madison, WI, USA) in a final volume of 25 μ l. The PCR amplification conditions consisted of 10 minutes initial denaturation at 95°C, followed by 35 cycles of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes before a final elongation at 72°C for 10 minutes. PCR product was separated by electrophoresis on a 3% agarose gel and visualized under UV light with SybrSafe stain. Genotyping calls were made by independently by two trained researchers. The 2R, 3R, and 5R alleles were classified as low activity alleles (LA) and the 3.5R and 4R were classified as high activity alleles (HA).

TPH2 rs4570625 was amplified using a Taqman SNP Genotyping Assay (Applied Biosystems, Foster City, CA). PCRs were performed in 5 μ l reactions containing 20ng DNA, 1X Taqman Master Mix, and 2X Taqman primers/probes. PCR amplification conditions consisted of 10 minutes initial denaturation at 95°C, followed by 50 cycles of 95°C for 15 seconds and then 60°C for 1 minute. Reactions were run on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). End point FAM and VIC fluorescence levels were analyzed using ABI Sequence Detection Software v1.2.3 (Applied Biosystems, Foster City, CA) and genotype calls were made based on the level of fluorescence signal.

HTR1B rs13212041 was amplified using a Taqman SNP Genotyping Assay (Applied Biosystems, Foster City, CA). PCRs were performed in 5 μ l reactions containing 20ng DNA, 1X Taqman Master Mix, and 2X Taqman primers/probes. PCR amplification conditions consisted of 10 minutes initial denaturation at 95°C, followed by 40 cycles of 95°C for 15 seconds and then 60°C for 1 minute. Reactions were run on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). End point FAM and VIC fluorescence levels were analyzed using ABI Sequence Detection Software v1.2.3 (Applied Biosystems, Foster City, CA) and genotype calls were made based on the level of fluorescence signal.

Ten percent of samples were re-genotyped for each polymorphism to assess genotyping accuracy. No discrepancies were observed in the genotyping calls. The call rate for each polymorphism is as follows: 96.7% for 5-HTTLPR+rs25531, 98.8% for *MAOA* uVNTR, 96.1% for *TPH2* rs4570625, and 99.2% for *HTR1B* rs13212041. Table 3.1 presents the allele frequencies and test of Hardy-Weinberg Equilibrium (HWE) for each polymorphism. Rs25531 was not within HWE, however this is likely the result of the G/G genotype having a sample size size ($N = 9$), as opposed to an error in genotyping. Our reported allele frequencies for rs25531 were

consistent with those reported by the National Center for Biotechnology Information (NCBI; MAF = 0.09).

Statistical Analyses. The first PRS (tPRS; transcriptional efficiency PRS) used for our analyses was based on transcriptional efficiency (Chen & Miller, 2008; Hu et al., 2006; Jensen et al., 2009; Sabol et al., 1998). Genotypes associated with lower transcriptional activity (5-HTTLPR+rs25531 S'/_; *MAOA* uVNTR LA/_; rs13212041 T/T; rs4570625 T/_) were given a score of 1 and genotypes associated with higher transcriptional activity were given a score of 0 (5-HTTLPR+rs25531 L'/L'; *MAOA* uVNTR HA/HA; rs13212041 C/_; rs4570625 G/G). In order to conserve power, heterozygous genotypes were grouped with the homozygous genotype associated with lower transcriptional activity. This grouping is supported by previous literature examining the association between the genetic polymorphisms and both transcriptional activity and aggressive behavior. The tPRS was calculated by averaging the score of all four polymorphisms. An average score was used rather than a sum score in order to include individuals who did not have a complete genetic profile (91.9% 4/4 genotypes; 7.2% 3/4 genotypes; 0.8% 2/4 genotypes; 0.1% 1/4 genotypes).

Our second PRS (dPRS; data derived PRS) was developed based on the association between each polymorphism and self-reported aggression from our data. In order to build the dPRS, an analysis of variance was used to examine the interaction between the four genetic polymorphisms on self-reported aggression. Patterns of the significant pairwise comparisons of the highest order effect (4-way interaction) were examined using a least significant difference (LSD). The interaction between polymorphisms was examined, rather than considering only the main effect of each polymorphism, because previous inconsistencies in the literature have been found when the interaction between polymorphisms is included (Nobile et

al., 2007). Due to *MAOA* uVNTR being located on the X chromosome, men and women were analyzed separately. Buss-Perry Aggression Questionnaire scores were significantly skewed (skewness = 1.00); therefore log-transformed scores were used in the following analyses. Uncorrected p-values are reported.

The association between PRS (both tPRS and dPRS) and self-reported aggression (log scores) as moderated by self-reported exposure to childhood trauma was examined with SPSS software v.23 (IBM Corporation, Armonk, NY) using PROCESS model 1 (Hayes, 2013; conceptual diagram is presented in Figure 3.1), including age, race (coded as 0 = White, 1 = non-White) and all predictor (PRS and childhood trauma exposure)*demographic (age and race) interactions as covariates. A quadratic term was not included in the model because the correlation between PRS and reported exposure to childhood trauma was not significant. For significant interaction effects, the PROCESS model reports the simple effect of PRS at the mean of childhood trauma exposure scores in addition to one standard deviation above and below the mean. Again, due to *MAOA* uVNTR being located on the X chromosome, men and women were analyzed separately. Uncorrected p-values are reported.

Results

Building the dPRS. For men, there were four significant pairwise comparisons when examining the 4-way interaction. Men who had a *MAOA* uVNTR LA-allele, heterozygous genotype of rs4570625, and were homozygous for the T-allele of rs13212041, reported lower aggression if also homozygous for the L'-allele of 5-HTTLPR+rs25531 compared to having at least one S'-allele (L'/S' $p = .036$, S'/S' $p = .044$; see Figure 3.2a). Men who were homozygous for the L' of 5-HTTLPR+rs25531, and had the heterozygous genotype for both rs4570625 and rs13212041, reported higher aggression if they also had a *MAOA* uVNTR HA-allele

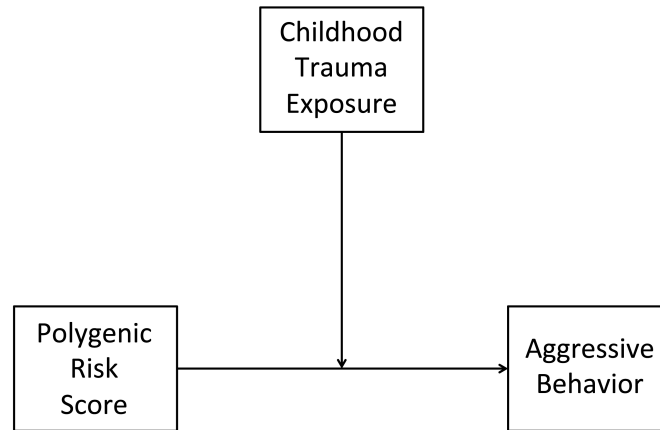


Figure 3.1: Conceptual diagram of SPSS PROCESS model 1

compared to a *MAOA* uVNTR LA-allele ($p = .026$; see Figure 3.2b). Men who were homozygous for both the S'-allele of 5-HTTLPR+rs25531 and the T-allele of rs13212041, and had the heterozygous genotype for rs4570625, reported lower aggression if they also had a *MAOA* uVNTR HA-allele compared to a *MAOA* uVNTR LA-allele ($p = .029$; see Figure 3.2c). Men who were homozygous for both the S'-allele of 5-HTTLPR+rs25531 and the T-allele of rs13212041, and had a *MAOA* uVNTR LA-allele, reported lower aggression if they were also homozygous for the G-allele of rs4570625 compared to if they had the heterozygous genotype ($p = .028$; see Figure 3.2d). Based on the pattern of the pairwise comparisons, the genotypes 5-HTTLPR+rs25531 S'/__, *MAOA* uVNTR LA, rs13212041 T/T, rs4570625 T/__, were associated with higher reported aggression and 5-HTTLPR+rs25531 L'/L', *MAOA* uVNTR HA, rs13212041 C/__, rs4570625 G/G were associated with lower aggression. These results were consistent with the tPRS.

For women, there were seven significant pairwise comparisons when examining the 4-way interaction. Women who were homozygous for a *MAOA* uVNTR

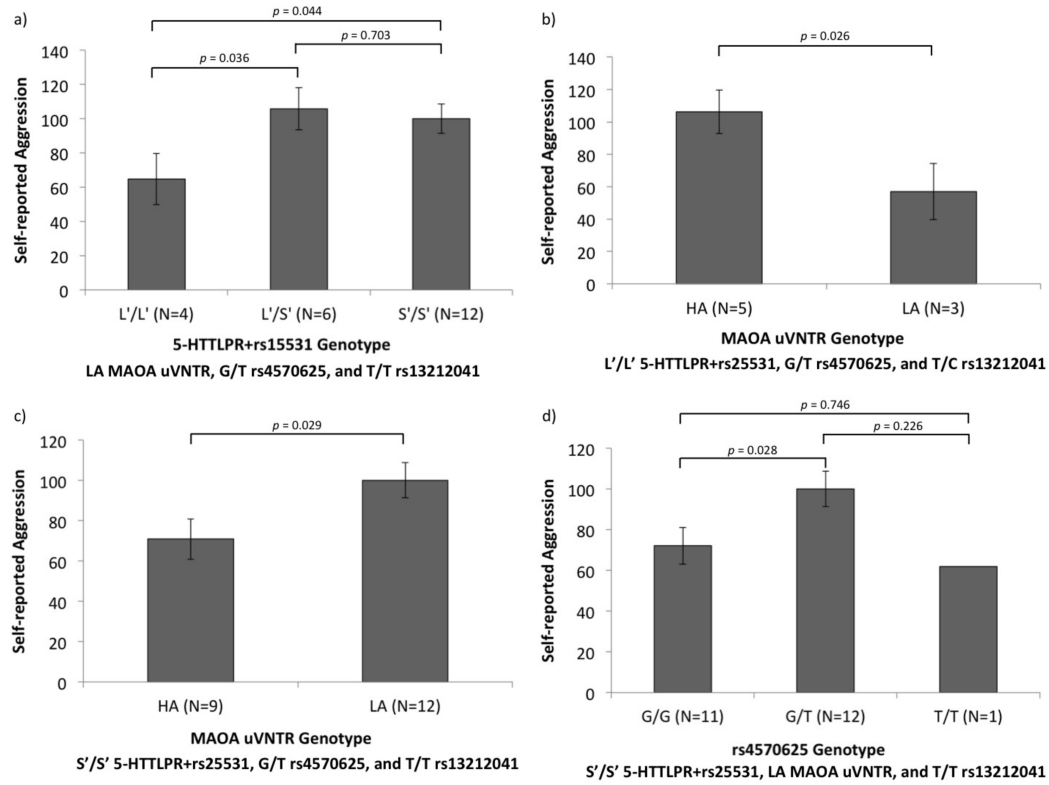


Figure 3.2: Significant pairwise comparisons of the 4-way interaction between *SLC6A4* 5-HTTLPR+rs25531, *MAOA* uVNTR, *TPH2* rs4570625, and *HTR1B* rs13212041 on self-reported aggression in men.

HA-allele, the G-allele of rs4570625, and the C-allele of rs13212041, reported higher aggression if they had the L'/L' genotype of 5-HTTLPR+rs25531 compared to if they were heterozygous for the S'-allele (no women were homozygous for the S'-allele; $p = .027$; see Figure 3.3a). Women who had a heterozygous genotype for *MAOA* uVNTR, and were homozygous for both the G-allele of rs4570625 and the T-allele of rs13212041, reported lower aggression if they had the L'/L' genotype of 5-HTTLPR+rs25531 compared to if they had the heterozygous genotype ($p = .019$; see Figure 3.3b). Women who were homozygous for the L'-allele of 5-HTTLPR+rs25531, the G-allele of rs4570625, and the T-allele of rs13212041, reported higher aggression if they were also homozygous for the *MAOA* uVNTR

LA-allele compared to having at least one HA-allele (HA/LA $p = .001$, HA/HA $p = .011$; see Figure 3.3c). Women who were homozygous for the S'-allele of 5-HTTLPR+rs25531 and the T-allele of rs13212041, and had the heterozygous genotype for rs4570625, reported higher aggression if they were also homozygous for a *MAOA* uVNTR HA-allele compared to having the heterozygous genotype ($p = .018$; see Figure 3.3d). Women who were homozygous for L'-allele of 5-HTTLPR+rs25531, HA-allele of *MAOA* uVNTR, and C-allele of rs13212041, reported higher aggression if they were also homozygous for the G-allele of rs4570625 compared to if they had the heterozygous genotype (no women were homozygous for the T-allele; $p = .045$; see Figure 3.3e). Women who were homozygous for L'-allele of 5-HTTLPR+rs25531, HA-allele of *MAOA* uVNTR, and G-allele of rs4570625, reported higher aggression if they were also homozygous for the C-allele of rs13212041 compared to if they had at least one T-allele (T/C $p = .046$; T/T $p = .004$; see Figure 3.3f). Women who were homozygous for both the L'-allele of 5-HTTLPR+rs25531 and the G-allele of rs4570625, and had a heterozygous genotype for *MAOA* uVNTR, reported lower aggression if they were also homozygous for the T-allele of rs13212041 compared to having the heterozygous genotype (no women were homozygous for the C-allele; $p = .040$; see Figure 3.3g). Based on the pattern of the pairwise comparisons, genotypes associated with higher reported aggression were given a score of 1 (5-HTTLPR+rs25531 L'/L'; *MAOA* uVNTR HA/HA; rs13212041 C/C; rs4570625 G/G) and genotypes associated with lower aggression were given a score of 0 (5-HTTLPR+rs25531 S'/-; *MAOA* uVNTR LA/-; rs13212041 T/-; rs4570625 T/-). A total dPRS was calculated by averaging the score of all four polymorphisms.

Association between PRS and Aggression. Because the dPRS and tPRS were identical in men, only one set of analyses is presented. There was no significant

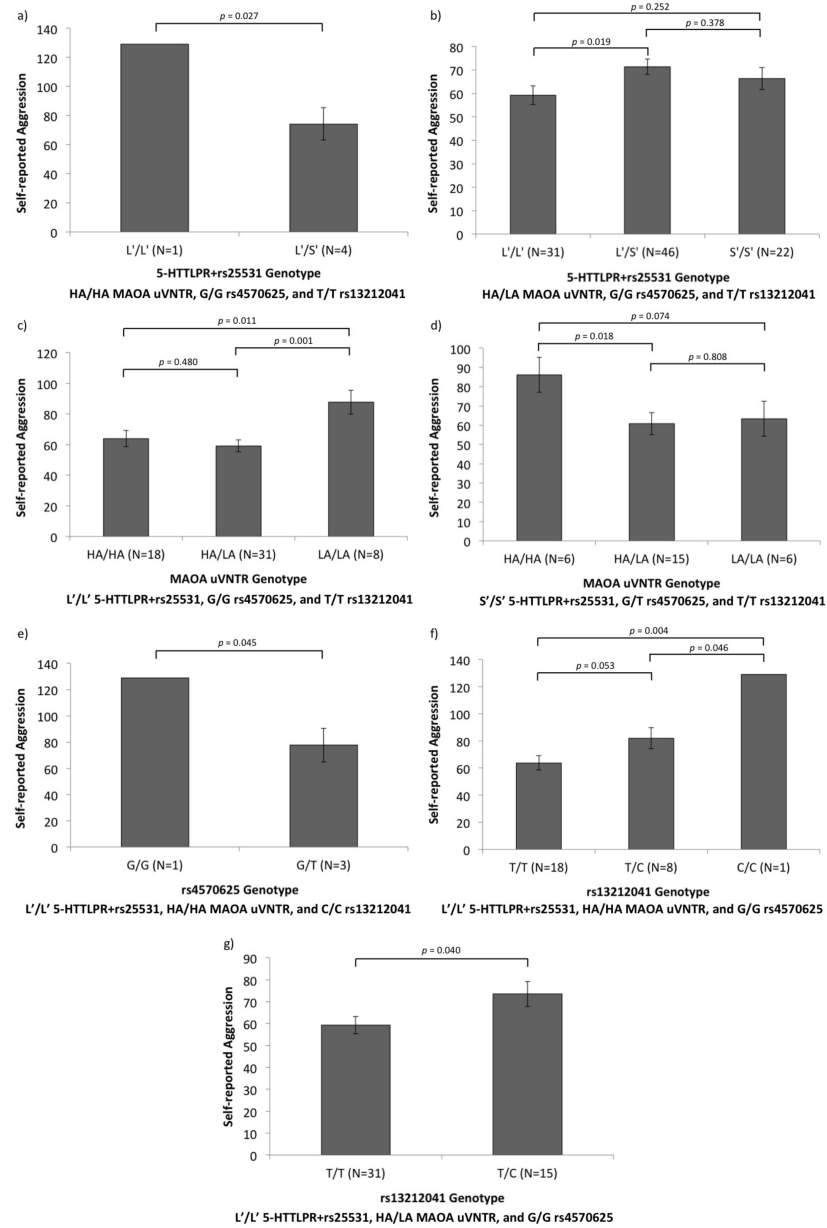


Figure 3.3: Significant pairwise comparisons of the 4-way interaction between *SLC6A4* 5-HTTLPR+rs25531, *MAOA* uVNTR, *TPH2* rs4570625, and *HTR1B* rs13212041 on self-reported aggression in women.

effect of PRS on self-reported aggression. Nor did self-reported childhood trauma exposure moderate the association between PRS and self-reported aggression.

Results of the full model are reported in Table 3.2.

For women, using the tPRS, there was a marginally significant moderating effect of self-reported childhood trauma exposure on the association between tPRS and self-reported aggression ($b = 0.198, p = 0.052$). Examination of the conditional effects of tPRS on aggression at varying levels of childhood trauma exposure revealed that tPRS was not significantly associated with aggression at any level of reported childhood trauma exposure (see Figure 3.4a). Results of the full model are reported in Table 3.2.

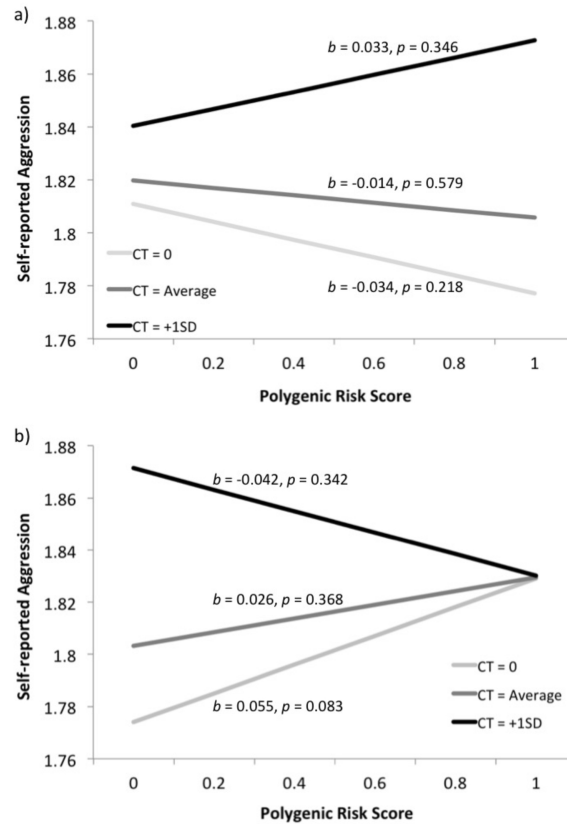


Figure 3.4: Conditional effects of polygenic risk scores on self-reported aggression as moderated by self-reported exposure to childhood trauma in women using the a) tPRS and b) dPRS. Self-reported aggression scores are presented as log scores.

Using the dPRS, there was a significant moderating effect of self-reported childhood trauma exposure on the association between dPRS and self-reported

aggression ($b = -0.288$, $p = 0.034$). There was also a significant effect of exposure to childhood trauma ($b = 0.291$, $p < 0.001$), such that at low polygenic risk (dPRS = 0), higher levels of reported exposure to childhood trauma was associated with higher reported aggression. Examination of the conditional effects of dPRS on aggression at varying levels of childhood trauma exposure revealed that dPRS was not significantly associated with aggression at any level of reported childhood trauma exposure (see Figure 3.4b). Results of the full model are reported in Table 3.2.

Discussion

Consistent with our first hypothesis, for men, alleles associated with reduced transcriptional efficiency were more consistently associated with higher self-reported aggression. However, inconsistent with our hypothesis, for women, alleles associated with greater transcriptional efficiency were more consistently associated with higher self-reported aggression. While there is previous literature to support an association between alleles resulting in greater transcriptional efficiency and aggression (Barr et al., 2003; Brune et al., 2006; Hessler et al., 2008; Tikkanen et al., 2009; Verhoeven et al., 2012), which was our rationale for examining this association rather than solely using a tPRS, it is interesting that the results between men and women are in opposition. In general, serotonin function is more robustly associated with aggression in men than in women (Manuck, Flory, Ferrel, Mann, & Muldoon, 2000), but to our knowledge only one study found an effect in the opposite direction between men and women when examining an association with aggressive behavior. Specifically, the 5-HTTLPR L-allele was associated with higher delinquency in men, while the S-allele was associated with higher delinquency in women (Åslund et al., 2012), which supports a gender difference. However, in the current study 5-HTTLPR L-allele was associated with higher aggression in women, while the

S-allele was associated with higher aggression in men. Given that genetic susceptibility to negative outcomes is dependent on the context of the environment (Belsky & Pluess, 2009), it is possible that a direct examination of genetic susceptibility is misleading. Because the effect of genotype may be conditional on the context of the environment, the pattern of the effect of genotype might vary as a result of the range of environmental factors (e.g. childhood trauma) within the population studied, potentially contributing to differences in reported findings.

As describe earlier, polygenic risk scores are typically developed based on transcriptional efficiency or GWAS analyses. However, given the inconsistencies in previous literature regarding which allele is associated with increased aggression, we created a PRS (dPRS) following Derringer and colleagues (2010; 2012), based on the association between genetic polymorphisms and aggression within our own data. The interaction between polymorphisms was also examined to account for any moderating effects between polymorphisms. However, the dPRS should be considered with caution, due to the small number of individuals with each genotype combination. Therefore, the possibility of a Type I error needs to be considered when interpreting the significant pairwise comparisons used to build the dPRS.

Our results using a tPRS, consistent with previous research (Bouvette-Turcot et al., 2015; Byrd & Manuck, 2014; Reif et al., 2007; Weder et al., 2009), indicate a significant moderating effect of childhood trauma on the association between polygenic risk and aggression. When examining the moderating effect of childhood trauma in women, consistent with our second hypothesis, there was a positive association between tPRS and self-reported aggression for women who reported experiencing high levels of childhood trauma, although this simple effect was not significant. However, inconsistent with our hypothesis that there would also be a positive association for women reporting low levels of childhood trauma exposure, a

nonsignificant negative association was observed. In the context of the Differential Susceptibility Model, we had expected that low exposure to childhood trauma would still be considered a negative environment (as opposed to a positive environment), and therefore, polygenic risk would still be associated with aggression even at low levels of reported exposure to childhood trauma. However, if we assume that children who experience low levels of childhood trauma, if any, are also likely to experience positive environments (e.g. parental monitoring, social support), then the Differential Susceptibility Model would actually suggest a negative association between polygenic risk and aggression, which is consistent with our results using a tPRS. Indeed previous literature suggests a negative correlation between negative environmental factors (e.g. physical abuse, neglect) and positive environmental factors (e.g. parental monitoring, social support) (Kort-Butler, Tyler, & Melander, 2011; Runtz & Schallow, 1997). The pattern of our results is also consistent with previous gene-by-environment literature that has found a negative association between childhood trauma and aggression for individuals who have a susceptible genotype (i.e. *MAOA* LA allele, 5-HTTLPR S allele) and no association between childhood trauma and aggression for the non-susceptible genotype, with a cross-over pattern indicated by the data (Bouvette-Turcot et al., 2015; Caspi et al., 2002; Enoch et al., 2013; Frazzetto et al., 2007).

Results with the dPRS were opposite to that of the tPRS in women, which is to be expected given that the alleles that correspond to higher transcriptional efficiency corresponded to lower polygenic risk in the tPRS, but corresponded to higher polygenic risk in the dPRS. The majority of our sample (78%) reported low levels (score ≤ 0.10) of exposure to childhood trauma ($M = 0.10$, $SD = 0.23$; range = 0-2.13), which means the majority of individuals (66.8%) reported experiencing traumatic events never or rarely, and an additional 11.2% (score = 0.10) reported

experiencing one traumatic event occasionally. Again, if we assume low levels of exposure to childhood trauma are correlated with other positive environments then it is not surprising that an examination of genetic risk alone would indicate an association in the opposite direction than expected. Therefore, overall our results in women suggest that the tPRS is associated with individual differences in aggression in a manner consistent with the Differential Susceptibility Model.

While the moderating effect of environmental factors, such as childhood trauma, explain the pattern of our results in women, it does not explain why the dPRS in men was consistent with theory. Nor does it explain why there was no significant moderating effect of childhood trauma in men. Serotonin (Manuck et al., 2000), and the LA-allele of *MAOA* uVNTR in particular (Byrd & Manuck, 2014), are more strongly associated with aggression in men compared to women, which suggests that a significant effect should have been present for men in our analyses. Although exposure to childhood trauma is a risk factor of aggressive behavior in both men and women (Gratz, Paulson, Jakupcak, & Tull, 2009; McKinney et al., 2009), women are more vulnerable to the effect of childhood trauma (Dornfeld & Kruttschnitt, 1992; Foster, Kuperminc, & Price, 2004). Therefore, the additive risk of susceptible genotypes and exposure to childhood trauma would suggest that an effect should have been present in both men and women. When considering the effect size of the interaction between PRS and experiences of childhood trauma, there was consistency across men ($r = 0.07$) and women ($r = 0.09$). However, the sample size for men ($N = 236$) was less than half the sample size for women ($N = 574$). For an effect size of 0.10, a sample size of 781 is required to have 80% power. Therefore, it is likely that the lack of a significant effect and fewer significant pairwise comparisons when building the dPRS in males is the result of our analyses being underpowered.

Table 3.1
Allele Frequency and Hardy-Weinberg Equilibrium Statistics for Study #1

	Genotype Frequency	Allele Frequency	Hardy-Weinberg Equilibrium (HWE)	
5-HTTLPR	L/L = 246 L/S = 389 S/S = 165	L = 0.55 S = 0.45	$\chi^2 = 0.24$	$p = 0.624$
rs25531	A/A = 703 A/G = 92 G/G = 9	A = 0.93 G = 0.07	$\chi^2 = 8.40$	$p = 0.004$
rs4570625	G/G = 467 G/T = 280 T/T = 46	G = 0.77 T = 0.23	$\chi^2 = 0.22$	$p = 0.639$
rs13212041	T/T = 508 T/C = 277 C/C = 33	T = 0.79 C = 0.21	$\chi^2 = 0.39$	$p = 0.532$
MAOA uVNTR men	3 = 89 3.5 = 2 4 = 143 5 = 2	3R = 0.38 3.5R = 0.01 4R = 0.61 5R = 0.01		
MAOA uVNTR women	2/2 = 1 2/3 = 3 2/4 = 2 3/3 = 77 3/3.5 = 2 3/4 = 257 3/5 = 4 3.5/4 = 7 4/4 = 217 4/5 = 8 5/5 = 1	2R = 0.01 3R = 0.36 3.5R = 0.01 4R = 0.61 5R = 0.01	$\chi^2 = 0.13$	$p = 0.718$

Note: HWE cannot be calculated for MAOA in men because the polymorphism is located on the X chromosome. HWE is presented for women, which is calculated from grouped genotypes based on transcriptional activity.

Table 3.2
Estimates from the Exposure to Childhood Trauma Moderation Model of Polygenic Risk on Aggression

	<i>Men</i>	<i>Women tPRS</i>	<i>Women dPRS</i>
<i>Model Variables</i>			
Constant	1.903	1.811	1.774
Exposure to Childhood Trauma	0.044	0.087	0.291***
Polygenic Risk Score	-0.031	-0.034	0.055
Childhood Trauma*Polygenic Risk	0.294	0.198 [#]	-0.288*
Age	-0.009	-0.001	-0.002
Race (White vs. non-White)	-0.001	0.126**	0.009
Childhood Trauma*Age	0.006	-0.014**	-0.015**
Childhood Trauma*Race	-0.180	-0.049	-0.054
Polygenic Risk*Age	0.006	0.002	0.007
Polygenic Risk*Race	0.073	-0.134*	0.126 [#]
<i>Model Fit</i>			
R ²	0.081	0.110	0.108
MSE	0.021	0.017	0.017
F (df)	2.213 (9, 226)	7.705 (9, 564)	7.618 (9, 564)
p	0.022	<0.001	<0.001

[#] $p < .10$, $p < .05$, $p < .01$, $p < .001$

CHAPTER 4: MEDIATION OF ACUTE PERCEIVED STRESS (STUDY #2)

Genetic polymorphisms of serotonin genes, and exposure to childhood trauma, have both been shown to be associated with HPA dysregulation (Brummett et al., 2008; Frodl & O’Keane, 2013). Dysregulation of the HPA axis subsequently alters response to stressors (Kuhlman et al., 2015; Trickett et al., 2014), and activation of the HPA axis increases the likelihood of engaging in aggressive behavior (Kruk et al., 2004). Exposure to stress has also been shown to moderate the association between genetic polymorphisms of serotonin genes and aggression (Conway et al., 2012; Schwandt et al., 2010; Verona et al., 2006). However, the mediating effect of acute stress on the association between genetic variation and aggression, as moderated by childhood trauma, has yet to be examined. Additionally, only about one quarter of replication studies support significant findings, suggesting that a large number of cGxE studies may be false positives (Duncan & Keller, 2011). Therefore, replication of cGxE findings, prior to publication, is important. The goals of this second study were to (1) examine the moderating effect of childhood trauma on the association between PRS and aggression (specific aim #2) in order to replicate the first study, and (2) examine the mediating effect of acute stress on the association between PRS and aggression as moderated by childhood trauma (specific aim #3). This chapter includes three sections. The first section describes the participants, measures, genotyping and analytical approaches taken to address the second and third specific aims. The second section reports the quantitative findings. The first set of analyses tested our hypothesis that at low reported exposure to childhood trauma there will be a positive association between PRS and aggression, and that the association will be more positive as the level of reported exposure to childhood

trauma increases. The second set of analyses tested our hypothesis that individuals with high PRS and higher reported exposure to childhood trauma are expected to report higher aggression via acute stress. The final section summarizes the findings in the context of our research hypotheses and previous literature.

Methods

Participants. Undergraduate students ($N = 765$; 56.3% women; 80.1% White; age $M = 19.53$ [$SD = 2.36$; range = 17-48]) from a Midwestern university were recruited from either (1) the Psychology Department's subject pool or (2) the University's Criminal Justice Department. Students recruited from the Psychology Department were provided the following study description:

Drinking, fighting, and breaking the rules. All of these behaviors vary from person to person, but why that is remains unclear. In this study we will be examining some of the psychological, social, and genetic influences on these behaviors. Participants in this study will complete a computerized survey that includes questions about involvement in and attitudes towards alcohol use, violence, and other anti-social behaviors. The survey also asks some questions about your childhood experiences, your personality, and your habits. After completing the questionnaire, you will also be asked to provide some saliva for genotyping. Participation will take approximately one (1) hour, and you will be compensated with two (2) research credits. YOU MUST BE 17 YEARS OLD OR OLDER TO PARTICIPATE IN THIS STUDY.

Psychology Department students earned two course credits for an hour of participation. Contact information of students recruited from the Criminal Justice Department was obtained from the academic advisor of the University's Criminal

Justice Department. Students were sent the following email:

INVITATION TO PARTICIPATE IN A STUDY OF GENES, MIND,
AND SOCIAL BEHAVIOR. As a UNL Criminal Justice major, you have
an opportunity to earn \$20 dollars by participating in a research study
organized by the School of Criminology and Criminal Justice.

Participating students will be asked to complete a questionnaire and to
provide a saliva sample at the UNL Center for Brain, Biology, and
Behavior located at the east section of the Memorial Stadium. The
entire session lasts approximately 45 minutes. Any information you
provide will be confidential. Your personal information will be collected
only for compensation and recruitment purposes and will be destroyed
after such compensation is given or recruitment is complete. Your
personal information (name, contact information, etc.) will not be
included in the responses you give in the study. At this time, we would
like to know if you are interested in participating in this study. If you
are, please respond to this email and write "YES" in the subject line.
We will then contact you to schedule an appointment. If you are unsure
about your willingness to participate but wish to find out more about
the study, please contact a member of our research team by responding
to this email with subject line "MAYBE." Thank you and best of luck
with your studies!"

Students, who responded with interest to the email and participated in the study,
received \$20 for their one hour of participation. The study included completing
questionnaires on a desktop computer and donating a saliva sample using
Oragene-Discover (OGR-500) self-collection kit (DNA Genotek, Inc., Ottawa,

Ontario, Canada) for genotyping purposes. The study was approved by the IRB and all participants gave written informed consent.

Measures. The Buss Perry Aggression Questionnaire (BPAQ), as described in Chapter 3 (page 40-41) was used to assess trait aggression (Buss & Perry, 1992). Items were measured on the original 5-point scale (from 1, “Extremely Uncharacteristic of Me”, to 5, “Extremely Characteristic of Me”), where higher scores suggest higher levels of aggression.

Our measure of direct and indirect aggression was adapted from the original Direct/Indirect Aggression Scale (Björkqvist, Lagerspetz, & Kaukiainen, 1992). The adapted measure consisted of 24 self-report items comprised of 3 subscales: Direct Physical Aggression (e.g. “Hit someone”), Direct Verbal Aggression (e.g. “Yelled or argued with someone”), and Indirect Aggression (e.g. “Ignored someone”). Participants were asked to respond on a 5-point scale (from 1, “Never”, to 5, “Very Often”) how often in the last 90 days they had done particular aggressive behaviors. Items were averaged to create three subscale scores, where higher scores correspond to higher amounts of aggression.

Reactive vs. proactive aggression was adapted from the original Reactive-Proactive Aggression Questionnaire (Raine et al., 2006). The adapted measure consisted of 23 self-report items comprised of two subscales: Reactive Aggression (e.g. “Yelled at others when they annoyed you”) and Proactive Aggression (e.g. “Yelled at others so they would do things for you”). Participants were asked to respond on a 3-point scale (from 1, “Never”, to 3, “Often”) how often in the last 90 days they had done particular aggressive behaviors. Items were averaged to create two subscale scores, where higher scores correspond to higher amounts of aggression.

The Childhood Trauma Questionnaire (CTQ) used in this study is a short form

of the original 70-item assessment of child abuse and neglect (Bernstein & Fink, 1998). The questionnaire is a 28-item measure comprised of physical (e.g. “People in my family hit me so hard that it left me with bruises or marks”), sexual (e.g. “Someone tried to touch me in a sexual way or tried to make me touch them”), and emotional abuse (e.g. “I thought that my parents wished I had never been born”), as well as physical (e.g. “I had to wear dirty clothes”) and emotional neglect (e.g. “I felt loved”-reverse scored) when growing up. Items are measured on a 5-point scale (from 1, “Never True”, to 5, “Very Often True”) producing a total sum score and sum scores for each subscale, where higher scores indicate the greater the severity of maltreatment. The questionnaire also has a three-item Minimization/Denial validity scale (e.g. “There was nothing I wanted to change about my family”) to detect the underreporting of maltreatment. For these three items, 1 point is added to the score for each item endorsed with a score of 5.

The Perceived Stress Scale is a 10-item self-report measure that asks about feelings and thoughts during the last month in order to gauge current levels of perceived stress (Cohen, Kamarck, & Mermelstein, 1983). Items are measured on a 5-point scale (from 1, “Never”, to 5, “Very Often”) producing a total sum score, where higher scores are higher levels of perceived stress. Example questions include: “In the last month, how often have you felt nervous and stressed?”, “In the last month, how often have you been angered because of the things that were outside of your control?”, and “In the last month, how often have you felt that you were on top of things”-reversed scored.

Genotyping. DNA was extracted from saliva following the DNA Genotek OGR-500Kit ethanol precipitation protocol prepIT-L2P reagent (DNA Genotek, Inc., Ottawa, Ontario, Canada). DNA concentration was determined using the NanoDrop2000 (Fisher Scientific, Inc., Pittsburgh, PA) and all samples were diluted

to 20ng/ μ l.

Genotyping methodologies described in Chapter 3 (pages 41-44) were used here to genotype 5-HTTLPR+rs25531, *MAOA* uVNTR, *HTR1B* rs13212041. *TPH2* SNP (rs6582071), which is in complete linkage disequilibrium (i.e. alleles are inherited together) with rs4570625, was amplified using a Taqman SNP Genotyping Assay (Applied Biosystems, Foster City, CA). *TPH2* rs6582071 data was used instead of rs4570625 because the genotyping for rs6582071 had already been completed at the time of our analyses, and due to complete linkage disequilibrium we are able to infer that the minor allele of rs6582071 is always inherited with the minor allele of rs4570625. PCRs were performed in 5 μ l reactions containing 20ng DNA, 1X Taqman Master Mix, and 2X Taqman primers/probes. PCR amplification conditions consisted of 10 minutes initial denaturation at 95°C, followed by 45 cycles of 95°C for 15 seconds and then 60°C for 1 minute. Reactions were run on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). End point FAM and VIC fluorescence levels were analyzed using ABI Sequence Detection Software v1.2.3 (Applied Biosystems, Foster City, CA) and genotype calls were made based on the level of fluorescence signal.

Ten percent of samples were re-genotyped for each polymorphism to test genotyping accuracy. No discrepancies were observed in the genotyping calls. The call rate for each polymorphism is as follows: 98.3% for 5-HTTLPR+rs25531, 99.5% for *MAOA* uVNTR, 98.6% for *TPH2* rs6582071, and 98.7% for *HTR1B* rs13212041. An average score was used to create the PRS rather than a sum score in order to include individuals who did not have a complete genetic profile (96.5% 4/4 genotypes, 2.5% 3/4 genotypes, 0.8% 2/4 genotypes, 0.1% 1/4 genotypes). Table 4.1 presents the allele frequencies and test of HWE for each polymorphism. All polymorphisms were within HWE.

Table 4.1

Allele Frequency and Hardy-Weinberg Equilibrium Statistics for Study #2

	Genotype Frequency	Allele Frequency	Hardy-Weinberg Equilibrium (HWE)	
5-HTTLPR	L/L = 229 L/S = 366 S/S = 157	L = 0.55 S = 0.45	$\chi^2 = 0.23$	$p = 0.632$
rs25531	A/A = 661 A/G = 94 G/G = 4	A = 0.93 G = 0.07	$\chi^2 = 0.11$	$p = 0.740$
TPH2 rs6582071	G/G = 407 G/T = 282 T/T = 65	G = 0.73 T = 0.27	$\chi^2 = 2.56$	$p = 0.110$
HTR1B rs13212041	T/T = 467 T/C = 253 C/C = 35	T = 0.79 C = 0.21	$\chi^2 = 0.01$	$p = 0.920$
MAOA uVNTR men	2 = 1 3 = 125 3.5 = 1 4 = 201 5 = 3	2R = 0.003 3R = 0.38 3.5R = 0.003 4R = 0.61 5R = 0.01		
MAOA uVNTR women	2/4 = 2 3/3 = 56 3/4 = 189 3/5 = 5 3.5/3.5 = 3 3.5/4 = 7 3.5/5 = 4 4/4 = 154 4/5 = 10	2R = 0.002 3R = 0.36 3.5R = 0.02 4R = 0.59 5R = 0.02	$\chi^2 = 0.06$	$p = 0.807$

Note: HWE cannot be calculated for MAOA in men because the polymorphism is located on the X chromosome. HWE is presented for women, which is calculated from grouped genotypes based on transcriptional activity.

Statistical Analyses. The association between genetic risk (both dPRS and tPRS as determined in Study #1) and self-reported aggression as moderated by self-reported exposure to childhood trauma was examined with SPSS software v.23

(IBM Corporation, Armonk, NY) using PROCESS model 1 (Hayes, 2013), including age, race (coded as 0 = White, 1 = non-White) and all predictor (genetic risk and experiences of childhood trauma)*demographic (age and race) interactions as covariates. A quadratic term was not included in the model because the correlation between PRS and reported exposure to childhood trauma was not significant. The mediating effect of perceived stress was examined with SPSS software v.23 (IBM Corporation, Armonk, NY) using PROCESS model 8 (conceptual diagram is presented in Figure 4.1). Reactive-Proactive Aggression Questionnaire (reactive aggression subscale skewness = 0.93; proactive aggression subscale skewness = 3.33) and Direct-Indirect Aggression Scale (direct physical aggression subscale skewness = 4.41; direct verbal aggression subscale skewness = 1.13; indirect aggression subscale skewness = 1.29) scores were significantly skewed. Therefore, log transformed scores were used for our analyses. For significant interaction effects, the PROCESS model reports the simple effect of PRS at the mean of childhood trauma exposure scores in addition to one standard deviation above and below the mean. Men and women were analyzed separately using the previously determined tPRS and dPRS, because *MAOA* uVNTR is located on the X chromosome. Uncorrected p-values are reported.

With PROCESS, a bootstrap approach (Shrout & Bolger, 2002) is implemented to examine indirect effects. Bootstrapping provides an empirical approximation of sample distributions of indirect effects to provide confidence intervals of estimates, and is the preferred method for testing indirect effects for multiple reasons (e.g. power is maximized, no assumptions about shape of the sample distribution) (Preacher, Rucker, & Hayes, 2007; Shrout & Bolger, 2002). If zero does not fall within the confidence interval, one can conclude that an indirect effect is different from zero. Bias-corrected bootstrap was performed with 1000 resamples drawn to derive the 95% confidence intervals.

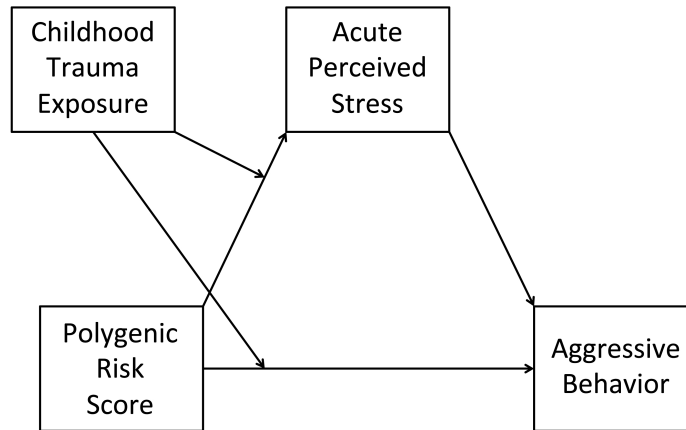


Figure 4.1: Conceptual diagram of SPSS PROCESS model 8.

Results

Association between PRS and Aggression. Again, because the dPRS was identical to the tPRS in men, only one set of analyses is presented. There was a significant effect of self-reported exposure to childhood trauma, such that at low polygenic risk (PRS = 0), higher reported exposure to childhood trauma was associated with higher BPAQ scores ($b = 1.157$, $p = 0.004$), verbal aggression ($b = 0.007$, $p = 0.004$), and reactive aggression ($b = 0.004$, $p = 0.049$). There was also a significant moderating effect of exposure to childhood trauma on the association between PRS and proactive aggression ($b = 0.003$, $p = 0.040$). Examination of the conditional effects revealed that higher PRS was associated with lower proactive aggression, at lower levels of reported exposure to childhood trauma ($b = -0.028$, $p = 0.048$); complete pattern of results is presented in Figure 4.2. Full model results are presented in Table 4.2.

Using the tPRS in women, there was a significant moderating effect of exposure to childhood trauma on the association between tPRS and physical aggression ($b =$

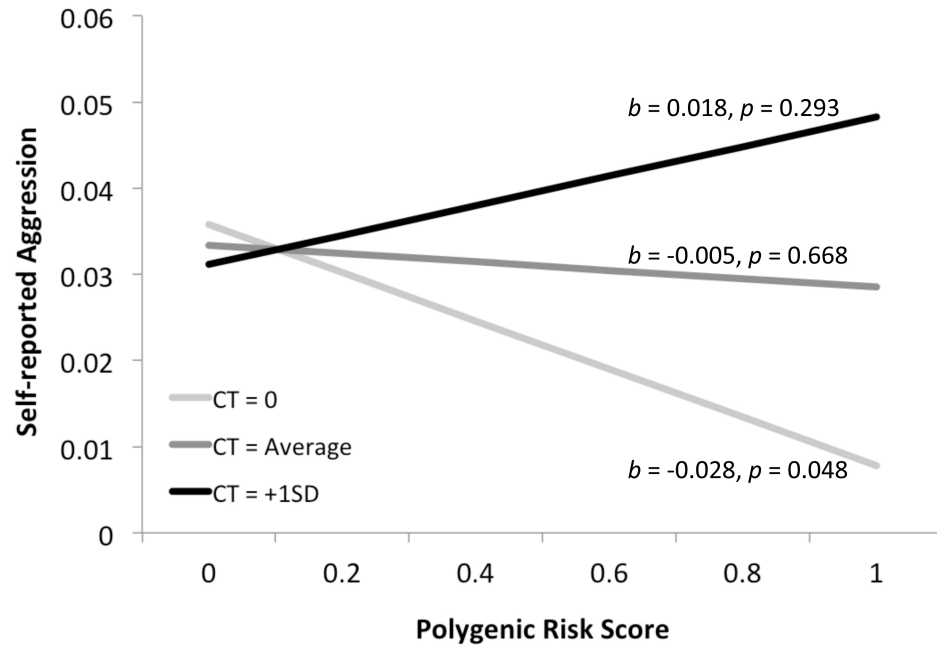


Figure 4.2: Conditional effects of polygenic risk scores on self-reported proactive aggression as moderated by self-reported exposure to childhood trauma in men. Self-reported aggression scores are presented as log scores.

0.004, $p = 0.006$). Examination of the conditional effects revealed that higher tPRS was associated with higher physical aggression, at higher levels of reported exposure to childhood trauma ($b = 0.040$, $p = 0.016$); complete pattern of results is presented in Figure 4.3a. There was also a significant moderating effect of exposure to childhood trauma on the association between tPRS and reactive aggression ($b = 0.007$, $p = 0.003$). Examination of the conditional effects revealed that higher tPRS was associated with lower reactive aggression, at lower levels of reported exposure to childhood trauma ($b = -0.046$, $p = 0.038$), and increased tPRS was associated with higher reactive aggression, at higher levels of reported exposure to childhood trauma ($b = 0.062$, $p = 0.025$); complete pattern of results is presented in Figure 4.3b. Finally, there was a significant moderating effect of exposure to childhood trauma

Table 4.2

Estimates for Men from the Exposure to Childhood Trauma Moderation Model of PRS on Aggression

<i>Model Variables</i>	Buss-Perry Aggression	Physical Aggression	Verbal Aggression	Indirect Aggression	Reactive Aggression	Proactive Aggression
Constant	60.346	0.036	0.238	0.169	0.118	0.036
Exposure to Childhood Trauma	1.157**	0.001	0.007***	0.005 [#]	0.004	-0.0003
Polygenic Risk Score	0.270	-0.007	-0.017	-0.015	0.010	-0.028*
Childhood Trauma*Polygenic Risk	-0.134	0.002	-0.004	-0.002	0.0005	0.003*
Age	-0.968	-0.004	-0.018 [#]	-0.016 [#]	-0.010	-0.008*
Race (White vs. non-White)	5.121	0.012	-0.049	-0.078	-0.047	-0.025
Childhood Trauma*Age	-0.183*	-0.0003	0.0003	-0.0002	-0.0001	0.0003
Childhood Trauma*Race	-1.302***	-0.004**	-0.006*	-0.004	-0.003	0.001
Polygenic Risk*Age	2.605	0.006	0.010	0.015	0.006	0.008 [#]
Polygenic Risk*Race	9.968	0.054	0.141	0.129	0.090	0.035
<i>Model Fit</i>						
R ²	0.119	0.045	0.070	0.067	0.096	0.145
MSE	309.767	0.007	0.021	0.014	0.007	0.002
F (df)	4.572 (9, 306)	1.661 (9, 319)	2.686 (9, 319)	2.525 (9, 319)	3.757 (9, 319)	6.017 (9, 319)
p	<0.001	0.098	0.005	0.008	<0.001	<0.001

[#] $p < .10$, $p < .05$, $p < .01$, $p < .001$

Note: Buss-Perry Aggression is from the BPAQ; Physical, Verbal, & Indirect Aggression are from the Direct-Indirect Aggression Scale; Reactive & Proactive Aggression is from the Reactive-Proactive Aggression Questionnaire.

on the association between tPRS and proactive aggression ($b = 0.003$, $p = 0.001$). Examination of the conditional effects revealed that higher tPRS was associated with higher proactive aggression, at higher levels of reported exposure to childhood trauma ($b = 0.036$, $p = 0.003$); complete pattern of results is presented in Figure 4.3c. Full model results are presented in Table 4.3.

Using the dPRS in women, there was an effect of self-reported exposure to childhood trauma on BPAQ scores ($b = 0.928$, $p < 0.001$), physical aggression ($b = 0.002$, $p < 0.001$) verbal aggression ($b = 0.006$, $p < 0.001$), indirect aggression ($b = 0.003$, $p = 0.030$), reactive aggression ($b = 0.005$, $p < 0.001$), and proactive aggression ($b = 0.003$, $p < 0.001$), such that, at low polygenic risk (dPRS = 0), higher reported exposure to childhood trauma was associated with higher aggression. There was also a moderating effect of childhood trauma exposure on the association between dPRS and physical aggression ($b = -0.003$, $p = 0.013$).

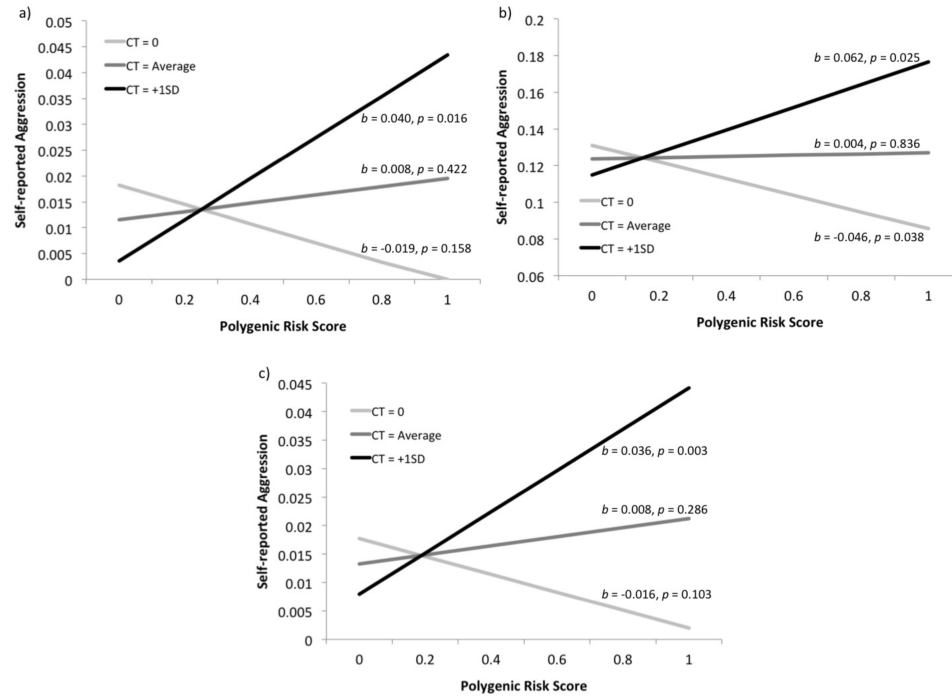


Figure 4.3: Conditional effects of tPRS on self-reported a) direct physical aggression, b) reactive aggression, and c) proactive aggression, as moderated by self-reported exposure to childhood trauma in women. Self-reported aggression scores are presented as log scores.

Examination of the conditional effects revealed that higher dPRS was associated with lower physical aggression, at higher levels of reported exposure to childhood trauma ($b = -0.040, p = 0.025$); complete pattern of results is presented in Figure 4.4a. Additionally, there was a moderating effect of childhood trauma exposure on the association between dPRS and reactive aggression ($b = -0.005, p = 0.013$). Examination of the conditional effects revealed that higher dPRS was associated with lower reactive aggression, at higher levels of reported exposure to childhood trauma ($b = -0.054, p = 0.045$); complete pattern of results is presented in Figure 4.4b. Finally, there was a moderating effect of childhood trauma exposure on the association between dPRS and proactive aggression ($b = -0.003, p < 0.001$). Examination of the conditional effects revealed that higher dPRS was associated

Table 4.3

Estimates for Women from the Exposure to Childhood Trauma Moderation Model of tPRS on Aggression

<i>Model Variables</i>	Buss-Perry Aggression	Physical Aggression	Verbal Aggression	Indirect Aggression	Reactive Aggression	Proactive Aggression
Constant	55.850	0.018	0.185	0.199	0.131	0.018
Exposure to Childhood Trauma	0.479	-0.001	0.001	0.0003	-0.001	-0.001
Polygenic Risk Score	-4.974	-0.019	-0.021	-0.033	-0.046*	-0.016
Childhood Trauma*Polygenic Risk	0.491	0.004**	0.005	0.003	0.007**	0.003**
Age	-1.653	-0.002	0.002	-0.008	-0.005	-0.0003
Race (White vs. non-White)	-5.836	0.014	-0.083	-0.103 [#]	-0.035	0.002
Childhood Trauma*Age	0.112	0.0000	-0.0001	0.0000	0.0000	0.0000
Childhood Trauma*Race	-0.380	-0.001	-0.002	-0.001	-0.001	-0.0001
Polygenic Risk*Age	0.120	-0.003	-0.010	-0.0003	-0.004	-0.002
Polygenic Risk*Race	15.944	-0.014	0.089	0.092	0.048	-0.005
<i>Model Fit</i>						
R ²	0.139	0.093	0.097	0.065	0.159	0.158
MSE	231.695	0.002	0.016	0.013	0.005	0.001
F (df)	6.987 (9, 390)	4.684 (9, 412)	4.911 (9, 412)	3.196 (9, 412)	8.664 (9, 412)	8.574 (9, 412)
p [#]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[#]*p*<.10, *p*<.05, *p*<.01, *p*<.001

Note: Buss-Perry Aggression is from the BPAQ; Physical, Verbal, & Indirect Aggression are from the Direct-Indirect Aggression Scale; Reactive & Proactive Aggression is from the Reactive-Proactive Aggression Questionnaire.

with lower proactive aggression, at higher levels of reported exposure to childhood trauma ($b = -0.032$, $p = 0.008$). Also higher dPRS was associated with higher proactive aggression, at lower levels of reported exposure to childhood trauma ($b = 0.021$, $p = 0.038$), complete pattern of results is presented in Figure 4.4c. Full model results are presented in Table 4.4.

Mediation of Acute Perceived Stress. For men, the 95% confidence intervals of all indirect effect examined included zero. Meaning that acute perceived stress did not mediate the association between PRS and aggression at any amount of reported exposure to childhood trauma.

For women using the tPRS, polygenic risk was indirectly associated with BPAQ scores via acute perceived stress, such that higher tPRS was associated with higher aggression scores, at higher levels of reported exposure to childhood trauma [95% CI: 0.545, 10.676]. The tPRS was also indirectly associated with verbal and indirect

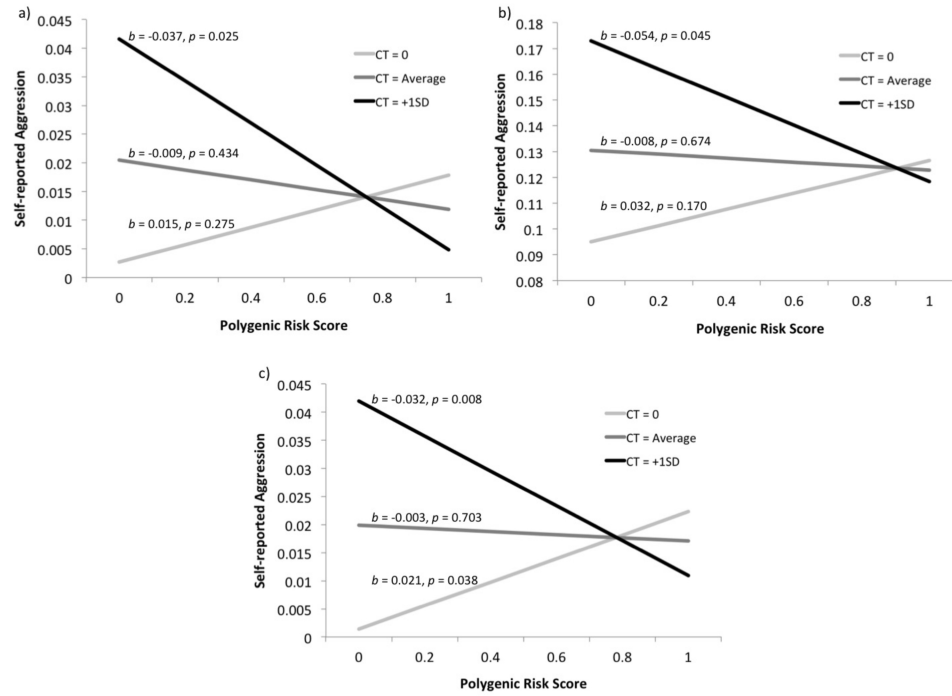


Figure 4.4: Conditional effects of dPRS on self-reported a) direct physical aggression, b) reactive aggression, and c) proactive aggression, as moderated by self-reported exposure to childhood trauma in women. Self-reported aggression scores are presented as log scores.

aggression, such that higher tPRS was associated with higher verbal and indirect aggression scores, at higher levels of reported exposure to childhood trauma [95% CI: 0.007, 0.073; 0.006, 0.068 respectively]. The tPRS was also indirectly associated with reactive and proactive aggression, such that higher tPRS was associated with higher reactive and proactive aggression scores, at higher levels of reported exposure to childhood trauma [95% CI: 0.004, 0.050; 0.002, 0.015 respectively].

For women using the dPRS, polygenic risk was indirectly associated with verbal aggression via acute perceived stress, such that higher dPRS was associated with lower verbal aggression, at higher levels of reported exposure to childhood trauma [95% CI: -0.066, -0.002]. The dPRS was also indirectly associated with proactive aggression via acute perceived stress, such that higher dPRS was associated with

Table 4.4

Estimates for Women from the Exposure to Childhood Trauma Moderation Model of dPRS on Aggression

<i>Model Variables</i>	Buss-Perry Aggression	Physical Aggression	Verbal Aggression	Indirect Aggression	Reactive Aggression	Proactive Aggression
Constant	52.347	0.003	0.170	0.178	0.095	0.001
Exposure to Childhood Trauma	0.928***	0.002***	0.006***	0.003*	0.005***	0.003***
Polygenic Risk Score	2.810	0.015	0.011	0.010	0.032	0.021*
Childhood Trauma*Polygenic Risk	-0.464	-0.003*	-0.004	-0.001	-0.005*	-0.003***
Age	-1.590*	-0.004 [#]	-0.006	-0.007	-0.009*	-0.002
Race (White vs. non-White)	7.617 [#]	-0.0002	-0.015	-0.038	0.001	-0.004
Childhood Trauma*Age	0.012	0.0000	-0.0001	0.0000	0.0000	0.0000
Childhood Trauma*Race	-0.268	-0.0004	-0.001	-0.0004	-0.0002	-0.0001
Polygenic Risk*Age	0.254	0.002	0.008	-0.002	0.004	0.001
Polygenic Risk*Race	-20.741	0.023	-0.056	-0.021	-0.040	0.019
<i>Model Fit</i>						
R ²	0.141	0.090	0.093	0.059	0.152	0.159
MSE	231.014	0.002	0.016	0.013	0.005	0.001
F (df)	7.136 (9, 390)	4.533 (9, 412)	4.685 (9, 412)	2.874 (9, 412)	8.227 (9, 412)	8.658 (9, 412)
p [#]	<0.001	<0.001	<0.001	0.003	<0.001	<0.001

[#]p<.10, p<.05, p<.01, p<.001

Note: Buss-Perry Aggression is from the BPAQ; Physical, Verbal, & Indirect Aggression are from the Direct-Indirect Aggression Scale; Reactive & Proactive Aggression is from the Reactive-Proactive Aggression Questionnaire.

lower proactive aggression, at higher levels of reported exposure to childhood trauma [95% CI: -0.014, -0.001].

Discussion

Consistent with our second hypothesis, there was a positive association between tPRS and self-reported aggression in women who reported higher exposure to childhood trauma. There was also a negative association between tPRS and self-reported aggression in both men and women who reported lower exposure to childhood trauma. Although the effect at low levels of childhood trauma is inconsistent with our hypothesis, the results replicate the pattern of the effect found in our first study. The pattern of results for the dPRS is the inverse of the tPRS for women, which is to be expected based on how the scores were calculated. Again the tPRS results are consistent with the Differential Susceptibility Model and previous literature (Bouvette-Turcot et al., 2015; Caspi et al., 2002; Enoch et al., 2013;

Frazzetto et al., 2007), suggesting that alleles corresponding to lower transcriptional efficiency should be considered as the susceptible allele when using polygenic risk scores. These findings reinforce the approach of using transcriptional efficiency as an indicator of risk, which has been taken by previous studies to examine the association between polygenic risk and aggression (Foshee et al., 2015; Simons et al., 2012; Stuart et al., 2014). Furthermore, our results suggest that the lack of examining environmental modifiers in studies of genetic risk may partly account for some of the inconsistencies within the literature in regards to which allele is more likely to confer risk for aggressive behavior. Therefore, future studies to examine the association between genetic variation and aggression should consider including measures of environmental factors (e.g. childhood trauma) shown by previous literature to moderate the association.

In Study #1 we found no significant effect of our PRS on aggression in men, regardless of childhood trauma exposure. However, in the current study (Study #2) there was a significant moderating effect in men for proactive aggression. It is possible that the presence of this effect is due to the inclusion of a proactive measure of aggression because there was no significant effect on aggression as measured by the Buss-Perry Aggression Questionnaire in either study. However, the presence of a significant effect in the current study could also be the result of an increased sample size. There were nearly 100 more men in our current study ($N = 329$) compared to Study #1 ($N = 236$). The effect size from Study #1 of the non-significant interaction between PRS and trait aggression ($r = 0.07$) and the effect size of the interaction between PRS and proactive aggression in the current study ($r = 0.12$) are both relatively small effects. Furthermore, the size of the interaction effect in men is also consistent with women in the current study for the significant effects found with physical aggression ($r = 0.13$), reactive aggression ($r = 0.10$), and

proactive aggression ($r = 0.16$), suggesting that exposure to childhood trauma moderates the effect of the PRS on aggression equivalently, in both men and women. The presence of a significant effect may also be the result of using an alternative measure of childhood trauma in the current study. In Study #1 we used the Traumatic Antecedents Questionnaire, which measured experiencing physical abuse or sexual abuse, witnessing aggression between other individuals, and experiencing other traumas (e.g. involved in a serious accident, serious illness). The current study used the Childhood Trauma Questionnaire, which measured experiences of abuse in addition to experiences of maltreatment. Because these measures are not on the same scale it is difficult to compare the levels of reported childhood trauma exposure between Study #1 and Study #2. However, it is possible that including a measurement of exposure to childhood maltreatment contributed to the presence of a significant effect in the current study that was not present in Study #1. Indeed there are several studies that have found a significant moderating effect of childhood maltreatment on the association between genetic variation and aggression (Byrd & Manuck, 2014; Caspi et al., 2002; Weder et al., 2009), suggesting that the polymorphisms included in our PRS are potentially more susceptible to the effect of childhood maltreatment than other forms of childhood trauma.

Also consistent with our third hypothesis, there was a significant mediating effect of acute perceived stress, such that women with high tPRS who reported high levels of exposure to childhood trauma reported more aggression via acute perceived stress. The mediating effect of acute perceived stress was present for all self-report measures of aggression except physical aggression demonstrating the robustness of the effect. Exposure to an acute stressor activates what is referred to as the “fight-or-flight” mechanism, however in women there is evidence to suggest that exposure to an acute stressor results in a “tend-and-befriend” response, where

women are more likely to seek out social support and increase maternal behavior when exposed to stress (Taylor et al., 2000). The “tend-and-befriend” hypothesis is further supported by sympathetic arousal not being associated with hostility in women (Girdler, Jamner, & Shapiro, 1997). However, the mediating effect of acute stress in women was only found to be present in individuals with high tPRS and high exposure to childhood trauma. Exposure to childhood trauma has been associated with hostile, inattentive, or role reversal parenting (Alexander, Teti, & Anderson, 2000; Lyons-Ruth & Block, 1996; Ruscio, 2001) and negative views of one’s parenting (Banyard, 1997). Childhood trauma was also associated with lower social support, resulting in lower psychological adjustment in both men and women (Runtz & Schallow, 1997), which could result in women not relying on social interaction as a coping mechanism for stress if they have been exposed to higher levels of childhood trauma. Therefore, while women in general may become more social following exposure to an acute stressor, women at the highest risk for aggression (i.e. high genetic risk, higher exposure to childhood trauma) may be more likely to respond aggressively to acute stress.

Sympathetic arousal has been associated with hostility in men (Girdler et al., 1997) promoting the “fight-or-flight” response to stress. Males have also been shown to have greater cortisol response to psychological stressors (Kudielka & Kirschbaum, 2005), although meta-analysis data suggests that cortisol response does not vary by sex (Dickerson & Kemeny, 2004). However, inconsistent with our third hypothesis, a mediating effect of acute perceived stress was not present in men. Although our findings are consistent with one other study that found an association between stress and aggression (i.e. intimate partner aggression) in women but not men (Mason & Blankenship, 1987), the majority of previous literature supports an association between increased stress and increased anger and aggression in men

(Tobin, Graziano, Vanman, & Tassinary, 2000; Verona, Reed, Curtin, & Pole, 2007). The low levels of reported stress may partially account for why a mediating effect was not present in men. Indeed, perceived stress reported by men in our sample was significantly lower than the level of perceived stress reported by women ($p = 0.001$). The effect of stress on aggression in men may also be dependent on the type of acute stress. Masculine gender role stress in particular (stress resulting from physical inadequacy, expressing “tender” emotions, being placed in subordination to women, having their intellectual control threatened, and failing in work and sex), was associated with higher self-reported aggression and violence in men (Jakupcak, 2003; Jakupcak, Lisak, & Roemer, 2002), and may therefore want to be considered in future studies.

While the mediating effect of acute perceived stress in women was consistent across the majority of self-report measures of aggression (no effect for physical aggression), the results were not as consistent across measures of aggression for the moderating effect of childhood trauma. In women an effect was only present in reports of physical, reactive, and proactive aggression, with only an effect for proactive aggression present in men. This inconsistency in results may be partly due the relatively low levels of aggression and childhood trauma overall reported by our sample. Therefore, greater exposure to childhood trauma may be necessary, in men particularly, in order to observe a moderating effect of childhood trauma consistently across measures of aggressive behavior. The mixed results across self-report measures indicates the importance of using multiple measures of aggression when examining gene-by-environment effects, especially when considering the low rate of replication in gene-by-environment research (Duncan & Keller, 2011). It is also possible that the inconsistency of the moderating effect of childhood trauma exposure across various types of aggression is due to genetic risk

and childhood trauma exposure contributing differently to various types of aggression. Research regarding the differences in contributors to various types of aggressive behavior is limited; however, genetic correlation between reactive and proactive aggression indicates that different genetic factors contribute to these two types of aggression (Tuvblad, Raine, Zheng, & Baker, 2009), even though genes account for about 40% of the variance in both reactive and proactive aggression (Baker, Raine, Liu, & Jacobson, 2008; Brendgen, Vitaro, Boivin, Dionne, & Perusse, 2006). Furthermore, a meta-analysis of the effect of serotonergic neurotransmission on aggression revealed that the differences in effect size are partly due to the type of aggression, with an effect being present in offensive or predatory aggression but not defensive aggression (Carrillo et al., 2009).

CHAPTER 5: POLYGENIC RISK, STRESS, AND AGGRESSION IN A HIGH DRINKING POPULATION (STUDY #3)

The purpose of the current study was to replicate Study #2 by examining the mediation effect of acute stress on the association between PRS and aggression as moderated by childhood family environment stress (specific aim #3), using an experimental design in which, aggression was measured with a behavioral paradigm following a stress manipulation. Additionally, a large percentage of aggressive behavior co-occurs with alcohol use (Greenfeld, 1998; Reingle et al., 2014). Given the association between genetic variation affecting serotonin neurotransmission (Fish et al., 2008; Foshee et al., 2015; Hallikainen et al., 1999) and alcohol-related aggression, in addition to stress being associated with increased likelihood of alcohol use (Cooper et al., 1992; Hutchinson et al., 1998; Park et al., 2004), an additional goal of this study was to examine whether the mediation effect of acute stress on the association between PRS and aggression as moderated by childhood family environment stress generalized to alcohol-related aggression (specific aim #4). This chapter includes three sections. The first section describes the participants, measures, stress manipulation, genotyping and analytical approaches taken to address the third and fourth specific aims. The second section reports the quantitative findings. The first set of analyses tested our hypothesis that individuals with high PRS and higher reported exposure to childhood family environment stress are expected to be more aggressive via stress reactivity. The second set of analyses tested our hypothesis that individuals with high PRS and higher reported exposure to childhood family environment stress are expected to report higher alcohol-related aggression via acute stress. The final section summarizes the findings in the context of our research hypotheses and previous literature.

Methods

Participants & Procedures. Undergraduate students were recruited from a Midwestern university through the Psychology Department's subject pool to participate in the study based on their drinking behaviors. The specific details of recruitment are described in the following section. Participants earned four research credits or \$20 for two hours of participation that involved completing three sets of self-report questionnaires, participation in a stress paradigm, participation in a behavioral paradigm to measure aggression, and donation of three saliva samples for genotyping and cortisol measurement. Participation occurred between 12-6pm to account for diurnal levels of cortisol. The study was approved by the local IRB and all participants gave written informed consent.

Recruitment. Participants were recruited based on their drinking behavior as measured by NIAAA recommend alcohol use questions or the Alcohol Use Disorders Identification Test (AUDIT) (Saunders, Aasland, Babor, De La Fuente, & Grant, 1993) via one of three methods: (1) Department Mass Screening, (2) an online study presented as "Stress and Social Interactions", or (3) direct phone or email screening of individual that requested to participate in the in-lab study "Genes, Stress, and Social Interactions". The details of each recruitment method are presented in the following sections.

Individuals that met eligibility were contacted weekly via email, phone call, or text message. The email message to recruit those eligible to the in-lab study was:

Hello [NAME], My name is [RESEARCHER] and I'm a researcher in the psychology department here at UNL.

Have you ever noticed that your behavior is different when you're stressed as opposed to when you're relaxed? You are being invited to

participate in a research study that examines the relationship between genes, stress and social interactions. The study takes about 2 hours and you will receive 4 In-Lab SONA research credits or \$20 for your participation. You are being contacted based on your enrollment in a Psychology course, responses an online study, and your indication of willingness to participate in future research. Would you be willing to participate? If you would like to participate in this study, then please respond with 3 of the listed times found below that are open for you. If you're not available at any of the listed times but would still like to participate you can simply respond to this email with your interest and I will attempt to arrange a time that will work for you.

A list of available appointments within the next two weeks were provided in the email.

The phone call message to recruit those eligible to the in-lab study was:

Hello, My name is [RESEARCHER] and I'm a researcher in the psychology department at UNL. I'm conducting a study that examines the relationship between genes, stress, and social interactions. The study last two hours and you will receive 4 in-lab SONA research credits for participating. I'm calling you based on your enrollment in a Psychology course, your responses an online study, and your indication of willingness to participate in future research. Would you be interested in participating? [When an individual answered the phone]/ If you're interested in participating in this study you can call or text me at [PHONE NUMBER] [When the call went to voicemail].

The text message to recruit those eligible to the in-lab study was: "UNL study

recruitment for 4 SONA credits. Learn more about genes, stress, and social interactions. Please call/text [RESEARCHER] ([PHONE NUMBER]) if you're interested."

Those that responded to these messages with interest in the in-lab study were scheduled to participate. Once scheduled, participants received the following email as a confirmation:

I've scheduled you for the appointment time of [APPOINTMENT DATE/TIME]. It is very important that you arrive on time, as the session will take the full 2 hours. This appointment will take place in [LOCATION]. During your appointment we will be collecting saliva samples so it is important that you refrain from eating a major meal within 60 minutes of the start of your appointment. Please also refrain from consuming alcohol, caffeine, nicotine, and prescription/over-the-counter medications within 12 hours prior to your appointment. Let me know if you have any questions.

Department Mass Screening Recruitment. At the beginning of each semester the Psychology Department recruited undergraduate students to complete a mass screening. Roughly 500-700 students completed this screening each semester. The NIAAA recommended alcohol use questions and the AUDIT were included in this screening for recruitment purposes. Undergraduate students who reported (1) binge drinking at least 2-3 times a month over the past 12 months (NIAAA recommended alcohol use questions) or (2) who reported drinking at least 2-4 times a month, drinking at least 3-4 drinks on a typical drinking day, and having six or more drinks at least less than monthly (AUDIT), and who agreed to be contacted for future studies were eligible to participate in the in-lab study.

Stress and Social Interactions - Online Study Recruitment. The online questionnaire study recruitment method was available through the Department's online resource for research participation. The study had the following description: "How do you perform under stress? Do you respond differently to those around you when in a stressful situation? In this study we are interested in examining some of the factors that may influence individual differences in response to stress." This online study consisted of taking self-report questionnaires, which included NIAAA recommended alcohol use questions. Undergraduates were given 1 research credit for 30-minutes of participation, and those who reported binge drinking at least 2-3 times a month over the past 12 months and who agreed to be contacted for future studies were eligible to participate in the in-lab study.

Direct Screening Recruitment. Individuals interested in participating in the in-lab study "Genes, Stress, and Social Interactions" based on its description:

Have you ever noticed that your behavior is different when you're stressed as opposed to when you're relaxed? In this study we will be examining some of the psychological and genetic influences on stress & social interactions. The study includes a brief phone screen and one laboratory session. The laboratory session takes about 2 hours and you will receive 4 in-lab SONA research credits -OR- \$20 for your participation

could contact the research via email or phone in order to be screened for eligibility. Screening included responding to the NIAAA recommended alcohol use questions. Participants who reported binge drinking at least 2-3 times a month over the past 12 months were scheduled to participate in the in-lab study.

In-lab Study Measures. Participants were asked their age, the gender, and race/ethnicity they most strongly identify with. Participants were allowed to select multiple responses for race and ethnicity if they identified strongly with multiple categories. Participants ($N = 182$; 58.8% women) were on average 19.65 years old ($SD = 1.90$, range = 17-30) with the majority identifying as Caucasian (94.5%); 4.4% Hispanic (e.g., Mexican American, Latino), 2.2% Black or African American, 2.2% Asian, 0.5% American Indian or Alaska Native, and 0% Native Hawaiian or other Pacific Islander.

The Drinking Motives Questionnaire used is a revised scale comprised of 20 self-report items that measure the frequency of various reasons why individuals might be motivated to drink alcohol (Cooper, 1994). Items were measured on a 6-point scale (from 1, “Never”, to 6, “Almost always”) producing a sum score for each of the four motives: Social (e.g. “How often would you say you drink to be sociable?”), Coping (e.g. “How often do you drink to forget your worries?”), Enhancement (e.g. “How often do you drink to get high?”), and Conformity (e.g. “How often do you drink to be liked?”). Only the coping subscale was used in our analyses.

The 6 recommended alcohol questions provided by NIAAA (National Institute on Alcohol Abuse and Alcoholism) were used in this study. These questions assess drinking patterns within the last 12 months, focusing on how often they have anything to drink, number of drinks on a typical drinking day, binge drinking, maximum number of drinks, and the frequency of drinking the maximum number of drinks. In addition to these 6 recommend questions we asked the age in which they had their first drink (not including sips) and the age in which they first got drunk.

The Buss Perry Aggression Questionnaire (BPAQ), as described in Chapter 4

(page 60), was used to assess aggression.

The Alcohol-Related Aggression Questionnaire is a 28-item self-report measure of aggression in the context of alcohol use (McMurrin et al., 2006). Items are measured on a 4-point scale (from 1, “Always false for me”, to 4, “Always true for me”) producing a total sum score, where higher scores suggest higher amounts of alcohol-related aggression. Example questions include: “I get aggressive if I drink too much”, “The more I drink, the more argumentative I get”, and “The more I drink, the more likely I am to jump to conclusions”.

The Displaced Aggression Questionnaire (DAQ) is a self-report measure of displaced trait aggression (Denson, Pedersen, & Miller, 2006). This is a 31-item measure comprised of three subscales: Angry Rumination (e.g. “I keep thinking about events that angered me for a long time”), Behavioral Displaced Aggression (e.g. “When angry, I have taken it out on people close to me”), and Revenge Planning (e.g. “If someone harms me, I am not at peace until I can retaliate”). Items are measured on a 7-point scale (from 1, “Extremely uncharacteristic of me”, to 7, “Extremely characteristic of me”) producing average subscale and total scores, where higher scores suggest higher levels of aggression.

Our measure of direct and indirect aggression was adapted from the original Direct/Indirect Aggression Scale, as reported in Chapter 4 (page 60). Items were also used to examine how often these acts co-occurred with alcohol use. If participants responded to a particular behavior with any value greater than 1 they were asked to report what percentage of the time they had done that particular behavior when drinking alcohol. Response options were in 10% intervals (i.e. 0%, 10%, 20%, 30%, etc.). A separate average score was created where the original response option was multiplied by the percentage under alcohol use, resulting in higher scores corresponding to higher alcohol-related aggression.

The Childhood Trauma Questionnaire (CTQ) was previously described in Chapter 4 (page 60-61).

The Index of Family Relations (IFR) assesses the magnitude of a problem that family members have in their relationships as perceived by the responder (Hudson, 1997). The IFR is a 25-item self-report measure, where items are measured on a 7-point scale (from 1, “None of the time”, to 7, “All of the time”). Example items include: “I can really depend on my family”-reverse scored, “I feel left out of my family”, and “Members of my family argue too much”. After reversing positive items, a total sum score is calculated. The sum score is reduced by 25 and divided by 1.5 to produce a range of 0-100. Lower scores suggest a relative absence of problems while a cutoff of 30 indicates that there may be a clinically significant problem, and scores above 70 are indicative of severe stress or the possibility of violence.

The Perceived Social Support/Conflict Scale was used in the Midlife Development in the U.S. (MIDUS) study (National Institute on Aging, 2002) and is used to measure both positive and negative interactions with three separate groups: 1) spouse or partner (12-items); 2) other members of the family (10-items); and 3) friends (8-items). Positive interaction items (e.g. “How much do they understand the way you feel about things”) are measured on a 4-point scale (from 1, “A lot”, to 4, “Not at all”) and negative interaction items (e.g. “How often do they criticize you”) are measured on a different 4-point scale (from 1, “Often”, to 4, “Never”). Positive interaction items are reverse scored before all items are average separately for each group (spouse, family, and friends) so that higher scores reflect greater support.

The Perceived Stress Scale was previously described in Chapter 4 (page 61).

Trier Social Stress Test. Men and women participants were separately assigned to either the stress or no stress (control) condition of the Trier Social Stress Test (TSST) (Kirschbaum, Pirke, & Hellhammer, 1993). Prior to the beginning of the task participants were asked how happy, sad, stressed, angry, and nervous they felt on a scale of 1-10, with 1 being not at all and 10 being extremely, in order to evaluate participants' baseline stress level. The procedures for these conditions are presented below.

Participants assigned to the stress condition were given the following instructions:

You will now give a speech to a panel of graduate students trained to assess how outgoing, gregarious, and comfortable you are in situations in which you must project yourself as an expert. You are to imagine that you are applying for your ideal job. You've dreamed about working in this job for as many years as you can remember. You've just seen an advertisement for this perfect job and decide to apply. After submitting your application, you have been invited for an interview. The job pays a very large salary. You are competing against a lot of other candidates, and the final selection will be made based on your ability to convince the interviewers of how your experiences, abilities, and education make you a better candidate than the others. You will try to convince this panel of interviewers that you are the best candidate for the position. In addition, you will be asked to perform a mental math test, which will give us additional information about your working memory capacity. You will have 5 minutes to prepare a detailed speech. After the preparation time has elapsed, you will deliver your speech to these

interviewers. Your speech should explain why you should get the job. Remember, you should try to perform better than all of the other participants. These examiners are specially trained to monitor and rate your speech for its believability and convincingness, and they will compare your performance to that of the others who perform this task. Also you will be videotaped during the task so that the examiners can go over the videotape carefully and rate the contents of your speech as well as your nonverbal behavior.

Participants were given a piece of paper and a pen to use during the initial 5-minute preparation period, but were told they were not required to use it. After the 5-minute preparation period, participants in this stress condition were taken to an adjoining room, where two panelists were seated in professional attire. They were instructed to stand on an X across the room and were made aware of the camera that would be recording them. In actuality no video recordings were taken. Participants were then instructed to give their speech. If participants finished their speech in less than 5 minutes, they were told, "You still have time, please continue". If participants stopped a second time before 5 minutes had elapsed they were asked questions from a standard list (e.g. "Why do you think you are better qualified than the other applicants?", "What kind of leading qualities do you have?", "What can you constructively add to a team?", "What do your employees appreciate about you most?") until the end of the 5-minute period. After the completion of the speech participants were asked to count backwards from 2023 in steps of 17. They were instructed to count backwards as quickly and as accurately as possible and were told that if they made a mistake it would be pointed out to them (i.e. "Error, 2023") and they would have to start over. Participants performed this task for 5

minutes. In addition to being told to start over after a mistake (i.e. “Error, 2023”), participants were also occasionally told, “You need to go faster”. At the end of this task participants were told, “You can let the researcher know you’re finished”. During this task, one of the panelists was in responsible for relaying all instructions and pretending to take notes on the participant’s performance. The second panelist remained quiet while maintaining constant eye contact with the participant. Both panelists kept a neutral expression and tone during the task.

Participants in the no-stress control condition were given the following instructions:

Today you will be a member of the control group. You will now be asked to spend 5 minutes think about how you would respond to the question what is your favorite movie and why? You will not be asked to share your response after the 5-minute period but rather we just want you to spend time thinking about how you would answer the question. If you prefer you can think about your favorite, book, TV show or food rather than a movie. Once the 5 minutes have passed I will ask you to read from a standard script for 5 minutes. You will not be judged on your reading ability. As a member of the control group, we need you to be speaking out loud for 5 minutes and having everyone read from the same script allows for greater control in our methods. After reading for 5 minutes, I will then have you count by fives, so 5, 10, 15, 20 and so on. If at any point you’d prefer to start over from the beginning you may feel free to do so. You will perform this task for 5 minutes as well.

Participants in this no-stress control condition were given a piece of paper and a pen to use during the initial 5-minute period to respond to the question “What is

your favorite movie and why?” but were told they were not required to use it. After 5-minutes had elapsed the researcher re-entered the room and participants read from a standard script [one of the following articles from the Association for Psychological Science Observer magazine: “The Importance of Divergent Thinking for Research in Graduate School and Beyond” (Ledwidge, 2014), “Mining the Unconscious” (Hassin, 2014), or “Mapping Mindsets” (Kitayama, 2013)] for 5-minutes. Participants then counted by 5 for an additional 5 minutes.

After the completion of these tasks participants were asked how happy, sad, stressed, angry, and nervous they felt on a scale of 1-10, with 1 being not at all and 10 being extremely, in order to evaluate how stressful the experience was for them by comparing to their baseline response to this question. In order to further evaluate how stressful the experience was participants were also asked: (1) To what extent did you want to leave that situation?, (2) To what extent did you feel uncomfortable in that setting?, and (3) To what extent did you feel tension in your body? on a scale of 1-10, with 1 being not at all and 10 being extremely. Finally they were asked how stressful they felt the experience was on a scale of 1-10 with 1 being not at all and 10 being the most intense experience tolerable.

Saliva Collection. At the beginning of the study participants rinsed out their mouth by swishing and swallowing a small amount of water for 10-15 seconds. Saliva samples were collected using the passive drool method. The first saliva sample was collected as a baseline measurement of cortisol approximately 10 minutes after participants had rinsed out their mouth and immediately prior to participation in the TSST. The second saliva sample was collected immediately after completion of the TSST. The final saliva sample was collected approximately 20 minutes after the TSST. A total volume of 2mL of saliva was collected within a 10 minute or less time frame. Participants were stopped after 10 minutes even if they had not reach 2mL

of saliva. Saliva samples were stored on ice until all three samples were collected and then they were moved to a -20°C freezer until all behavioral data was collected.

Point Subtraction Aggression Paradigm. Participants completed the point subtraction aggression paradigm (PSAP), adapted from Cherek and colleagues (Cherek, Moeller, Schnapp, & Dougherty, 1997) as a behavioral measure of aggressive responding. Inquisit software was used to run this aggression paradigm. Prior to starting the paradigm participants were instructed:

In just a minute, you will be playing a computer game with an online opponent. The purpose of this game is to get as many points as possible. Once I've started the game, the first few screens will provide you with instructions on how to play. Please read all instructions carefully so that you completely understand how the game will work.

Once the paradigm was started on the computer screen participants were shown the following instructions:

Today, you will be earning points by working at the computer. You will be participating with others in this study. These other people will have a similar computer set-up and are located at a different facility. The computer set-up includes three letters "A", "B", "C" that appear on the screen. When each session starts, the letters "A", "B", "C", a point counter (located at the top of the screen) and a press counter (located at the bottom of the screen) appear. The point counter will be at 0.

Pushing key "A" on your keyboard will make the letters "B" and "C" go off the screen and turn letter "A" blue. The press counter will start counting how often you press the key. Pushing key "A" on your

keyboard 100 times will make the letter “A” go off the screen and 1 point will be added to your point counter. The point counter will turn green and enlarges for about 1s. After about 1s the letters “A”, “B”, “C”, will reappear on the computer screen. At that point you can continue to press key “A” or switch to key “B” or “C” on your keyboard. During the session, the point counter may turn red and become larger and 1 point will be subtracted from your point counter. After the point is subtracted, the counter will return to its normal size. This means that one of the other persons has subtracted a point from your counter by pushing the key “B” on his keyboard. Every point that this person subtracts from your counter is added to his counter.

If you push key “B” on your keyboard, the letters “A” and “C” will go off the screen and the letter “B” will turn blue. After you have pushed key “B” 10 times, the letter “B” will go off the screen and one point will be subtracted from the other person’s counter. After about one second, the letters “A”, “B”, and “C” will come back on the computer screen. You can continue to press key “B” and subtract additional points from the other person or switch to key “A” or “C” on your keyboard. If you subtract a point from the other person, it will not be added to your counter. Remember, points that are subtracted from your counter by the other person are added to that person’s counter.

If you push key “C” on your keyboard, letters “A” and “B” will go off the screen and the letter “C” will turn blue. After you have pushed key “C” 10 times, the letter “C” will go off the screen and your earnings displayed on the counter will be protected from point subtractions

initiated by the other person for some period of time. After about one second, the letters “A”, “B”, and “C” will come back on the screen. You can continue to press key “C” or switch to key “A” or “B”.

If you have any questions regarding the task ask them now. If you are ready to start the task, press “C”.

The task lasted for 20 minutes. Participants were provoked by randomly having a point subtracted at varying intervals (between 6-90 seconds). Pressing button “B” to subtract a point from their opponent was defined as aggressive. Pressing button “C” was defined as escape, because it protected the participant’s earnings from point subtractions initiated by their opponent. Protection from their opponent lasted for 75 seconds during which no additional points were subtracted from the participant. At least one point had to be subtracted from the participant before each protection interval could be initiated. This contingency was important because it ensured that participants could not avoid point subtractions.

After the completion of the PSAP, participants were verbally asked how happy, sad, stressed, angry, and nervous they felt on a scale of 1-10, with 1 being “not at all” and 10 being “extremely”, in order to evaluate their feelings after participating in the task. In order to check the manipulation of the task, participants were also asked how many individuals they thought they played during the game and to describe their opponent(s).

Debriefing. After the completion of the study, participants were debriefed about the purposes of the study and were asked if they felt okay to leave.

Genotyping. DNA was extracted from 0.5mL of saliva using the PUREGENE DNA extraction kit (Qiagen). DNA concentration was determined using the NanoDrop2000 (Fisher Scientific, Inc., Pittsburgh, PA) and all samples

were diluted to 20ng/ μ l.

5-HTTLPR uVNTR was amplified using PCR forward and reverse primers: 5'-GGCGTTGCCGCTCTGAATGC-3' and 5'-GAGGGACTGAGCTGGACAACCAC-3'. The PCRs were performed in 25 μ l reactions containing 20ng of DNA, 1X GoTaq Master Mix (Promega, Madison, WI, USA), and 10 μ M of each primer. The PCR amplification conditions were followed as previously described (Wendland et al., 2006). PCR product was separated by electrophoresis on a 2.5% agarose gel and visualized under UV light with SybrSafe stain. Two trained researchers made genotyping calls independently. Due to faint DNA bands following restriction enzyme digest, there was poor inter-rater reliability (79.6%) among rs25531 polymorphism genotyping calls in addition to a large percentage of samples unable to be scored (24.7%). As a result, rs25531 genotype was not included in the PRS for this study.

Genotyping methodologies described in Chapter 3 (pages 41-43) were used to genotype *MAOA* uVNTR, *HTR1B* rs13212041, and *TPH2* rs4570625. Ten percent of samples were re-genotyped for each polymorphism to test genotyping accuracy. No discrepancies were observed in the genotyping calls. The call rate for each polymorphism is as follows: 89.0% for 5-HTTLPR, 76.4% for *MAOA* uVNTR, 90.1% for *TPH2* rs4570625, and 95.1% for *HTR1B* rs13212041.

The tPRS as described in Chapter 3 (page 45) was used as our measure of genetic risk. The total tPRS was calculated by averaging the score of all four polymorphisms in order to include individuals who did not have a complete genetic profile (70.9% 4/4 genotypes, 15.9% 3/4 genotypes, 7.7% 2/4 genotypes, 3.9% 1/4 genotypes). Table 5.1 presents the allele frequencies and test of Hardy-Weinberg Equilibrium (HWE) for each polymorphism. All polymorphisms were within HWE.

Table 5.1

Allele Frequency and Hardy-Weinberg Equilibrium Statistics for Study #3

	Genotype Frequency	Allele Frequency	Hardy-Weinberg Equilibrium (HWE)	
5-HTTLPR	L/L = 45 L/S = 80 S/S = 37	L = 0.52 S = 0.48	$\chi^2 = 0.02$	$p = 0.888$
TPH2 rs4570625	G/G = 94 G/T = 57 T/T = 13	G = 0.75 T = 0.25	$\chi^2 = 1.07$	$p = 0.301$
HTR1B rs13212041	T/T = 101 T/C = 61 C/C = 11	T = 0.76 C = 0.24	$\chi^2 = 0.19$	$p = 0.663$
MAOA uVNTR men	3 = 18 4 = 40	3R = 0.31 4R = 0.69		
MAOA uVNTR women	3/3 = 6 3/4 = 31 3.5/4 = 1 4/4 = 41 4/5 = 1	3R = 0.27 3.5R = 0.01 4R = 0.72 5R = 0.01	$\chi^2 = 0.00$	$p = 1.000$

Note: HWE cannot be calculated for MAOA in men because the polymorphism is located on the X chromosome. HWE is presented for women, which is calculated from grouped genotypes based on transcriptional activity.

Cortisol Assays. Saliva samples were thawed at room temperature and then centrifuged at 3000rpm for 15 minutes then pipetted into test wells. Cortisol levels were measured using Salimetrics Cortisol ELISA Kits following the manufacturer's protocol (Salimetrics, LLC, Carlsbad, CA) at the Center for Brain, Biology, and Behavior Endocrinology Laboratory at the University of Nebraska-Lincoln. Cortisol levels were assessed from a 25 μ l sample. Each sample was assayed in duplicate and the average score was used for each sample. All saliva samples were within the desired pH range (3.5-9.0). The assays had good reproducibility as indicated by the

maximum intra- and inter-assay coefficients of variation obtained, were 2.66% and 6.72%, respectively.

Statistical Analysis. All statistical analyses were run in SPSS v.23 software (IBM Corporation, Armonk, NY). A composite score of childhood family environment stress was determined using factor analysis with standardized scores from the CTQ, IFR, and family subscale of the Perceived Social Support/Conflict Scale. Regression scores obtained from a 1-factor solution using maximum likelihood and Promax rotation were used as an index of childhood family environment stress.

Cortisol levels for each participant were log transformed and winsorized prior to calculating reactivity to stress. Cortisol reactivity was calculated by subtracting the cortisol level immediately following participation in the TSST from the cortisol level prior to receiving task instructions. Stress and anger reactivity were calculated by subtracting the self-reported value of each emotion immediately following participation in the TSST from the self-reported value prior to receiving task instructions.

Based on the results of the previous two studies, only the tPRS is used in the following analyses. The association between genetic risk, childhood family environment stress, acute stress and behavioral aggression was examined using PROCESS model 12 (Hayes, 2013; conceptual diagram is presented in Figure 5.1), including age, race (coded as 0 = White, 1 = non-White) and all predictor (genetic risk and experiences of childhood trauma)*demographic (age and race) were included as covariates. The association between genetic risk, childhood family environment stress, acute stress and alcohol-related aggression was examined using PROCESS model 22 (Hayes, 2013; conceptual diagram is presented in Figure 5.2), including age, race (coded as 0 = White, 1 = non-White) and all predictor (genetic risk and experiences of childhood trauma)*demographic (age and race) were

included as covariates. Men and women were analyzed separately because MAOA uVNTR is located on the X chromosome. A quadratic term was not included in either model because the correlation between PRS and reported exposure to childhood stress was not significant. Uncorrected p-values are reported for all analyses.

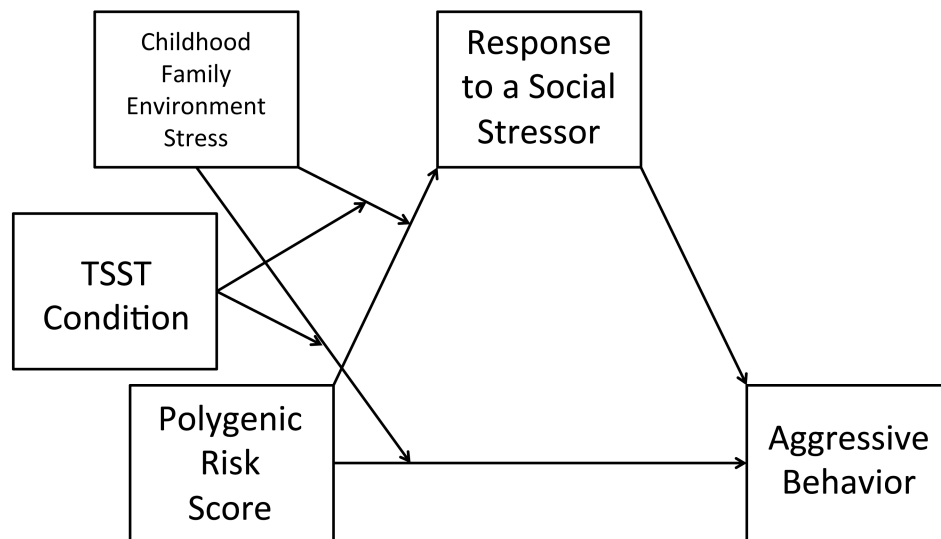


Figure 5.1: Conceptual diagram of SPSS PROCESS model 12.

For men in the TSST control condition, change in self-reported stress (skewness = 1.23) and anger (skewness = 1.48) scores were slightly skewed; outliers as determined by Tukey's hinges were winsorized, which eliminated the skewness. Direct/Indirect Aggression Scale scores (direct physical aggression subscale skewness = 5.36; direct verbal aggression subscale skewness = 2.68; indirect aggression subscale skewness = 4.09) and behavioral aggression (each TSST condition were examined separately; stress condition participants PSAP skewness = 1.80; control condition participants PSAP skewness = 1.23) were also skewed for men; therefore

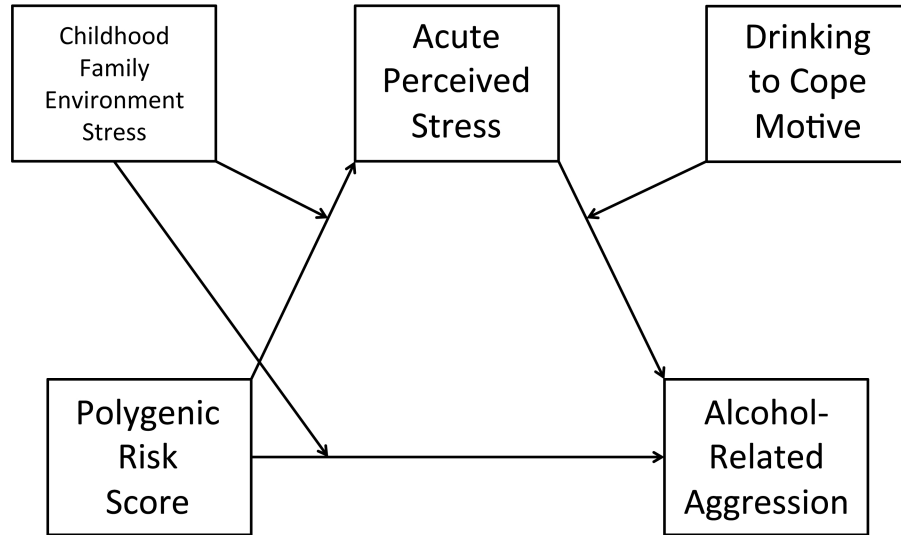


Figure 5.2: Conceptual diagram of SPSS PROCESS model 22.

log-transformed scores for these variables were used for the following analyses. For women, change in anger scores in the control condition (skewness = -1.08) and behavioral aggression for individuals who were in the stress condition (skewness = 1.11), were slightly skewed for individuals; outliers as determined Tukey's hinges were winsorized, which eliminated the skewness. Direct/Indirect Aggression Scale scores were also skewed for women (direct physical aggression subscale skewness = 5.61; direct verbal aggression subscale skewness = 3.40; indirect aggression subscale skewness = 3.65); therefore log-transformed scores for these variables were used for the following analyses. The type of transformation implemented was selected based on whether the skewness of the data was the result of a few outliers (winsorizing) or if the entire distribution was skewed (log-transformation).

Results

Childhood Family Environment Stress Factor. The childhood family environment stress factor accounted for 73% of the variance across the three scales (82.2% of variance for IFR, 79.6% of variance for Family Perceived Social Support, and 57.2% of variance for CTQ). Scores from each questionnaire also significantly loaded onto the factor using ± 0.3 as a cutoff (IFR = .907; Family Perceived Social Support = -.892; CTQ = .756). Factor scores were significantly and positively correlated with IFR ($r = .952, p < .001$) and CTQ scores ($r = .794, p < .001$), as well as significantly and negatively correlated with Family Perceived Social Support scores ($r = -.936, p < .001$). Therefore higher factor scores correspond to higher amounts of childhood family environment stress.

Trier Social Stress Test. There were no significant group differences in descriptive statistics, self-reported exposure to childhood stress, self-reported aggression, baseline cortisol, or baseline stress (Table 5.2). Following the stress manipulation, self-reported stress and cortisol level were significantly higher for individuals who participated in the stress condition, compared to individuals who participated in control condition, controlling for use of alcohol, caffeine, medication, nicotine, and birth control within the 12 hours prior to participation as well as if they had eaten or performed any vigorous physical activity in the hour prior to participation, see Table 5.2. Average cortisol levels for each TSST condition across the three collection time points (i.e. baseline, immediately following TSST, 20 minutes after TSST) are shown in Figure 5.3.

Point-Subtraction Aggression Paradigm. Correlations between self-report measures of aggression, and aggression as measured by the PSAP, are presented in Table 5.3, for males and females separately. About four-fifths of the

Table 5.2

Differences between Participants in the Control and Stress Conditions of the Trier Social Stress Test

	Control Condition	Stress Condition	<i>p</i>
Race (% non-White)	6.6%	4.4%	.518
	Mean (SE)	Mean (SE)	
Age (mean centered)	-.002 (.208)	.01 (.192)	.969
Polygenic Risk Score	.53 (.030)	.56 (.025)	.453
Childhood Family Environment Stress	1.28 (.109)	1.16 (.093)	.400
Buss-Perry Aggression Questionnaire Scores	66.09 (1.685)	64.64 (1.873)	.565
Displace Aggression Questionnaire Scores	2.76 (.108)	2.63 (.099)	.397
Perceived Stress Scale Scores	27.94 (.593)	27.55 (.620)	.646
Baseline Salivary Cortisol (µg/dL)	.24 (.016)	.25 (.016)	.441
Baseline Self-report Stress Level	5.48 (.303)	5.43 (.300)	.898
Baseline Self-report Anger Level	1.30 (.122)	1.43 (.121)	.432
Salivary Cortisol (µg/dL) immediately after TSST	.22 (.017)	.30 (.017)	.001
Self-report Stress Level immediately after TSST	4.97 (.317)	7.37 (.314)	<.001
Self-report Anger Level immediately after TSST	1.40 (.265)	4.12 (.262)	<.001
“To what extent did you want to leave the situation?”	3.46 (.282)	8.10 (.279)	<.001
“To what extent did you feel uncomfortable?”	2.51 (.235)	8.50 (.233)	<.001
“To what extent did you feel tension in your body?”	3.48 (.302)	7.92 (.300)	<.001
“How stressful do you feel the experience was?”	2.78 (.235)	6.86 (.233)	<.001
Change in Cortisol pre- to post-TSST	-.52 (.290)	1.94 (.287)	<.001
Change in Stress pre- to post-TSST	.11 (.255)	2.69 (.253)	<.001
Change in Anger pre- to post-TSST	-.07 (.035)	.16 (.034)	<.001
Salivary Cortisol (µg/dL) 20 minutes post TSST	.21 (.019)	.34 (.019)	<.001
Self-report Stress Level post PSAP	4.47 (.321)	4.08 (.318)	.390
Self-report Anger Level post PSAP	2.46 (.264)	2.47 (.261)	.987

Note: TSST = Trier Social Stress Test; PSAP = Point Subtraction Aggression Paradigm

participants (80.8%) believed the manipulation that the PSAP was against another individual, while the remaining participants knew they were playing a computer. Aggression as measured by the PSAP did not significantly differ ($p = .862$) between individuals who believed they were playing a computer ($M = 45.11$, $SD = 33.27$) compared to individuals who believed they were playing an actual person ($M = 46.20$, $SD = 32.90$). Therefore, a task manipulation check variable was not included in our analyses.

Association between Genetic Risk and Behavioral Aggression in

Men. Three separate models were used to test the mediating effect of acute stress

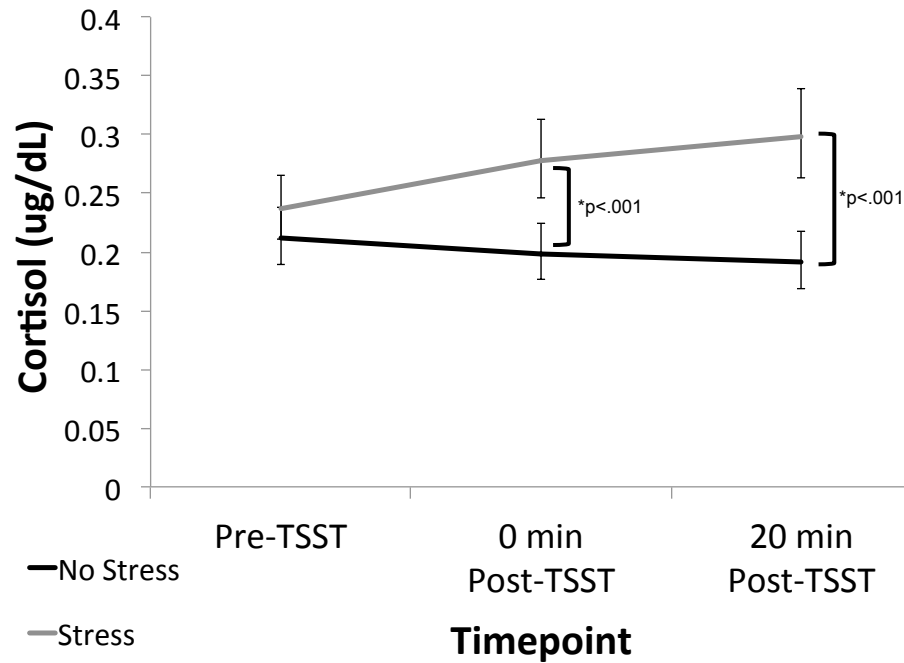


Figure 5.3: Change in cortisol from pre- to post-participation in the Trier Social Stress Test, controlling for any medication, nicotine, alcohol, birth control, and caffeine use within 12 hours of participation, as well as eating or vigorous physical activity within 1 hour of participation.

Table 5.3
Correlations between self-reported and behavioral aggression measures

	1	2	3	4	5	6	7	8	9	10
1. Buss-Perry Questionnaire	-	.822***	.456***	.546***	.502***	.368**	.283*	.310**	.161	-.017
2. Displaced Aggression Questionnaire	.758***	-	.329**	.450***	.545***	.265*	.127	.244*	.207 [#]	-.019
3. Direct Physical Aggression Scale	.346***	.320**	-	.614***	.312**	.234*	.635***	.618***	.219 [#]	.216 [#]
4. Direct Verbal Aggression Scale	.612***	.601***	.490***	-	.579***	.141	.270*	.609***	.197 [#]	.186
5. Indirect Aggression Scale	.371***	.475***	.317**	.626***	-	.089	.137	.235*	.546***	-.024
6. Alcohol-Related (AR) Aggression Questionnaire	.547***	.549***	.271**	.522***	.493***	-	.320**	.343**	.238*	.228 [#]
7. AR Direct Physical Aggression Scale	.178 [#]	.268**	.718***	.455***	.463***	.345***	-	.729***	.485***	.214 [#]
8. AR Direct Verbal Aggression Scale	.349***	.432***	.492***	.754***	.637***	.515***	.669***	-	.457***	.281*
9. AR Indirect Aggression Scale	.197*	.268**	.244*	.384***	.647***	.419***	.355***	.595***	-	.023
10. PSAP Aggressive Responses	-.015	-.083	.070	.003	.072	<.001	-.014	.020	.067	-

Note: Correlational data for men are presented above the diagonal and data for women is presented below the diagonal

[#]p < .10, *p < .05, **p < .01, ***p < .001

on the association between PRS and aggression as moderated by childhood family environment stress, with (1) self-reported stress reactivity, (2) self-reported anger reactivity, and (3) cortisol reactivity, as measures of stress in response to the TSST. Results from the statistical model using self-reported stress reactivity are presented in Table 5.4. The self-report stress reactivity model did not explain a significant

amount of variance in either stress reactivity or behavioral aggression. Results from the statistical model using self-reported anger reactivity are presented in Table 5.4. The self-reported anger reactivity model explained a significant amount of variance in anger reactivity, however the only significant predictor was the covariate age ($b = -.625$, $p = .045$). Additionally, this model did not explain a significant amount of variance in behavioral aggression. Results from the statistical model using cortisol reactivity are presented in Table 5.4. The cortisol reactivity model explained a significant amount of variance in cortisol reactivity, however no individual predictors were significant. Additionally, this model did not explain a significant amount of variance in behavioral aggression.

Table 5.4
Model Estimates of the Association between Genetic Risk, Childhood Family Environment Stress, Acute Stress and Behavioral Aggression (PSAP) in Men

	Model 1		Model 2		Model 3	
	Stress Reactivity	PSAP Aggression	Anger Reactivity	PSAP Aggression	Cortisol Reactivity	PSAP Aggression
<i>Model Variables</i>						
Constant	-1.667	3.084	0.140	2.950	-0.099	2.947
Polygenic Risk Score (PRS)	1.970	1.642	-0.597	1.801	0.034	1.802
Childhood Family Environment (CFE)	0.647	0.569	-0.211	0.621	-0.087	0.619
PRS * CFE	-0.901	-1.416	0.724	-1.489	0.206	-1.484
TSST Condition	2.819	0.579	1.429	0.807	0.610	0.822
PRS * TSST Condition	-1.748	-1.732	1.714	-1.872	-0.091	-1.876
CFE * TSST Condition	0.327	-0.687	1.952	-0.660	0.029	-0.660
PRS * CFE * TSST Condition	-0.369	1.317	-2.980	1.285	-0.179	1.283
Age	-0.294	0.045	-0.625*	0.020	-0.046	0.020
Race (White vs. non-White)	14.858	-16.752*	24.724	-15.536*	4.586	-15.435*
PRS * Age	0.156	-0.236	0.765	-0.223	0.046	-0.222
PRS * Race	-25.468	34.929*	-53.479	32.837*	-8.784	32.648*
CFE * Age	0.028	0.013	0.153	0.016	0.032 [#]	0.016
CFE * Race	5.226	-10.386*	15.718	-9.954*	2.175	-9.909*
Reaction to TSST	-	0.081	-	-0.001	-	-0.026
<i>Model Fit</i>						
R ²	0.256	0.223	0.452	0.187	0.433	0.187
MSE	4.311	0.626	3.733	0.655	0.121	0.655
F (df)	1.426	1.084	3.420	0.871	3.174	0.872
	(13, 54)	(14, 53)	(13, 54)	(14, 53)	(13, 54)	(14, 53)
p	0.178	0.393	0.001	0.593	0.001	0.592

[#] $p < .10$, $p < .05$, $p < .01$, $p < .001$

Association between Genetic Risk and Behavioral Aggression in

Women. The same three models analyzed with men, were used with women to examine the mediating effect of acute stress on the association between PRS and aggression as moderated by childhood family environment stress. Results from the statistical model using self-reported stress reactivity are presented in Table 5.5. The self-reported stress reactivity model explained a significant amount of variance in stress reactivity. TSST condition significantly predicted stress reactivity, such that individuals in the stress condition reported a larger change in stress from baseline to post-stress test, compared to individuals in the control condition ($b = 4.675$, $p = .005$). There was also a significant interactive effect of childhood family environment stress and stress condition ($b = -3.066$, $p = .013$), in addition to a significant interactive effect of PRS, childhood family environment stress, and TSST condition ($b = 4.550$, $p = .017$); the pattern of effects is shown in Figure 5.4a & 5.4b. The self-reported stress reactivity model however, did not explain a significant amount of variance in behavioral aggression.

Results from the statistical model using self-reported anger reactivity are presented in Table 5.5. The self-reported anger reactivity model explained a significant amount of variance in anger reactivity. TSST condition significantly predicted anger reactivity, such that individuals in the stress condition reported a larger change in anger from baseline to post-stress test, compared to individuals in the control condition ($b = 4.311$, $p = .002$). There was also a significant interactive effect of PRS, childhood family environment stress, and TSST condition ($b = 3.815$, $p = .019$); the pattern of effects is shown in Figure 5.4c & 5.4d. The self-reported anger reactivity model however, did not explain a significant amount of variance in behavioral aggression.

Table 5.5

Model Estimates of the Association between Genetic Risk, Childhood Family Environment Stress, Acute Stress and Behavioral Aggression (PSAP) in Women

	Model 1		Model 2		Model 3	
	Stress Reactivity	PSAP Aggression	Anger Reactivity	PSAP Aggression	Cortisol Reactivity	PSAP Aggression
<i>Model Variables</i>						
Constant	-0.527	31.552	0.283	31.015	-0.002	31.498
Polygenic Risk Score (PRS)	-0.384	26.799	-0.313	27.279	0.047	26.558
Childhood Family Environment (CFE)	0.210	6.526	-0.312	7.074	-0.079	6.884
PRS * CFE	-0.544	-11.228	0.066	-11.404	-0.019	-11.212
TSST Condition	4.675**	8.292	4.311**	1.616	0.040	8.675
PRS * TSST Condition	-2.519	-29.173	-3.506	-23.592	0.104	-29.907
CFE * TSST Condition	-3.066*	-13.050	-1.767 [#]	-10.449	0.042	-13.588
PRS * CFE * TSST Condition	4.550*	20.162	3.815*	14.304	0.128	20.161
Age	0.096	-9.259 [#]	0.056	-9.342 [#]	0.011	-9.294 [#]
Race (White vs. non-White)	-1.958	92.693**	-3.103	97.663**	0.329	91.076**
PRS * Age	0.045	11.399	-0.187	11.718	0.008	11.369
PRS * Race	0.400	-99.836 [#]	3.563	-105.762*	-0.890 [#]	-96.043 [#]
CFE * Age	-0.238	0.522	-0.198	0.826	-0.033	0.632
CFE * Race	1.170	2.274	0.065	2.303	0.124	1.899
Reaction to TSST	-	0.118	-	1.677	-	4.207
<i>Model Fit</i>						
R ²	0.386	0.201	0.472	0.212	0.313	0.202
MSE	4.423	704.206	3.233	695.079	0.064	703.129
F (df)	4.448	1.637	6.331	1.744	3.230	1.650
	(13, 92)	(14, 91)	(13, 92)	(14, 91)	(13, 92)	(14, 91)
p	<0.001	0.084	<0.001	0.060	<0.001	0.081

[#]p<.10, *p<.05, **p<.01, ***p<.001

Results from statistical model using cortisol reactivity are presented in Table 5.5. The cortisol reactivity model explained a significant amount of variance in cortisol reactivity; however, there were no significant individual predictors. The cortisol reactivity model also did not explain a significant amount of variance in behavioral aggression.

Association between Genetic Risk and Alcohol-Related Aggression in Men. Four separate models were used to examine the mediating effect of acute stress on the association between PRS and alcohol-related aggression as moderated by childhood family environment stress, with the (1) Alcohol-Related Aggression Questionnaire, (2) Alcohol-Related Direct Physical Aggression Scale, (3) Alcohol-Related Direct Verbal Aggression Scale, and (4) Alcohol-Related Indirect

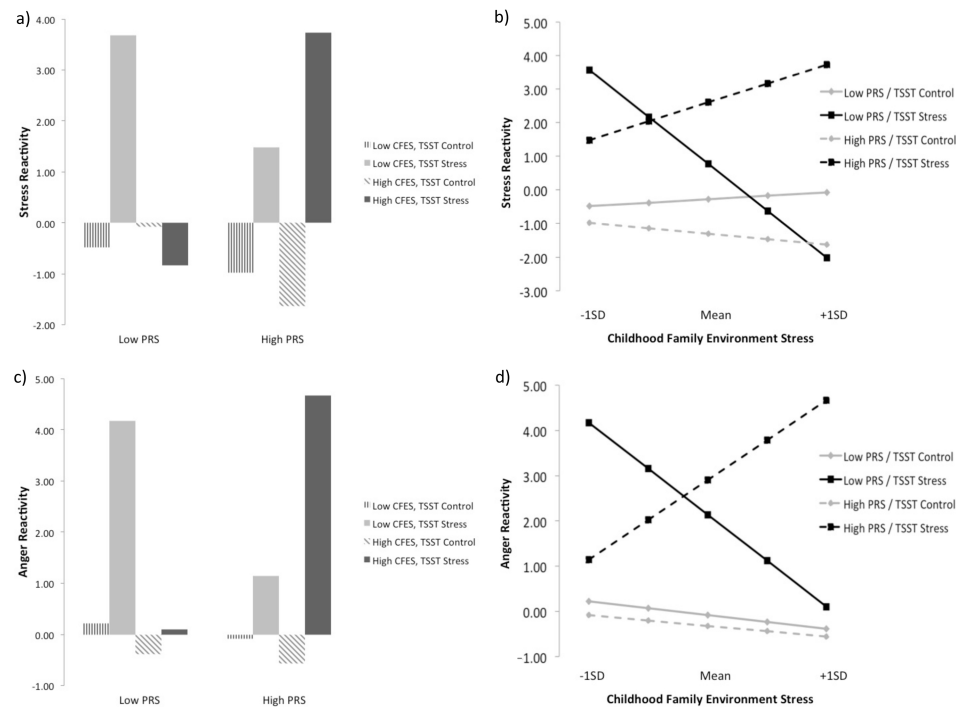


Figure 5.4: Conditional effects of polygenic risk scores as moderated by self-reported childhood family environment stress and Trier Social Stress Test condition on self-reported stress reactivity in women. b) Conditional effects of self-reported childhood family environment stress as moderated by polygenic risk scores and Trier Social Stress Test condition on self-reported stress reactivity in women. c) Conditional effects of polygenic risk scores as moderated by self-reported childhood family environment stress and Trier Social Stress Test condition on self-reported anger reactivity in women. d) Conditional effects of self-reported childhood family environment stress as moderated by polygenic risk scores and Trier Social Stress Test condition on self-reported anger reactivity in women.

Aggression Scale as measures of alcohol-related aggression. Results from all four statistical models are presented in Table 5.6. None of the four models explained a significant amount of variance in either self-reported acute perceived stress or alcohol-related aggression.

Association between Genetic Risk and Alcohol-Related Aggression in Women. The same four models analyzed in men, were used in women to examine the mediating effect of acute stress on the association between PRS and

Table 5.6
Model Estimates of the Association between Genetic Risk, Childhood Family Environment Stress, Acute Perceived Stress and Alcohol-Related Aggression in Men

	Model 1		Model 2		Model 3		Model 4	
	Acute Stress	Alcohol-Related (AR) Aggression	Acute Stress	AR Direct Physical Aggression	Acute Stress	AR Direct Verbal Aggression	Acute Stress	AR Indirect Aggression
<i>Model Variables</i>								
Constant	22.499	0.707	22.434	0.135	22.434	0.176	22.434	-0.004
Polygenic Risk Score (PRS)	1.520	-0.224	1.690	-0.100	1.690	-0.196	1.690	-0.156
Childhood Family Environment (CFE)	3.217	-0.060	3.238	-0.039	3.238	-0.077	3.238	-0.012
PRS * CFE	-2.915	0.203	-2.970	0.047	-2.970	0.083	-2.970	0.009
Age	0.926	-0.035	0.936	-0.003	0.936	-0.001	0.936	-0.020
Race (White vs. non-White)	-31.605	-1.866	-31.465	-0.180	-31.465	-0.097	-31.465	-0.682
PRS * Age	-2.352	0.122	-2.275	0.025	-2.275	0.007	-2.275	0.061
PRS * Race	51.845	4.506	51.325	0.289	51.325	0.013	51.325	1.480
CFE * Age	0.046	-0.016	0.034	-0.002	0.034	-0.003	0.034	-0.004
CFE * Race	-9.782	-1.677	-9.543	0.289	-9.543	0.028	-9.543	-0.444
Acute Perceived Stress (APS)	-	0.027	-	-0.003	-	<0.001	-	0.004
Drinking to Cope Motive (DCM)	-	0.107	-	-0.015	-	0.005	-	0.011
APS * DCM	-	-0.003	-	0.001	-	0.001	-	<0.001
<i>Model Fit</i>								
R ²	0.183	0.268	0.185	0.128	0.185	0.117	0.185	0.177
MSE	35.629	0.115	35.035	0.012	35.035	0.074	35.035	0.027
F (df)	1.417 (9, 57)	1.648 (12, 54)	1.460 (9, 58)	0.672 (12, 55)	1.460 (9, 58)	0.609 (12, 55)	1.460 (9, 58)	0.988 (12, 55)
p	0.202	0.106	0.185	0.771	0.185	0.825	0.185	0.472

$p < .10$, $p < .05$, $p < .01$, $p < .001$

alcohol-related aggression, as moderated by childhood family environment stress.

Results from the statistical model using the Alcohol-Related Aggression

Questionnaire (ARAQ) are presented in Table 5.7. The ARAQ model explained a significant amount of variance in self-reported acute perceived stress; however, no individual predictors were significant. The ARAQ model also explained a significant amount of variance in alcohol-related aggression. Acute perceived stress significantly predicted alcohol-related aggression, such that higher levels of acute perceived stress was associated with higher alcohol-related aggression ($b = .030$, $p = .002$). Drinking to cope motives also significantly predicted alcohol-related aggression, such that higher drinking to cope motives was associated with higher alcohol-related aggression ($b = .132$, $p < .001$). Lastly, there was a significant interactive effect of acute perceived stress and drinking to cope motives ($b = -.004$, $p = .003$); the pattern of this effect is shown in Figure 5.5a.

Results from the statistical model using the Alcohol-Related Direct Physical

Table 5.7
Model Estimates of the Association between Genetic Risk, Childhood Family Environment Stress, Acute Perceived Stress and Alcohol-Related Aggression in Women

	Model 1		Model 2		Model 3		Model 4	
	Acute Stress	Alcohol-Related (AR) Aggression	Acute Stress	AR Direct Physical Aggression	Acute Stress	AR Direct Verbal Aggression	Acute Stress	AR Indirect Aggression
<i>Model Variables</i>								
Constant	27.294	0.311	27.294	-0.114	27.294	-0.466	27.294	-0.321
Polygenic Risk Score (PRS)	-2.128	0.077	-2.128	-0.032	-2.128	0.028	-2.128	-0.055
Childhood Family Environment (CFE)	1.327	0.062	1.327	-0.007	1.327	0.040	1.327	0.021
PRS * CFE	0.687	-0.094	0.687	-0.003	0.687	-0.062	0.687	-0.051
Age	-0.449	0.106 [‡]	-0.449	0.014	-0.449	0.068	-0.449	0.020
Race (White vs. non-White)	-5.634	0.325	-5.634	-0.097	-5.634	-0.055	-5.634	0.016
PRS * Age	-0.099	-0.095	-0.099	-0.012	-0.099	-0.075	-0.099	-0.018
PRS * Race	11.956	-0.911 [#]	11.956	-0.141	11.956	-0.588	11.956	-0.271
CFE * Age	-0.424	-0.028	-0.424	-0.005	-0.424	-0.020	-0.424	-0.005
CFE * Race	-1.106	0.301 [‡]	-1.106	0.189	-1.106	0.420***	-1.106	0.153 [#]
Acute Perceived Stress (APS)	-	0.030**	-	0.006**	-	0.019*	-	0.015*
Drinking to Cope Motive (DCM)	-	0.132***	-	0.009	-	0.061*	-	0.066**
APS * DCM	-	-0.004**	-	<0.001	-	-0.002 [‡]	-	-0.002**
<i>Model Fit</i>								
R ²	0.250	0.356	0.250	0.487	0.250	0.314	0.250	0.196
MSE	21.246	0.070	21.246	0.003	21.246	0.045	21.246	0.027
F (df)	3.510 (9, 95)	4.230 (12, 92)	3.510 (9, 95)	7.264 (12, 92)	3.510 (9, 95)	3.502 (12, 92)	3.510 (9, 95)	1.864 (12, 92)
p	0.001	<0.001	0.001	<0.001	0.001	<0.001	0.001	0.049

[#]p<.10, p<.05, p<.01, p<.001

Aggression Scale (ARDPA) are presented in Table 5.7. The ARDPA model explained a significant amount of variance in self-reported acute perceived stress; however, no individual predictors were significant. The ARDPA model also explained a significant amount of variance in alcohol-related aggression. Acute perceived stress significantly predicted alcohol-related aggression, such that higher levels of acute perceived stress was associated with higher alcohol-related aggression ($b = .006$, $p = .008$).

Results from the statistical model using the Alcohol-Related Direct Verbal Aggression Scale (ARDVA) are presented in Table 5.7. The ARDVA model explained a significant amount of variance in self-reported acute perceived stress; however, no individual predictors were significant. The ARDVA model also explained a significant amount of variance in alcohol-related aggression. Acute perceived stress significantly predicted alcohol-related aggression, such that higher levels of acute perceived stress was associated with higher alcohol-related aggression

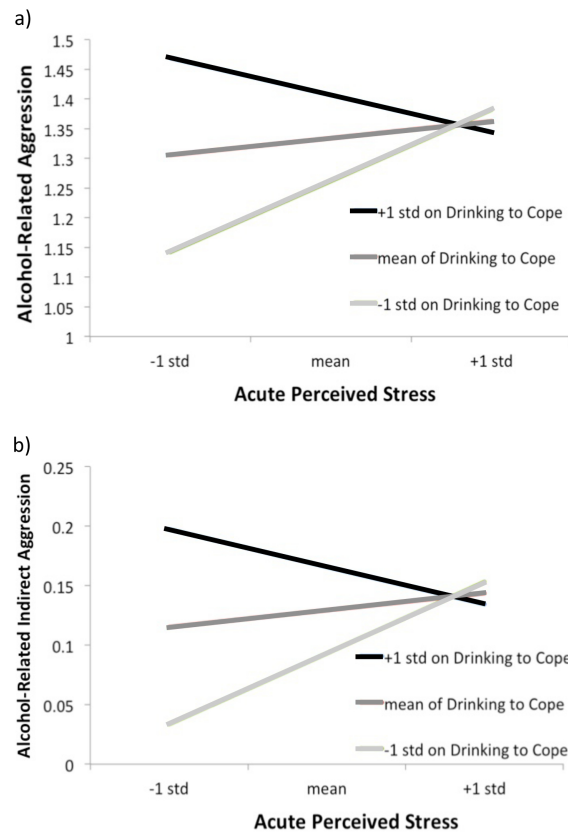


Figure 5.5: Conditional effects of self-reported acute perceived stress on self-reported a) alcohol-related aggression, and b) alcohol-related indirect aggression (log scores), as moderated by self-reported drinking to cope with stress in women.

($b = .019$, $p = .018$). Drinking to cope motives also significantly predicted alcohol-related aggression, such that higher drinking to cope motives was associated with higher alcohol-related aggression ($b = .061$, $p = .040$).

Results from the statistical model using the Alcohol-Related Indirect Aggression Scale (ARIA) are presented in Table 5.7. The ARIA model explained a significant amount of variance in self-reported acute perceived stress; however, no individual predictors were significant. The ARIA model also explained a significant amount of variance in alcohol-related aggression. Acute perceived stress significantly predicted alcohol-related aggression, such that higher levels of acute perceived stress was

associated with higher alcohol-related aggression ($b = .015, p = .014$). Drinking to cope motives also significantly predicted alcohol-related aggression, such that higher drinking to cope motives was associated with higher alcohol-related aggression ($b = .066, p = .004$). Lastly, there was a significant interactive effect of acute perceived stress and drinking to cope motives ($b = -.002, p = .008$); the pattern of this effect is shown in Figure 5.5b.

Discussion

Consistent with results from Study #2 in women, higher PRS was associated with higher reported acute stress response (i.e. increase in self-reported stress and anger), and the association was exacerbated by higher self-reported exposure to childhood family stress. However, inconsistent with our hypothesis there was no direct or indirect association between PRS and aggression as moderated by childhood family stress. One other study found no association between serotonin levels and aggression measured by the PSAP (Zhou et al., 2006), which is similar to our lack of association between genetic variation involved in serotonin neurotransmission efficiency and aggression. However, others have reported an association between tryptophan depletion (Bjork, Dougherty, Moeller, & Swann, 2000; Marsh, Dougherty, Moeller, Swann, & Spiga, 2002) and lower serotonergic function (Fulwiler, Eckstine, & Kalsy, 2005) with increased aggression using the PSAP, suggesting that our findings may be the result of a Type II error.

The lack of association with behavioral aggression may also potentially be the result of the behavioral paradigm we used. While the behavioral aggression as measured by the PSAP has been associated with reported aggressive behavior (Cherek, Moeller, Schnapp, & Dougherty, 1997; Golomb, Cortez-Perez, Jaworski, Mednick, & Dimsdale, 2007), there was no correlation between self-report measures of aggression and behavioral aggression as measured by the PSAP in the current

study. Overall the average number of aggressive responses during the PSAP ($M = 45.99$, $SD = 32.88$) was lower (difference of about 100) than other studies using the PSAP that did find a correlation between PSAP and self-reported aggression (Bjork et al., 2000; Carré & McCormick, 2008; Dougherty, Bjork, Marsh, & Moeller, 1999), which is surprising considering the modifications made to the PSAP in the current study. In the original PSAP points are randomly subtracted from the participant (provocation) every 6-120 seconds and protection intervals lasted 250 seconds, however in the current study provocation could occur every 6-90 seconds and protection intervals only lasted 75 seconds. Increasing the frequency of provocation, increased aggressive responding in the PSAP (Cherek, Moeller, Schnapp, & Dougherty, 1997), and similar modifications have been made by others to increase aggressive responding (Bjork et al., 2000; Fulwiler et al., 2005; Geniole, Carré, & McCormick, 2011; Marsh et al., 2002). A large portion of participants (19.2%) were aware that they were playing a computer, but aggression did not vary as a result of this knowledge, suggesting that the low aggressive responding of our sample is not the result of our manipulation. Given that only 16.4% of our sample described their opponent with aggressive adjectives (e.g. aggressive, annoying, competitive, fast, sneaky), it would suggest that the PSAP, with our current modifications, is not a good measure of aggression in our sample and should either be modified further (e.g. increasing rate of provocation, offering monetary incentive) or other behavioral paradigms should be considered.

Alpha-amylase is a marker for adrenergic activity during stress (van Stegeren, Wolf, & Kindt, 2008). Furthermore, alpha-amylase reactivity to stress modifies the association between cortisol reactivity to stress and aggression (Allwood, Handwerker, Kivlighan, Granger, & Stroud, 2011; Gordis, Granger, Susman, & Trickett, 2006). However, results have been inconsistent regarding the pattern of the

interaction between cortisol and alpha-amylase reactivity. Gordis and colleagues (2006) found that among individuals with low alpha-amylase reactivity there was a positive association between cortisol and problem behaviors (e.g. aggressive behavior, rule-breaking behavior, social problems). On the other hand, Allwood and colleagues (2011) found that among individuals with low alpha-amylase reactivity there was a negative association between cortisol and aggression. While the pattern of the interaction between alpha-amylase and cortisol reactivity in predicting aggression may be unclear, another explanation for the lack of association between cortisol reactivity and aggression in the current study may be due to our lack of examining alpha-amylase, because cortisol reactivity was only associated with aggression in individuals with low alpha-amylase reactivity to stress.

Although we found an association between our PRS, and both self-report stress and anger reactivity, as moderated by childhood family stress, there was no association between PRS and cortisol reactivity (small effect; females $r = 0.02$; males $r = 0.03$). An examination of the interactive effect of *DRD4* and 5-HTTLPR on cortisol reactivity showed a blunted response to TSST for individuals with a *DRD4* 7R-allele and 5-HTTLPR L'/L' genotype (Armbruster et al., 2009), suggesting that it may be important to include *DRD4* in our PRS when examining the association between genetic risk, childhood family stress, and cortisol reactivity. Furthermore, individuals with the *DRD4* 7R-allele who had also been exposed to high prenatal stress showed an attenuated cortisol secretion, and in individuals carrying a *DRD4* 7R-allele there was also a positive association between prenatal stress and self-reported aggression, while there was no association for those without a *DRD4* 7R-allele (Buchmann et al., 2014), demonstrating a similar crossover effect that we found in our study. Therefore, the inclusion of *DRD4* 7R-allele may also be important when examining the mediating effect of cortisol reactivity on the

association between genetic risk, childhood stress exposure, and aggression.

Similar to the results of Study #2, PRS and childhood family stress were not associated with acute stress response in men. Again, this could be the result of men and women responding differently to specific types of stress (Jakupcak, 2003; Jakupcak et al., 2002). Consistent with the hypothesis that men do not respond as robustly to the type of stressor used in our study, for women the effect of the TSST on stress and anger reactivity was stronger for women (effect size; $r = 0.29$ & $r = 0.31$, respectively) than men ($r = 0.13$ & $r = 0.07$, respectively), suggesting that situations particularly stressful to men (e.g. gender role threat) should be considered in future studies. While cortisol reactivity to the TSST is equivalent across men and women (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004b), women have shown higher heart rate (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004a) and self-reports of fear, irritability, and confusion (Kelly, Tyrka, Anderson, Price, & Carpenter, 2008) compared to men, further supporting the use of an alternative stressor when examining the mediating role of acute stress in men.

Also consistent with our fourth hypothesis, in the current sample of women, who endorse frequent binge drinking, self-reported acute stress predicted self-reported alcohol-related aggression. Inconsistent with our hypothesis however, self-reported drinking to cope with stress did not strengthen the effect between acute stress and alcohol-related aggression. Instead, alcohol-related aggression was high if women endorsed either high stress, regardless of whether they used alcohol to cope with stress or not, or high use of alcohol to cope with stress, regardless of whether they reported high or low levels of stress. It was only in women who reported low levels of drinking to cope with stress that there was a positive association between stress and aggression. Our results are consistent with previous literature to demonstrate a positive association between acute stress and aggression (Hasan, Begue, & Bushman,

2013; Mason & Blankenship, 1987; Nosjean et al., 2015). Results are also consistent with findings from Study #2, in which a positive association between self-reported acute perceived stress and self-reported aggression was found, but only in women.

Inconsistent with our fourth hypothesis, there was no direct or indirect effect via acute stress of our PRS or childhood family stress on alcohol-related aggression. The lack of significant effect is not surprising when considering that our sample size is about one-fourth of our previous studies (Study #1 & Study #2). Because the current study included a behavioral manipulation, a smaller sample size was used, and in an attempt to increase the power of our analyses for self-reported alcohol-related aggression, only individuals with higher alcohol patterns were included. However, the interactive effect for both men ($r = 0.11$) and women ($r = 0.12$), while consistent with our previous two studies, suggests that our analyses were underpowered. It is also likely that we have decreased power in the current study compared to our previous two studies, because a large proportion of our sample (29.1%) were unable to be genotyped for all four polymorphisms due to low amplification. *MAOA* uVNTR was the most frequently missing polymorphism. *MAOA* uVNTR is the most commonly studied polymorphism in regards to aggressive behavior (Byrd & Manuck, 2014) out of the four polymorphisms included in our PRS. Therefore, the missing genotype information poses a considerable issue of statistical power and interpretability when comparing our results to our two previous studies.

CHAPTER 6: GENERAL DISCUSSION

Broad Summary of Findings

To our knowledge, there has been no previously published research that has examined the association between a PRS and aggression, as moderated by childhood stress exposure and mediated with acute stress. Overall, the results from the three current studies support an association between a PRS (comprised of *TPH2* rs4570625, *SLC6A4* 5-HTTLPR+rs25531, *HTR1B* rs13212041, and *MAOA* uVNTR), exposure to childhood stress, and aggressive behavior consistent with the Differential Susceptibility Model. Suggesting that alleles associated with lower serotonin neurotransmission efficiency are associated with increased risk for engaging in aggressive behaviors, in part by, increasing an individual's susceptibility to the effect of negative environmental factors, such as childhood stress. In our studies specifically, individuals with higher PRS showed greater susceptibility to the environment, such that under conditions of higher childhood stress, higher PRS was associated with higher aggression, and under conditions of lower childhood stress, higher PRS was associated with lower aggression. Our findings are consistent with the three studies to examine the effect of a PRS on aggression (Foshee et al., 2015; Simons et al., 2012; Stuart et al., 2014), in that higher polygenic risk scores (corresponding to lower neurotransmission efficiency) were associated with higher aggression. Our findings also expand upon these previous studies (Simons et al., 2012; Stuart et al., 2014) by including additional polymorphisms, *HTR1B* rs13212041 and *TPH2* rs4570625, involved in regulating serotonin neurotransmission. The combination of genetic polymorphisms with different functions that are all associated with aggression represents a systems approach [joint association with overlap in downstream function; (Plomin et al., 2009)], when

investigating the quantitative nature of aggression.

Our study is the first to demonstrate that in women an association between genetic risk (as measured by our PRS) and aggression was mediated by acute perceived stress. Women with higher PRS and higher reported childhood family stress reported higher aggression via acute stress. The mediating role of stress suggests that exposure to childhood family stress, when an individual is more susceptible to their environment based on their ability to regulate serotonin transmission efficiently, likely results in HPA-axis dysregulation and subsequently a greater response to acute stress. While acute stress did not mediate the association between our PRS, childhood family stress, and aggression in men, this may be the result of men being less stressed overall or the type of stress measured. Further replications would therefore, greatly benefit from multiple measures of acute stress, in addition to having a large enough sample size to encompass a larger range of self-reported acute stress.

The association between our PRS, childhood family stress, and aggression also did not generalize to alcohol-related aggression. However, in Study #3, self-reported acute stress was associated with self-reported alcohol-related aggression. Both the Study #1 and Study #2 found an association between PRS, reported exposure to childhood trauma, and acute stress in women, and the lack of this association in Study #3 is potentially due to the small sample size. Therefore, a replication of Study #3 with a larger sample size is recommended before concluding that the lack of an association between our PRS, childhood family stress, and alcohol-related aggression is not a Type II error.

Our results are consistent with the framework of the theoretical models considered to design our research questions. Genetic factors (e.g. PRS) interact with environmental factors (e.g. childhood family environment stress) to predict

aggression (General Aggression Model), such that certain alleles are proposed to be more susceptible to the environment (Differential Susceptibility Model & Diathesis Stress Model), and that particularly in adverse environments (e.g. higher exposure to childhood trauma), individuals who are more susceptible to the environment, based on genetic risk, are more likely to be aggressive (Differential Susceptibility Model & Diathesis Stress Model).

Limitations

Although four polymorphisms that alter gene transcription were included in our PRS, which is a larger number of polymorphisms than either of the previous two studies to examine the association between a PRS specific to serotonergic genes and aggression (Simons et al., 2012; Stuart et al., 2014), additional polymorphisms need to be considered in order to account for a larger portion of the variance in aggressive behaviors accounted for by genetics. Additionally, our inclusion of *HTR1B* rs13212041 and *TPH2* rs4570625 in the PRS can be criticized (Dick et al., 2015) because, even though the polymorphisms alter gene transcription, previous literature investigating the association between these two polymorphisms and aggression, as moderated by exposure to childhood stress, is limited.

Another potential polymorphism that regulates serotonin neurotransmission efficiency and has been investigated regarding aggressive behavior, which could be included in future PRS, is the *HTR2B* rs6437000. *HTR2B* codes for the serotonin 2b (5-HT_{2B}) receptor, which regulates serotonin release presynaptically through the serotonin transporter (Callebert, 2006; Doly et al., 2008; Launay, Schneider, Loric, Prada, & Kellermann, 2006). *Htr2b* knockout mice are more impulsive compared to wildtype mice (Bevilacqua et al., 2010). Additionally, a *HTR2B* stop codon (Q20*) exclusive to Finnish individuals, is shown to block expression of the 5-HT_{2B} receptor protein, and violent offenders, 94% of whom committed their crimes under the

influence of alcohol, carrying the Q20* were the most impulsive compared to violent offenders who did not carry the Q20* (Bevilacqua et al., 2010). The A-allele of *HTR2B* SNP rs6437000, which is predictive of lower protein expression in Finnish individuals, is associated with higher aggressive behavior and brain activation in the amygdala during a behavioral paradigm (Bevilacqua et al., 2011). Therefore, rs6437000 may be a strong candidate to consider when examining the association between genetic variation in serotonin system genes and aggressive behavior.

Other neurotransmitter systems outside of serotonin should also be considered for future polygenic risk scores when examining the association with childhood stress exposure, acute stress, and aggression. Foshee and colleagues (2015) investigated genetic polymorphisms that regulate dopamine signaling (i.e. *DAT1*, *DRD2* rs1799732 & rs1800497, *DRD4* VNTR, *COMT* rs4680) and had previously been associated with emotional and motivational aspects of behavior (Nikolova et al., 2011) regarding their effect on alcohol-related dating violence, and found that boys with more low activity alleles perpetrated more dating violence. Secondly, the FKBP5 protein is crucial in terminating HPA axis activation (Binder et al., 2004). The *FKBP5* GATT haplotype of polymorphisms rs3800373, rs9296158, rs1360780, and rs9470080 has been associated with (1) greater mRNA levels (Binder et al., 2004), (2) prolonged cortisol response to stress (Ising et al., 2008), and (3) increased aggressive behavior in individuals who experienced higher amounts of childhood trauma (Bevilacqua et al., 2012). Lastly, *GABRA2* influences receptor sites for GABA, which is the major inhibitory neurotransmitter in the central nervous system (Whiting et al., 1999). GABA antagonists enhance brain activity in the amygdala, insula, and striatum (Phillips, Drevets, Rauch, & Lane, 2003). The G-allele of *GABRA2* SNP rs279858, which results in lower mRNA levels (Lieberman, Kranzler, Joshi, Shin, & Covault, 2015), has been associated with

higher intimate partner hostility, for individuals who were exposed to high levels of harsh parenting, and lower intimate partner hostility, for individuals who were exposed to low levels of harsh parenting (Simons et al., 2013). The G-allele of *GABRA2* SNP rs279858 was also more common in individuals showing high levels of externalizing behavior (e.g. delinquency, aggression) that remained high across adolescence (12-22 years old), and the effect was stronger in individuals who experienced low parental monitoring (Dick et al., 2009). Also, patients diagnosed with alcohol dependence were more likely to demonstrate aggressive behavior if they carried at least of G-allele of rs279858 (Strac et al., 2015). The inclusion of polymorphisms that affect neurotransmission of systems other than serotonin, such as the examples listed in this paragraph, would therefore potentially be beneficial to consider in future polygenic risk scores.

Although the selection of polymorphisms that alter gene regulation and are previously associated with aggressive behavior and susceptibility to stress has been previously used to build a PRS (Foshee et al., 2015; Stuart et al., 2014), this approach is also criticized (Dick et al., 2015) given that GWAS data suggests a priori selection of candidate genes is poor (i.e. polymorphisms investigated in cGxE research are usually not significant in GWAS) (Kendler, 2013; Sullivan, Daly, & O'Donovan, 2012), especially when considering the issue of publication bias (i.e. authors are more likely to submit and publishers are more likely to accept studies with statistically significant findings). However, no polymorphisms have been found to be significantly associated with aggression-related behaviors in the limited GWAS literature. It can also be argued that, due to GWAS becoming more cost efficient in recent years, our first study should have been a GWAS, in order to select the polymorphisms to include in our PRS. However, GWAS require thousands of individuals in order to have enough power to detect a significant effect, and given

that the few GWAS studies to examine aggressive behaviors have yielded no significant findings, the use of polymorphisms that alter gene regulation is a viable alternative.

Additionally, we did not use previous effect sizes or meta-analysis data to weight the contribution of each polymorphism to the PRS, which is recommended (Cumming, 2014). Indeed a comparison of weighted and unweighted models suggests that weighted genetic risk scores are superior (Che & Motsinger-Reif, 2013). There have been two meta-analyses to examine the effect of 5-HTTLPR and *MAOA* uVNTR on aggression (Ficks & Waldman, 2014; Vassos et al., 2014); however, the results between these two studies were inconsistent and there is no meta-analysis data available for *HTR1B* rs13212041 and *TPH2* rs4570625. Therefore, while we acknowledge that our approach to building a PRS based on polymorphisms that alter gene regulation is more controversial, we were restricted by the lack of previous GWAS and meta-analysis data. So although we recommend the addition of other polymorphisms that alter gene regulation or function in future research, if possible the development of a PRS should also consider GWAS and meta-analysis data. Lastly, if polymorphism are selected based on previous cGxE research then selecting polymorphisms that directly alter gene regulation also raises concerns due to the increased evidence that intronic variants also regulate gene expression (Greenwood & Kelsoe, 2003; Payton et al., 2016; Rose, 2008; Ziller et al., 2013).

An additional issue with the approach we used to build our PRS is the characterization of each polymorphism. Although our approach was strengthened by initially examining the 4-way interaction between these polymorphisms in order to assign which allele is the risk allele, in the final PRS individuals who carry two copies of the minor allele were grouped with individuals who had a heterozygous genotype. The grouping of minor allele carriers is often used to conserve statistical

power; however, this approach potentially renders results as unclear by assuming a dominant effect of the minor allele, which does not allow for the investigation of recessive or additive effects (Dick et al., 2015).

Although our statistical analyses were improved by accounting for factors that could contribute to spurious results, there are several limitations to our statistical analyses as well. In order to account for the skewness of self-report measures of aggression, data was log-transformed and winsorized. However, log-transformations can produce misleading results (i.e. multiplicative effects of predictors appear additive) (Dick et al., 2015). The use of ± 1 standard deviation in order to examine the pattern of the interaction between our PRS and childhood stress is also criticized, with some recommending the use of ± 2 standard deviations (Roisman et al., 2012). However, the skewed nature of exposure to childhood stress in our sample makes including an examination at ± 2 standard deviations difficult, because the number of individuals reporting high levels of exposure to childhood stress, who also have either very low or very high polygenic risk scores, is extremely small and therefore, the inclusion of these sparse or non-existent data points could generate misleading regression plots (i.e. pattern of the effect is different from the true effect) (Dick et al., 2015). Lastly, we did not include interaction terms between the mediator of stress and either the PRS or the environmental factor of childhood stress (e.g. childhood trauma). Even though our model was based on our research questions, if an interaction exists between the mediator and either the PRS or childhood stress then the indirect effects of PRS on aggression may be biased (i.e. strength of indirect effect may differ at varying levels of the moderator). Therefore, when interpreting our indirect effects, it is important to be aware of the statistical assumption that the indirect effect does not vary as a result of an interaction between stress and either the PRS or childhood stress.

Both the Differential Susceptibility and Diathesis Stress Models propose that individuals at the highest risk for negative outcomes, such as engaging in aggressive behavior, are individuals with susceptible/vulnerable genotypes, who are exposed to a negative environment (e.g. exposure to childhood maltreatment, no family social support). The Differential Susceptibility Model also proposes that individuals with susceptible/vulnerable genotypes, who are exposed to a positive environment, are the most likely to achieve positive outcomes. Although the pattern of our results appears to be more consistent with the Differential Susceptibility Model, this was determined by a visual inspection. A better approach would be to examine regions of significance (Roisman et al., 2012). However, in order to properly test whether results of cGxE research support the Differential Susceptibility or Diathesis Stress Model, there should be (1) a consensus among researchers to first determine the range of interest for environmental factors, and (2) a large enough sample size to properly test for regions of significance (Roisman et al., 2012). As mentioned in the previous paragraph, the use of ± 2 standard deviations on the environmental factor to examine the simple effects of biological factors on an outcome is preferred by some (Roisman et al., 2012). However, if the environmental factor is skewed, then the examination of regions at the furthest point from the mean will include sparse data points, which raises concern for the validity of any effect found (Dick et al., 2015). Additionally, because both the Differential Susceptibility and Diathesis Stress Models span the range of positive and negative environments, an examination of regions of significance, to compare results to these models, would be potentially biased if only one type of environment (i.e. positive or negative) was included in a study. Study #1 and Study #2 of this dissertation included only a measure of negative environment exposure (i.e. childhood trauma), which was significantly skewed, and in Study #3, although it included positive environment exposure (i.e.

family social support), there was no significant association between our PRS and aggression at any level of the environment factor (i.e. childhood family stress). For these reasons, we did not statistically examine whether our results support the Differential Susceptibility Model or Diathesis Stress Model. Therefore, our visual inspection that our results are consistent with the Differential Susceptibility Model should not be interpreted as support for the Differential Susceptibility Model over the Diathesis Stress Model.

As noted earlier in our discussion, the use of an independent replication strengthens the current findings. However, it has been noted that the sample size of the replication should be substantially better powered, or false-positive findings are likely to be perpetuated (Button et al., 2013). The only findings to fully replicate were with women between Study #1 and Study #2, which were equivalent in sample size, suggesting that we still need to consider the possibility of a false positive and therefore our results should be considered cautiously. The pattern of results for the interaction between our PRS and exposure to childhood stress, while consistent with the Differential Susceptibility Model, also needs to be considered with caution as a result of our sample size, because interactions typically produce a crossover pattern when sample sizes are small (Dick et al., 2015). All of these statistical issues can be improved in future studies with increased sample size, and particular attention given to selecting individuals more equivalently across the range of possible scores for childhood stress exposure and aggressive behavior.

Broader Implications

Current prevention and intervention programs are typically applied with the assumption that individuals will benefit from treatment equally, a “one size fits all” approach (Dick & Hancock, 2015). However, the risk factors that contribute to an individual’s likelihood of participating in aggressive behaviors will vary from person

to person. Therefore, using cGxE research to better inform the development of targeted prevention and intervention programs for quantitative traits is promising. Although cGxE research is still in its early stages, particularly for aggressive behaviors, there are still several examples of treatment programs informed by genetic profiles that demonstrate increased effectiveness.

Individuals with higher genetic risk have shown greater improvement in risky behaviors classified as early alcohol use, marijuana use, & sexual intercourse (Brody, Beach, Philibert, Chen, & Murry, 2009) and child externalizing & adolescent problem behavior (Albert et al., 2015), suggesting that individuals with higher genetic risk who are provided with early prevention programs may be at a decreased risk for later engaging in problem behaviors. The interaction between level of nicotine dependence and genetic risk related to dopamine neurotransmission, predicted likelihood of relapse during smoking cessation treatments (McGeary et al., 2012), suggesting that treatment programs may benefit from a priori knowledge of genetic risk, in order to adjust treatment to increase effectiveness for individuals at highest risk for relapse. Similarly, for women with an A-allele of apolipoprotein A-1 gene, there was a positive association between polyunsaturated fatty acid intake and cholesterol, while there was a negative association between polyunsaturated fatty acid intake and cholesterol for women with a G/G genotype (Ordovas et al., 2002), suggesting that treatments to lower cholesterol may benefit from a priori knowledge of genetic information, in order to provide the best course of treatment. Overall these examples suggest that a priori knowledge of genetic risk may be beneficial in informing who may benefit the most from certain treatments, or how treatments should be implemented.

As our understanding of the biological function of genetic polymorphisms increases, we are also able to develop treatment programs that counter-effect these

polymorphisms. Individuals who were given a weight management program personalized to their genetic profile had a larger reduction in BMI and improved glucose levels compared to individuals given a standard weight management program (Arkadianos et al., 2007). Several recent studies have shown that genetic polymorphisms affect response to cancer treatment (Cocca, Bedognetti, Bianca, Gasparini, & Girotto, 2016; Liang et al., 2016; Yuan et al., 2015), suggesting that medications tailored to an individual's genetic profile may be more effective. Genetic profiles can also inform day-to-day nutrition in order to prevent later disease. Currently the Food Standards Agency (FSA) recommends 200 μ g of folic acid each day to keep homocysteine levels below risk, but individuals with a *MTHFR* C677T T/T genotype require higher folate intake (400-600 μ g) (Görman, Mathers, Grimaldi, Ahlgren, & Nordström, 2013). Cruciferous vegetables consumption has been associated with protection against lung cancer, but only in individuals null for *GSTM1* and/or *GSTT1* genes (Brennan et al., 2005). Glucosinolate intake has also been associated with reduced cancer risk for individuals null for both *GSTM1* and *GSTT1* genes (Steinbrecher et al., 2010). These examples suggest that intervention and prevention efforts may benefit from a priori genetic information, particularly when there is an understanding of the biological function of the polymorphisms.

Intervention and prevention efforts aimed at reducing aggression are not currently at a point where tailoring to genetic profiles would be useful, because our understanding of the association between genetic variation and aggressive behavior is still limited. However, based on our results, it is possible that individuals with higher genetic risk, if they also experience childhood stress, may benefit from early prevention programs aimed at reducing aggressive behavior. Furthermore, early prevention programs that focus on adaptive strategies to coping with stress and frustration may be particularly useful in reducing aggressive behavior, especially in

women. Additional research is needed before implementation of such programs can take place.

Directions for Future Research

It was our intention that our examination of the moderating effect of childhood stress on the association between a PRS and aggression, via stress, across three studies, have independent replications to account for the possibility of a Type I error (i.e. Study #2 replicates Study #1, Study #3 replicates Study #2). Using an independent replication strengthened our findings that exposure to childhood stress (e.g. childhood trauma) moderates the association between our PRS and aggressive behaviors. However, in Study #3 we did not replicate the mediating role of acute stress (i.e. no effect found in Study #3) that was found, in women, in Study #2. Additionally, whether the association between our PRS, exposure to childhood stress, acute stress, and aggression generalizes to alcohol-related aggression, particularly in individuals who drink to cope with stress, was not replicated because it was investigated initially in our final study (Study #3). Therefore, one immediate direction of future research would be to replicate Study #3 with some alterations, detailed in the following paragraphs, to improve statistical power.

An a priori selection of individuals at both high and low ends of the distribution for the variables of interest (e.g. childhood stress exposure, aggression) would allow for the testing of simple effect patterns at the extreme ends of the distribution (± 2 standard deviations), while avoiding misleading regression plots. This method was successfully implemented in Study #3 to recruit individuals who reported high frequency binge drinking, which demonstrates the capability of using this approach to recruit individuals of interest. Although this approach would increase the time to achieve an appropriate sample size, it would address a critical concern of performing regression-type analyses with skewed distributions (Dick et al., 2015).

An alternative behavioral aggression paradigm should be used in future experimental studies as well, because the PSAP resulted in low levels of aggression compared to other samples that have used the PSAP (Bjork et al., 2000; Cherek, Moeller, Schnapp, & Dougherty, 1997; Dougherty et al., 1999). Other currently used, validated behavioral aggression paradigms that could be selected are the Taylor Aggression Paradigm or the Hot Sauce Paradigm. In the Taylor Aggression Paradigm, which is the most widely used behavioral paradigm to measure aggression, participants are told they are playing a competitive reaction time task where the loser (i.e. slower to respond) of each trial receives a shock/noise blast. The participant chooses the duration and intensity of shock for their opponent at the beginning of each trial, and their selection is used as the measure of aggression. Participants are actually playing this task against a computer, where they will automatically lose about half the trials in order to receive a shock/noise blast (i.e. provocation) (Taylor, 1967). The Hot Sauce Paradigm involves a participant being provoked by another individual (e.g. giving an unpleasant beverage, writing an insulting essay opposing the political views of the participant, taking away money), followed by the participant choosing an amount of hot sauce that the other individual, who has indicated they do not like spicy food, must allegedly consume. The amount of hot sauce chosen by the participant is used as the measure of aggression (Lieberman, Solomon, Greenberg, & McGregor, 1999). Regardless of the behavioral paradigm chosen for future studies, laboratory aggression paradigms provide insufficient attention to the individual's motivation for their apparent aggressive behavior (Ritter & Eslea, 2005), and because aggression is typically defined as having an intention to cause harm, future studies should include post-hoc measures of individual's reasoning for their behavior during behavioral aggression paradigms. Additionally, prior to the inclusion of a behavioral paradigm to examine

the association between genetic risk and aggression, an independent study of the effectiveness of the behavioral paradigm should be considered. Although the PSAP is previously associated with reported levels of aggressive behavior (Cherek, Moeller, Schnapp, & Dougherty, 1997; Golomb, Cortez-Perez, Jaworski, Mednick, & Dimsdale, 2007), aggressive responding on this paradigm was not correlated with self-report measures of aggression in Study #3, which raises concerns of validity in our population.

As noted in our limitations, the method of selecting polymorphisms from candidate genes that alter gene regulation is a somewhat limited approach, especially without weighting the contribution (Dick et al., 2015). Future polygenic risk scores, built from candidate genes, should consider only including polymorphisms that alter gene regulation or function and have been widely studied so that their contribution to the PRS can be weighted by effect sizes determined from meta-analyses. Even though the use of widely studied polymorphisms will limit the number of polymorphisms able to be included in the PRS, and inhibit the discovery of new polymorphisms that may contribute to aggression, the results will be more interpretable. Alternatively, as other techniques become available for building a PRS they should be considered against the criticisms of a priori selection of a small number of polymorphisms. One potential approach, taken by Derringer and colleagues (2010), included the use of the Illumina Human IM Bead Chip to genotype all single nucleotide polymorphisms within dopamine-related genes as identified from the literature. Polymorphisms ($N = 273$) were individually tested for their association with sensation seeking, and the 12 polymorphisms with significant associations were included in a PRS, weighted by their unique contribution to sensation seeking, as determined in the individual analyses. The PRS significantly predicted sensation seeking above and beyond a covariates only

model. This same approach was later examined with cocaine dependence symptoms, which identified four SNPs that accounted for 0.55% of the variance in cocaine dependence symptoms (Derringer et al., 2012). While the approach by Derringer and colleagues (2012) is limited by the testing of a PRS in the same sample that was used to identify significant polymorphisms, the use of chip technology to genotype en masse is preferred compared to a priori selection of a small number of polymorphisms based on previous literature (Dick et al., 2015).

Our examination of acute stress included the self-report Perceived Stress Scale and the use of the TSST, however alternative methods of inducing stress should also be considering when examining the mediating role of acute stressors, especially in the context of gender differences. In an examination of cortisol response across acute stress laboratory research, stress paradigms that combine public speaking and cognitive tasks (e.g. TSST) had the largest effect on cortisol response, whereas the use of noise exposure or emotion induction did not affect cortisol response (Dickerson & Kemeny, 2004). Tasks, such as the TSST, that involve a motivated performance (i.e. active performance situations) with social-evaluative threat (e.g. evaluative audience, recording of performance for subsequent evaluation) and uncontrollability (e.g. performing under time constraints, completing impossible tasks) also have the largest effect on cortisol response (Dickerson & Kemeny, 2004). The effect of laboratory paradigms on cortisol response reported by Dickerson & Kemeny (2004) would support the use of the TSST, however women report higher levels of fear and irritability, although they did not have a greater cortisol response, following the TSST compared to men (Kelly et al., 2008). Therefore, if men need to experience higher levels of stress to induce aggression, the TSST may not be the best laboratory paradigm to use. However, the use of an alternative laboratory paradigm poses considerable difficulty, because the TSST is the standard for inducing

psychological stress, and is comprised of all the components (e.g. motivated performance, social-evaluative threat, uncontrollability) that have the largest effect on cortisol response (Dickerson & Kemeny, 2004). Although gender role stress is associated with self-reported aggression (Jakupcak, 2003; Jakupcak et al., 2002), to our knowledge, there are currently no behavioral paradigms that induce gender role stress. Stress resulting from social exclusion is also a potential mediating acute stress factor on the association between genetic risk and aggression, because stress resulting from social exclusion also biases individuals to be more aggressive (Behrendt, 2011). Male students were more aggressive after being socially excluded if they carried the *MAOA* LA-allele (Gallardo-Pujol et al., 2013). Furthermore, both men and women with the *MAOA* LA-allele showed increased rejection-related distress, as measured by activation in the dorsal anterior cingulate cortex, following social exclusion (Eisenberger, Way, Taylor, Welch, & Lieberman, 2007). Social exclusion also increases hostile cognitive bias, which is subsequently associated with increased aggression (DeWall, Twenge, Gitter, & Baumeister, 2009). Therefore, social exclusion may be of particular interest when examining the effect of acute stress, and may also be a better inducer of stress in men compared to the TSST.

In order to further investigate our fourth specific aim using self-report measures of alcohol-related aggression, a larger sample size is needed. However, in a replication using a behavioral aggression paradigm, an alcohol manipulation component could be added in order to examine the association with alcohol-related aggression in a smaller sample. The use of an alcohol manipulation would allow testing of the direct effect of alcohol intoxication on aggression following acute stress, as opposed to the correlational relationship using self-report. Our hypothesis that alcohol use in order to cope with stress increases the likelihood of aggressive behavior, is supported by Hoaken and colleagues (2003), who found that alcohol

resulted in lower stress reactivity, as measured by heart rate and blood pressure, and greater aggressive responding during the Taylor Aggression Paradigm. Additionally, self-reports of alcohol consumption as a means of coping increased the likelihood of engaging in alcohol-related aggression among university students (Mihic et al., 2009). However, in accordance with the Alcohol Myopia Model, stress induced individuals have also been shown to be less aggressive after consuming alcohol, presumably due to being emotionally distracted by the stressor (Phillips & Giancola, 2008). However, stress induction involved informing students that they would have to give a speech about what they disliked about their bodies while being videotaped, followed by individuals consuming an alcohol or placebo beverage and participating in the Taylor Aggression Paradigm. Because participants were not told until after participating in the aggression paradigm that they would not give a speech, acute stress can be viewed as continuing through the aggression paradigm rather than solely preceding the aggression paradigm. Therefore, an inclusion of alcohol manipulation following stress induction and preceding an aggression paradigm would also allow for a comparison between the Alcohol Myopia Model and the Alcohol Stress-Response Dampening Theory on the threat detection system.

Lastly, although the General Aggression Model informed the current studies, the current studies do not include measures of the cognition aspect of an individual's present internal state, or appraisal and decision making processes (impulsive vs. thoughtful action). The current studies, although reasonably so, also do not encompass all possible factors contributing to the other components of the General Aggression Model (e.g. person input, situation input, biological factors, environmental factors). Therefore, as the association between genetic risk, childhood stress exposure, acute stress, and aggression is better understood, additional factors that fit within the General Aggression Model should be included.

For example, social exclusion increases hostile cognitive bias (DeWall et al., 2009), therefore hostile cognitive bias should be investigated as a cognitive aspect of the internal state that potentially mediates the association between genetic risk and aggression. Additionally, peer deviance during childhood is also associated with increased risk for aggression, particularly for individuals homozygous for the *BDNF* (brain-derived neurotrophic factor) met variant (Kretschmer, Vitaro, & Barker, 2014), which has also been associated with increased HPA axis activity following stress induction (Colzato, Van der Does, Kouwenhoven, Elzinga, & Hommel, 2011). It is beyond the scope of this discussion to include a comprehensive review of all possible factors contributing to the likelihood of aggressive behavior, but it should be noted that moving forward research should consider a more comprehensive examination of the association between genetic risk and aggression as informed by the General Aggression Model.

Conclusions

The purpose of the studies presented in this dissertation was to investigate the contribution of a polygenic risk score, childhood stress, and acute stress to individual differences in engaging in aggressive behavior, including alcohol-related aggression. In accordance with theory, our novel findings indicate that individuals with a higher polygenic risk score, higher exposure to childhood stress, and higher acute stress are more likely to engage in aggressive behavior, particularly in females. Our findings support that future research, should continue to examine the interaction between biological and environmental modifiers on aggression in the context of situational risk factors. This dissertation overcomes several limitations in cGxE research by, (1) including independent replications, (2) using a polygenic risk score that accounts for the quantitative nature of aggressive behavior, and (3) accounting for factors in analyses that can produce spurious results. As research

investigating the underlying genetic architecture of aggressive behavior moves forward, special attention should also be given to the increased scrutiny on cGxE research. Hopefully, by better understanding the biological circuits that contribute to aggression and by elucidating the mechanisms between genetic risk and aggression, it will be possible to identify individuals most at risk for engaging in aggressive behavior, in order to provide early intervention and prevention. In addition to, potentially providing more personalized interventions that could result in increased effectiveness of reducing aggressive behavior.

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