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Oxytocin Genetic and Epigenetic Variation: Association with Social Adversity and Behavioral and Health Outcomes

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Oxytocin Genetic and Epigenetic Variation: Association with Social Adversity and Behavioral and Health Outcomes

Advisors: Jessica Sales, PhD and Patricia Brennan, PhD

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Abstract

Oxytocin Genetic and Epigenetic Variation: Association with Social Adversity and Behavioral and Health Outcomes

By: Erica Lauren Smearman

Exposure to environmental stress can increase an individual's risk for behavioral problems, psychopathology, and chronic health conditions. Importantly individuals with different genetic profiles vary in their responsiveness to these environments, and thus their likelihood of developing adverse outcomes. Given the social nature of many stressful environments, genetic factors related to the oxytonergic system may be relevant to environmental differential susceptibility. Oxytocin is a hormone important for perceiving social cues. Therefore, the oxytonergic system may influence perception of, and thus sensitivity towards, both nurturing and adverse social environments. While much of the initial human oxytocin work draws from positive social environments, more recent work has incorporated adverse social contexts. This dissertation expands this literature, exploring genetic differences in the oxytocin receptor gene (sequence variation and methylation patterns) in the association between adverse social environments and behavioral and health outcomes, including responsiveness to intervention.

First, in a prospective cohort of youth (N=404), we tested the role of *OXTR* genetics in the association between interpersonal conflict and conduct and antisocial behaviors at age 15 and 20, finding that individuals with the rs53576 GG genotype engaged in more disordered behavior when exposed to high levels of interpersonal conflict. Second, in a sample of adults (N=393), we assessed the role of *OXTR* DNA methylation in the association between abuse and psychopathology, finding that individuals with specific *OXTR* CpG methylation patterns reported higher levels of depression and anxiety when exposed to abuse, compared to those without those patterns. *OXTR* sequence variation and methylation were also considered together, with *OXTR* genotypes associating with methylation of nearby CpG sites. Third, in a sample of parent-youth pairs (N=191), we explored the role of *OXTR* genetics on responsiveness to a family-based intervention. Youth with the rs53576 GG genotype again showed the greatest sensitivity to environmental context, exhibiting the shortest telomeres when exposed to high parent-youth conflict and randomized to the control intervention condition, and telomere lengths similar to the non at-risk group when randomized to the intervention.

The combined findings suggest a role for *OXTR* in sensitivity to the social environment and in the prediction of behavioral and health outcomes.

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CHAPTER 1
Introductory Literature Review

Childhood and lifetime adversity can result in high levels of psychosocial stress that increase an individual's risk for behavioral problems, psychopathology, and chronic health conditions (Harkonmäki, Korkeila, & Vahtera, 2007; Miller, Chen, & Parker, 2011; Raposa, Hammen, & Brennan, 2014). Research has provided strong evidence that behavioral and health trajectories begin to diverge at a young age, with differences noted in blood pressure and immune response to allergens as early as childhood (Cohen, Evans, Krantz, & Stokols, 1980; Flaherty et al., 2006; Shonkoff, 2010; Wright et al., 2004). Research into both biological and behavioral mechanisms that underlying the development of behavioral and health outcomes will give insight into risk and avenues for public health interventions.

Psychosocial stress, such as that resulting from exposure to social adversity or abusive environments, can have a variety of impacts. Studies have suggested that individuals exposed to high levels of psychosocial stress may be more likely to engage in risky behaviors, which can place them at increased risk for poor physical and mental health outcomes and lead to interpersonal problems with others (Aiello, Simanek, & Galea, 2010; Brody, Chen, & Kogan, 2010; Cohen, 1995). Furthermore, behavioral responses that may be used as maladaptive coping mechanisms, such as smoking and poor dietary habits, can put individuals at increased risk for poor health due to these additional exposures (Raposa, Bower, & Hammen, Najman & Brennan, 2014; Rutter, Caspi, & Moffitt, 2003).

In addition to changes in behavior, a number of biological changes can occur during stress. These biological responses are multifaceted and include genetic, epigenetic, neuroendocrine and immune processes. For example, exposure to stress can result in activation of the hypothalamic-pituitary-axis, resulting in heightened exposure to cortisol, as well as heightened activation of the sympathetic ("fight or flight") nervous system. While these responses are critical and important for an effective response to brief exposures to stressful contexts, when

stress is experienced chronically, the continuous high levels of cortisol and sympathetic activation can lead to wear and tear of the body over time (Brody, Yu, Chen, & Miller, 2013b; Cohen, Janicki-Deverts, & Miller, 2007; Ziemssen & Kern, 2007). Recent research has also suggested that exposure to stressful contexts during sensitive times of development may result in more distinct biological changes, whereby the stress "gets under the skin" and influences differential trajectories for later health and behavior (Miller et al., 2011; Segerstrom & Miller, 2004). This model of the Biological Embedding of Childhood Adversity suggests that factors such as epigenetic markers, protein modifications, and tissue changes may be influenced by stressful contexts, and result in differences between individuals that can be systematically and objectively assessed following stress and linked to later poor health (Miller et al., 2011).

When considering mechanisms that underlie these findings, a critical observation to note is that individuals vary in their responsiveness to the environment, and thus their likelihood of developing adverse outcomes following exposure. Understanding factors that influence an individual's level of sensitivity or resiliency towards environmental contexts is particularly important for public health as it has the potential to provide insights into factors that may be incorporated into prevention programs or that may be used to target prevention efforts towards individuals who will evidence the greatest impact.

Much of this effort has been driven by an increase in gene-by-environment studies, whereby individuals with certain genotypes may be more at risk for certain outcomes following environmental exposures. Following initial groundbreaking studies by Caspi and colleagues suggesting a moderating role of genetics on risk for adverse behavioral outcomes, such as the role of *MAOA* on maltreatment and antisocial behaviors (Caspi et al., 2002), and of *5-HTTLPR* on stressful life events and depressive symptoms (Caspi et al., 2003), many studies proceeded to explore the role of genetic differences in risk.

Genetics

Genetic differences refers to differences in the sequence of DNA. DNA consists of all of our genes as well as "non-gene" areas that are less understood though may be involved in gene regulation. DNA utilizes just four basepairs: guanine (G), adenine (A), thymine (T) and cytosine (C). These basepairs function as a sort of "alphabet" and are linked together using carbohydrate and phosphate groups as a backbone structure. Therefore, all DNA, and all genes, consists of a certain string of these four letters.

The string of basepairs that make up a gene can differ slightly between individuals. For example, where one individual may have a "G" in the sequence, another individual may have an "A". Given that every person has two copies of each gene (one from each parent), an individual's genotype for that location could be: GG, AG, or AA. In behavioral research, the genetic differences that are studied are commonly those that result from these single basepair differences or single nucleotide polymorphisms (SNPs). These SNPs can occur in regions of the gene that actually code for protein ("exons"), in regions of the gene that do not code for protein though may influence gene regulation ("introns"), or in other regions of DNA, which can include areas that are important for regulating gene expression such as gene promoter regions. Therefore, the impact of a certain SNP can depend on where it is located. *5-HTTLPR,* for example, occurs in the promoter region of the serotonin transport gene, and is thought to influence the expression level of the serotonin transporter.

While the biological functions of many SNPs studied in behavioral research are not yet known, exploring associations between SNPs and behavioral outcomes, and the role of the environment in evidencing these outcomes, may provide important insight into genes that are important in behavior and health. SNP associations that continue to be replicated should be considered for more detailed biological research exploring the mechanism by which these associations may occur.

Differential Susceptibility

Historically, psychological and behavioral research has largely been focused on understanding the influence of adverse contexts on pathologic outcomes. Out of this base, the largely predominant diathesis-stress model was used to conceptualize an individual's predisposition or risk for psychopathology following exposure to adversity (Monroe & Simons, 1991; Zuckerman, 1999). Under this model, individuals with certain predispositions are thought to be at higher risk for poor outcomes and thus more likely to evidence poor outcomes if they encounter a provoking stressful context. This model has been utilized in the context of gene-environment (GxE) interactions, whereby individuals with specific genotypes may be at increased risk for poor behavioral and health outcomes following exposure to adversity (Monroe & Simons, 1991; Zuckerman, 1999). However, more recent work has shown that this conceptualization may be too narrow and too heavily focused on negative contexts. Broadening this lens, and providing enhanced explanation for recent findings assessing more positive contexts and outcomes, Belsky and colleagues proposed the theory of Differential Susceptibility (Belsky & Pluess, 2009; Belsky et al., 2009). Applied to the GxE literature, the theory of Differential Susceptibility suggests that genotypes previously conceptualized as 'risk' may actually result in increased susceptibility to the environment - both good and bad (Belsky et al., 2009). According to this theory, individuals who are susceptible should evidence greater risk for maladaptive outcomes in the context of adversity, similar to the diathesis-stress model, but should also evidence more positive outcomes in the context of nurturing and supportive environments.

Epigenetics

When assessing the role of genetics in behavioral and health outcomes, another component that is important to consider is epigenetics. While an individual's DNA sequence is generally consistent between all cells in the body and across the lifespan, the specific genes that are made or "expressed" does differ. This process allows cells to differentially express genes that are distinct to their cell type, and it is regulated through a number of factors including transcription factors and epigenetic modifications. The term "epigenetics" refers to chemical

modifications of the DNA structure that change the accessibility of genes to transcription machinery, thus altering the amount of a gene that is able to be expressed without changing the DNA sequence (Bird, 2002; Klose & Bird, 2006; Tamashiro & Moran, 2010). The regulation of gene expression by transcription factors and epigenetic modifications is a dynamic process that has the potential to respond to environmental cues. One of the most commonly studied epigenetic changes is the addition of methyl groups to the promoter region of a gene. Generally, the addition of these methyl groups occurs at CpG sites, locations where a cytosine and guanine basepair occur next to each other in the DNA sequence and are joined by a phosphate bond. When occurring in the promoter region of a gene, these methyl groups generally function to block the binding of transcription factors and machinery that would otherwise facilitate gene expression (Bird, 2002). Therefore, the addition of methyl groups in promoter regions tends to reduce gene expression. However, it has been increasingly recognized that methyl groups that bind outside of CpG sites can also influence expression, and the role of methylation in non-promoter regions, such as within the gene body, may be more nuanced, with some studies reporting increased expression linked with increased methylation (Ehrlich and Lancey, 2013).

Differential Susceptibility and Epigenetics

Given the role of epigenetics in gene regulation, it is plausible that individual differences in methylation patterns may underlie some variability in risk and resilience. Individuals with certain methylation patterns may be at increased susceptibility to their environment compared to those with different patterns. Therefore, while genotype differences have been the primary focus of the differential susceptibility literature to date, epigenetics may be an important factor to consider as well.

Epigenetics and the Environment

While epigenetics may serve as a moderator between environmental exposures and behavioral and health outcomes, one of the exciting features of epigenetics is its documented ability to change in response to the environment. Beginning with landmark studies by Meaney

and colleagues, it has been shown that some early life experiences have the ability to alter epigenetic programming of certain genes, especially those related to the stress response system and social interactions (e.g. Szyf, Weaver, Champagne, Diorio, & Meaney, 2005). In these rodent studies, exposure to varying levels of maternal care (high and low nurturance through levels of licking and grooming and arched back nursing) as well as early adversity paradigms, such as maternal separation, resulted in differences in the methylation levels of a number of genes (Champagne, 2010; Champagne, 2008). Importantly, these exposures, and methylation levels, were associated with differences in behavioral outcomes such as fearfulness, social aggression, and future maternal behavior.

Human studies have recently begun to explore this question by assessing the presence of methylation differences among individuals exposed to varying degrees of early adversity. Intriguingly, initial studies suggest that this pattern of epigenetic "embedding of stress" may hold, with different methylation patterns found in genes such as the glucocorticoid receptor (McGowan et al., 2009) and serotonin transporter (Beach, Brody, Todorov, Gunter, & Philibert, 2010b), across individuals with varying degrees of exposure to childhood abuse.

However, the degree to which epigenetic patterns can change across the lifespan, and the degree to which different genes are impacted, is currently relatively unknown. Therefore, epigenetic patterns of certain genes may serve as moderators between the environment and behavioral outcomes, whereas others may serve as mediators, in which case the environment influences epigenetic patterns, which in turn influence risk for later outcomes.

Role of Oxytocin

A number of genes have been explored for association with adverse contexts and behavioral and health outcomes. One area that has more recently become recognized in this literature is the oxytonergic system. Given the social nature of many of adverse contexts, such as abusive relationships and interpersonal stress oxytocin may be a particularly fruitful area to explore. Oxytocin (OT) is a neurohormone that is produced in the hypothalamus and is

transported to the posterior pituitary where it is released. It is best known for its role in lactation and child birth but has more recently been recognized for its role in social behaviors in both humans and animals (Carter, 2003; Young & Wang, 2004).

While our understanding of OT is still developing, a number of studies have begun to explore the role of OT in rodent social behavior, the levels of OT in human subjects, and impact of intranasal OT administration on human behavior. In rodent research, raising young rodents in both socially deprived and socially enriched environments has resulted in alterations in the production of OT as well as its binding to receptors (Veenema, 2012). Social deprivation models have been found to result in a variety of social behaviors. These behaviors vary between rodent species but can include increased aggression in male rodents (Veenema, 2012; Veenema & Neumann, 2008) as well as impaired maternal care and earlier sexual acceptance in some female rodents strains (Cameron et al., 2008). It is thought that deprivation may prime the rodent for adversity, potentially explaining the changes in aggression and stimulating mechanisms to increase reproductive success in the face of adverse conditions (Veenema, 2012). While these findings are likely due to a complex milieu of biological and environmental factors, the role of OT in this process is becoming increasingly recognized (Carter, 2003; Young & Wang, 2004).

When exploring the role of OT in human studies, it is important to understand that OT levels naturally vary in healthy populations and OT data is largely limited to peripheral measures (blood, saliva, urine) since cerebrospinal fluid is difficult to obtain. These studies have generally found an association between increased levels of blood OT and higher feelings of trust and lower levels of anxiety (Bartz, Zaki, Bolger, & Ochsner, 2011a; Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011). In addition, studies have revealed lower blood OT levels in patients with autism-spectrum disorders and psychiatric disorders such as depression and schizophrenia (Ozsoy et al., 2009). Intranasal OT administration has been used in a variety of studies to attempt to elucidate its influence. Studies have provided mixed results, but in general demonstrate enhanced facial recognition, increased willingness to trust others in social games, increased positive

communication in couple conflict resolution, and improved performance in emotion recognition, especially among those with autistic features (though the type of emotion most improved varied by study) (Meyer-Lindenberg et al., 2011).

While oxytocin was originally seen as facilitating prosocial behaviors, recent studies suggest that the effects of oxytocin on human behavior are more complex, and may be dependent on environmental context. For example, intranasal administration of oxytocin heightens feelings of envy during a gambling game (Shamay-Tsoory et al., 2009), and results in reduced trust and cooperation among individuals sensitive to rejection (Bartz, Simeon, Hamilton, Kim, Crystal, et al., 2011b). Oxytocin administration also increases ethnocentric behavior, resulting in greater preference toward in-group members and derogation toward out-group members (De Dreu, 2012), which overlaps with findings in the animal literature where oxytocin actually promotes aggression towards intruders (Pedersen, 2004). In a recent review of the oxytocin literature, Bartz et al. (2011) proposed the social salience hypothesis, suggesting that rather than increasing trust or positive affiliation, oxytocin may increase an individual's sensitivity to, and thus potential reactivity towards, the social environment. More recent data further supports the role of oxytocin in heightening the salience of social cues, with reports of oxytocin administration in humans increasing recognition of positive facial expressions (Marsh, Yu, Pine, & Blair, 2010) and the salience of socially positive and negative (but not socially neutral) stimuli, where specific brain regions such as the ventral tegmental area have been implicated (Groppe et al., 2013). According to the Social Sensitivity hypothesis, higher levels of oxytocin may result in beneficial outcomes in familiar and nurturing contexts, but may also result in more adverse outcomes in socially adverse environments given that increased attention to social cues could be problematic when those cues are negative or adverse (Bartz, Zaki, Bolger, & Ochsner, 2011a).

Oxytocin must bind to the oxytocin receptor in order to have its effect. Therefore, variation in oxytocin receptor and its expression may also underlie variations in social behavior. The oxytocin receptor is located on chromosome 3p25 with four exons and three introns (Gimpl

& Fahrenholz, 2001). Oxytocin receptors are expressed in certain areas of the brain and are also expressed in the periphery, such as on the cardiac and vascular muscles where binding generally lowers heart rate and reduces vascular tone (Kimura, 2003). Animal literature has provided compelling evidence that differences in expression levels of the oxytocin receptor in certain regions of the brain, such as those involved in reward and parenting behaviors, may underlie individual and species differences in social behaviors (Young & Wang, 2004). Furthermore, animal "knockout" studies, where the animals are missing the oxytocin receptor completely, have been associated with aberrant social and emotional behaviors, such as reduced nurturing and maternal behaviors (Nishimori et al., 2008), and lack of memory of recently encountered individuals (Ferguson, Young, Hearn, & Matzuk, 2000). These studies highlight the importance of the oxytocin receptor in the development and display of social behaviors.

Oxytocin Genetics

While the functional role of SNPs within the human *OXTR* gene are yet to be fully elucidated, initial studies have begun to test for the presence of associations between *OXTR* SNPs and traits such as trust (Krueger, Parasuraman, Iyengar, Thornburg, Weel, Lin, Clarke, McCabe, & Lipsky, 2012) empathic concern (Montag et al., 2012; Schneiderman, Kanat-Maymon, Ebstein, & Feldman, 2013; Wu, Li, & Su, 2012), and autistic behaviors (Jacob et al., 2007). More recent work has extended this literature to incorporate the role of environmental context and has highlighted the importance of considering the environment in the association between *OXTR* genetics and behavioral outcomes (Bradley et al., 2011; McQuaid, McInnis, Stead, Matheson, & Anisman, 2013).

***OXTR* rs53576**

One of the most commonly studied *OXTR* SNPs is rs53576, which is located in the 3rd intron of the gene and has both G and A alleles (Gimpl & Fahrenholz, 2001). Initial studies of this polymorphism suggest an overall positive influence of the G allele on social behaviors, such as higher levels of prosocial and trusting behaviors compared to those with the A allele (Kogan et

al., 2011; Krueger et al., 2012). However, the majority of this initial research examined the main effects of *OXTR* polymorphisms on social behavior in healthy, non-clinical samples. More recent reports incorporating environmental context suggest that those carrying a G-allele may demonstrate increased risk of maladaptive behaviors in the presence of social adversity (Bradley et al., 2011; Sturge-Apple et al., 2012). Research suggests that individuals with the G-allele are more susceptible to the environment, both good and bad. This increased susceptibility may be through the mechanism of increased salience of, and thus potential reactivity towards, social cues (Bartz, Zaki, Bolger, & Ochsner, 2011a). For example, Bradley et al. (2011) found that individuals with the G allele evidenced greater levels of emotion dysregulation in the context of early life abuse, compared to those with the A allele. Furthermore, those with the G allele were found to have greater sensitivity to an infant cry (Riem, Bakermans-Kranenburg, & Pieper, 2011) and Sturge-Apple et al. (2012) showed that mothers with the G allele were more influenced by the quality of their inter-partner relationship in their display of sensitivity towards their children – among those with the G-allele, those with a positive inter-partner relationship exhibited the highest level of maternal sensitivity while those with high inter-partner conflict exhibited the lowest level of sensitivity. These effects suggest that G carriers may be more attentive to social cues, and thus may be more vulnerable in the context of adverse social environments, making them more likely to develop adverse behavioral outcomes.

***OXTR* Epigenetics**

As the GxE literature continues to grow, the rise in behavioral epigenetic research has added additional insights and components to consider. The animal literature has provided strong evidence that the expression levels of the oxytocin receptor in certain areas of the brain may underlie individual and species differences in social behaviors (Insel & Young, 2001). Given epigenetics role in regulating gene expression, exploring epigenetic differences between individuals may give insight into biology underlying human behavioral differences. Importantly, methylation of certain CpG sites in both the promoter region and 3rd intron of the *OXTR* gene

have been associated with expression levels of the receptor (Harony-Nicolas et al., 2014; Kusui et al., 2001; Mizumoto, Kimura, & Ivell, 1997). Therefore, it is plausible that methylation patterns may be useful for studying variations in human social behaviors. In social contexts, individuals with DNA methylation patterns linked to high expression may evidence increased perception and responsivity to social cues, resulting in more positive – or more negative – outcomes, depending on the context.

OXTR **Epigenetics in Humans**

Initial studies have tested for associations between *OXTR* methylation and human behavioral traits. These studies have provided preliminary evidence for associations between methylation of certain *OXTR* CpG sites and traits as autistic symptoms (Gregory et al., 2009), perception of biological motion (Jack, Connelly, & Morris, 2012), and callous and unemotional behaviors (Dadds et al., 2013).

Interestingly, to date, no study has looked at an interaction between the environment and *OXTR* epigenetics on behavioral outcomes, though there have been calls for research exploring this question (Jack et al., 2012; Kumsta, 2013). In addition, there has been limited animal research assessing environmental exposures and methylation of *OXTR.* An initial human study suggests that there may be small rapid changes in *OXTR* methylation following exposure to stressful contexts. Unternaehrer et al., (2012) investigated dynamic changes in DNA methylation of CpG sites across the majority of *OXTR* exon 3 and found that average methylation status increased following the Trier Social Stress Test (TSST) and then decreased below pre-TSST levels 90-min post-test. Therefore, the methylation level appeared to undergo rapid changes, at least to a degree. However, it is yet unknown how early experiences influence *OXTR* epigenetics, and the degree to which epigenetic programming of the *OXTR* gene can change over time and in response to experiences. Given this, an important question that yet to be addressed is whether *OXTR* methylation may serve as a mediator to later behavior, or whether it may function more as

a moderator (assuming associations with expression level) in the link between early adversity and poor adult health.

Prevention & *OXTR*

While exploring biological mechanisms underlying risk is important, prevention is a critical component to public health. Intervention programs can themselves have a social component, particularly those focused on improving relationships and social interactions. Therefore, this literature on differential susceptibility may extend into the prevention literature as well. Specifically of question is whether individuals with ”more sensitive” genotypes, such as the rs53576 GG genotype (Bartz, Zaki, Bolger, & Ochsner, 2011a; Bradley et al., 2011), will be more responsive to both adverse environments and to prevention programs. Indeed, recent studies have suggested that genetics may underlie some of the variability seen in an individual's responsiveness to an intervention program. For example, Brody et al. (2014) explored the role of genetic variation in the dopamine transporter on intervention outcomes, Sales et al. (2014) explored the role of 5-HTTLRP in intervention responsiveness, and Brody and colleagues have also explored the role of multilocus effects on these intervention outcomes (Brody, Chen, & Beach, 2013a). However, no study to date has explored the role of *OXTR* genetics in responsivity to intervention programs. Exploring the role of *OXTR* in these associations may be particularly informative for extending the *OXTR*, GxE, and prevention literatures.

Summary

The literature surrounding oxytocin's role in the link between environmental exposures and behavioral and health outcomes is becoming increasingly recognized. While initial studies of oxytocin and *OXTR* suggested an overall positive influence, more recent work has suggested a more nuanced and "social sensitivity" role for oxytocin. Within the *OXTR* rs53576 literature, the G allele was initially associated with more positive, pro-social behaviors, such as higher levels of prosocial and trusting behaviors compared to those with the A allele (Kogan et al., 2011; Krueger., 2012). However, more recent reports incorporating environmental context suggest that

those carrying a G-allele may demonstrate increased risk of maladaptive behaviors in the presence of social adversity (Bradley et al., 2011; Sturge-Apple et al., 2012). Whether these findings will continue to hold, and rs53576 GG individuals will show increased sensitivity to both positive and adverse contexts, will be explored within this dissertation.

In addition to the genetic literature, the *OXTR* epigenetics literature continues to grow, with research supporting a role of *OXTR* epigenetics in *OXTR* regulation and human behavioral outcomes. While these initial studies are promising, no study to date has incorporated the role of adverse environments, exploring whether adverse environments may impact *OXTR* methylation levels, or may interact with these levels to predict future behavioral and health outcomes.

This dissertation seeks to expand upon these fields by exploring the role of oxytocin genetics and epigenetics in the link between social adversity and behavioral and health outcomes. A number of contexts and outcomes will be addressed throughout the 3 chapters, including 1.) Assessing the role of OXTR genetics in the link between exposure to interpersonal stress and behavioral outcomes, 2.) Assessing the role of *OXTR* genetics and epigenetics in the link between abuse during childhood and mental health outcomes and 3.) Assessing the role of *OXTR* genetics in responsiveness to a parent-child intervention program on a biomarker of health, telomere length. Each chapter will go into depth on the specific context and outcomes of interest, as well as the proposed role for *OXTR* genetics.

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CHAPTER 2
Social stress and the oxytocin receptor gene interact to predict antisocial behavior in an at-risk cohort

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Social stress and the oxytocin receptor gene interact to predict antisocial behavior in an at-risk cohort

ABSTRACT

Polymorphisms in the oxytocin receptor gene (*OXTR*) are commonly associated with prosocial behaviors in the extant literature, yet their role in antisocial behaviors has rarely been explored, particularly during the transition from adolescence to early adulthood. We examined a prospective cohort (N=404), collecting youth, mother and clinician reports of conduct disordered and antisocial behavior at age 15 and 20. The oxytocin receptor rs53576 polymorphism was hypothesized to interact with social stress to predict antisocial outcomes. Structural equation modeling (SEM) results revealed a significant main effect at age 15 (p=.025); those with the G allele exhibited higher levels of conduct problems. SEM revealed a significant gene-by-environment interaction at age 20 (p=.029); those with the G allele who experienced high social stress exhibited higher levels of antisocial behavior. Heterozygous (AG) grouping models were compared and parameter estimations supported G dominant groupings. These novel findings suggest that rs53576 polymorphisms may influence social salience and contribute to risk for antisocial outcomes, particularly under conditions of high social stress.

INTRODUCTION

Conduct problems and antisocial behaviors place significant social and financial burden on society (Moffitt, Caspi, Harrington, & Milne, 2002), and as a result, the identification of etiological factors has been the focus of intense empirical scrutiny for over a century. Current theories suggest that both biological and social risk factors contribute to antisocial behavior (Moffitt, 1993; Raine, 2002), and recent studies suggest that particular genotypes may interact with the social environment to predict aggression, conduct problems, and antisocial outcomes (Brennan et al., 2011; Caspi et al., 2002).

In the human literature, one potentially relevant gene that has not garnered much attention in the prediction of antisocial outcomes is the oxytocin receptor gene. This omission is surprising given the links between the oxytocin hormone and behavior relating to social interaction and affiliation highlighted in the empirical literature and in the popular press (Bartz, Zaki, Bolger, & Ochsner, 2011; Yong, 2012). Existing theory and human and animal studies support positive associations between oxytocin and prosocial behaviors such as maternal-infant bonding (Gordon et al., 2008; Lim & Young, 2006), empathy (Barraza & Zak, 2009), and trust (Gonzaga, Turner, Keltner, Campos, & Altemus, 2006), making it potentially relevant in the prediction of antisocial behaviors as well. Two reports to date have examined associations between *OXTR* polymorphisms and antisocial outcomes, and these focused only on children with persistent, severe aggressive behavior (Beitchman et al., 2012; Malik, Zai, Abu, Nowrouzi, & Beitchman, 2012).

While the popular press may dub oxytocin "the love hormone," recent studies suggest that the effects of oxytocin on human behavior are more complex, and are dependent on environmental context. For example, intranasal administration of oxytocin heightens feelings of envy during a gambling game (Shamay-Tsoory et al., 2009), and results in reduced trust and cooperation among individuals sensitive to rejection (Bartz, Simeon, et al., 2011). Oxytocin administration also increases ethnocentric behavior, resulting in greater preference toward

ingroup members and derogation toward outgroup members (De Dreu et al., 2010). In a recent review of the oxytocin literature, Bartz et al. (2011) proposed the social salience hypothesis, suggesting that rather than increasing trust or positive affiliation, oxytocin may increase an individual's sensitivity to, and thus potential reactivity towards, the social environment. Neuroimaging data further supports the role of oxytocin in heightening the salience of socially positive or negative but not socially neutral stimuli, and specific brain regions such as the ventral tegmental area have been implicated (Groppe et al., 2013).

Although originating from the oxytocin hormone literature, the social salience hypothesis appears to be consistent with findings in the genetic literature as well, and in particular, findings concerning the rs53576 polymorphism of the oxytocin receptor gene (*OXTR*). Similar to the oxytocin hormone literature, initial candidate gene studies of rs53576 reported more positive, prosocial outcomes among certain individuals with the G allele, compared to those with the A allele, such as higher levels of prosocial and trusting behaviors (Kogan et al., 2011; Krueger et al., 2012), increased empathy (Rodrigues et al., 2009), and greater sensitivity to an infant cry (Riem et al. 2011). Many of the initial genetic studies were conducted in healthy samples and a recent meta-analysis casts doubt on direct effects of *OXTR* genotypes on prosocial behavior (Bakermans-Kranenburg & .van IJzendoorn, 2013). While the biological function of the rs53576 polymorphism has yet to be delineated, results from gene by environment interaction studies of *OXTR* highlight the importance of the social context and suggest that individuals with the G allele may be better characterized as more attentive to social cues, rather than prosocial in nature. For example, Bradley et al. (2011) found that people with the G allele, compared to AA individuals, exhibited heightened emotion dysregulation following exposure to childhood abuse (Bradley et al., 2011). Individuals with the G allele were more physiologically responsive than AA individuals to social support in a stressful context (Chen et al., 2011), and more likely to seek out social support during stress, though only if it was culturally appropriate (Kim et al., 2010). These interaction effects suggest that G carriers may be more attentive to social cues, and thus may be

more vulnerable in the context of adverse social situations. Given that even in normal adult populations, hypersensitivity to social cues is linked to higher levels of negative affect and greater fluctuations in negative affect and self-esteem, increased social salience combined with a negative social environment could readily lead to antisocial behavior, particularly in an at-risk population.

Thus, the current study sought to examine whether the rs53576 polymorphism interacts with social stress to predict to conduct problems and antisocial behaviors in a high-risk sample of youth. Specifically, the sample was considered high-risk because a high proportion of youth were exposed to maternal depression earlier in life. To our knowledge, there are no published studies examining this specific gene-environment interaction with respect to antisocial outcomes in humans. The behavioral genetics literature suggests that genetic effects may differentially impact the manifestation of adolescent and adult antisocial behaviors (Rhee & Waldman, 2002). Therefore, our study sought to examine whether the *OXTR* rs53576 polymorphism interacted with current social stress to predict conduct and antisocial behaviors at two different developmental time points: age 15 and age 20. We hypothesized a gene by environment interaction such that individuals with rs53576 G allele would be more likely to evidence conduct and antisocial behaviors in the presence of current social stress. We further explored developmental differences by comparing findings for outcomes at age 15 versus age 20.

METHODS

Participants

Participants were 404 Caucasian youth (n= 237 females) drawn from a large prospective birth cohort of children (N=7223) born between 1981 and 1984 at the Mater Misericordiae Mother's Hospital in Brisbane, Australia (Najman et al., 2005). A sample of 815 mothers and children were recruited from the original birth cohort on the basis of varying histories of maternal depression measured longitudinally through age five (Hammen & Brennan, 2001). Approximately two-thirds of the children were chosen on the basis of medium to high levels of

maternal self-reported depression, and one-third was chosen on the basis of low levels of maternal self-reported depression. This "high-risk" sample of children was assessed at age 15 (N=815) and again at age 20 (N=747), and DNA was collected at ages 22-25 (N=512). Of the youth eligible for DNA collection based on their previous participation in either the age 15 or age 20 follow-ups, 63 could not be located and 173 either actively or passively refused participation in the DNA collection.

Inclusion criteria for the current study included participation in the age 15 follow up, the availability of genetic data for analysis, and Caucasian ethnicity due to differences in oxytocin receptor gene allele frequencies noted across ethnic groups (e.g., Kim et al., 2010). Participants included in the current study did not differ from the overall birth cohort in terms of mother education ($t(df=7164)=-.76$, $p=.45$), family income ($t(df=6747)=.09$, $p=.93$), or number of siblings ($t(df=6667)=-.87$, $p=.38$). However, there were significantly more females in the current study (59%) than in the original birth cohort (48%) ($c^2(N=7223)= 22.36$, $p<.001$). A similar pattern was noted when comparing participants in the current sample to those in the high-risk depression cohort established at age 15 (N=815). The two samples were similar in terms of maternal education ($t(df=808)=.199$, $p=.84$), family income ($t(df=764)=.215$, $p=.83$), and number of siblings ($t(df=810)=-.891$, $p=.84$) but differed with respect to gender ($c^2(N=815)=32.57$, $p<.001$), with more females in the current sample. Sample descriptions are provided in Table 1.

Procedures

Multi-method assessments of youth behavior and family functioning, based on clinical interviews and questionnaires, were conducted at ages 15 and 20. Family interviews were conducted in the participants' homes by teams of two graduate-level students who were blind to mother's psychiatric history. Mothers and their children were interviewed separately. Mothers provided written informed consent for themselves and their children at age 15, and the youth also provided verbal assent. For subsequent visits, all participants provided written informed consent. All procedures were approved by the University of California, Los Angeles (UCLA) Institutional

Review Board, Emory University Institutional Review Board, and the University of Queensland Ethics Review Committee.

Measures

Youth conduct problems at age 15. At age 15, youth completed the Achenbach Youth Self-Report (YSR), which contains 112 items that assess the frequency with which the youth engages in various maladaptive behaviors in the last six months. Mothers completed the Achenbach Child Behavior Checklist (Achenbach & Edelbrock, 1983), which consists of a similar set of 113 maladaptive behaviors items. Responses for items on both measures are coded as 0 (Never/Rarely), 1 (Sometimes), and 2 (Almost Always). The YSR and CBCL are widely used, empirically validated measures with high internal consistency (α=0.78-0.97) and adequate test-retest reliability (e.g., r=0.83 for 15-18 year-olds; Achenbach, 1991). DSM-oriented conduct disorder symptom scales were calculated from the CBCL and YSR as specified by Achenbach, Dumenci and Rescorla (2003). Sample items on the conduct disorder scale include whether the child is mean to others, gets in fights or arguments, threatens others, destroys or vandalizes property, breaks rules, lies, or swears. Youth (YSR) and maternal (CBCL) reports of conduct disorder demonstrated adequate internal reliability (α=0.80 and α= 0.87, respectively).

Clinical research interviewer ratings of Conduct Disorder were based on a semi-structured interview data using the Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Epidemiological Version (KSADS-E; Orvaschel, 1995). The KSADS-E was administered to youth during home visits and a clinical research team assigned consensus diagnoses of Conduct Disorder (present versus absent) based on reviews of each recorded interview. Seventy-five of the KSADS-E interviews were selected for reliability ratings by a second clinician blind to original diagnoses. All inter-rater reliability ratings were acceptable (κ>0.7).

Youth Antisocial Behavior at age 20. At age 20, youth completed the Adult Self Report (ASR; Achenbach & Rescorla, 2003) and mothers completed the Adult Behavior Checklist

(ABCL; Achenbach & Rescorla, 2003). Similar to the YSR completed at age 15, the 126-item ASR measures the participant's self-reported frequency of maladaptive behaviors using a scale of 0 (Not true), 1 (Somewhat or sometimes true), and 2 (Very true or often true). The ABCL is completed by an informant, in this case the mother, and measures similar constructs to the CBCL collected at age 15. Example items on the antisocial personality problems subscales of ASR and ABCL include whether the youth argues a lot, is mean to others, breaks rules, fights with others and threatens others. Item responses on the DSM oriented subscales for Antisocial Personality problems were summed for both the ASR and the ABCL. Adequate internal reliability in these scales was noted (youth-report ASR α=.83; maternal-report ABCL α=.90).

Clinician ratings of antisocial behavior were based on the administration of the Structured Clinical Interview for DSM-IV Axis II Disorders (SCID-II; First et al., 1997). Counts of antisocial personality disorder symptoms (e.g. "do you often find that you have to lie to get what you want?"), coded as "present" by the interviewer, were summed to reflect the clinician-rated variable of antisocial behavior at age 20. Consistent with the standard administration of the SCID-II (First et al., 1997), individuals who did not endorse the necessary amount of relevant pre-interview screener items, which assess past conduct problems, a criteria for ASPD, were not administered the SCID-II Antisocial Personality Disorder Questions and thus received a zero for symptom count.

Social stress at age 15 and 20. Youth were administered the UCLA Life Stress Interview at ages 15 and 20, which has been utilized in previous research with diverse populations (Hammen, 1991), including multiple studies with adolescents and young adults (Adrian & Hammen, 1993; Rao, Hammen, & Daley, 1999). The interviewer inquired about the youth's ongoing chronic stress over the last six months, and rated the severity using 5-point, behaviorally anchored scales in each of several domains: social life, close friendships, romantic relationships/dating interests, relationships with family members, academic performance, occupational experiences, personal health, and health of close family members. In the current

study, the social life domain score was used to capture current social problems in peer relationships (e.g. “are there people you can go out with,” “are you lonely,” “how often do you get invited to social activities,” etc.). Scores on this scale ranged from 1 to 5, with higher scores indicating greater stress. For chronic stress at age 15, interclass correlation coefficients ranged from 0.63 to 0.94, and for age 20, ranged from 0.72 to 0.88 (Hammen, Hazel, Brennan, & Najman, 2012).

Youth Early Adversity. Given the high-risk nature of the sample and noted associations between early childhood adversity and conduct problems (Chronis et al., 2007; Fergusson, Horwood, & Lynskey, 1994; Greenberg, Speltz, Deklyen, & Jones, 2001), early adversity was included to assess its potential independent contribution to antisocial outcomes. Youth early adversity was determined using information provided by the mother at pregnancy, birth, 6-month, and 5-year assessments. This variable has been used in our previous work (e.g., Hazel, Hammen, Brennan, & Najman, 2008), and reflects the following adversities: maternal Axis I diagnosis prior to age 5 (primarily maternal depression), financial hardship, parental discord, maternal stressful life events, serious childhood illness, and maternal separation from partners. The continuous variables were recoded as a binary variable above or below the 33rd percentile, and the remaining variables were coded for presence or absence. Variables were then summed to reflect the total number of adversities, with scores ranging from 0 (no adversity) to 6 (all of the aforementioned adversities were present).

Genotyping. Participants who agreed to participate in blood collection were mailed consent forms, questionnaires, and a blood collection pack. Blood was drawn at local pathology clinics, and the samples were then transported to the Genetic Epidemiological Laboratory of the Queensland Institute of Medical Research, where the DNA was stored and extracted. For the current study, aliquots of DNA were shipped to UCLA for genotyping by the UCLA Social Genomics Core of the USC/UCLA Biodemography Center. Individual status on the *OXTR* rs53576 polymorphism was assayed by a commercial TaqMan Genotyping Assay (Applied

Biosystems, Foster City, CA) performed on an iCycler real-time PCR instrument (BioRad, Hercules, CA), following the manufacturer's specified protocol. Test-retest reliability of duplicated samples yielded a total genotyping error rate less than 1 percent. Genotype distributions were GG=180(44.6%), AG=173(42.8%), and AA=51(12.6%), and were in Hardy-Weinberg equilibrium, $\chi^2(2, 404)=0.87$, p=ns.

Data analysis strategy

Structural equation modeling (SEM; Arbuckle, 2008) with AMOS 20.0 was used to test our primary hypothesis that the *OXTR* genotype and current levels of social stress would interact to predict conduct and antisocial behavior outcomes. Dependent measures were latent factors of youth, maternal, and clinician rated conduct and antisocial behavior outcomes at age 15 and 20. Independent variables (genotype and current social stress) were centered to reduce problems of multicollinearity, and centered variables were multiplied to create interaction terms. When hypothesized interaction terms were significant, a median split on current social stress was used to interpret the direction of the interaction effect.

As indicated above, extensive previous research (Chronis et al., 2007; Fergusson et al., 1994; Greenberg et al., 2001) justified the inclusion of early adversity as a potential confound. Given the well-known gender differences in rates of conduct and antisocial behaviors, gender was also tested as a potential covariate. Early adversity and gender were significantly associated with the outcomes (p<.05) and were therefore retained in all models.

Early adversity and mother and youth ratings of conduct problems and antisocial behaviors were square root transformed to provide normalized distributions. Table 2 presents the correlations between all study variables. Significant correlations between predictors were accounted for in the structural equation models. *OXTR* genotype was not correlated with any of the covariates nor social stress at age 15 and age 20 (p>.05).

Because the biological function of the rs53576 polymorphism has yet to be delineated and the literature is mixed in regards to heterozygote (AG) grouping, the implications for AG

grouping are not well understood. We initially tested all models using only homozygous (GG and AA) individuals. Follow up analyses compared G-dominant (GG/AG versus AA) and A-dominant (GG versus AG/AA) allelic groupings to the homozygous grouping models.

Model fit was assessed using the χ^2 index, the comparative fit index (CFI) and the root-mean-square of approximation (RMSEA) and its 90% confidence interval. The χ^2 index tests the discrepancies between the population covariance and the covariance predicted by the model, where a non-significant χ^2 indicates a good, non-discrepant fit (Hooper, Coughlan, & Mullen, 2008). The CFI compares the model of interest with the independence model while taking the sample size into account. CFI values range from 0-1 with those over 0.95 indicating an adequate fit (Hu & Bentler, 1999). RMSEA compares how well the model estimates fit the population covariance matrix (Browne, Cudeck, Bollen, & Long, 1993), where values less than 0.5 indicate a good fit (Hu & Bentler, 1999; Kline, 2010).

RESULTS

Age 15 Conduct Problems. Despite good model fit ($\chi^2(17, N=231)=11.97$, $p=.80$, CFI=1.0, RMSEA=.00 (90% CI=.00-.04)), our hypothesis that *OXTR* would interact with social stress to predict conduct problems at age 15 was not supported, as the interaction term was non-significant ($p=.79$). Correlated predictors for this model included early adversity and social stress, the interaction term and genotype, and the interaction term and social stress. Removing the interaction term, the model fit remained adequate ($\chi^2(13, N=231)=11.58$, $p=.56$, CFI=1.0, RMSEA=.00 (90% CI=.00-.06)), and the main effect of *OXTR* genotype on conduct problems was significant, with GG individuals exhibiting higher levels of conduct problems compared to AA individuals at age 15 ($\beta=.15$, $p=.05$). Correlated predictors for this model included early adversity and social stress.

Age 20 Antisocial Problems. As predicted, there was a significant interaction between *OXTR* rs53576 genotype and current social stress to predict antisocial behavior at age 20 ($\beta=.21$, $p=.014$, see Figure 1). The *OXTR* and social stress interaction model provided adequate fit

(χ^2(16, N=231)=23.0, p=.11, CFI=.92, RMSEA=.04 (90% CI=.04-.08)). Correlated predictors in this model included early adversity and social stress, gender and social stress, the interaction term and genotype, and the interaction term and social stress. A median split on current social stress revealed that GG individuals were more likely than AA individuals to exhibit antisocial behaviors in the presence of high social stress (β=.32, p=.020) but not low social stress (β=.10; p=.10; see Figure 1). Both low and high social stress models provided adequate fit (Low Social Stress: χ^2(9, N=111)=2.63, p=.98, CFI=1.0, RMSEA=.00 (90% CI=.00-.00); High Social Stress: χ^2(9, N=111)=11.28, p=.26, CFI=.94, RMSEA=.05 (90% CI=.00-.12)). No correlations between predictors were included as they were non-significant.

Allelic Grouping. Heterozygous groupings (G-dominant and A-dominant) were compared to the homozygous models described above, including the main effect of *OXTR* on conduct problems at age 15 and the interaction of *OXTR* and social stress at age 20 (see Table 3). Correlated predictors in the original models above were retained. All Age-15 models provided good fit (G-Dominant Grouping: χ^2(13, N=404)=7.75, p=.86, CFI=1.0, RMSEA=.00 (90% CI=.00-.03)); A-Dominant Grouping: χ^2(13, N=404) = 12.55, p=.48, CFI=1.0, RMSEA=.00 (90% CI=.00-.05)). The association between *OXTR* and conduct problems remained significant in the G-dominant grouping (β=.13; p=.03) but lost significance when using A-dominant grouping (β=.01; p=.93). All Age-20 interaction models provided adequate fit (G-Dominant Grouping: χ^2(16, N=404)=16.98, p=.39, CFI=.99, RMSEA=.01 (90% CI=.00-.05); A-Dominant Grouping: χ^2(16, N=404)=28.12, p=.031, CFI=.93, RMSEA=.04 (90% CI=.01-.07)). Again, the G-dominant grouping was more consistent with the homozygous model, such that the interaction remained significant for the G-dominant grouping (β=.13; p=.03), but lost significance for the A-dominant grouping (β=.07; p=.23). A median split on current social stress, similar to that described above, further supported a G-dominant grouping, indicating that social stress was positively associated with antisocial behavior only among GG/AG individuals (see Figure 2).

DISCUSSION

Our study assessed whether the *OXTR* rs53576 polymorphism interacts with current social stress to predict conduct problems and antisocial behaviors at two developmental time points. Previous research suggests a mechanism of social sensitivity for rs53576 where those with the G allele, compared to the A allele, experience heightened social salience (Bartz, Zaki, et al., 2011; Kumsta & Heinrichs, 2013). The majority of published research examines main effects of *OXTR* polymorphisms on social behavior in healthy, non-clinical samples. More recent reports (Bradley et al., 2011; Sturge-Apple, Cicchetti, Davies, & Suor, 2012) have also suggested that those carrying a G-allele may demonstrate increased risk of maladaptive behaviors in the presence of social adversity. Results from the current study lend additional support to this hypothesis. At age 20, the *OXTR* polymorphism interacted with current levels of social stress such that G carriers exhibited higher levels of antisocial behaviors only in the presence of high social stress, while AA individuals did not significantly differ on antisocial behavior ratings across social stress levels.

While there is limited research on the influence of *OXTR* on antisocial outcomes, previous literature demonstrates the important role of the interaction between a negative social environment and a specific genotype in the development of antisocial behavior (Caspi et al., 2002). Given that the current study is the first to report associations between *OXTR* rs53576 and antisocial/conduct-disordered behavior in adolescents, including a gene-by-environment interaction, these findings require replication before any clinical implications can be drawn. Though preliminary, our findings suggest that certain individuals carrying the G-allele may be particularly sensitive to social stress and that the role of social support and peer-based interventions could ameliorate the risk of persistent antisocial behavior in these young adults.

Our age 20 findings are consistent with Dodge's social cognitive model of aggression. Dodge and colleagues (e.g., see Dodge & Pettit, 2003 for review) and others have published a body of literature that supports associations between social cognitive biases, such as a propensity to perceive threat and hostility, and increased likelihood of conduct disorder. They also suggest

that individuals engaging in delinquent behavior often utilize a more limited behavioral repertoire (Dodge & Crick, 1990), so that when faced with social stress, they may be more likely to engage in antisocial behavior, even when their peers may have developed more adaptive behavioral strategies as they transition into adulthood. Heightened social salience (as evidenced by G allele carriers) may therefore lead to increased sensitivity to perceived negative cues from the social environment, contributing to negative, acting out behavior in young adults facing high social stress.

A significant proportion of our sample was already at risk for negative behavioral outcomes, given the maternal history of depression; and while early adversity also predicted antisocial behavior at age 20, the gene-by-environment interaction with current levels of social stress predicted negative outcomes above and beyond the influence of early adversity. This finding may suggest that while extensive empirical support for early intervention exists, intervention in young adults aimed at decreasing social stress and/or reducing an individuals' sensitivity to negative social cues could also be effective in reducing antisocial outcomes. Future studies should assess the unique and combined influences of early and current social adversity on antisocial outcomes and whether they interact with *OXTR*, in order to identify individuals at highest risk for negative outcomes in adulthood.

At age 15, only a genetic main effect was demonstrated, such that individuals with the G-allele showed significantly higher levels of conduct problems, regardless of their current levels of social stress. This finding was unexpected and contradicts a recent study looking at several oxytocin-related genes in children (Malik et al., 2012). This recent study looked only at childhood-onset, severe, persistent aggression and failed to find significant effects for the rs53576 polymorphism. Developmental context may be useful in explaining our results. As per Moffitt (1993), empirical data suggest that antisocial behavior is more normative in mid-adolescence, in large part because it is highly socially rewarding. It might be the case then, that 15 year olds who are more sensitive to social cues (i.e., the G-allele carriers) might also be more sensitive to social

rewards for delinquency, and more likely to display conduct problems overall. Another possible explanation for our age 15 findings is the high-risk nature of our cohort, which may have restricted our ability to examine the moderating effects of current social stress. Furthermore, the mean score for social stress was slightly lower at age 15 than at age 20, potentially suggesting that at age 15, lower stress levels mitigated an interaction effect. Further research is necessary to fully understand implications for developmental theory, prevention, or treatment.

The rs53576 polymorphism has not been well studied in adolescence with the majority of previous studies reporting an average participant age of college age or older (Krueger et al., 2012; Rodrigues, Saslow, Garcia, John, & Keltner, 2009). Our study identified differential genetic influences at age 15 and age 20, highlighting the importance of studying such gene by environment interactions in a developmental context using longitudinal data. The different findings between these age groups support the suggestion by Rhee and Waldman (2002) that genes may differentially influence antisocial behaviors in adolescence versus adulthood. The limited published data on *OXTR* polymorphisms during the adolescence strongly suggests a need for replication in other prospective samples.

The biological function of allelic variance in rs53576 is not known, resulting in a lack of a clearly defined grouping method for heterozygous individuals. A majority of previous studies have grouped AG individuals with AA individuals, primarily due to sample size limitations. In the current study, we used an empirical approach to test the influence of heterozygote grouping, first conducting all analyses using only homozygous individuals and then re-analyzing the data to compare G-dominant (GG/AG versus AA) versus A-dominant (GG versus AG/AA) groupings. Our results indicated that the G-dominant grouping (GG/AG versus AA) was more consistent with the homozygous models. This finding highlights the importance of addressing grouping in future studies, which may help delineate the biological function of *OXTR* polymorphisms.

Despite the preliminary nature of these data, the current study makes a novel contribution to the etiology of antisocial behavior across an important developmental period, highlighting

OXTR's role in conduct and antisocial behaviors. These findings should be interpreted, however, in the context of the following limitations: 1.) The sample is relatively small in comparison to other GxE designs, which highlights the concern for spurious findings and need for replication (Duncan & Keller, 2011). These findings should be considered preliminary pending replication. The sample is on the larger end of studies assessing behavioral associations with *OXTR*, which often range from 100 to 400 subjects (Krueger et al., 2012; Rodrigues et al., 2009; Saphire-Bernstein, Way, Kim, Sherman, & Taylor, 2011); and the subsample of AA individuals is sufficiently large to assess research questions surrounding allelic grouping, a limitation expressed in a number of previous studies. Furthermore, as with all gene-behavior association studies, our candidate gene accounts for only a small percent of the variance of the outcome. Antisocial behavior has been reported to have a heritability of 0.5 (Rhee & Waldman, 2002). Therefore, our results similarly reflect the concept of missing heritability, whereby individual genes account for a small proportion of the expected overall inherited variance (Manolio et al., 2009). Other influences that may be important to consider in future studies are epistatic and epigenetic phenomena (Slatkin et al., 2009). 2.) Different clinical questionnaires were used to evaluate conduct problems at 15 (e.g. YSR) and antisocial behaviors at 20 (e.g. ASR). These differences were due to measurement variation in the child and adolescent versus adult versions of the questionnaires and interviews. However, the questionnaires tap into similar behavioral constructs and there is significant item overlap. 3.) It is possible that the clinician completed SCID-II measure of antisocial behavior symptom count may underrepresent age 20 antisocial behaviors for youth who did not meet the necessary amount of relevant screener questions. Therefore it is possible that those individuals who were given a score of 0 may have endorsed some of antisocial questions had they been administered this assessment regardless of screener criteria. However, standard protocol for SCID-II administration and antisocial personality disorder assessment were followed (First et al., 1997) making these findings more translatable to what may be found in clinical practice. Furthermore, given the use of SEM for data analysis, the potential gap arising

from the standard administration of this measure should be captured through the incorporation of the youth and maternal Achenbach measures included in the antisocial latent construct 4.) The time point at which the genetic data was collected (age 25) may have contributed to increased attrition. While minimal differences between the larger at-risk cohort and those who contributed genetic data do not have direct implications for our findings (i.e., greater number of females), collecting genetic data when the adolescents were still living at home may have increased follow-up rates and reduced potential sampling bias. 5.) Social stress was collected concurrently with behavioral outcomes at both time points, which tempers our ability to make causal inferences in our hypothesized associations. However, our data collection strategy is consistent with our hypothesis that current social stress would be influential in changing behavior in genetically at-risk youth.

The following methodological and statistical considerations strengthen the preliminary findings reported in the current study: 1.) Using a multi-method approach including self-report, parent-report, and clinician report data, enhanced the validity of the behavioral constructs while reducing sampling bias. 2.) The use of SEM strengthened our analytical approach by reducing the inherent error that exists when using a single scale to approximate a construct and thus allowed for a more robust test of our hypotheses. 3.) The longitudinal, prospective nature of the data facilitated our ability to meaningfully interpret differences at age 15 versus 20 and allowed us to incorporate important covariates, such as early adversity measured prospectively, to help tease apart environmental influences on antisocial behavior. 4.) The use of a data driven method for allelic grouping makes an innovative contribution to the *OXTR* literature.

Although significant research efforts have sought to understand the transition from childhood to adolescence, the period between adolescence and adulthood has been surprisingly understudied. The present findings shed light on the role of *OXTR* in the etiology of conduct disorder and antisocial behavior and highlight the importance of studying gene by environment interactions in a developmental context.

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Table 2.1. Descriptive Statistics By *OXTR* rs53576 Genotype

Genotype	**GG** N, % or Mean (SD)	**AG** N, % or Mean (SD)	**AA** N, % or Mean (SD)	**Total** N, % or Mean (SD)
Number of Participants	180	173	51	404
Number of Males, N, %	71, 39.4%	75, 43.4%	21, 42.2%	167, 41.3%
Low Income at Baseline	55, 32.7%	67, 39.9%	17, 40.5%	139, 34.4%
Parental Education, N, %	96, 53.6%	100, 58.1%	33, 64.7%	229, 56.7%
Early Life Adversity	1.5 (1.4)	1.9 (1.5)	2.0 (1.8)	1.7 (1.5)
Age 15 Social Stress	2.3 (0.5)	2.4 (0.5)	2.2 (0.4)	2.3 (0.5)
Age 20 Social Stress	2.5 (0.8)	2.5 (0.9)	2.4 (0.9)	2.5 (0.9)

Note: Amount of missing data varied and ranged from 0 to 42 missing data points for Early Adversity (12 GG, 23 AG, and 7 AA). Low Income represents the percentage of participants with family incomes under $10,400 Aus per year, Parental Education represents the number and percentage with the equivalent of a high school degree or less, Early Life Adversity is a sum of number of adversities experienced in childhood ranging from 0 to 6, and Social Stress is derived from the chronic stress interview and ranges from 1 (no stress) to 5 (exceptional social stress).

Table 2.2. Correlations Between Variables

Variables	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
1.Gender	-.02	.09	-.15**	-.02	.13**	.13*	.10	.11*	.11*	.13**
2.Early Adversity	1	.15**	.19**	-.09	.12*	.22**	.09	.12**	.24**	.20**
3.Age 15 Stress		1	.33**	-.03	.09	.15**	.09	.08	.15**	.06
4.Age 20 Stress			1	.02	.13*	.18**	.06	.16**	.15**	.11*
5.Genotype				1	.02	.02	-.01	.03	.10	.05
6.Youth Report 15					1	.44**	.26**	.39**	.35**	.17**
7.Mother Report 15						1	.33**	.34**	.50**	.25**
8.Clinician Report 15							1	.19**	.18**	.11*
9.Youth Report 20								1	.46**	.25**
10.Mother Report 20									1	.23**
11.Clinician Report 20										1

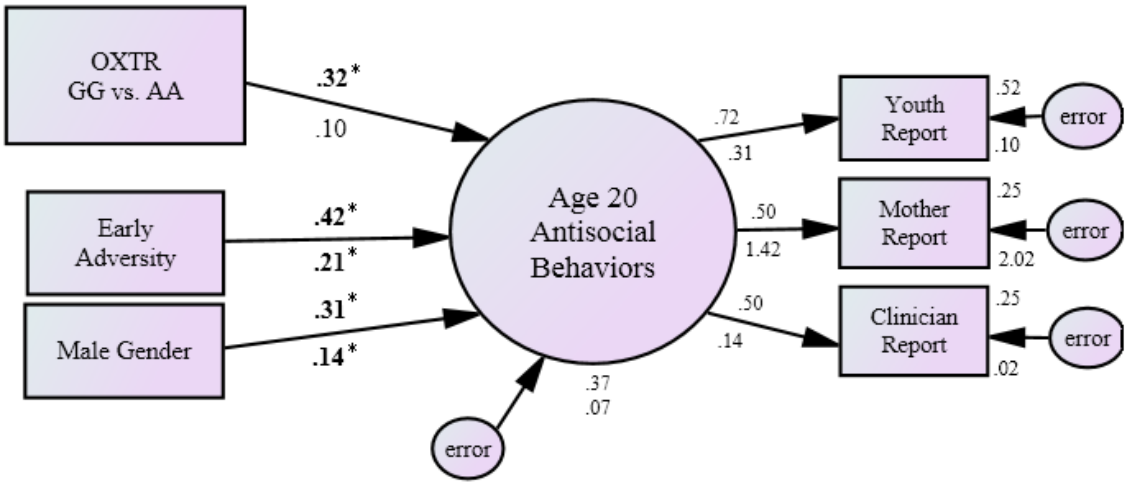
Note: * $p < .05$, ** $p < .01$. Gender is coded as 1 = male and 0 = female. Early adversity and mother and youth ratings of conduct problems and antisocial behaviors are square root transformed.

Table 2.3. Exploring Allelic Grouping. *OXTR* Beta Weight & P-Value in the Homozygous, G Dominant, and A Dominant Models

Conduct Problems at Age 15: Direct Effects Model		
Allelic Grouping	***OXTR B***	**p-value**
Homozygous Model (GG vs. AA)	B = .15	p = .05*
G Dominant Model (GG/AG vs. AA)	B = .13	p = .03*
A Dominant Model (GG vs. AA/AG)	B = .01	p = .93
Antisocial Behaviors at Age 20: Interaction Model		
Allelic Grouping	***OXTR* x Social Stress *B***	**p-value**
Homozygous Model (GG vs. AA)	B = .23	p = .02*
G Dominant Model (GG/AG vs. AA)	B = .16	p = .03*
A Dominant Model (GG vs. AA/AG)	B = .07	p = .23

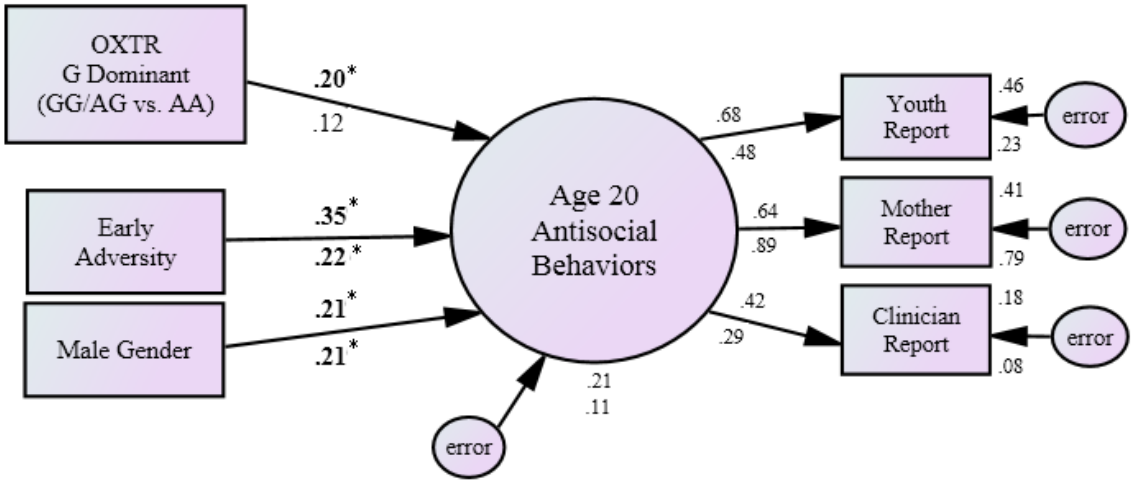
Note: * = $p < .05$. Standardized beta weights are presented.

Figure 2.1. The Influence of the *OXTR* rs53576 Polymorphism on Antisocial Behaviors at age 20 in the Presence of High versus Low Social Stress: Homozygous Grouping (GG vs AA)



Note: The sample was split into high and low social stress groups using the median as a cut point. Standardized betas are presented; those above the arrow are under conditions of high social stress, those below are under conditions of low social stress. In the path model, bolded and starred (*) beta weights are significant at $p<.05$. Given no significant intercorrelations identified between the predictors, none were included in the model. *OXTR* genotypes were coded as GG carrying = 1 and AA = 0 and were centered on the mean. Early adversity is a square root transformed combination score of adverse early life events where higher values represent greater adversity.

Figure 2.2. The Influence of the *OXTR* rs53576 Polymorphism on Antisocial Behaviors at age 20 in the Presence of High versus Low Social Stress: Heterozygous Grouping (GG/AG vs AA)



Note: The sample was split into high and low social stress groups using the median as a cut point. Standardized betas are presented; those above the arrow are under conditions of high social stress, those below are under conditions of low social stress. In the path model, bolded and starred (*) beta weights are significant at $p<.05$. Given no significant intercorrelations identified between the predictors, none were included in the model. *OXTR* genotypes were coded as GG or G carrying = 1 and AA = 0 and were centered on the mean. Early adversity is a square root transformed combination score of adverse early life events where higher values represent greater adversity.

CHAPTER 3
Oxytocin receptor genetic and epigenetic variation: association with child abuse and adult psychiatric symptoms

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Oxytocin receptor genetic and epigenetic variation: association with child abuse and adult psychiatric symptoms

ABSTRACT:

Childhood abuse can alter biological systems and increase risk for adult psychopathology. Epigenetic mechanisms, alterations in DNA structure that regulate the gene expression, are a potential mechanism underlying this risk. While abuse associates with methylation of certain genes, particularly those in the stress response system, no study to date has evaluated abuse and methylation of the oxytocin receptor (*OXTR*). However, studies support a role for *OXTR* in the link between abuse and adverse adult outcomes, showing that abuse can confer greater risk for psychiatric symptoms in those with specific *OXTR* genotypes. Our study therefore sought to (1) assess the role of epigenetics in the link between abuse and psychopathology and (2) to begin to integrate the genetic and epigenetic literature by exploring associations between *OXTR* genotypes and DNA CpG methylation. Data on 18 *OXTR* CpG sites, 44 SNPs, childhood abuse, and adult depression and anxiety symptoms were assessed in 393 African American adults (age = 41±12.8). Overall, 68% of genotypes associated with methylation of nearby CpG sites, with a subset surviving multiple test correction. Child abuse associated with higher methylation of two CpG sites yet did not survive correction or serve as a mediator of psychopathology. However, abuse interacted with CpG methylation to predict psychopathology. These findings suggest a role for *OXTR* in understanding the influence of early environments on adult psychiatric symptoms.

INTRODUCTION

Exposure to abuse during childhood is a strong predictor of increased risk for adverse adult outcomes such as psychopathology (Mancini, Van Ameringen, & MacMillan, 1995; Bradley, Binder, & Epstein, 2008; Raposa, Hammen, Brennan, O'Callaghan, & Najman, 2014). Mechanisms by which early adversity results in these outcomes has been a topic of intense research and has been explored from different biological perspectives (Miller, Chen, & Parker, 2011; Shonkoff, 2010). However, not all individuals exposed to adverse contexts will develop poor outcomes, suggesting that certain factors, such as genetic predispositions, may place individuals at varying risk (Belsky et al., 2009). Recent studies have suggested that the oxytocin system may be important to consider in these processes given the social context of abuse and the early environment.

Oxytocin is a neurohormone that is involved in social behaviors and the development of bonds (Bartz, Zaki, Bolger, & Ochsner, 2011; Insel & Young, 2001). Recent studies suggest that oxytocin functions to increase the salience of social cues and thus influences an individual's sensitivity to the social environment (Bartz et al., 2011). Given this, oxytocin can result in both enhanced positive or negative outcomes depending on the level of nurturance or adversity in the environment (Bartz et al., 2011). Recent gene-environment studies assessing *OXTR* SNPs (single nucleotide polymorphisms) have provided support for this concept, whereby genotypes that associate with positive behaviors such as empathy (Wu, Li, & Su, 2012) and trust (Krueger et al., 2012) also confer greater risk for emotion dysregulation (Bradley et al., 2011) and depressive symptoms (McQuaid, McInnis, Stead, Matheson, & Anisman, 2013; Myers et al., 2014) following childhood abuse. The role of *OXTR* genetics in the association between early adversity and later psychopathology is supported by additional studies reporting that *OXTR* SNPs can moderate the association between early life adversity and adult psychopathology such as depression and anxiety (Myers et al., 2014; Thompson, Parker, Hallmayer, Waugh, & Gotlib, 2011).

In addition to this moderating role, early life experiences have also been found to influence the development of the oxytocin system. For example, women exposed to early life abuse have lower levels of oxytocin in their cerebrospinal fluid (Heim, Young, Newport, & Mletzko, 2008), and rodents exposed to low levels of nurturing during development have lower oxytocin receptors levels in certain areas of the brain (Francis, Champagne, & Meaney, 2000; Nair & Young, 2006). One mechanism by which these early environments can "get under the skin" and influence biological systems is epigenetics, the term applied to structural modifications that can regulate gene expression without changing the DNA sequence. One of the most commonly studied epigenetic modifications is the addition of a methyl group to DNA (Bird, 2002). DNA methylation primarily occurs at a CpG site, in which cytosine and guanine basepairs occur consecutively and are linked by a phosphate bond. When this addition occurs in the promoter region of a gene, it can block the binding of factors that regulate gene expression, and thus reduce overall expression of the gene (Klose & Bird, 2006).

Much of the excitement surrounding epigenetics stems from the ability of the environment to alter methylation patterns of certain genes, such as those involved in the stress response system (McGowan et al., 2009; Szyf & Bick, 2012; Szyf, Weaver, Champagne, Diorio, & Meaney, 2005). This ability points to epigenetics as a possible mechanism by which adverse early environments can result in lasting biological changes that may increase an individual's risk for poor adult outcomes (Meaney, 2010; Szyf & Bick, 2012; van Ijzendoorn, Bakermans-Kranenburg, & Ebstein, 2011). Initial human studies exploring *OXTR* methylation have identified associations between *OXTR* methylation and certain behaviors, such as autistic behaviors (Gregory et al., 2009) and unemotional and callous behaviors (Dadds et al., 2013). However, no study to date has evaluated *OXTR* methylation for association with the early environment or adult psychopathology. When considering the associations between the early environment and *OXTR* methylation, it is important to note that the environment may influence DNA methylation to different extents across different genes. Given the lack of both human and animal studies to guide

this question, our study sought to evaluate whether *OXTR* methylation may mediate or moderate the association between abuse and adult psychopathology.

To date, genetic and epigenetic risk factors have primarily been studied separately, though there are correlations between them. Initial evidence suggests that some SNPs associate with the degree of methylation of nearby CpG sites (Bell et al., 2012; Smith et al., 2014). It is therefore plausible that these associations may provide a mechanism by which certain SNPs associate with behavioral and health outcomes. Indeed DNA methylation may be the mechanism underlying some gene-environment interactions. Integrating both SNPs and DNA methylation into conceptual models of abuse and adult psychopathology will provide a more comprehensive understanding of biological processes underlying the risk for adverse outcomes following childhood abuse.

METHODS

Participants

We evaluated African American subjects recruited as part of a larger study investigating the influence of genetic and environmental factors on the development of post-traumatic stress disorder in a predominantly African American, urban population of low socioeconomic status (Gillespie et al., 2009). Research participants were approached in the waiting rooms of the primary care clinic or obstetrical-gynecological clinic of a large, urban, public hospital in Atlanta, GA while either waiting for their medical appointments or while waiting with others who were scheduled for medical appointments. Subjects willing to participate provided written informed consent and participated in a verbal interview and blood draw for genetic and methylation data collection. As we have previously reported (Gillespie et al., 2009), this cohort is characterized by high rates of interpersonal violence and psychosocial stress. The majority of subjects have been exposed to one major trauma during their lifetime, and the number of traumatic experiences in childhood and adulthood predict psychiatric symptom severity in adulthood (Binder, Bradley, Liu, & Epstein, 2008; Bradley et al., 2008). All subjects who had *OXTR* methylation data were

included in the current study (N = 393). Subjects were an average age of 41 ± 12.8 years (range 18 – 77), the majority were female (70.7%), and all subjects were African-American and lived in urban neighborhoods of low socioeconomic status in Atlanta, GA. Study procedures were approved by the Institutional Review Board of Emory University School of Medicine and the Grady Health Systems Research Oversight Committee.

Measures

Demographic information including subject age, sex, and race was provided on a self-administered form. The Childhood Trauma Questionnaire (CTQ), a retrospective self-report inventory, was used to assess physical, sexual, and emotional abuse during childhood based on the established scores for mild, moderate, and severe abuse for each type (Bernstein & Fink, 1998; Bernstein, Stein, Newcomb, & Walker, 2003; Fink, Bernstein, Handelsman, Foote, & Lovejoy, 1995). In this study, the CTQ was used to create a composite score to classify each participant based on a history of any type of abuse (physical, sexual, or emotional), resulting in 3 categories: none or mild (N = 200), moderate (N = 100), or severe (N = 89). Lifetime exposure to traumatic events was assessed using the Traumatic Events Inventory (Bradley et al., 2008; Schwartz, Bradley, Sexton, Sherry, & Ressler, 2005), which measures lifetime (childhood and adulthood) exposure to trauma such as natural disaster, serious accident or injury, and physical or sexual assault. Depressive symptoms were indexed using the 21-item Beck Depression Inventory (BDI), a widely used continuous measure of depressive symptoms (Beck, Steer, & Brown, 2005). Similarly, anxiety symptoms were assessed as a continuous score from the Hamilton Anxiety Scale (HAM-A) (Maier, Buller, Philipp, & Heuser, 1988). The correlation between depression and anxiety symptoms in this cohort is 0.51 ($p < .001$).

DNA Extraction & Assays

Whole blood was collected in EDTA tubes for DNA extraction. The DNA was quantified using the PicoGreen (Invitrogen) and the quality was checked on an agarose gel. Genotyping was performed using the Omni-Quad 1M or the Omni Express BeadChip (Illumina). The majority of

SNPs assessed were consistent on both arrays. However, the sample size is reduced for some SNPs due to their inclusion on only one array ($95 < N < 312$). PLINK was used to perform quality control analyses such that SNPs that had a call rate < 95%, a minor allele frequency (MAF) < .05, or significant deviation from Hardy-Weinberg proportions ($p < .00001$) were excluded, as were samples with > 5% missing data. From this data, we evaluated 44 SNPs within and near the *OXTR* gene (Supplementary Table 1).

One microgram of DNA was bisulfite-treated for cytosine to thymine conversion using the EZ DNA Methylation-Gold kit (Zymo Research). The DNA was then whole-genome amplified, fragmented, and hybridized to the HumanMethylation450 BeadChip (Illumina). The BeadChips were scanned using a BeadStation 500GX, and the methylation level (beta value) was calculated for each queried CpG locus using the Methylation Module of BeadStudio software. Beta values were set to missing (no call) if detection p-values exceeded .001. CpGassoc (Barfield, Kilaru, Smith, & Conneely, 2012) was used to remove samples with probe detection call rates <95% and those with an average intensity value of either <50% of the experiment-wide sample mean or <2,000 arbitrary units (AU). In addition, CpG sites with missing data for >10% of samples were excluded from analysis. Finally, probes with known SNPs or that cross hybridize between autosomes and sex chromosomes were also removed (Chen et al., 2013). No samples warranted removal. From this data, we evaluated 18 CpG sites within *OXTR*. The probe sequence of one CpG site, cg00078085, contains a common SNP, rs73132856. This SNP was not evaluated in our study, and the CpG site does not associate with abuse or adult psychiatric symptoms.

Statistical Analysis

Data from leukocyte subtypes (GEO GSE35069) was used to identify CpG sites that are tissue-specific for certain cell types, and the method described by Houseman and colleagues was used to estimate the proportion of granulocytes and lymphocytes in our whole blood DNA samples (Houseman, Accomando, & Koestler, 2012.; Koestler et al., 2013; Sun et al., 2013).

Age, sex, cellular heterogeneity (percent of granulocytes and lymphocytes in the blood sample), and positional effects (sample chip and row) associate with methylation of one or more *OXTR* CpG sites. Therefore, these variables were included as covariates in all analyses.

One-way ANOVAs were used to test for mean differences in covariates and psychiatric symptoms across abuse categories. The association between each SNP within or near *OXTR* (chr3:8,788,096-8,841,641; hg19) and the proportion of methylation at each CpG site was examined using regressions, where methylation was modeled as a linear function of the number of reference alleles (0, 1, or 2), adjusting for the covariates of age, sex, cellular heterogeneity, and positional effects, as described above.

To evaluate the association between child abuse and methylation of *OXTR*, methylation of each CpG site was modeled as a linear function of a reported history of abuse during childhood (none to mild, moderate or severe), adjusting for the same covariates. To begin to incorporate genetic-epigenetic associations into these analyses, the models were retested while including SNPs that associate with the CpG site after multiple test correction as an additional covariate. To explore whether CpG methylation may mediate the association between abuse and psychiatric symptoms, CpG sites that associated with abuse were subsequently tested for association with depression and anxiety symptoms, adjusting for covariates.

To test whether DNA methylation may moderate the relation between abuse and psychiatric symptoms, an interaction term for abuse and methylation of each CpG site was included in models predicting psychiatric symptoms. For visual representation, we explored the direction of these interactions by using simple slope procedures and graphing the association between abuse and psychiatric symptoms for one standard deviation above and below the mean CpG methylation level for the associated CpG site. To integrate the genetic-epigenetic associations into these models, the interaction analyses were repeated while controlling for one of each of the SNPs that associate with the CpG site of interest after multiple test correction. Because interaction analyses can yield spurious results when the variance of a quantitative

outcome differs by level of the environmental variable (Voorman et al., 2011, Almli et al., 2014), top interaction results were tested for statistical robustness by computing robust standard errors using the "sandwich" package in R (Zeileis, 2004). Finally, since studies traditionally test interactions between SNPs and the environment on behavioral outcomes, we tested abuse × SNP interactions on psychiatric symptoms, adjusting for sex, and repeated these analyses controlling for associated CpG sites.

We present results for all nominal associations ($p < .05$) as a resource for investigators interested in specific SNPs or CpG sites along with the Bonferroni threshold that considers the number of independent tests performed. To determine the number of tests performed, we examined pairwise correlation between each CpG site and its nearest neighbors and identified significant correlations across all but one of the adjacent CpGs. Based on the direction and significance of the correlation across the gene (Supplementary Table 3), we estimate 5 methylation blocks in *OXTR*. We performed a similar analysis for the SNPs included in this study. PLINK was used to assess linkage disequilibrium (LD), suggesting 11 LD blocks in this cohort and 27 independent genetic tests. These estimates were used to calculate the appropriate number of independent tests considered for our Bonferroni correction for i) associations between SNPs and methylation (135 tests, $\alpha = 3.7\times10^{-4}$) ii) associations between methylation and a trait (5 tests, $\alpha = 0.01$) and iii) associations between SNPs and a trait (27 tests, $\alpha = .0019$).

RESULTS

Study Cohort

Approximately half of the subjects in this cohort were exposed to physical, emotional, or sexual abuse at moderate (25.7%; $n = 100$) or severe (22.9%; $n = 89$) levels (Table 1). Across these categories, there were no differences in age or blood sample cellular proportions; however females reported a history of moderate or severe abuse during childhood at a higher rate compared to males ($p < .05$). Therefore, sex was included as a covariate in all models. Within the categories of moderate or severe abuse, 24.9% of the sample experienced physical abuse ($n = 97$),

23.5% experienced emotional abuse (n = 91), and 45% experienced sexual abuse (n = 130). Those who reported moderate or severe abuse also experienced greater levels of trauma over the lifespan and had higher levels of adult depression and anxiety symptoms (p <. 001; Table 1). For the *OXTR* methylation data, average methylation levels varied across CpG sites, with sites located immediately upstream of the gene or within the 3rd intron of the gene tending to show higher overall levels of methylation compared to other CpG sites across *OXTR* (Supplementary Table 2).

Genetic-epigenetic associations in *OXTR*

We evaluated the association between genotypes for each SNP and the methylation level of 18 CpG sites within the *OXTR* gene (Figure 1; Table 2) for all pairwise combinations. Overall, the majority of SNPs associated with at least one CpG site (N = 30 of 44; 68.2%; p < .05), with an average distance of 10.5 kilobases (kb) between associated SNP-CpG pairs. Conversely, the majority of CpG sites associated with genotypes of one or more SNPs (N = 14 of 18; 77.8%; p < .05). Four of these pairs survive correction for multiple testing (Table 2). For example, individuals with a GG genotype (versus AG or AA genotypes) at rs2301261 had higher DNA methylation levels at cg00247334 and cg25140571, two CpG sites in the *OXTR* promoter.

***OXTR* methylation as a mediator of abuse and psychiatric symptoms**

A history of abuse during childhood predicted higher methylation of 2 CpG sites (cg04523291 and cg02192228) located in exon 3 of *OXTR* (.011 < p < .017). While the proximity of these sites is of note and their methylation is highly correlated with each other (r = 0.882, p < .01), neither association survived correction for multiple testing. Furthermore, in testing a mediation model, neither CpG site predicted depression and anxiety symptoms (p > .05), suggesting that *OXTR* methylation does not mediate the association between abuse and psychopathology. Neither of these CpG sites was associated with nearby SNPs when accounting for multiple test correction (Table 2). Therefore, no subsequent models were performed to test the association between abuse and methylation while accounting for CpG-associated SNPs. However,

including any of the nominally associated SNPs as covariates did not substantially change the results (data not show).

OXTR methylation as a moderator of abuse and psychiatric symptoms

Depression and anxiety symptoms were more common among those with a history of abuse (Table 1) but did not directly associate with *OXTR* methylation in our sample. However, abuse did interact with methylation of multiple *OXTR* CpG sites to predict psychiatric symptoms (Table 3). Five CpG sites interacted with abuse to predict depression and three of these also interacted to predict anxiety. Furthermore, these 3 overlapping sites survived multiple test correction for both outcomes ($p < 0.01$). The direction of the interaction was dependent upon CpG location. For example, individuals with lower methylation of cg08535600 in exon 1, located in the promoter region of the gene, reported higher depressive ($p = 5.4x10^{-4}$) and anxiety ($p = 7.5x10^{-5}$) symptoms compared to those with higher methylation at this site, if they report a history of abuse (Figure 2 A-B). Alternatively, those with higher methylation of cg11589699, located in the 3rd intron of the gene, report more depressive ($p = 5.5x10^{-4}$) and anxiety ($p = 7.6x10^{-4}$) symptoms, compared to those with lower methylation, if they also report a history of abuse (Figure 2 C-D). Methylation levels of CpG sites located in intron 3 correlate positively with each other and negatively with the sites located in exon 1 (Supplementary Table 3).

Of the five CpG sites that interact with abuse to predict depression, one site, cg00385883, associates with two SNPs after multiple test correction. When controlling for each of the two associated SNPs in abuse × methylation models, the interaction between abuse and methylation remained significant ($p = .008$ for rs7629329 and $p = .022$ for rs237897), though only nominally for rs237897 (corrected $\alpha = 0.01$). Finally, because interaction analyses can produce spurious results if the variance of a quantitative outcome differs by level of the environmental variable (Voorman et al., 2011, Almli et al., 2014), we also present p-values from a robust version of the interaction test (Zeileis, 2004) and note that our results remain consistent (Table 3).

Integration of _OXTR_ SNPs and methylation

Traditionally, studies evaluate the interaction of SNPs and an environmental exposure to predict outcomes; in this study, we identified 6 SNPs within or near *OXTR* that interact with abuse to predict psychiatric symptoms (Supplementary Table 4). Interpretation of these results should proceed with caution given the number of tests, as none remained significant after correction for multiple testing (27 independent tests for each outcome, $\alpha = .0019$). Two of the SNPs (rs9817913 and rs237852) do not associate with any CpG sites in our study, and the other 4 (rs237837, rs3901926, rs9860869, rs237889) do not remain associated with study CpG sites after multiple test correction. However, inclusion of nominally associated CpG sites in the models did not substantially change the abuse × SNP interaction results (data not shown). Furthermore, though rs3901926 and rs237889 interacted with abuse to predict depression and or anxiety symptoms and associated with CpG sites in the study (Table 2), those CpG sites did not interact with abuse in any model tested. However, rs9860869 interacted with abuse to predict anxiety symptoms and nominally associated with CpG cg02192228, a CpG site that was directly associated with abuse. Finally, rs237837 interacted with abuse to predict anxiety symptoms, and nominally associated with a CpG site (cg11589699) that interacted with abuse to predict both depression and anxiety symptoms.

DISCUSSION

Our study explored the role of *OXTR* epigenetics in the link between abuse during childhood and adult psychopathology. Much of the excitement around epigenetics surrounds its documented ability to change in response to the environment. Within the oxytocin receptor gene, we found that abuse during childhood associates with higher methylation at two CpG sites, both located within exon 3 of the gene. However, this finding did not reach significance when accounting for multiple testing nor did these sites serve as a mediator of psychopathology. This finding is intriguing given the relative lack of human and animal studies assessing this question to date, despite the rapidly growing literature assessing the role of the early environment on methylation patterns of a number of genes (e.g. Beach, Brody, Todorov, Gunter, & Philibert,

2010; F. A. Champagne, 2008; McGowan et al., 2009). However, it is plausible that *OXTR* methylation may still be responsive to the environment. A recent study found that the environment may be able to influence small rapid changes in *OXTR* methylation. Unternaehrer et al. (2012) investigated dynamic changes in DNA methylation of CpG sites across the majority of *OXTR* exon 3 and found that average methylation status increased following the Trier Social Stress Test (TSST) and then decreased below pre-TSST levels 90-min post-test. While the influence of stress on methylation of other regions of this gene was not explored in Unternaehrer et al., it is of interest that methylation within this segment of DNA, which overlaps with the span containing CpG sites nominally associated with abuse during childhood in our study, may dynamically change in response to environmental exposures to stressful environments. Of question is the degree to which methylation of this site may be influenced by the environment, and whether methylation may become fixed following stressful exposures during developmentally sensitive time-periods, or whether it may continue to respond to stress over time. For example, it is possible that our nominal associations may be more representative of exposure to current stressful environments rather than those experienced during childhood. Future research should incorporate thorough assessments of both early and concurrent stress to explore the question of timing on methylation and begin to address the limitations of the current study and literature to date surrounding this question.

Given the early nature of this research, while *OXTR* CpG methylation did not serve as a mediator to psychopathology, we did find that it served as a moderator for abuse and psychopathology. Abuse interacted with methylation at specific *OXTR* CpG sites to predict depression and anxiety symptoms. Three of the five CpG sites that interacted with abuse to predict depression symptoms survived Bonferroni correction, and these were the same three sites found to predicted anxiety outcomes. Interestingly, the pattern of the interaction differed depending on the area of the gene where the CpGs were located. For CpG sites within exon 1, part of the *OXTR* promoter region, individuals with lower levels of methylation reported higher

depression and anxiety symptoms if they experienced abuse during childhood. For sites located in intron 3, within the gene body, an opposite pattern was observed – individuals with higher methylation reported higher depression and anxiety symptoms if they also reported abuse. Interestingly, methylation at these sites grouped together; the significant sites in exon 1 were correlated with each other and negatively correlated with sites in intron 3. In other words, individuals who tended to have lower methylation of the CpG sites in exon 1 also tended to have higher methylation in intron 3. In our study, lower methylation of the CpG sites within exon 1, and higher methylation of the CpG sites within intron 3, associated with greater risk for psychopathology following abuse.

The function of these CpG sites is currently unknown. Methylation of promoter regions is often conceptualized as blocking or reducing expression of a gene, with lower methylation facilitating gene expression, though the role of methylation within the gene body is still relatively unclear and high gene body methylation has been reported for genes that are highly expressed (Maunakea et al., 2010). Previous reports have suggested that methylation across similar regions of *OXTR* may associate with *OXTR* reglation and expression (Harony-Nicolas et al., 2014; Mamrut et al., 2013; Mizumoto et al., 1997) however, additional mechanistic research is warranted. Future studies that explore the regulatory role of CpG sites across these locations, and specifically at the sites examined in this study, will be important for the current study as well as future *OXTR* epigenetics research.

Exploring the interplay between genetics, epigenetics and the early environment is relatively new, yet it has the potential to provide additional insight into the development of behavioral and health outcomes. When incorporating epigenetics into conceptual models of complex behavioral outcomes, an initial question to address is how to integrate genetic and epigenetic variation. Our study identified a number of associations between *OXTR* SNPs and DNA methylation of specific *OXTR* CpG sites. On average, SNPs were a moderate distance away from their associated CpG sites (~10.5 kb). This is consistent with patterns observed across the

genome (Smith et al., 2014), and suggests that DNA methylation may provide a functional link between genetic variation and gene expression (Bell et al., 2012). In this study, we observed individual SNPs interacting with abuse to predict psychiatric symptoms and DNA methylation associating with abuse and psychiatric symptoms. In some cases, genetic or epigenetic variation associated independently, though there was also overlap between SNPs that interact with abuse and CpG sites that associate with abuse. This may suggest a potential mechanistic link underlying some G × E findings, yet continues to allow for additional mechanisms underlying these associations.

Three SNPs associate with CpG sites after correction for multiple tests (rs2301261, rs237897 and rs7629329). These SNPs have been examined in the *OXTR* literature previously, and one of these SNPs, rs7629329, associated with expression of *OXTR* in human brain tissue (Myers et al., 2014), which may suggest a possible role of methylation in this finding. However, the majority of reports, which primarily assess SNP associations with autistic or social behaviors, have not found associations with these SNPs (Campbell et al., 2011; Wu et al., 2012), and few studies have assessed interactions between these SNPs and early adversity (Loth et al., 2013; Myers et al., 2014). It is possible that the literature has not evaluated behaviors important for these SNPs or their associated CpG sites. It is also possible these SNP-CpG associations may be specific to our sample or to the African American population. Lastly, the functional significance of these SNPs and their associated CpG sites in regulating *OXTR* is not well understood.

Two of the most commonly studied *OXTR* SNPs are rs53576 and rs2254298. The mechanism through which these SNPs may influence behavioral outcomes has yet to be elucidated. We identified one associated CpG site for each SNP, though our sample size was limited for these analyses (N~100). While these SNPs did not interact with abuse to predict psychiatric symptoms in our cohort ($p>.05$), CpG sites associated with these SNPs did interact with abuse to predict adult psychiatric symptoms. Individuals with the G allele of rs53576 had higher methylation at cg00385883, and individuals with higher methylation at this site reported

nominally higher depression symptoms if they also had a history of childhood abuse. A recent report by McQuaid et al. (2013) showed that individuals with the rs53576 G allele had higher depressive symptoms following exposure to childhood maltreatment. For rs2254298, individuals with the G allele had higher methylation at cg11589699, and higher methylation at this site was found to associate with both increased depression and increased anxiety symptoms among those reporting abuse during childhood. The rs2254298 GG genotype has previously been associated with both unipolar depression and adult attachment anxiety (Costa et al., 2009), and Thompson and colleagues reported an interaction between this SNP and early adversity (conceptualized as a mother with major depression), though the AG genotype was associated with the highest depression and anxiety outcomes (Thompson, Parker, Hallmayer, Waugh, & Gotlib, 2011). It may be possible that rs53576 and rs2254298 associate with psychiatric outcomes through *OXTR* methylation. Replication and further research exploring this possibility are warranted.

Limitations

While this study provides new insight into the interplay between genetic and epigenetic variation in *OXTR*, the biological consequences of child abuse, and the interaction between abuse experienced in childhood and methylation on adult psychiatric symptoms, there are a number of important limitations to address. First, abuse during childhood was assessed retrospectively with a self-report measure that does not include detailed information on the abuse and it's psychological impact. However, the CTQ is a well-validated measure that has been used previously in the literature to assess abuse during childhood. Additional insight will likely be gained by including a more detailed assessment of the abuse timing and type on the development of biological changes and adverse adult outcomes.

Second, we sought to test associations between events in early life and DNA methylation in adulthood. The degree to which methylation remains fixed following sensitive developmental time periods, or continues to change in response to the environment throughout life, is still a topic of debate and is not fully known. While retrospective cross-sectional samples can begin to

highlight genes that may be important for investigating the question of timing, prospective longitudinal studies will be critical for providing insight into how stressful experiences may influence epigenetic programming over time.

Sample size is an important concern for both genetic and epigenetic studies, and our sample size is limited, particularly for the subsample with genotype data for rs53576 and rs2254298, two of the most commonly studied *OXTR* SNPs. Therefore, these associations should be considered preliminary prior to replication. However, for methylation analyses, our sample is considerably larger than any previous studies of *OXTR* methylation, which often have less than 100 participants.

The majority of human *OXTR* methylation studies to date have assessed methylation of sites within the first intron of the gene (Robert Kumsta, 2013). Our study includes data on two CpG sites within the first intron, though comparability between previous findings and our study is limited given different areas covered. Future work should assess the role of abuse on methylation of CpG sites previously reported and continue to assess CpG sites across the gene, particularly those across the promoter and within intron 3, to provide a more comprehensive understanding of *OXTR* regulation.

The range and variance of methylation differs across CpG sites in this gene. While the functional significance of this is of yet unknown, it may have implications for statistical testing and interpretation, as CpG sites with low variance will have attenuated covariance with other variables and a reduced likelihood of achieving association. Furthermore, when interpreting high versus low methylation of specific CpG sites on behavioral outcomes, absolute levels of high and low methylation will be limited by the range and average methylation of CpG site of interest, and can differ from the levels of other CpG sites.

Our study was limited to utilizing peripheral tissue. *OXTR* methylation should ideally be assessed in the tissues that are known to express *OXTR* and directly involved in psychiatric symptoms. While initial post-mortem studies have reported *OXTR* methylation and expression

differences in the brain tissue of those with and without autism (Gregory et al., 2009), no studies to date have looked at associations between abuse and *OXTR* methylation in human brain tissue, though differences by abuse exposure have been reported for other genes (McGowan et al., 2009). How well methylation of peripheral tissues can be used to study methylation changes in response to the environment or in association with behavioral outcomes is currently a topic of debate, though there is some evidence that methylation in peripheral tissues may be comparable to that of brain (Smith et al., 2014). Further understanding will be paramount for interpreting and conducting human methylation studies given this important yet practical limitation.

Lastly, our study did not evaluate gene expression and thus cannot explore whether differences in methylation of our study's CpG sites may be influential for *OXTR* regulation and expression. Previous research has shown functional significance of areas within the *OXTR* promoter region and intron 3 (Harony-Nicolas et al., 2014; Kusui et al., 2001; Mizumoto, Kimura, & Ivell, 1997). Furthermore, a recent study reported that a number of *OXTR* SNPs associate with *OXTR* expression level in human brain tissue (Myers et al., 2014). Eight of the SNPs found to associate with expression were present in our dataset, and all of these SNPs associate with methylation of a nearby CpG site in our sample (p-value = 0.046 to $1.0x10^{-4}$), which may point to a possible role of methylation in this finding. Additional research on the functional significance of SNPs and methylation across this gene, and in particular sites that associate with behavioral outcomes, is warranted.

Conclusions

Interest in the role of genetic and epigenetics influences on behavioral outcomes has grown quickly (Meaney, 2010). Our study suggests that genetic variation may associate with methylation of CpG sites, further supporting the possibility that this link may be a potential mechanism by which some SNPs influence behavioral outcomes. However, our study also supports the role of additional mechanisms underlying genetic associations, as many of our models did not substantially change when controlling for associated SNPs or CpG sites.

Studies have called for the inclusion of *OXTR* in research assessing epigenetic responses to early life stress (Jack et al., 2012; Robert Kumsta, 2013). Our study supports the potential for this association, identifying nominally higher methylation levels among CpG sites in an area of the gene where methylation has previously been found to dynamically change in response to a stress-provoking task, the TSST (Unternaehrer et al., 2012). Further, while *OXTR* SNPs have been found to interact with early adversity to predict psychiatric outcomes, our study found that methylation of CpG sites within *OXTR* may similarly interact with abuse to predict these outcomes. Oxytocin plays an important role in social behaviors, and research on *OXTR* genetics and epigenetics will continue to provide important insight into how the oxytonergic system may underlie and influence human behavioral development.

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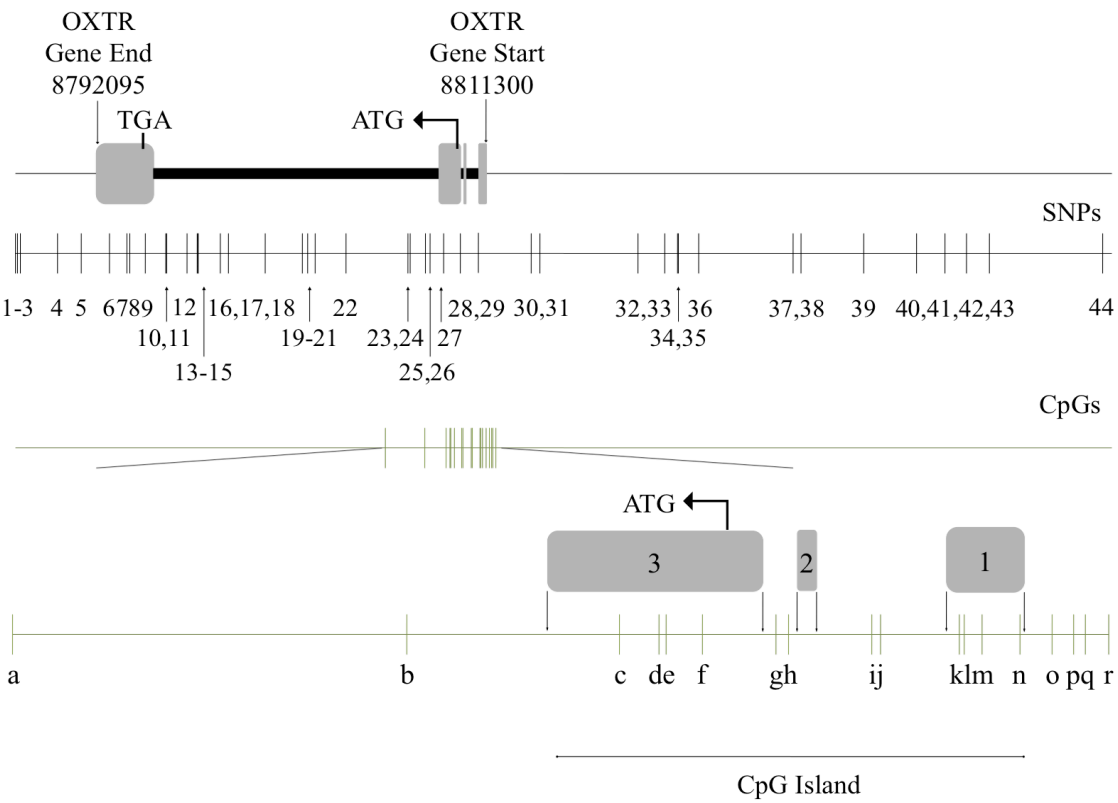
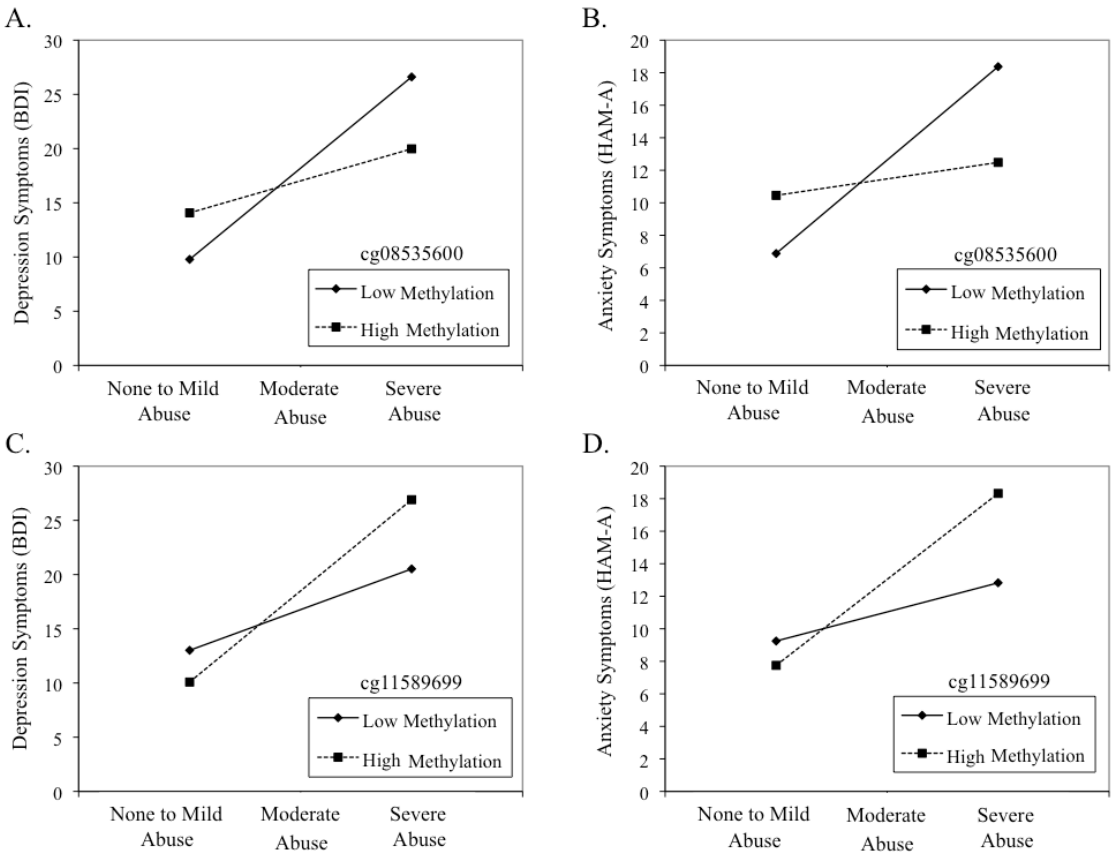
Figure 3.1. *OXTR* structure and variant location.



Note. The oxytocin receptor, located on chromosome 3p25, has four exons and three introns that span 17kb. Only SNPs and CpG sites assessed in this study are depicted. Table 2 provides a key for CpG abbreviations, and Supplementary Table 1 provides a key for SNP labels.

Figure 3.2. *OXTR* methylation moderates the association between childhood abuse and adult psychiatric symptoms.



Note. These figures display the pattern of interaction between abuse and CpG site methylation for CpG sites that associate with both depression and anxiety after Bonferroni correction. (A) Association between abuse and depression for one standard deviation above and below the mean methylation of cg08535600, located in exon 1. (B) Association between abuse and anxiety for one standard deviation above and below the mean methylation of cg08535600. (C) Association between abuse and depression for one standard deviation above and below the mean methylation of cg11589699, located in intron 3. (D) Association between abuse and anxiety for one standard deviation above and below the mean methylation of cg11589699.

Table 3.1. Study Variables Among those with None to Mild, Moderate, or Severe Abuse

	Physical, Emotional, or Sexual Abuse			p-value
	None to Mild (N=200)	Moderate (N=100)	Severe (N=89)	
Age (Mean, SD)	41.3 (13.2)	40.1 (13.2)	42.4 (11.3)	0.46
Females (N, %)	130 (65.0%)	77 (77.0%)	68 (76.4%)	0.040
% Granulocytes	61.9 (10.2)	62.2 (12.4)	62.1 (10.9)	0.89
% Lymphocytes	15.0 (5.8)	15.1 (8.3)	14.2 (5.0)	0.56
Lifetime Trauma	3.8 (2.9)	5.5 (2.8)	8.1 (3.4)	<.001
BDI Score	12.1 (10.5)	16.4 (11.6)	23.5 (14.0)	<.001
HAM-A Score	8.8 (7.9)	11.1 (8.2)	15.1 (9.9)	<.001

Note: Percent granulocyte and lymphocyte represents the percent of that cell type in the blood sample used for methylation analyses, and represents the cellular heterogeneity within the sample. Lifetime Trauma is the number of traumatic events experienced or witnessed upon time of study participation. Sample range was 0-16. BDI is the Beck Depression Inventory and HAM-A is the Hamilton Anxiety Rating Scale. These scales range from 0-58 for BDI and 0-37 for HAM-A, with higher scores represent higher depression or anxiety symptoms, respectively.

Table 3.2. Association between Single Nucleotide Polymorphisms and Methylation of Specific CpG Sites

				Model Statistics			SNP Predictor Statistics		
CpG site	**CpG position**	**SNP**	**SNP position**	**R-squared**	**F statistic**	**p-value**	**Beta**	**t-statistic**	**p-value**
cg14483142	8811758 (r)	*Not associated with any tested SNPs*							
cg17036624	8811601 (q)	*Not associated with any tested SNPs*							
cg00247334	8811543 (p)	rs2301261	8810896 (29)	0.089	(7, 310) = 4.23	$1.8x10^{-4}$	-0.24	-4.27	$2.6x10^{-5}$*
		rs6777726	8813494 (30)	0.066	(7, 309) = 3.03	0.004	-0.18	-3.20	0.002
		rs237888	8797095 (15)	0.060	(7, 310) = 2.76	0.009	0.16	2.90	0.004
		rs2268495	8807535 (24)	0.054	(7, 309) = 2.45	0.019	0.15	2.61	0.010
		rs237899	8808515 (26)	0.054	(7, 310) = 2.49	0.017	-0.14	-2.55	0.011
		rs237902	8809184 (27)	0.171	(7, 94) = 2.56	0.019	0.23	2.32	0.023
cg25140571	8811437 (o)	rs2301261	8810896 (29)	0.093	(7, 310) = 4.46	$9.6x10^{-5}$	-0.22	-4.02	$7.5x10^{-5}$*
		rs3901926	8834933 (42)	0.068	(7, 309) = 3.17	0.003	0.15	2.74	0.006
		rs237888	8797095 (15)	0.064	(7, 310) = 2.95	0.005	0.14	2.45	0.015
		rs2139184	8795494 (10)	0.060	(7, 309) = 2.78	0.008	-0.12	-2.19	0.029
		rs237885	8795543 (11)	0.059	(7, 310) = 2.70	0.010	0.12	2.08	0.038
		rs918316	8798181 (16)	0.059	(7, 310) = 2.70	0.010	-0.12	-2.08	0.038
		rs237899	8808515 (26)	0.059	(7, 310) = 2.70	0.010	-0.12	-2.08	0.039
cg23391006	8811279 (n)	rs11476	8788198 (2)	0.274	(7, 94) = 4.70	$1.7x10^{-4}$	0.27	2.85	0.005
cg09353063	8811092 (m)	*Not associated with any tested SNPs*							
cg17285225	8811004 (l)	rs11131149	8802851 (21)	0.220	(7, 309) = 12.18	$<1.0x10^{-6}$	-0.13	-2.42	0.016
		rs6443206	8820075 (33)	0.275	(7, 94) = 4.71	$1.6x10^{-4}$	0.22	2.34	0.022
cg08535600	8810980 (k)	rs237895	8807423 (23)	0.251	(7, 94) = 4.16	0.001	-0.3	-3.11	0.003
cg00078085	8810592 (j)	rs237889	8802483 (20)	0.059	(7, 309) = 2.71	0.010	-0.13	-2.37	0.018
		rs2268495	8807535 (24)	0.056	(7, 308) = 2.53	0.015	-0.12	-2.11	0.036
		rs237885	8795543 (11)	0.055	(7, 309) = 2.52	0.016	-0.12	-2.08	0.038
		rs6793234	8826396 (37)	0.054	(7, 309) = 2.45	0.019	0.11	1.98	0.049
cg03987506	8810549 (i)	*Not associated with any tested SNPs*							
cg19619174	8810139 (h)	rs11476	8788198 (2)	0.260	(7, 94) = 4.37	$3.4x10^{-4}$	0.33	3.46	0.001

Table 3.2 Continued

				Model Statistics			SNP Predictor Statistics		
CpG site	**CpG position**	**SNP**	**SNP position**	**R-squared**	**F statistic**	**p-value**	**Beta**	**t-statistic**	**p-value**
cg12695586	8810077 (g)	rs237899	8808515 (26)	0.080	(7, 310) = 3.77	0.001	-0.18	-3.18	0.002
		rs7628723	8832470 (40)	0.223	(7, 94) = 3.56	0.002	-0.25	-2.57	0.012
		rs11476	8788198 (2)	0.212	(7, 94) = 3.34	0.003	0.23	2.31	0.023
		rs237925	8821742 (36)	0.065	(7, 310) = 3.02	0.004	0.13	2.26	0.025
		rs7629329	8788336 (3)	0.061	(7, 309) = 2.80	0.008	-0.12	-2.11	0.036
		rs7632031	8818757 (32)	0.063	(7, 310) = 2.92	0.006	0.12	2.11	0.036
cg27501759	8809715 (f)	rs237925	8821742 (36)	0.087	(7, 310) = 4.14	$2.3x10^{-4}$	0.15	2.70	0.007
		rs237897	8808285 (25)	0.083	(7, 215) = 2.69	0.011	0.17	2.53	0.012
		rs6443206	8820075 (33)	0.170	(7, 94) = 2.54	0.020	0.20	2.03	0.045
cg02192228	8809536 (e)	rs237925	8821742 (36)	0.068	(7, 310) = 3.14	0.003	0.19	3.42	0.001
		rs180789	8813927 (31)	0.058	(7, 310) = 2.65	0.011	-0.16	-2.90	0.004
		rs9860869	8820740 (35)	0.048	(7, 310) = 2.16	0.037	0.13	2.25	0.025
		rs6791619	8829872 (39)	0.046	(7, 307) = 2.06	0.047	-0.13	-2.23	0.026
cg04523291	8809501 (d)	rs237925	8821742 (36)	0.057	(7, 310) = 2.59	0.013	0.18	3.15	0.002
		rs180789	8813927 (31)	0.046	(7, 310) = 2.10	0.043	-0.15	-2.57	0.011
cg15317815	8809306 (c)	rs237925	8821742 (36)	0.051	(7, 310) = 2.34	0.025	0.19	3.33	0.001
cg00385883	8808259 (b)	rs237897	8808285 (25)	0.170	(7, 215) = 6.11	$2.0x10^{-6}$	-0.29	-4.52	$1.0x10^{-5}$*
		rs7629329	8788336 (3)	0.124	(7, 309) = 6.13	$1.0x10^{-6}$	0.21	3.94	$1.0x10^{-4}$*
		rs237899	8808515 (26)	0.115	(7, 310) = 5.65	$4.0x10^{-6}$	0.19	3.57	$4.1x10^{-4}$
		rs13093809	8788096 (1)	0.105	(7, 310) = 5.07	$1.8x10^{-5}$	0.17	3.01	0.003
		rs237895	8807423 (23)	0.179	(7, 94) = 2.71	0.014	0.31	3.03	0.003
		rs53576	8804371 (22)	0.242	(7, 100) = 4.23	$4.3x10^{-4}$	-0.21	-2.27	0.025
		rs2301261	8810896 (29)	0.093	(7, 310) = 4.44	$1.0x10^{-4}$	0.12	2.22	0.027
cg11589699	8806317 (a)	rs75775	8820732 (34)	0.151	(7, 310) = 7.70	$<1.0x10^{-6}$	-0.17	-3.23	0.001
		rs2268495	8807535 (24)	0.151	(7, 309) = 7.70	$<1.0x10^{-6}$	0.16	3.06	0.002
		rs6791619	8829872 (39)	0.146	(7, 307) = 7.31	$<1.0x10^{-6}$	0.15	2.70	0.007
		rs237837	8841641 (44)	0.142	(7, 310) = 7.18	$<1.0x10^{-6}$	0.14	2.69	0.008
		rs237899	8808515 (26)	0.139	(7, 310) = 7.00	$<1.0x10^{-6}$	-0.13	-2.48	0.014
		rs2301261	8810896 (29)	0.138	(7, 310) = 6.95	$<1.0x10^{-6}$	-0.13	-2.41	0.016
		rs237911	8810008 (28)	0.231	(7, 94) = 3.72	0.001	-0.24	-2.38	0.019
		rs2254298	8802228 (19)	0.221	(7, 94) = 3.52	0.002	-0.21	-2.12	0.037
		rs11476	8788198 (2)	0.217	(7, 94) = 3.45	0.003	-0.20	-2.02	0.046

Note for Table 3.2. For each CpG site, associations are shown if both the overall model and the SNP predictor reach p<.05. The table is organized by CpG position, starting with those upstream of the gene (larger values given it's location on the negative DNA strand), and provides the actual position of each CpG (and SNP) followed in parentheses by the position label for Figure 1. Note that cg00078085 has a SNP, rs73132856, in its probe sequence. There were 135 tests in total (27 independent SNPs for each of 5 regions of methylation), leading to a Bonferroni corrected p-value of $p < 3.7x10^{-4}$.
* indicates p-values maintaining significance after multiple test correction. All analyses above controlled for age, sex, cellular heterogeneity, and positional effects (sample chip and row). Standardized betas are presented. Note that sample size varies based on SNP inclusion from two genotyping arrays used in the study; sample size for each analysis can be determined by adding 1 to the second degrees of freedom in the F-statistic.

Table 3.3. Association between Childhood Abuse and Depression and Anxiety Symptoms: Potential Moderating Role of *OXTR* CpG site Methylation

Abuse×CpG Interaction Model	Depression				Anxiety				Methylation level with Stronger Association	
	Beta	t-statistic	p-value	p-robust	Beta	t-statistic	p-value	p-robust	Depression	Anxiety
cg23391006 (n)	-0.90	-2.28	0.023	0.028	--	--	0.67	0.69	Lower	--
cg17285225 (l)	-0.50	-3.16	$1.7x10^{-3}$*	$2.1x10^{-3}$	-0.48	-2.80	0.005*	0.001	Lower	Lower
cg08535600 (k)	-0.56	-3.49	$5.4x10^{-4}$*	$1.3x10^{-3}$	-0.70	-4.01	$7.5x10^{-5}$*	$1.2x10^{-6}$	Lower	Lower
cg00385883^{+}(b)	6.70	2.54	0.012	0.016	--	--	0.10	0.09	Higher	--
cg11589699 (a)	6.28	3.49	$5.5x10^{-4}$*	$9.8x10^{-4}$	6.58	3.40	$7.6x10^{-4}$*	$4.8\ x10^{-5}$	Higher	Higher

Note: The interaction between abuse and CpG site methylation was tested in a linear regression predicting depression or anxiety symptoms controlling for age, sex, cellular heterogeneity, and positional effects. Models are shown if both the interaction variable (abuse x CpG methylation) and the overall model reach p<.05. The table is organized by CpG position, start with those more upstream in the gene, with the position label for Figure 1 provided in parentheses after the cgID. The first three CpG sites are within the exon 1, the last two are within intron 3. Standardized beta values are presented. * indicates interactions maintaining significance after multiple test correction (α=0.01). "p-robust" indicates p-values estimated via a robust sandwich estimator. The direction of the interaction was explored using simple slope procedures assessing the association

CHAPTER 4
Variation in the Oxytocin Receptor Gene Moderates the Protective Effects of a Family-Based Prevention Program on Telomere Length

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Variation in the Oxytocin Receptor Gene Moderates the Protective Effects of a Family-Based Prevention Program on Telomere Length

ABSTRACT

Parent-child relationships with high levels of conflict and low levels of warmth and support are associated with later adverse behavioral and physiological child outcomes. These outcomes include shorter telomere lengths, the repetitive sequences at the ends of chromosomes that have been utilized as a biomarker for chronic stress. Our research group furthered this by exploring telomere length outcomes following a family-based prevention program and identified reduced telomere shortening five years post-intervention among those originally exposed to non-supportive parenting and randomized to the intervention condition. However, not all individuals respond equally, and a growing literature suggests genetic sensitivity to one's environment, with variations in the oxytocin receptor gene (*OXTR*) potentially influencing this sensitivity.

We utilized data from African American youths (mean age 17) randomized to intervention (n=100) or control condition (n=91) with baseline assessments of genetic status and non-supportive parenting and 5-year follow-up assessments of telomere length. We found a significant 3-way interaction between non-supportive parenting, intervention condition and *OXTR* rs53576 genotype. *OXTR* GG individuals, who are suggested to be more sensitive to their social environment, exhibited significantly more variability, evidencing the shortest telomeres when exposed to non-supportive parenting and randomized to the control condition, and similar telomere lengths to non at-risk groups when randomized to the intervention. In contrast, those with the A allele showed no statistical difference in telomere lengths across parental and intervention conditions. These findings highlight the importance of individual differences and potential role of genetic status in moderating the relationship between environmental contexts and biological outcomes.

INTRODUCTION

Exposure to chronic stressors such as parent-child relationships with high levels of conflict and low levels of warmth and emotional support have repeatedly been associated with later adverse behavioral and physiological outcomes (Miller, Chen, & Parker, 2011; Repetti, Taylor, & Seeman, 2002). Indeed, this link between exposure to chronic stressors in youth and later poor outcomes has become well-established and has resulted in a call for increased attention to public policy and interventions designed to reduce exposure to these conditions or their effects (Shonkoff, 2010). However, questions remain about how to best measure the impact of such interventions, especially given that outcomes of interest such as improved health and behavior can occur a number of years in the future. Telomeres have emerged as a possible option for addressing this question.

Telomeres are repetitive sequences on the ends of chromosomes that serve as protective caps. During cell division, the ends of the chromosome are not replicated resulting in shorter telomeres following cell division (Blackburn, 2005), though telomere length can be increased by the enzyme telomerase, allowing for some dynamic regulation (Blackburn, 2005). Interestingly, in addition to age, telomere shortening has repeatedly been reported under conditions of chronic psychosocial stress (Epel et al., 2004), including stress that occurs during childhood (Cohen et al., 2013; Mitchell et al., 2014; Price, Kao, Burgers, Carpenter, & Tyrka, 2013; Shalev et al., 2013). Furthermore, telomere shortening has also been predictive of future poor health (Cohen et al., 2013; Epel et al., 2006; Fitzpatrick et al., 2007) and mortality (Bakaysa et al., 2007; Cawthon, Smith, O'Brien, & Sivatchenko, 2003). While the mechanisms by which this shortening occurs are still being explored, including the potential for increased cell replication or weakened telomerase activity (Choi, Fauce, & Effros, 2008; Epel et al., 2010; Tomiyama et al., 2012), investigating telomere length in the context of stress and long-term behavioral and health outcomes may have implications for intervention research. If intervention programs can result in

reduced telomere shortening, this may suggest a positive impact of interventions at a biological level and may forecast a greater likelihood of improved mortality and future health.

Recent pilot studies suggest that preventative interventions may have protective effects on telomere length (Daubenmier et al., 2012; Epel, Daubenmier, Moskowitz, Folkman, & Blackburn, 2009; Jacobs et al., 2011) and spurred the incorporation of telomere length assessment in the Adults in the Making (AIM) prevention program (Brody, Chen, & Kogan, 2010; Brody, Yu, Chen, Kogan, & Smith, 2012). AIM is an efficacious family-centered, skill-based prevention program for African American adolescents in the rural south primarily aimed at building family stress-buffering processes. At age 17, when youth were initially randomized to the 6-week intervention or control conditions, primary caregivers provided data on parental support including degree of conflict and level of warmth and emotional support provided to the youth. At age 22, five years after the intervention, telomere length was indexed from youth peripheral blood mononucleocytes.

Among youth exposed to high conflict and low warmth and emotional support at baseline, participation in the AIM intervention program impacted telomere length across 5 years. Exposure to these environments was associated with significantly shorter telomere length at the 5-year follow up; however, this effect was found only for those randomized to the control condition (Brody, Yu, Beach, & Philibert, 2015). Participation in the intervention appeared to attenuate this relationship, with those in the intervention evidencing similar telomere lengths to the non at-risk group.

The AIM-telomere length protective finding suggests that participation in efficacious intervention programs may have the potential to intervene on stress processes at a biological level and promotes the role of intervention programs among youth living in high conflict or stressful environments. While prevention programs may benefit all youth to a degree, there is a growing appreciation and literature on individual differences in sensitivity to one's environment, both positive and negative (Belsky & Pluess, 2009). These differences may be partly explained by

genetic predispositions. Certain genes, especially those involved in neurotransmission, may influence one's sensitivity to environmental contexts, with individuals evidencing greater behavioral and phenotypic outcomes following environmental exposures (Belsky & Pluess, 2009; Belsky, Bakermans-Kranenburg, & van Ijzendoorn, 2007). Conditions with both negative and positive environments may provide a unique opportunity to explore these questions of genetic sensitivity.

Within the telomere literature, the hypothesis of genetic sensitivity was recently applied with a report showing that exposure to disadvantaged environments was associated with reduced telomere length at age 9, but that this association was moderated by genotype (Mitchell et al., 2014). Children with certain serotonin and, to a lesser degree, dopamine genotypes were reported to have the shortest telomeres when exposed to disadvantaged environments, compared to those without these genotypes. Studies have also applied this hypothesis to prevention programs, finding that genetics may influence the association between intervention participation and the degree of behavioral and phenotypic outcomes (Brody et al., 2014; Rutter, 2005; Sales, DiClemente, Brody, Philibert, & Rose, 2014), yet no study to date has explored whether genetics may moderate the impact of prevention programs on telomere length outcomes.

Although a number of genotypes have been included in the sensitivity literature, due to the social nature of parent-child relationships and family-based intervention programs, genetic variation in the oxytonergic system, a system important for bonding and perception of social cues (Bartz, Zaki, Bolger, & Ochsner, 2011; Olff, Frijling, Kubzansky, & Bradley, 2013), may be particularly relevant when studying the impact of these environmental contexts. One of the most commonly studied polymorphisms in the oxytocin receptor gene (*OXTR*) is rs53576, which results from an adenine (A) / guanine (G) transition in the 3rd intron. Interestingly, while the G allele was initially associated with more positive prosocial outcomes, such as trust (Krueger et al., 2012) and empathy (Rodrigues, Saslow, Garcia, John, & Keltner, 2009), in negative social contexts, individuals with the G allele have been found to display more adverse outcomes, such

as greater emotional dysfunction (Bradley et al., 2011), decreased maternal sensitivity (Sturge-Apple, Cicchetti, Davies, & Suor, 2012), and increased antisocial behaviors (Smearman et al., 2014). Furthermore, those with the G allele, compared to the AA genotype, were found to display significantly higher levels of resilience and positive affect when raised in high stability and warmth environments, yet significantly lower levels of resilience and affect when raised in low stability and low warmth environments (Bradley, David, Wingo, Mercer, & Ressler, 2013). These findings support the concept of genetic sensitivity and more specifically, the Social Sensitivity hypothesis for oxytocin, where individuals with the rs53576 G-allele are thought to be more perceptive of social cues and thus more sensitive to, and responsive towards, the social environment (Bartz et al., 2011; Olff et al., 2013). In this regard, individuals with the G-allele may be more impacted by non-supportive family environments, yet may also be more responsive to family-based prevention interventions.

Our study sought to expand upon this literature and the initial AIM study finding by evaluating the role of *OXTR* genetic status on the effect of intervention participation on later telomere length. A unique benefit of intervention programs is the ability to directly modify the environment in a controlled setting. This control allows for more direct testing of the association between environmental exposure and phenotypic outcomes and the ability to test for moderators of this association (Brody et al., 2014; Brody, Chen, & Beach, 2013a; Rutter, 2005). Therefore, our study proposed a three-way interaction incorporating non-supportive parenting, intervention condition, and *OXTR* genetic status on 5-year follow up telomere lengths among AIM study participants. Aligned with the Social Sensitivity hypothesis for oxytocin, we hypothesized that individuals with the *OXTR* GG genotype would evidence the shortest telomeres when exposed to high levels of non-supportive parenting and the control intervention condition, and longer telomere lengths when randomized to the intervention, while individuals with the A-allele would be less impacted, evidencing relatively similar telomere lengths across all conditions.

METHODS

Participants

Participants were drawn from 367 African American youth who participated in the Adults in the Making (AIM) trial. Participants were in high school at the start of the study, 63.6% lived in single-mother-headed households, and 56.1% of participants were female. The majority of the youths' caregivers (78.7%) completed high school or earned a GED and median family income was $2,012 per month, which can be described as working poor and is representative of the sampled population (Boatright 2005). Of the 367 who participated, 216 agreed to telomere length assessment at age 22 and a total of 191 had both telomere and genetic data. Using two-factor multivariate analyses, there were no differences between the original and final samples on any demographic or study variables other than gender; the final sample had a lower percentage of females ($p<.05$). Gender was controlled for in all analyses. Of those in the final sample, 100 were randomized to the intervention condition and 91 to the control condition. Caregivers provided informed consent for their own participant and the participation of minor youth and youth provided assent, if under 18, or informed consent for participation. Families were compensated $100 at each assessment.

AIM intervention

The AIM intervention program is modeled after an existing family-based, skills-training intervention designed to mitigate the negative impact of life stress on rural African American adolescents through enhancement of family-based buffering processes (Brody et al., 2004). The program consists of six consecutive weekly sessions held in community facilities with separate, concurrent training sessions followed by joint parent-youth sessions during which families practice the skills learned in the separate sessions (Brody et al., 2012). Parenting sessions were designed to promote provision of instrumental support, emotional support and communication, problem-focused coping, and occupational and educational mentoring. Youth sessions were designed to promote the development of goal setting and future orientation, to identify sources of support for goal attainment, and to cope with barriers and racial discrimination. The separate and

concurrent sessions each lasted 1 hour resulting in a total of 12 hours of prevention training for each youth and parent.

Data Collection Procedures

Demographic and parenting variables were collected in the participants' home at baseline at youth age 17 (M = 17.7, SD = 0.72). Telomere length and additional health variables, including body mass index (BMI) and blood pressure, were measured at a 5-year follow up at age 22 (M = 22.02, SD = 0.98). Two African American field researchers visited the family's home and worked separately with the parent and youth. Interviews were conducted in a private location with no other family members present.

Measures

Non-supportive parenting. Non-supportive parenting was measured through self-report using measures assessing parent-child conflict and parental warmth and emotional support. Parent-child conflict was measured using parent report on two scales. The first, the Ineffective Arguing Inventory (IAI; (Kurdek, 1994), consisted of statements describing parent-child conflict with response scales ranging from 0 (*disagree strongly*) to 4 (*agree strongly*); α = .82. Examples include, "You and your child go for days being mad at each other" or "You and your child's arguments are left hanging and unsettled." The second, the Arguing subscale from the Discussion Quality Scale (DQS, Brody et al., 1998), consisted of four statements regarding the frequency with which parents and youth argued over choice of friends, school or job, youth sex, and alcohol and other drugs, with response scales ranging from 1 (*never*) to 4 (*always*); α = .69 The IAI and DQS items were highly correlated ($r = .51$, $p < .001$) and were summed to form an indicator of parent-child conflict.

Parental warmth and emotional support was also measured using parental report on two scales. The first, the emotional support subscale from the Family Support Inventory (FSI; (Wills, Vaccaro, & McNamara, 1992), consisted of five items with parental rating scales ranging from 0 (*not at all true*) to 5 (*very true*); α = .79. Examples include "My child can trust me as someone to

talk to," and "When my child feels bad about something, I will listen." The second, a revised version of the Emotional Support subscale from the Carver Support Scale (CSS; (Carver, Scheier, & Weintraub, 1989), consisted of four items with parental rating scales ranging from 1 (*not at all true*) to 5 (*very true*); α = .77 Examples include, "My child discusses his/her feelings with me," and "My child gets sympathy and understanding from me." The CSS and FSI were highly correlated ($r = .67$, $p < .001$) and were summed to form an indicator of parental support. Parent-child conflict and parental warmth and support scores were standardized, and parental warmth and support was subtracted from parent-child conflict to create a measure of non-supportive parenting. High values indicated high parent-child conflict and low levels of warmth and emotional support.

Genotyping. Youths' DNA was obtained using Oragene™ DNA kits (Genetek, Calgary, AB, Canada). Youths rinsed their mouths with tap water and deposited 4 ml of saliva in the Oragene sample vial. The vial was sealed, inverted, and shipped via courier to a central laboratory in Iowa City, where samples were prepared according to the manufacturer's specifications. Genotyping of *OXTR* rs53576 was determined for each youth using Applied Biosystems' TaqMan SNP Genotyping technology. Of the sample, 63.9% ($n = 122$) were GG, 30.4% ($n = 58$) were AG, and 5.7% ($n = 11$) were AA. None of the alleles deviated from Hardy-Weinberg equilibrium, $\chi^2(1) = 1.31$, p = ns and no gender or intervention differences could be detected ($\chi^2 = 0.137$; df = 2, $p = 0.93$ for gender; $\chi^2 = 2.156$; df = 2, $p = 0.34$ for intervention status). Given the limited sample size of AA individuals, *OXTR* rs53576 alleles were grouped and coded as GG (1) vs. AA/AG (0).

Telomere length. At the 5-year follow up, certified phlebotomists visited the participant's home to draw a blood sample for telomere assessment. After the blood was drawn into serum separator tubes, it was frozen and delivered to the Psychiatric Genetics Lab at the University of Iowa for assaying. Telomere assessment of this sample has been described previously (Brody et al., 2015). Briefly, mononuclear (e.g. lymphocyte) cell pellets were

generated using Ficoll separation (see (Philibert, Beach, & Brody, 2012) and the resulting lymphocyte cell pellets were prepared using a Qiagen QIAamp DNA Prep Kit according to the manufacturer's instructions. Telomere/standard (T/S) ratios were then calculated for each sample using a minor adaption of the improved quantitative polymerase chain reaction (PCR) method that (Cawthon, 2009) developed, where DNA is amplified using a set of primers specific for either telomeric sequence or a single-copy-number standard gene (albumin).

Demographic and control variables. Intervention status and gender. Intervention condition was coded as randomization to intervention participation (1) or control condition (0). Gender was coded as male (1) and female (0).

Family socioeconomic status (SES) risk. SES risk has previously been associated with shorter telomere length outcomes (Price et al., 2013) and therefore was included in the study. Family SES risk was measured using six dichotomous variables that assessed presence or absence of the following characteristics: family poverty based on federal guidelines, primary caregiver unemployment, receipt of Temporary Assistance for Needy Families, primary caregiver single parenthood, primary caregiver education level less than high school graduation, and caregiver-reported inadequacy of family income. The scores were summed to create the SES risk score. This technique has previously been used to forecast biomarkers of stress in African American adolescents (Brody et al., 2015).

Youth blood pressure and body mass index (BMI). At the 5-year follow up, resting blood pressure was monitored with a Critikon Dinamap Pro 100 (Critikon; Tampa, FL). Three readings were taken every 2 minutes, and the average of the last two readings were used. To create a single score, systolic and diastolic blood pressure scores were standardized and summed. Weight and height of each participant were then measured and used to calculate BMI (weight in kilograms divided by the square of height in meters) to control for the potential role of obesity in telomere length (Buxton et al., 2011).

Statistical Analysis

Chi-square and t-tests were used to compare study variables across the intervention and control conditions. To test the study hypotheses, three regression models were executed. The models testing gene by environment interactions followed the conventions of Aiken and West (Aiken & West, 1991). Specifically, non-supportive parenting was mean centered and interaction terms were calculated by creating a product term of the variables of interest (non-supportive parenting, intervention condition and/or *OXTR* genotype). The first two models were intended to replicate the previous telomere findings in the subset of individuals with *OXTR* genetic data (see (Brody et al., 2015). The first model tested the association between non-supportive parenting at age 17 and telomere length at age 22. The second model tested the protective effects of the intervention program by incorporating an interaction term between non-supportive parenting and intervention condition (PxI) on telomere length. The third model extended this further by including *OXTR* genetic status creating a three-way interaction between non-supportive parenting at age 17, intervention condition, and *OXTR* genetic status (PxIxG) on telomere length to test whether individuals with the GG genotype may show greater differences in telomere lengths across the non-supportive and intervention conditions. All analyses controlled for the covariates of gender, family SES risk at age 17, BMI at age 22, and blood pressure at age 22. To further explore and visualize the direction of significant interactions, graphs were created using simple slope procedures. Estimate levels and slopes of telomere length outcomes for low (-1 SD) and high (+1 SD) non-supportive parenting were graphed for each intervention and genotype group. Groups for PxI interaction include the intervention and control conditions. Groups for the PxIxG interaction include intervention GG genotype, intervention A+ genotype, control GG genotype, and control A+ genotype. All tests were performed using SPSS 22.

RESULTS

Descriptive statistics

More males were randomized to the intervention compared to control condition ($\chi^2(1) = 6.76$, $p < .01$), but the groups did not differ on any other study variables ($p > .05$). Descriptive statistics and correlations among study variables are presented in Table 1.

Non-supportive parenting, intervention condition, and telomere length

While the direct association between non-supportive parenting and telomere outcomes was only significant at $p = .07$ (Table 2, Model 1; $\beta = -.131$), an interaction emerged when taking intervention condition into account. Replicating previous findings, exposure to non-supportive parenting at age 17 forecasted shorter telomere length at age 22, but this association was attenuated for those who participated in the AIM intervention (Table 2, Model 2; $\beta = .223$, $p < .05$). Among those exposed to high levels of non-supportive parenting, only those randomized to the *control* condition evidenced significantly shorter telomeres at age 22 (Figure 1.A, simple-slope = -.072, $p < .01$), while those randomized to the intervention evidenced similar telomere lengths to those with more supportive, non at-risk parenting scores at baseline (Figure 1.a, simple-slope = .006, p = ns).

Non-supportive parenting, intervention condition, and *OXTR* genotype on telomere length

OXTR genetic status moderated the effects of non-supportive parenting and intervention condition on telomere length across 5 years (Table 2, Model 3, $\beta = .453$, $p < .01$). Visualizing this 3-way interaction in Figure 1.b, the association between parenting and telomere length is presented for low (-1 SD) and high (+1 SD) non-supportive parenting for each genotype. *OXTR* rs53576 GG individuals, who are suggested to be more sensitive to the social environment, evidenced the shortest telomeres when exposed to non-supportive parenting and randomized to the control condition (Figure 1.B, simple slope = -.107, $p < .001$), and similar telomere lengths to the supportive parenting group when randomized to the intervention (Figure 1.B, simple slope = .033, p = ns). In contrast, AG/AA individuals showed no statistically significant difference in telomere lengths across all parenting and intervention conditions. In summary, the finding of protective effects of the AIM intervention on non-supportive parenting and telomere length was found for

the *OXTR* GG individuals, while those with the A allele showed similar telomere lengths across all conditions.

DISCUSSION

The current study identified a significant 3-way interaction whereby *OXTR* genetic status moderated the association between non-supportive parenting and exposure to a family-based prevention intervention program on long-term telomere length outcomes. Adolescent youth with the *OXTR* GG genotype were the most sensitive to both the parental support and intervention conditions, evidencing the shortest telomeres when exposed to high levels of non-supportive parenting (high parent-child conflict and low levels of warmth and emotional support), yet longer telomeres when exposed to these conditions but randomized to participate in the intervention. In contrast, youth with AA/AG genotypes showed no statistical difference in telomere lengths across all parental support and intervention conditions.

These findings can be interpreted in the context of genetic sensitivity and the Social Sensitivity hypothesis for oxytocin (Bartz et al., 2011). According to this hypothesis, oxytocin functions to increase the salience of social cues and thus influences an individual's sensitivity to the social environment, both positive and negative. Applied to the *OXTR* genetics literature, the rs53576 G allele has been conceptualized as conferring increased social sensitivity (Bartz et al., 2011). The current study further supports this interpretation and extends the *OXTR* literature by suggesting that differential phenotypic outcomes by environmental context may be present at the level of telomere length outcomes. In the current study, GG individuals evidenced greater variation in telomere length outcomes in association with environmental context. This finding suggests that the adverse social aspects of high conflict and low warmth parent-child environments, and the positive aspects of intervention participation and parental engagement in socioemotional and instrumental support development may be particularly relevant and impactful for these individuals.

While intriguing, there are some important points to consider when interpreting the *OXTR* genetic findings. First, the biological function of allelic variation of rs53576, or of genotypes in linkage disequilibrium, is still not known. The presence of rs53576 in the large 3rd intron of the gene may point to potential influence on gene regulation (Gimpl & Fahrenholz, 2001; Mizumoto, Kimura, & Ivell, 1997) but research exploring mechanistic questions is needed. Following this, grouping of the heterozygote AG individuals has primarily been influenced by sample size limitations, with the majority of studies grouping AG with AA individuals due to a limited sample size of AA genotypes. While A-dominant (AG/AA) grouping can facilitate appropriate statistical testing and social sensitivity has been reported for both G-dominant (Bradley et al., 2011; 2013) and A-dominant (Sturge-Apple et al., 2012) grouping, it is plausible that grouping may influence the associations found (Smearman et al., 2014) and supports further exploration of allelic grouping and underlying biological mechanisms.

According to the Social Sensitivity hypothesis, it may be expected that those with the GG genotype would evidence the longest telomeres when exposed to highly supportive parenting and intervention participation, above those of the AA/AG genotype. However, telomere lengths across these groups were not statistically different. This may be due to limitations of the study measures or current sample. The parenting scales may have not been as sensitive on the higher end of positive parenting; however, while conflict was primarily focused on adverse interactions, warmth and emotional support focused on more positive interactions. Sample size is an important consideration for genetic interaction studies, as discussed in detail below, and it is also possible that the differences may become apparent in a larger sample. However, it is plausible that this finding is representative of the sample. This sample is drawn from a rural community of primarily working poor who may be experiencing additional sources of stress that could limit the upper end of telomere length outcomes. While SES risk was included in the study, those with the GG genotype may be influenced by other potential sources of stress even if parenting-based stressors are not present or are addressed through the intervention. Finally, it is also possible that those

with the GG genotype may be more impacted by negative social environments on telomere length outcomes than positive environments, with telomere lengths in positive environments resembling lengths of those with the A allele. These questions could be addressed through additional research and thorough assessments of social stress and positive social environments on long-term telomere length outcomes across *OXTR* genotypes.

The current study is not without limitations. The study was conducted with an African American sample of relatively low SES. Therefore, these results may have limited generalizability. However, few studies have utilized African Americans, particularly African American youth, in assessments of telomere length outcomes (Mitchell et al., 2014) and *OXTR* genetic moderation on phenotypic outcomes (adult samples: Bradley et al. 2011; Bradley et al. 2013). Therefore, the study provides an important addition to the literature by utilizing this population.

As with all gene-environment studies, sample size limitations are an important consideration. The current sample size is relatively small, highlighting concern for spurious findings (Duncan & Keller, 2011). Therefore, replication is warranted. Considering this, there are some unique aspects to utilizing this sample for an interaction analysis. Intervention programs allow for direct modification of the environment in a controlled setting, allowing researchers to more directly assess the relationship between varying environments and phenotypic outcomes (Brody et al., 2014; 2013b; Rutter, 2005). This direct modification provides a more controlled assessment of these relationships, as well as whether the relationships may be moderated by genetic status. Added to this, the current sample provides opportunity for exploration of both negative and intentionally more positive environments through non-supportive parenting assessments and direct implementation of a program intended to promote positive relationship development and family-based buffering processes. Future research drawing from larger samples will allow for replication and further exploration of these findings.

Finally, telomere assessment was collected at 5 years post intervention, in response to the growing literature in this field. While informative, this collection timing is limited and future research utilizing both pre and post telomere assessments should be conducted to more directly test questions of telomere length change over time, as well as the potential for genetic moderation of this change.

Conclusions

The telomere literature has continued to grow with numerous studies to date reporting associations between exposure to chronic stress and shorter telomere length outcomes (Price et al., 2013). In addition to these negative environments, positive social environments, such as healthy social ties (Uchino et al., 2012), and greater perceived social support (Carroll, Diez Roux, Fitzpatrick, & Seeman, 2013), have now repeatedly been associated with longer overall telomere lengths. Our research group previously reported that implementation of a prevention program aimed at promoting positive parent-child interactions and family-based stress buffering processes may result in greater overall telomere lengths among those originally exposed to stressful, non-supportive parenting environments (Brody et al., 2015). Extending this into the growing literature of genetic sensitivity to environmental contexts, the current study found that *OXTR* genetic variation associated with environmental contexts at this biological level. As hypothesized, individuals with the rs53576 GG genotype evidenced greater variability, showing the shortest telomere lengths across 5-years when exposed to non-supportive parenting and randomized to the control condition, yet similar telomere lengths to non at-risk groups when randomized to the intervention. In contrast, those with the A allele showed no statistical difference in telomere lengths across all parental support and intervention conditions. These findings highlight the role of prevention in attenuating the impact of stressful exposures yet the potential importance of individual differences in genetic sensitivity to these contexts.

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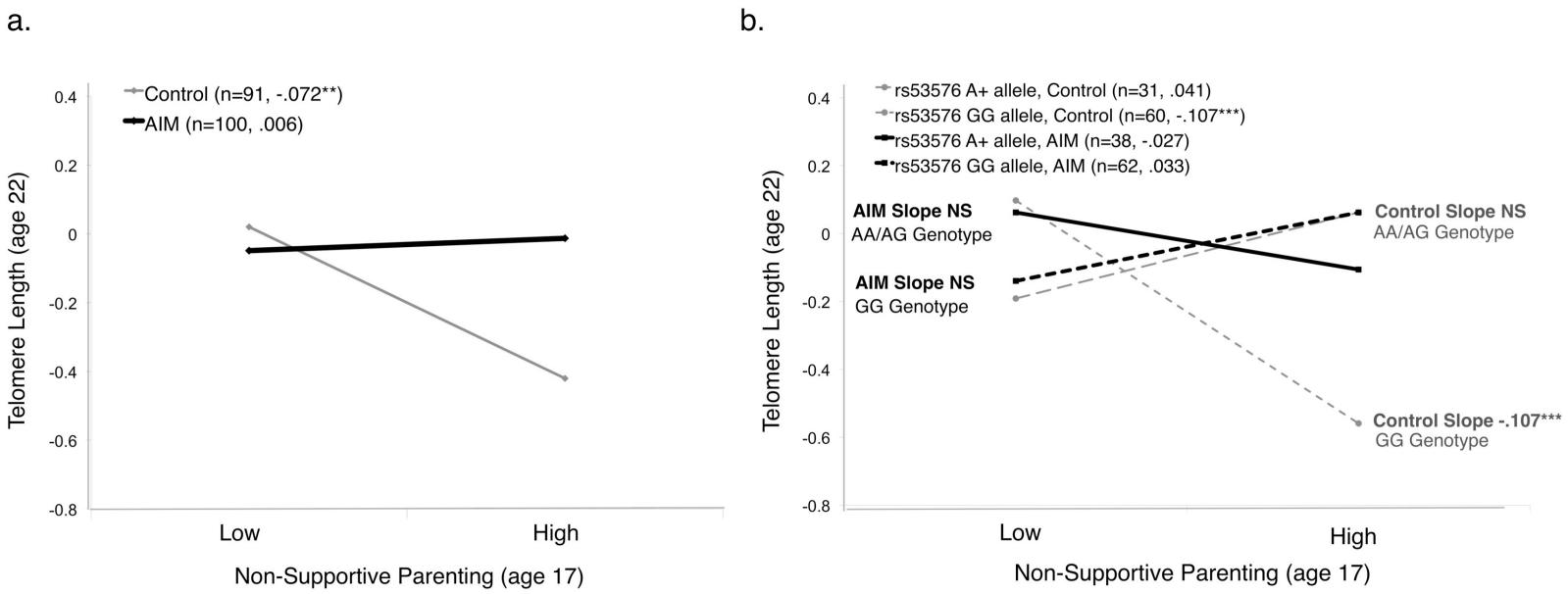
Figure 4.1. Non-Supportive Parenting and Telomere Length Outcomes by Intervention and Genotype Status



Note. Significant study interactions are depicted using simple slopes procedures for ±1 standard deviation of non-supportive parenting. Graphs display the association between non-supportive parenting and telomere length outcomes across 5 years by a. intervention condition and b. intervention condition and *OXTR* rs53576 genotype. Numbers in parentheses refer to simple slopes for each group. $^{**}p < .01$. $^{***}p < .001$.

Table 4.1. Descriptive Statistics and Correlations among Study Variable (N = 191)

Variables	1	2	3	4	5	6	7	8
1. Gender, male	-							
2. Family socioeconomic risk (age 17)	.017	-						
3. Intervention, AIM	-.188**	-.002	-					
4. Non-supportive parenting (age 17)	.055	.017	.013	-				
5. Telomere length (age 22)	-.057	.029	.107	-.135	-			
6. Body Mass Index (age 22)	-.093	-.080	.098	-.002	-.080	-		
7. Blood pressure (age 22)	.417***	.063	-.080	-.015	-.036	.422***	-	
8. rs53576 (A+ genotype vs. GG)	-.022	-.032	-.041	.059	-.045	-.104	-.105	-
Mean or N	73	1.94	0.52	0	-0.11	30.13	-0.02	122
SD or %	38%	1.21	0.50	3.05	0.76	10.03	1.88	64%

**$p < .01$. ***$p<.001$.

Table 4.2. Non-supportive Parenting, Intervention Status, and *OXTR* rs53576 as Predictors of Telomere Length

	Telomere Length (age 22)								
	Model 1			Model 2			Model 3		
Predictors	*B*	*SE*	β	*B*	*SE*	β	*B*	*SE*	β
1. Gender, male	-.113	.132	-.073	-.098	.131	-.063	-.128	.132	-.082
2. Family SES risk (age 17)	.013	.041	.023	.011	.040	.019	-.009	.041	-.017
3. Body Mass Index (age 22)	-.007	.006	-.098	-.009	.006	-.123	-.011	.006	-.149
4. Blood pressure (age 22)	.013	.038	.032	.015	.037	.038	.017	.037	.043
5. Non-supportive parenting (age 17)	-.032	.018	-.131′	-.072	.025	-.291**	.041	.051	.167
6. Intervention, AIM	-	-	-	.169	.110	.112	.043	.182	.028
7. rs53576 (A+ vs. GG)	-	-	-	-	-	-	-.166	.167	-.106
8. Parenting x AIM	-	-	-	.078	.036	.223*	-.069	.063	-.197
9. Parenting × rs53576	-	-	-	-	-	-	-.149	.060	-.487*
10. AIM x rs53576	-	-	-	-	-	-	.149	.226	.093
11. Parenting x AIM x rs53576	-	-	-	-	-	-	.209	.077	.453**

Note. *SE* = standard error. ′p = .07. *p < .05. **p < .01.

Model 1: $F(1, 185) = 3.257$, $p = .073$, $\Delta R^2 = .017$ (parenting main effect)

Model 2: $F(1, 183) = 4.812$, $p = .030$, $\Delta R^2 = .025$ (parenting x AIM)

Model 3: $F(1, 179) = 7.389$, $p = .007$, $\Delta R^2 = .037$ (parenting x AIM x rs53576)

CHAPTER 5
Summary & Conclusions

Early life adversity is known to have a significant impact on future health and well-being (Harkonmäki, Korkeila, & Vahtera, 2007; Miller, Chen, & Parker, 2011). There are a variety of proposed mechanisms by which early adversity may have these impacts and why individuals may be more or less at risk (Miller et al., 2011). A large portion of literature exploring these mechanisms has focused on the role of biological differences, such as differences in an individual's genetic code. In this case, an individual's genotype may influence the degree to which he or she responds to an adverse environment and develops a certain outcome. A number of genotypes have been studied, with particular focus on those involved in neurotransmission. These studies have suggested a pattern of differential susceptibility, where individuals with a specific genotype evidence greater phenotypic outcome following environmental exposures, both good and bad (Belsky & Pluess, 2009). Genotypes within the oxytocin receptor gene are suggested to confer this enhanced susceptibility to the environment. Specifically, the rs53576 SNP, one of the most commonly studied SNPs in *OXTR*, has been linked with differential sensitivity to the social environment (Bartz, Zaki, Bolger, & Ochsner, 2011). Individuals with the GG genotype evidence more prosocial and positive outcomes in positive environments (Krueger et al., 2012), yet also more maladaptive and negative outcomes in adverse social environments (Bradley, David, Wingo, Mercer, & Ressler, 2013; Bradley et al., 2011). The primary goal of the three studies presented here was to extend this literature, further exploring the role of *OXTR* genetics in socially adverse contexts, as well as in responsiveness to intervention programming.

The first and third study focused primarily on the interplay between environmental contexts and the *OXTR* rs53576 SNP. The first study (Chapter 2) provided support for the social sensitivity conceptualization of *OXTR* rs53576. This study tested an interaction between interpersonal stress and *OXTR* genotype on conduct and antisocial behaviors at two developmental time points, age 15 and 20. We found that GG individuals reported higher levels

of antisocial behaviors at age 20 if exposed to interpersonal stress. In contrast, AA individuals did not show this association; interpersonal stress was not associated with engagement in antisocial behaviors. These findings may suggest enhanced sensitivity, and thus responsively, to interpersonal stress among GG individuals. However, it is important to note that the direction of this effect cannot be determined given the cross-sectional nature of the data collection.

At age 15, we found a direct effect where GG individuals reported higher levels of conduct behaviors regardless of exposure to interpersonal stress. Conduct-like behaviors are more normative during this time period (Moffitt, 1993), therefore it is plausible that individuals who are more sensitive to the social environment, due to having the rs53576 G allele, may be more likely to engage in these behaviors. Replication is warranted, and future research that can delve into the behavioral mechanisms underlying this finding, such as whether individuals with the G-allele may be more sensitive to social pressures to engage in conduct like behaviors, will aid in interpretation.

The third study (Chapter 4) expanded the literature surrounding *OXTR* rs53576 and socially adverse contexts by additionally incorporating the role of intervention participation. The hypothesis of social sensitivity for GG individuals was again supported. In this study, parent-youth pairs were assessed at baseline on the degree of conflict in the parent-youth relationship as well as degree of emotional support and warmth. Parent-youth pairs were then randomized to participate in a family-based intervention program or a control condition. After 5-years, a follow up was conducted in which youth provided blood samples for telomere length assessments, a biomarker for exposure to chronic stress that is predictive of future poor health and mortality (Cawthon, Smith, O'Brien, & Sivatchenko, 2003; Epel et al., 2004). While participation in the intervention attenuated the link between exposure to high parent-youth conflict and shorter telomeres lengths across 5 years, suggesting an impact of the intervention at this biological level, these effects were moderated by *OXTR* genotype. Individuals with the GG genotype evidenced the shortest telomeres (more adverse) when exposed to high parent-youth conflict and

randomized to the control condition, yet longer telomeres when randomized to the intervention. In contrast, AA/AG individuals evidenced similar telomere lengths across all conditions. These findings suggest less environmental responsively among AA/AG individuals at this biological level of telomere length. This study, addressing both socially adverse and intentionally more positive contexts, suggests that GG individuals, compared to AA/AG individuals, exhibit the enhanced sensitivity to environmental contexts.

While the majority of work to date has explored differences in the DNA sequence in association with behavioral outcomes, genetics does not function alone and epigenetics is also important to consider. One of the most commonly studied epigenetic markers is DNA methylation, where a methyl group binds to DNA and can influence gene regulation and expression (Bird, 2002). Methylation is commonly recognized for its role in decreasing gene expression when occurring in the promoter region of a gene, though it may also associate with increased gene expression (Maunakea et al., 2010). Importantly, much of the excitement around epigenetics surrounds its documented ability to change in response to the environment, particularly for genes involved in the stress response system (McGowan et al., 2009; Szyf & Bick, 2012). While there is a newly growing literature exploring *OXTR* epigenetics and behavioral outcomes and traits, ours was the first to explore the role of early adversity and *OXTR* methylation with behavioral outcomes. Specifically, we tested whether *OXTR* methylation played a role in the link between abuse in childhood and adult depression and anxiety outcomes.

Our second study found that abuse during childhood was associated with higher methylation at two CpG sites, both located within exon 3 of the gene, but this finding did not survive correction for multiple testing. In addition, these sites did not serve as a mediator to depression and anxiety. While it is possible that the early environment may influence methylation patterns of this gene, this finding suggests that this effect may be limited for this gene. The *OXTR* epigenetics literature is relatively new, and it is currently unknown whether methylation of *OXTR* may respond to the environment. While one study suggests the potential for small rapid changes

in methylation of the 3rd exon of *OXTR*, the same location where we saw CpG sites associate with abuse exposure, it is possible that *OXTR* methylation is relatively stable across environmental exposures. Otherwise, it may be possible that *OXTR* methylation, particularly in the 3rd exon, is most responsive to current stressful contexts, and that our findings represent higher levels of current stress rather than an association with early stress.

While methylation did not serve as a mediator between abuse and mental health outcomes in our study, it is plausible that it could serve as a moderator, and this is indeed what was found. Abuse interacted with methylation of multiple CpG sites in *OXTR* to predict depression and anxiety outcomes. Interestingly, we found that lower levels of methylation in exon 1, located in the promoter region of *OXTR,* were associated with higher levels of depression and anxiety following abuse. A recently published study on human *OXTR* epigenetics suggests the potential for similar findings (Reiner et al., 2015). In this study, individuals with depression, compared to matched controls, had lower levels of methylation in the 1st exon of *OXTR* (Reiner et al., 2015). While this study did not report on levels of abuse or adversity among these groups, it is possible that these findings overlap given that lower exon 1 methylation levels associated with higher depression in our study if the individual was exposed to abuse. These studies suggest that *OXTR* epigenetics may be important to consider in the etiology of depression.

Exploring the interplay between our environment, our genetics, *and* our epigenetics is relatively new yet has the potential to provide additional insight into the development of behavioral and health outcomes. When incorporating epigenetics into the GxE literature, an initial question to address is how genetics and epigenetics function together. Our 2nd study (Chapter 3) identified a number of associations between *OXTR* SNPs and the methylation of specific *OXTR* CpG sites. These associations point to the potential for a mechanistic link for SNP-behavioral outcomes, whereby the association with methylation links SNPs to factors that may directly influence gene regulation and expression (Bell et al., 2012). However, while our study found overlap in genetic and epigenetic associations with abuse and psychiatric outcomes, genetic and

epigenetic differences also tracked independently, suggesting that other mechanisms also underlying SNP-behavioral associations.

Limitations

The current studies provide support for the social sensitivity hypothesis for oxytocin (Bartz et al., 2011), however these studies should be considered in the context of a number of limitations. The first critical limitation for this and all gene-environment work is sample size. Small sample sizes increase the risk for spurious findings (Duncan & Keller, 2011), highlighting the need for replication. While the sample size of each of our studies was relatively limited, the first study (Chapter 2) was on the higher range of current *OXTR* behavioral genetic studies, and the 2nd study (Chapter 3) utilized a sample that was considerably larger than much of the of *OXTR* methylation studies to date, which often have less than 100 participants (Kumsta, Hummel, Chen, & Heinrichs, 2013). Furthermore, our 3rd study's use of a randomized intervention design (Chapter 4) provides a unique opportunity for GxE interaction testing, given that the environment is modified in a more controlled setting. While each of these studies have unique strengths regarding their sample, and the findings add to the growing literature on the role of *OXTR* genetics and epigenetics, results should be interpreted with caution given this limitation. Future work that draws from larger samples, especially those assessing the interplay between genetics and epigenetics, will be critical for this field.

Another important consideration in the interpretation of these findings is the cross-sectional nature of much of the data. In the first study, interpersonal stress interacted with *OXTR* genotype at age 20 to predict antisocial behaviors. It is plausible that individuals who engage in antisocial behaviors may be more likely to report higher levels of interpersonal conflict, particularly if they are more sensitive to the social environment. Therefore, directionality of this association is not clear. In our epigenetics study, we found that abuse may influence methylation of CpG sites within the 3rd exon of *OXTR*. However, this association did not hold when

accounting for multiple testing. Of question is whether social adversity, such as abuse, may in fact influence methylation across *OXTR*, and whether these methylation changes may occur throughout life or during specific time periods. For example, while we found a nominal association with abuse, it is possible that these findings may better represent current social stress. The degree to which areas of this gene are responsive to environmental contexts, and whether this responsively varies across time, is still unknown. Longitudinal studies will be critical in advancing understanding and interpretation of cross-sectional epigenetic data. Importantly, studies are needed that address the degree to which epigenetic patterns may be malleable across the lifespan, compared to during specific developmental time points, and the degree to which specific genes are more or less responsive to environmental context. This research will shed light into how to best conduct and interpret studies addressing the interplay between environmental contexts, epigenetics, and behavioral and health outcomes.

Though a longitudinal design was implemented in the prevention study (Chapter 4), the outcome of interest, telomere length, was only assessed at the final follow-up 5 years post intervention. Therefore, while initial results suggesting that intervention participation may influence telomere length over time, and that this may be moderated by *OXTR*, this study could be strengthened by incorporating two time points of telomere length assessment. Future research that utilizes both pre- and post- assessments will allow for more direct tests of causality and the temporal nature of these findings. However, one strength of the current study is the use of a randomized controlled design. Therefore, while still limited, this design allows for telomere length outcomes to be more confidently attributed to intervention participation.

A third limitation across each study is the generalizability of study findings to the general population. The first study utilized a sample of Caucasian youth from Brisbane, Australia. The second drew from a cohort of African American adults from Atlanta, GA exposed to high levels of trauma and abuse. The third sample consisted of African American adolescents from rural Georgia, with data from parent-adolescent pairs. Importantly, while each study individually may

have limited generalizability, the continued support of the social sensitivity hypothesis for oxytocin and *OXTR* genetics across these various samples is of note. These samples span different races, geographic regions, and developmental time frames, thus lending added support to the hypothesis of social sensitivity. These findings suggest that the rs53576 G allele, and potential certain CpG methylation patterns across *OXTR,* may confer enhanced sensitivities across a variety of demographics and populations.

A final important limitation in the interpretation of this research is that the function of the majority of *OXTR* SNPs and CpG sites are as of yet unknown. In the case of *OXTR* rs53576, some studies suggest that rs53576 genotypes associate with differences in hypothalamic size and connectivity between the hypothalamus and amygdala, which may influence the rewarding and motivational aspects of social interactions (Tost et al., 2010; Wang et al., 2013). Our study extended the question of SNP-mechanism by exploring the potential for associations between SNPs and nearby CpG site methylation. It is possible that methylation may provide a mechanistic link between allelic variation and functional gene regulation. Indeed, our study found evidence of these associations within *OXTR*, supporting recent literature examining genome wide associations (Bell et al., 2012; A. K. Smith et al., 2014). In regards to rs53576, we found that the rs53576 G allele was associated with increased methylation at a CpG site within the 3rd intron of *OXTR*. Intriguingly, increased methylation at this site was subsequently associated with reports of higher levels of depression when exposed to abuse. The rs53576 G-allele itself has been linked with increased risk of depression following abuse (McQuaid, McInnis, Stead, Matheson, & Anisman, 2013), and a recent meta-analysis supports a role for *OXTR* rs53576 in depression outcomes, with the majority of studies finding an association (Jacondino, Borges & Gottlieb, 2014). Furthermore, a subsequent study has found an association between rs53576 and methylation within exon 1 of *OXTR* (Reiner et al., 2015). Therefore, it is plausible that these SNP-CpG methylation associations provide a potential mechanistic route through which some SNPs may have an effect.

While methylation is known to influence gene regulation and expression (Bird, 2002), our studies did not evaluate gene expression and cannot conclude whether the CpG's found to be significant in our study directly influence *OXTR* regulation. Previous work suggests functional significance of CpG sites within the *OXTR* promoter region as well as within intron 3 in both cell culture and initial human work (review: Kumsta et al., 2013). For example, Gregory et al. (2009) utilized human temporal cortex brain tissue and identified several CpG sites that may associate with *OXTR* expression. Specifically, higher methylation of these sites in peripheral blood samples was associated with lower *OXTR* mRNA in the temporal cortex (Gregory et al., 2009). Furthermore, cell line research supports a functional role of CpG methylation as well, suggesting that increased methylation in the promoter region of *OXTR* results in reduced expression (Kumsta et al., 2013). While this supports the potential for CpG sites in our study to serve a functional role in *OXTR* expression and regulation, future research directly testing this question will aid with interpretation of our study results.

A final concern to note is that appropriate allelic grouping of rs53576 is not currently known, and has been mixed in the literature. A number of studies have utilized G-dominant (Bradley et al., 2011; 2013) and A-dominant (Sturge-Apple, Cicchetti, Davies, & Suor, 2012) grouping. Our first study suggests that grouping may be important to consider as it may influence whether an association is identified as significant. However, previous GxE findings have suggested increased social sensitivity for the G-allele for both G-dominant and A-dominant grouping (Bradley et al., 2011; Sturge-Apple et al., 2012). Furthermore, in a number of studies, A-dominant grouping has allowed for appropriate statistical testing given the limited sample size of AA individuals in these studies. The biological impact of these allelic differences will provide increased insight into the question of appropriate allelic grouping, and grouping should be kept in mind when interpreting results. For example, it is plausible that the G-allele may result in greater expression of *OXTR* in certain areas of the brain, where GG individuals may have the highest expression (with some variability remaining due to the influence of other SNPs and regulatory

elements). Therefore, using allele count (0, 1 or 2 G alleles), as employed in the second study, may best align with the biological data. If this were the case, it may point to a mechanism by which all groupings (G dominant, A dominant, and allele count) have provided overall support for G-sensitivity, give that the GG or AG group overall could have higher expression. A recent study by Myers et al. (2014) provides initial data on the question of whether *OXTR* SNPs may associate with *OXTR* expression in human brain tissue. Though rs53576 wasn't tested in this study, the authors found associations between a number of *OXTR* SNPs and expression levels, supporting the potential for this mechanistic relationship. Both human and animal work that continues to explore associations between *OXTR* expression levels, particularly in brain regions related to reward or social behaviors, in association with rs53576 as well as other *OXTR* SNPs of interest will strengthen future study designs and interpretation.

Future Directions

There are a number of intriguing future avenues of research stemming from this work. First, much of the current work has explored socially adverse environments, such as abuse or interpersonal stress. However, a large portion of the animal literature has utilized maternal separation paradigms (Champagne, 2008; Meaney, Szyf, & Seckl, 2007), which may better represent neglectful environments. Whether socially adverse, versus socially neglectful, environments differentially interact with *OXTR* genetics and epigenetics is of yet unknown. While individuals who are more sensitive, such as those with the *OXTR* G-allele may be more sensitive to adverse social environments, they may be more resilient in environments with relatively low social interactions. For example, Thompson et al., (2014) found that rs53576 moderated the association between exposure to maternal depression and adolescence depressive symptoms. Intriguingly, among those exposed to maternal depression, it was those with the A-allele that then evidenced the greatest level of depressive symptoms in adolescence. It is possible that maternal depression represents a low level of social interaction, better reflecting animal models of maternal separation or neglect, whereby those with low oxytocin signaling may be

most impacted. On the other hand, even the low levels of social interaction may be sufficient for the G-allele individuals, and they may be less impacted. Future research that directly explores neglectful versus abusive conditions will help tease apart whether these environments have differential effects in regards to *OXTR*.

A second future direction is to more fully tease apart the nature and duration of stressful exposures. Numerous studies have reported an anxiolytic effect of oxytocin, where oxytocin is thought to calm the stress response system (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003) potentially through attenuated cortisol release (Neumann, 2002) and reduced amygdala activation (Domes, Heinrichs, & Glascher, 2007). This may initially appear to contradict the above studies and literature discussed, where higher oxytocin, or the *OXTR* G-allele, is thought to associate with more adverse outcomes following exposure to stressful environments. One potential avenue for exploring this question is to address the duration of the stressful exposure. It may be that oxytocin functions as an anxiolytic for short-term stressors, yet may result in more adverse outcomes when the stressor occurs over an extended time-period given the enhanced sensitivity to these contexts. A second avenue is to address the nature of the stressful exposure, specifically the degree to which it has a social component. For example, there may be different mechanisms by which oxytocin influences physiologic reactivity in the context of a non-social stressor compared to social-stress. Oxytocin is hypothesized to reduce fear and activation of the stress response system and it is also hypothesized to increase perception and salience of social cues (Bartz et al., 2011). It may be that oxytocin reduces response to stressful signals, but that socially relevant stress signals may lead to a heightened physiologic response through different neural pathways or mechanisms. Therefore, while oxytocin levels, or certain oxytocin genotypes, may reduce some forms of stress reactivity, they may lead to enhanced reactivity to distress signals that are relevant for bonding and interpersonal dynamics. Future work that emphasizes thoughtful inclusion of a variety of stressors, including social (such as interpersonal conflict) and non-social (such as a loud-noise) stressors, as well as short-term versus long-term stressors, will

help tease apart these questions and provide a better understanding of how the environment and oxytonergic signaling interact to influence behavioral and health outcomes.

Public Health Implications

The current work highlights the role of individual genetic and epigenetic differences underlying variation in sensitivity to stressful social environments. As such, there are important public health implications of this work. Findings from this work suggest a role for *OXTR* genetics in understanding mechanisms that underlie risk and resilience to adverse social environments. Importantly, these findings suggest that this sensitivity spans a variety of adverse social environments, including differences in timing (concurrent versus early life) and across individuals (peers and parents), with this work showing sensitivity to interpersonal stress, childhood abuse, and parent-youth relationships with high conflict and low warmth and support. Furthermore, this work extends these findings into the prevention literature. Individuals with the rs53576 GG genotype, which were found to be most sensitive to social adversity in the first study, were also most responsive to an intentionally more positive environment – a family based intervention program intended to improve parent-youth relationships. These findings are promising in that they suggest that individuals who may show the greatest risk to adverse social environments given their underlying oxytonergic biology, may also be the most positively impacted by intervention efforts. This thus highlights the importance of utilizing interventions to help attenuate the association between adversity and poor behavioral and health outcomes.

Conclusions

Exploring the interplay between our environment, our genetics, and our epigenetics has the potential to provide enhanced insight into the development of behavioral and health outcomes. The oxytocin system is an important candidate in this understanding. The current literature, and the studies covered in this dissertation, continue to support the proposed conceptualization of oxytocin as conferring enhanced sensitivity to the social environment (Bartz et al., 2011). In the studies presented here, the *OXTR* rs53576 G-allele, as well as certain *OXTR* methylation patterns,

were associated with enhanced sensitivity to a variety of adverse social environments, ranging from interpersonal stress, to abuse, to parent-youth relationships. Furthermore, the rs53576 G-allele was also associated with increased sensitivity to an intentionally more positive social environment, a family-based intervention program. The current studies add to a growing literature on the role of oxytocin genetics and epigenetics in the impact of social environments on behavioral and health outcomes.

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