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Genetic and Environmental Predictors of Empathy in Children at Risk for an Autism Spectrum Disorder

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GENETIC AND ENVIRONMENTAL PREDICTORS OF EMPATHY IN CHILDREN AT RISK FOR AN AUTISM SPECTRUM DISORDER

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Individuals with autism spectrum disorders (ASD) have difficulty empathizing with others. These empathy deficits are apparent from as early as 12 months of age and predict later ASD diagnosis and symptom severity; however, the factors that influence empathy development in children at risk for an ASD are not clear. In typically developing samples, genetic factors, such as the oxytocin receptor gene (OXTR), and environmental factors, such as early parent-child interactions, contribute to individual differences in empathy. In this study, I investigated the influence of OXTR variants and characteristics of early parent-child interactions on later empathic behavior in toddlers at high- and low-risk for an ASD. Additionally, the influence of OXTR on ASD symptom severity was explored within the high-risk group. ASD risk status was defined by the presence or absence of an older sibling with an ASD. Parent-child interaction was measured during free play sessions at 15 and 18 months of age. Empathy was measured through the children's responses to their parent's simulated distress at 24 and 30 months. ASD symptom severity was measured with the Autism Diagnostic Observation Schedule.

A dyadic parent-child interaction variable, affective mutuality, predicted later empathic behavior. By contrast, a parenting composite variable, emotional supportiveness, did not predict later child empathy. There were no significant main effects for either OXTR marker (rs53576 or rs2254298); however, the genotype for one

of the markers (rs53576) moderated the relation between affective mutuality and later empathic behavior. There was no direct relation between OXTR and ASD symptom severity. This is the first study to investigate predictors of empathy in a sample including children at heightened risk for an ASD. Findings suggest that genetics factors, such as OXTR, and early parent-child interactions interact to influence individual differences in empathy in children at both high- and low-risk for an ASD.

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CHAPTER 1: INTRODUCTION

Individuals with autism spectrum disorders (ASD) demonstrate difficulty empathizing with others (i.e., identifying with others' emotional experiences). These empathy deficits are apparent from as early as 12 months of age and predict later ASD diagnosis and symptom severity (Hutman et al., 2010; McDonald & Messinger, in press). While there is evidence to suggest that empathy development is influenced by specific genetic and environmental factors in typically developing children, the factors that influence the development of this essential ability in young children at risk for an ASD have not yet been investigated. The current study examined whether a specific genetic factor (i.e., the oxytocin receptor gene), and an environmental factor (i.e., early parent-child interactions) influenced the later empathic responding of children at high- and low-risk for an ASD.

Early Empathy Development in Typically Developing Children

The emergence of empathy has been well documented among typically developing children. In the first days of life, infants demonstrate precursors to empathy through reflexive crying in response to other infants' cries (Martin & Clark, 1982; Sagi & Hoffman, 1976). Personal distress in response to others' negative emotions is characteristic of pre-empathic behavior during the first year of life. Within the second year of life, children commonly transition from experiencing personal feelings of distress in response to another's distress to demonstrating concern for others (Zahn-Waxler, Radke-Yarrow, Wagner, & Chapman, 1992). By approximately 18 months of age, a majority of typically developing toddlers display concern about others' distress (e.g., sad look, "I'm sorry"), and are capable of a wide variety of helping behaviors (e.g., verbal or

physical comfort, sharing, distracting the person in distress; Knafo, Zahn-Waxler, Van Hulle, Robinson, & Rhee, 2008; Zahn-Waxler et al., 1992). The current study utilized a simulated distress paradigm to measure early empathic responding to parental distress during the third year of life.

Within this typical developmental course, however, there is variability in empathic abilities. These individual differences in empathy predict both optimal and problematic social outcomes. For example, empathy is positively related to prosocial behavior, social competence, and relationship satisfaction (Cramer, 2003; Eisenberg & Miller, 1987; Eisenberg, Miller, Shell, McNalley, & Shea, 1991; Saliquist, Eisenberg, Spinrad, Eggum, & Gaertner, 2009; Zhou et al., 2002). Conversely, lower levels of empathy place children at risk for adjustment problems, such as antisocial behavior (e.g., Hastings, Zahn-Waxler, Robinson, Usher, & Bridges, 2000; Miller & Eisenberg, 1988). The present study included a typically developing, as well as an at-risk group, to investigate predictors of individual differences in empathy.

Early Empathy Deficits in Autism Spectrum Disorders

Although the early development of empathy in typically developing children is well established, less is known about the development of empathy in the context of risk for an ASD. Multiple theories of autism, including the extreme male brain theory (Baron-Cohen, 2002), the mirror neuron hypothesis (e.g., Oberman & Ramachandran, 2007), and the 'theory of mind' theory (e.g., Baron-Cohen, Alan, & Frith, 1985) posit a central role for empathy deficits in the disorder. The latter theory proposes a prominent role of deficits in the ability to understand the perspectives of others, similar to the concept of cognitive empathy, in contributing to the pervasive social and communication deficits

present in individuals with ASDs (Baron-Cohen et al., 1985; White, Hill, Happe, & Frith, 2009).

A growing literature provides support for a central role of empathy deficits in emerging ASD. Specifically, multiple studies have found that preschool-aged children with ASDs display less empathic behavior, including decreased attention to the person in distress and less apparent concern, than children with developmental delays, intellectual disabilities, and mental-age matched typically developing children (Bacon, Fein, Morris, Waterhouse, & Allen, 1998; Charman et al., 1997; Sigman, Kasari, Kwon, & Yirmiya, 1992).

Notably, two recent studies investigated whether these deficits were present very early in development and prior to ASD diagnosis, in separate samples of children at heightened risk for an ASD. First, Hutman et al. (2010) found that children later diagnosed with an ASD paid less attention to and displayed less affective response to an examiner's distress than comparison children (high- and low-risk children with no later diagnosis), from as early as 12 months of age and after controlling for verbal abilities. Second, in a subset of the current sample, McDonald and Messinger (in press) found that high-risk children who responded more empathically to their parent's distress at 24 months of age exhibited lower levels of ASD symptomatology at 30 months. Despite mounting evidence for the presence and importance of empathy deficits in young children with emerging ASD, very little is known about possible contributors to the development of empathy within this population; however, studies of typical development have illuminated several possible factors that may influence empathy development.

Genetic and Environmental Influences on Empathy Development

In a longitudinal study of twins (Zahn-Waxler, Robinson, & Emde, 1992), both genetic and environmental components were implicated in the development of empathy in a typically developing sample. Responses to simulated distress were measured in monozygotic and dizygotic twins at 14 and 20 months of age. Findings indicated significant heritability at both ages for various empathy-related responses (e.g., empathic concern, unresponsive-indifferent behavior). Knafo, Zahn-Waxler, Van Hulle, Robinson, and Rhee (2008) extended the study's findings with a larger twin sample and the addition of older ages. They focused on the relative contributions of genetics and shared environment to the development of empathy. By 24 and 36 months of age, heritability was associated with one-third to almost one-half of the variation in children's empathic behaviors, with non-specific environmental factors believed to account for the remainder. While these studies demonstrate the relative importance of both genetic and environmental influences on typical empathy development, specific contributors to this development are less clear.

Genetic: Oxytocin Receptor Gene. One possible contributor to empathy is the oxytocin receptor gene (OXTR). Research with animals and humans suggests that oxytocin plays an important role in social behavior and social cognition, as well as the formation and maintenance of social relationships (Donaldson & Young, 2008; Ebstein et al., 2009). Oxytocin is synthesized in specialized cells of the hypothalamus, and works as both a neurotransmitter/neuromodulator and a peripheral hormone (MacDonald & MacDonald, 2010). Within the brain, oxytocin is released to axons connected directly and indirectly to several critical brain regions, including those important for social

functioning (e.g., amygdala, anterior cingulate cortex, insula, striatum; MacDonald & MacDonald, 2010). Initial studies in the prairie vole indicate an important role for oxytocin in facilitating pair bonding (Carter, Williams, Witt, & Insel, 1992), in addition to possibly having more far-reaching effects on social functioning (Donaldson & Young, 2008; MacDonald & MacDonald, 2010; Neumann, 2008). Research in humans is less clear, partially due to the methodological and ethical limitations of conducting experiments in humans; however, recent studies involving intranasal and intravenous transmission of oxytocin have illuminated several possible effects of oxytocin on behavior.

Oxytocin inhalation and infusion have been shown to improve a number of behaviors that are important for successful social functioning. For example, Kosfeld, Heinrichs, Zak, Fischbacher, and Fehr (2005) conducted a double-blind, placebo-controlled study of the relation between oxytocin inhalation and trust. Participants in the oxytocin group demonstrated higher levels of trust than the placebo group, findings which were only evident in a social context (Kosfeld et al., 2005). Interestingly, there is also evidence to suggest that oxytocin inhalation increases gaze to the eye region, which may be one mechanism through which oxytocin facilitates social functioning (Guastella, Mitchell, & Dadds, 2007).

Research has also suggested a role for oxytocin in empathy-related behaviors and abilities. Domes, Heinrichs, Michel, Berger, and Herpertz (2007) measured empathic perspective taking, or cognitive empathy, using the Reading the Mind in the Eyes Task. They found that empathic perspective taking abilities improved, relative to controls, after receiving a dose of oxytocin. Oxytocin had more of an assistive effect for participants on

the test items requiring more sophisticated perspective taking skills. Similarly, Bartz et al. (2010) found that oxytocin selectively improved empathic accuracy in a typically developing, adult male sample. Only individuals who reported lower social competence exhibited an increase in empathic abilities after oxytocin inhalation. In fact, after oxytocin administration, the individuals with lower social competence, who in the baseline condition had lower empathic accuracy, were indistinguishable from those with higher social competence in the experimental condition. This supports the potential utility of using oxytocin for treatment of the social deficits in ASD.

Multiple studies have investigated the role of OXTR in empathy, as well as related social behaviors. OXTR spans ~19 kb and comprises four exons, which contain the protein coding regions, and three introns, which are the spaces between exons and are not involved in coding for protein synthesis (Inoue et al., 1994). The current study focused on two relatively well-studied single nucleotide polymorphisms (SNP) located in intron 3. One of these OXTR SNPs, rs53576, has shown an association with empathy, with individuals with at least one A allele exhibiting poorer empathic perspective taking skills, as measured with the Reading the Mind in the Eyes Task, and lower dispositional empathy (i.e., self-reported trait empathy) than those with two G alleles (Rodrigues, Saslow, Garcia, John, & Keltner, 2009). Moreover, the A allele of this SNP was associated with less self-reported temperamental sociality, decreased gray matter in the hypothalamus, and increased functional coupling between the amygdala and hypothalamus (Tost et al., 2010). These findings suggest that genotypic variations in OXTR SNPs may signal differences in functioning in important areas of the "social brain." In addition, multiple SNPs and haplotypes (i.e., combinations of SNPs) of OXTR

have shown associations with prosocial behaviors, as measured in a social decision-making game (Israel et al., 2009), which may be motivated by empathy (de Waal, 2008). Despite mounting evidence for the importance of oxytocin for social behavior, less is known about the potential influence of OXTR on empathic behavior in a developmental context. This study investigated the role of OXTR in the early empathic behavior of children at high- and low-risk for an ASD.

Following research positing an important role for oxytocin in social behavior, several studies have found intriguing evidence in favor of oxytocin playing a role in autism. Modahl et al. (1998), for instance, found that children with autism (mean age = 8.1 years) had lower levels of plasma oxytocin than typically developing children (mean age = 8.8 years). Several experimental studies have demonstrated that oxytocin inhalation improves social cognition abilities in adults with an ASD (e.g., Andari et al., 2010; Hollander et al., 2007). Additionally, a genome-wide screen for potential candidate genes associated with ASDs provided evidence for linkage at a specific location on the genome (3p24-26), which includes the location of OXTR (Ylisaukko-oja et al., 2005).

Despite this promising evidence for the role of OXTR in ASDs, studies examining specific SNPs and haplotypes in relation to ASD diagnosis and symptomatology have produced conflicting findings. The methods of these studies have typically assessed for overtransmission of OXTR alleles and haplotypes within family trios, including a child with autism, and his or her mother and father. While multiple studies have found positive associations with OXTR and autism (Jacob et al., 2007; Lerer et al., 2008; Liu et al., 2010; Wu et al., 2005), others have not (Tansey et al., 2010). Further complicating the issue, the particular SNPs associated with autism have been

relatively inconsistent across studies. The association of some SNPs has been replicated, including rs53576 and rs2254298 (Jacob et al., 2007; Lerer et al., 2008; Wu et al., 2005), which have also been studied in relation to OXTR and other areas of social functioning (e.g., Chen, Barth, Johnson, Gotlib, & Johnson, 2011; Domes et al., 2007); however, there is some inconsistency with regard to the "risk" allele in different populations. Specifically, while two Asian samples found overtransmission of rs53576A and rs2254298A (Liu et al., 2010; Wu et al., 2005), the former SNP was not associated and, for the latter SNP, the G, rather than the A, allele was associated with risk in a Caucasian sample (Jacob et al., 2007).

In spite of these inconsistent findings on the specific polymorphisms associated with ASD in different populations, OXTR is a promising candidate for associations with both empathy and ASD symptomatology in an at risk sample. Some of the inconsistency in the genetic findings with OXTR and autism may be due to the methodological limitations of relating this gene to the complex and varied behavioral profile of autism, rather than to specific behaviors, such as empathy (Geschwind, 2011). In addition, many of these studies have related variations in OXTR to the presence/absence of an ASD, when this gene may be more important in explaining variability in ASD symptom severity. Accordingly, I explored the association between two well-studied OXTR SNPs, rs53576 and rs2254298, and a continuous measure of ASD symptom severity in a sample of children at high-risk for an ASD.

Environment: Parent-Child Interaction. In addition to the potential influence of OXTR variants on empathy, specific aspects of the environment are thought to influence the development of empathy. In particular, parents provide essential

socialization experiences for their developing children. This may be especially true for moral development, as morality is embedded in social interactions, which generally first occur within the parent-child relationship (Kochanska, 2002). Empathy has been theorized to form the basis of moral and altruistic behavior, by providing a strong internal motivation for identifying with and helping others (de Waal, 2008). Several longitudinal studies have suggested an important role of early parent-child interactions on empathy in typically developing children.

Several specific parenting behaviors, such as warmth and discourse about emotions, are associated with empathy development. Robinson, Zahn-Waxler, and Emde (1994) measured the relation between maternal warmth and empathic responding in typically developing toddlers. Maternal warmth was measured in the context of parent-child interactions, and was composed of ratings of the mother's warmth, as well as responsiveness to and tolerance of the child's expressions of need. Children of parents who displayed higher maternal warmth at 14 months of age had higher levels of empathic responding to an examiner at 20 months. Similarly, Zhou et al. (2002) found that a global rating of parental warmth, measured during a parent-child interaction, predicted later levels of children's empathy. The way that parents talk to their children about emotions also appears to be an important factor in empathy development. Specifically, parental labeling of emotions is associated with children's emotional concern for others, and parental explanations of the causes and consequences of emotions showed an association with more child attempts to understand others' emotions (Garner, 2003). In addition to these specific behaviors, two prominent developmental researchers, Kochanska and

Feldman, have studied and developed two related mechanisms that tie aspects of parent-child interactions to moral and empathy development.

Kochanska proposes that a specific pattern of parent-child interactions, termed mutually responsive orientation (MRO), plays an important role in children's moral development (Kochanska, 2002). MRO reflects a close, mutually binding, cooperative, and positive parent-child relationship. Kochanska and her colleagues have studied the relation between MRO and aspects of child conscience, including moral emotion (i.e., empathy and/or guilt), moral conduct, and moral cognition, in a series of longitudinal studies involving typically developing toddlers. Kochanska, Forman, and Coy (1999) found that higher maternal responsiveness to their child, an important component of MRO, at 9 months of age predicted higher levels of children's empathic responding to maternal distress at 22 months. Furthermore, MRO, measured at toddler and preschool ages, predicted later conscience development, including moral cognition in response to hypothetical scenarios (Kochanska & Murray, 2000). In a more recent study, MRO, measured at 9 to 22 months of age, had a direct effect on moral emotion (i.e., guilt; observed at 45 months), and indirect effects on moral conduct and moral cognition (observed at 56 months; Kochanska, Forman, Aksan, & Dunbar, 2005). Kochanska has reasoned that children whose early development is embedded within these warm and responsive dyads will more eagerly embrace their parents' values and be more likely to develop a strong conscience (Kochanska, 2002).

In a similar vein, Feldman has demonstrated the importance of affective synchrony, or the temporal matching of affective behavior during parent-child interactions, on children's later empathy development. Feldman (2007) conducted a

longitudinal study of typically developing children that spanned from infancy to early adolescence. She found that mother-infant affective synchrony measured in the first year of life (3 and 9 months) was directly associated with empathy in childhood and adolescence (6 and 13 years). Specifically, the higher the degree of mother-infant affective synchrony and mutual influence during face-to-face play in infancy, the more empathy was expressed by the child during mother-child conversations that occurred during middle childhood and adolescence. This affective matching may provide children with two important experiences relevant to empathy development. First, it provides children with a model for empathic behavior, demonstrating that another can feel what they feel. Second, it may provide children with an understanding that their own emotionally motivated actions can influence another person, which may promote the feelings of efficacy necessary for acting on a desire to help others.

Despite evidence suggesting an important contribution of early parent-child interactions to empathy in typically developing children, little is known about the impact of similar experiences in children with or at risk for atypical empathy development (e.g., ASDs). Although there is a shortage of research on parenting influences on empathy in the context of ASDs, research has shown that parents of children with or at risk for an ASD can influence the development of other skills, such as language. For example, Siller and Sigman (2002; 2008) found that children with autism who had parents who were more synchronized and responsive to their interests and behaviors developed better joint attention and language abilities many years later, as compared to those with parents showing lower initial levels of synchrony and responsivity. In addition, in a sample of children at risk for an ASD (partially overlapped with current sample), children with

emergent ASD who had parents who displayed higher levels of sensitive structuring during play at 18 months of age experienced greater growth in language abilities between 24 and 36 months (Baker, Messinger, Kelley, & Grantz, 2010).

Intervention studies have provided further evidence for the influence of parenting behaviors on the development of children with ASDs. For instance, interventions focused on increasing adult interactional synchrony and responsiveness have led to increases in child imitation abilities (Landa, Holman, O'Neill, & Stuart, 2011) and joint engagement (Kasari, Gulsrud, Wong, Kwon, & Locke, 2010), which are important components of social-emotional development.

The parent-child interaction factors that promote optimal development in typically developing children were expected to do the same in children at risk for an ASD in the current study. A study by Fenning, Baker, and Juvonen (2011), which examined the influence of parent-child emotion discourse on children's social-cognitive skills in a sample of typically developing children and children with developmental delays, provides a model for this hypothesis. They found a comparable developmental model across the two groups. Namely, the parenting variable, emotion discourse, predicted children's social cognitive abilities, which, in turn, predicted children's social skills similarly across the typically developing and developmental delay groups. In addition, the quality of parenting does not appear to differ between parents of children with an ASD and of children with other developmental disabilities and typically developing children (e.g., Baker et al., 2010; Siller & Sigman, 2002; van IJzendoorn et al., 2007). Alternatively, a biological constraint model of autism has been proposed, based on findings such as the lack of association between parent sensitivity and secure attachment

in children with an ASD, suggesting less susceptibility to parenting influences in young children with or at risk for an ASD (van IJzendoorn et al., 2007). The present study examined the influence of important early parent-child interaction variables, including dyadic affective mutuality and parental emotional supportiveness, on later empathic behavior in children at high- and low-risk for an ASD.

Gene x Environment Interactions. While it is important to assess the individual contributions of genetic and environmental factors to behavior, there is a substantial literature indicating that these variables often interact to produce phenotypic outcomes. Two prominent models of the effects of gene x environment interactions (G x E) on behavior are the dual-risk (or diathesis-stress) model and the differential susceptibility hypothesis.

The dual-risk model holds that particular genotypes, endophenotypes, or temperamental characteristics (which presumably reflect genetic variation) may denote risk or vulnerability for a suboptimal behavioral outcome, when combined with an environmental stressor. For example, several studies have found that variants in 5-HTTLPR, which is believed to be involved in coding for the serotonin transporter, moderated the effect of an environmental stressor. Individuals with at least one short allele were more at risk for depressive symptomatology in the context of stressful life events (Caspi et al., 2003) and maltreatment during childhood (Kaufman et al., 2006). Additionally, toddlers with at least one short allele were more at risk for developing an insecure attachment, when combined with lower maternal responsiveness (Barry, Kochanska, & Philibert, 2008). Specific to OXTR, Caucasian girls with an A allele on rs2254298 were more at risk for depressive symptoms in the context of high early

adversity (Thompson, Parker, Hallmayer, Waugh, & Gotlib, 2011). Studies such as these suggest that some individuals, based on their genetic makeup, appear to be resilient to the potential effects of environmental stressors, while others, based on their genetics, are vulnerable to these same stressors. Moreover, the negative behavioral outcomes, such as depression, seem to occur almost exclusively in individuals with both the "risk" allele and the environmental stressor, indicating that neither genetics nor environment alone appear to be sufficient in explaining these behavioral outcomes.

Alternatively, Belsky (e.g., 2009) has argued that these "risk" genotypes may in fact represent a differential susceptibility to one's environment, with certain individuals genetically predisposed to be more sensitive to their environmental circumstances. Belsky and Pluess (2009) argue that this model is more consistent with evolutionary logic. Given the uncertainty of which parenting practices would be most successful in promoting reproductive fitness in children, it would be biologically advantageous for parents to have children that vary in terms of their susceptibility to these practices. This would allow parents to have children who could receive maximum benefit from positive effects of these practices, or, conversely, be resilient to negative effects of these practices.

To investigate the differential susceptibility hypothesis, Belsky and Pluess (2009) reviewed and re-analyzed several G x E studies, finding support for this model. For example, they re-examined the landmark study by Caspi et al. (2003), which found evidence for a moderation effect with 5-HTTLPR, stressful life events, and depression. They discovered that the short allele group had the best and the worst outcomes, depending on the presence or absence of stressful life events. In addition, the 7-repeat variant of DRD4 has been identified as a genetic risk factor due to its relation to negative

outcomes, such as ADHD symptoms and high novelty-seeking. Consistent with the differential susceptibility hypothesis, longitudinal research has shown that children with the 7-repeat allele display the highest and the lowest externalizing behaviors, depending on the quality of parenting (Bakermans-Kranenburg & van IJzendoorn, 2006). To the author's knowledge, there have been no G x E studies involving OXTR, early parent-child interactions, and empathy; however, it is likely that there is an interplay between these factors in the development of this ability.

The current study explored whether the empathic behavior of young children at high- and low-risk for an ASD, due to sibling ASD status, is more affected by early parent-child interaction variables depending on the presence or absence of an A allele in OXTR rs53576 or rs2254298. Belsky, Bakermans-Kranenburg, and van IJzendoorn (2007) outlined several steps to determine whether a study provides evidence for this hypothesis. Evidence for differential susceptibility is assumed when there is moderation that includes a crossover effect (i.e., regression lines cross), and which consists of both negative and positive environmental circumstances. Thus, the "susceptible" group should have a slope that is significantly different from zero and steeper than the "nonsusceptible" group. More simply, a larger range of scores in a given outcome in the "susceptible" group, comprising the highest and lowest scores on the outcome, can also provide evidence for this hypothesis. If there is a significant gene x environment interaction in this study, these criteria will be utilized to determine whether findings provided evidence for the differential susceptibility hypothesis or were more consistent with the dual-risk model.

The Current Study

Since empathy appears to be an important predictor for diagnostic outcomes in children with emerging ASDs and facilitates positive social outcomes in typically developing children, it is essential to gain a better understanding of factors that may influence individual differences in empathy. This study examined the influence of specific genetic and environmental factors on the later empathic responding of children at high- and low-risk for an ASD. ASD risk status was defined by the presence or absence of an older sibling with a confirmed ASD diagnosis. Specifically, this study investigated whether phenotypic variations in empathic behavior during the third year of life were influenced by genotypic variations in two well-studied OXTR SNPs (rs53576 and rs2254298) and by aspects of early parent-child interactions, measured in the second year of life. Additionally, the current study examined whether aspects of early parent-child interactions had more or less of an influence on children's empathy depending on variations in polymorphisms of OXTR. Finally, this study preliminarily explored whether variations in OXTR were associated with ASD symptom severity. This study tested the following hypotheses (see Figure 1):

1. Children with at least one A allele in OXTR rs53576 are hypothesized to have lower levels of empathic behavior in the third year of life. Similarly, children with at least one A allele in OXTR rs2254298 are expected to have lower levels of empathy. Findings are expected to be comparable across ASD risk groups.
2. Children of dyads with higher affective mutuality and of parents with higher emotional supportiveness in the second year of life are hypothesized to have

higher levels of empathy in the third year of life. Findings are expected to be comparable across ASD risk groups.

3. The relation between levels of affective mutuality/emotional supportiveness and empathic responding are expected to be moderated by A allele presence/absence. Specifically, it is hypothesized that the relation between early parent-child interaction variables and empathy will be stronger for children with at least one A allele than children homozygous for the G allele. If an interaction is present, whether it provides differential support for the dual-risk model or differential susceptibility hypothesis will be assessed.
4. The influence of variations in OXTR on ASD symptom severity was preliminarily explored within the high-risk group only.

CHAPTER 2: METHOD

Participants

Participants were part of a larger longitudinal study examining the early social and emotional development of infants at high- and low-risk for developing an ASD. Several recruitment strategies were utilized: (1) obtaining referrals from a university-based autism service, (2) distributing a brochure at autism-related events and other functions to parents of infants, (3) mailing a brochure to parents of infants whose addresses and names were obtained from county birth records, (4) contacting child care programs, and (5) "word of mouth."

Infants were categorized as high-risk if they had one or more older siblings with an ASD diagnosis, and low-risk if they did not have any older siblings diagnosed with or showing research evidence of an ASD. Older sibling diagnoses were confirmed by an experienced licensed clinician based on DSM-IV-TR diagnostic criteria and results from the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000). An ASD screener, the Social-Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003), was completed by a parent for all older siblings. Older siblings in the low-risk group who received an elevated score (SCQ total ≥ 9) were administered an ADOS. Depending on the results of the ADOS, the younger sibling was placed in the low- or high-risk group. If the findings were inconclusive or if the older sibling assessment was not completed, the younger sibling was placed in an "unresolved" category. Children who were "unresolved" at the time of this study were excluded from analyses.

Inclusion criteria for the primary analyses (Hypotheses 1-3) included the presence of at least one successfully genotyped OXTR SNP, parent-child interaction data at 15 or

18 months of age, and empathy data at 24 or 30 months. A total of 77 participants (High-Risk $n = 46$, Low-Risk $n = 31$) met these inclusion criteria. See Table 1 for demographic information by ASD risk group. There were no significant associations between ASD risk group and gender, $\chi^2 (1) = 3.04$, $p = .08$, race/ethnicity, $\chi^2 (4) = .50$, $p = .97$, or maternal education, $\chi^2 (4) = .51$, $p = .97$; however, high-risk siblings had lower developmental scores than low-risk siblings (Early Learning Composite: $t(74) = 3.64$, $p < .01$).

For the secondary analyses (Hypothesis 4), which examined the relation between OXTR genotypes and ASD symptom severity, inclusion criteria included the presence of at least one successfully genotyped OXTR SNP, ASD symptom severity data at one of the clinical outcome visits (primarily from the 30-month age assessment), and high-risk status. A total of 45 children met these inclusion criteria. See Table 2 for participant information for this sub-sample.

Genetic Data Collection

Procedure. At one of the longitudinal assessments (ranging from the 15-month to the 4-6 year age assessments), genetic data were obtained through the collection of the children's saliva using Oragene DNA collection kits (DNA Genotek). Younger children's saliva (~4 years and under) was collected using sponges, and older children's saliva (~5 years and older) was collected through the children spitting directly into the container. Families of a subset of participants who had already completed the study or missed their genetic data collection visit were mailed kits to collect their child's saliva at home ($n = 14$). Genetic samples were sent for extraction and analysis at the John P. Hussman Institute for Human Genomics (HIHG) at the University of Miami, Miller School of Medicine. Additional consent was obtained for participation in this portion of the study.

Overview. Genotyping was conducted for the following OXTR SNPs: rs53576 (A/G) and rs2254298 (A/G). These SNPs were chosen due to their demonstrated associations with empathy and/or autism in previous studies (e.g., Jacob et al., 2007; Rodrigues et al., 2009; Wu et al., 2005). Due to the relatively small sample size, two well-studied SNPs were chosen to minimize the risk of type 1 error.

Saliva and DNA Extraction. Genetic material was extracted at HIHG using standard procedures recommended by DNA Genotek for samples that include DNA collected from young children through the use of sponges. The samples were incubated at 50°C for two hours in an air incubator. The free liquid was removed from the sponges by centrifugation in a 6 ml syringe suspended on a 15 µL conical centrifuge tube. The device was then centrifuged at 200 × g (e.g., 1,000 rpm in a Beckman Coulter Allegra® X-15R centrifuge) for 10 minutes at 20°C. The free saliva was then extracted using DNA Genotek prepIT-L2P protocol. The majority of the clear supernatant was transferred to a fresh microcentrifuge tube with a pipette, and 600 µL of room temperature ethanol was added to it and mixed by inversion. After the samples rested at room temperature for 10 minutes to fully precipitate, they were centrifuged at 15,000 x g for two minutes. After discarding the supernatant, 250 µL of 70% ethanol was added for one minute, and then removed. Finally, 100 µL of TE solution was added and the sample was vortexed for five seconds, then incubated at 50°C for one hour.

DNA Amplification. Due to low saliva yields (< 2µg) in approximately one-third (n = 31) of the total sample (n = 93), the extracted samples underwent multiple displacement amplification prior to genotyping (Qiagen, Repli-G Midi Kit). After preparing the buffers (D1 and N1), 2.5 µL of buffer D1 was mixed with the DNA by

vortexing, then briefly centrifuging. After incubating the samples at room temperature for three minutes, 5 μL of buffer N1 was similarly mixed with the samples. After thawing the remaining components, a master mix was prepared and briefly centrifuged. Then, 40 μL of the master mix was added to 10 μL of the denatured DNA and incubated at 30°C for 12 hours. Finally, the REPLI-g Mini DNA Polymerase was inactivated by heating the samples for three minutes at 65°C.

For samples with sufficient DNA yields (n = 60), both genomic and amplified DNA were sent for genotyping to assess for concordance between the genotypes obtained from the genomic versus the amplified DNA. The remaining 33 samples were genotyped using amplified DNA only.

Genotyping of SNPs. Genotyping was conducted using Taqman allelic discrimination assays from Applied Biosystems (ABI). Cycling was performed on GeneAmp PCR Systems 9700 thermocyclers, with conditions recommended by ABI. End-point fluorescence was measured on the ABI 7900 HT system. Genotype discrimination of experimental results was then conducted using ABI's 7900 HT Sequence detection Systems version 2.3 analysis software. To ensure genotyping accuracy, one negative and seven positive quality control samples per 96 well plate were included.

For rs53576, 84 samples were successfully genotyped and nine of the samples' genotypes were undetermined. For rs2254298, 88 samples were successfully genotyped, and five samples were undetermined. Participants who were undetermined for both SNPs were excluded from analyses. The relatively high level of participants with inconclusive genotypes is likely related to lower yields resulting from the predominant use of swabs,

rather than direct saliva collection, which was necessary with this study's young sample. Genotype concordance rates between genomic and amplified DNA were 100% for both SNPs, indicating reliable genotyping of the amplified DNA.

Assessment of Parent-Child Interaction

Procedure. At the 15- and 18-month age assessments, families visited the university laboratory. Prior to the session, parents were instructed to, "Play with [child's name] as you normally would at home." An array of age-appropriate toys were available to facilitate the play. The free play sessions lasted for five minutes. The interactions were videorecorded to allow for later behavioral coding.

Coding. Parent, child, and dyadic behaviors were rated using the National Institute of Child Health and Human Development (NICHD) Early Child Care Research Network (ECCRN) scales (e.g., 1999). Multiple research associates, blind to child genotypes and empathy ratings, rated the free play interactions. The following scales were coded: Parental Sensitivity, Respect for Autonomy, Positive Regard, and Affective Mutuality (dyadic code). These were rated on scales from 1 to 7, with a 1 corresponding to the absence of a given behavior and a 7 to the clear and abundant presence of a behavior. The latter scale, Affective Mutuality, which is a global rating of the quality of the dyadic interaction, was used independently in analyses, as it closely represents concepts shown to influence typical empathy development (e.g., Feldman, 2007; Kochanska, 2002). An Emotional Supportiveness composite score, which was calculated using the mean of the Parental Sensitivity, Respect for Autonomy, and Positive Regard parent ratings, was also utilized (see Baker et al., 2010). This composite score was determined to have good internal consistency in this sample (ICC = .85). See Table 3 for

more detailed information on these rating scales. The mean of the 15- and 18-month ratings were utilized in analyses.

Reliability. Inter-rater reliability estimates for the 15- and 18-month dyadic and parent ratings were good. Intra-class correlations were as follows: 15-month Affective Mutuality (.80), 18-month Affective Mutuality (.80), 15-month Emotional Supportiveness (.81), and 18-month Emotional Supportiveness (.88).

Assessment of Empathy

Procedure. At the 24- and 30-month age assessments, families visited the university laboratory. Prior to the procedure, a trained examiner gave the parent the following instructions for the empathy task: "After you and [child's name] play for a while, I will step into the room to alert you to begin pretending that you have something in your eye. Act like it really bothers you by saying 'Oh, I have something in my eye.' Carry on like this for a while, but don't say your child's name or suggest your child do anything to help you feel better." If the parent did not begin the empathy task at the first prompt, the examiner prompted the parent unobtrusively up to two times. The task lasted approximately one minute. It was terminated when the examiner re-entered the room and instructed the parent to tell the child that his or her eye felt better.

Coding. An empathy coding system, originally established for use with typically developing toddlers by Zahn-Waxler et al. (1992) and adapted by Young, Fox, and Zahn-Waxler (1999), was utilized for this study. Since this sample was at risk for language deficits, minor adaptations were made to this coding system to remove bias toward higher scores for verbal, rather than non-verbal, responses. Undergraduate research assistants, blind to ASD risk group status, genotypes, and parent-child interaction ratings, rated the

episodes. Each episode was given a global Empathy rating of 1 to 7, which captured the overall quality of the child's empathic responding. On this scale, a 1 corresponds to no signs of empathy or concern and a 7 to strong expressions of concern and caring behavior (McDonald & Messinger, in press; Young et al., 1999). The mean of the 24- and 30-month Empathy ratings were utilized in analyses. To ensure the quality of the parent performances, undergraduate research assistants rated the episodes for Credibility (1 – not believable, 2 – passable, 3 – particularly authentic) and Affective Intensity (1 – little or no affect, 2 – moderate level of affect, 3 – high affect and pain expressed; Young et al., 1999).

Reliability. Approximately 25% of the 24- and 30-month empathy episodes were double coded to assess inter-rater reliability. Reliability for the Empathy rating was high. The intra-class correlations, using absolute-agreement, were .90 at 24 months and .94 at 30 months.

With respect to parent performance, approximately 20% of the 24-and 30-month Empathy episodes were double coded. There was high agreement on Credibility (95%) and Affective Intensity (93%) ratings. All parents in this sample were rated as having at least a passable performance, and most with a moderate level of affect. These ratings are consistent with those reported by Young et al. (1999). Parent performance scores were not associated with corresponding 24- or 30-month Global Empathy ratings (24-month, Credibility, $r(97) = .01$, $p = .90$; 24-month, Intensity, $r(97) = -.04$, $p = .67$; 30-month, Credibility, $r(94) = .14$, $p = .29$; 30-month, Intensity, $r(94) = .02$, $p = .85$); thus, they were not included in analyses.

Assessment of ASD Symptom Severity

ASD Symptom Severity was measured in a continuous manner using ADOS severity scores. The ADOS is a play-based structured observational measure designed to elicit behaviors that are relevant to an ASD diagnosis (Lord et al., 2000). ADOS severity scores were calculated for each child based on the criteria presented in Gotham, Pickles, and Lord (2009). These criteria control for the age and language level of the child when determining severity. ADOS protocols were scored by experienced clinicians who achieved at least 80% reliability with a designated ADOS trainer. Only children in the high-risk group were included in these analyses. For children who did not have 30-month ADOS data (e.g., missed time point), ADOS scores collected at an alternate age assessment were used instead (24-month ADOS $n = 2$, 30-month ADOS $n = 38$, 36-month ADOS $n = 4$, 4-6 year ADOS $n = 1$), resulting in a total of 45 children included in the relevant analyses.

CHAPTER 3: RESULTS

Preliminary Analyses

Missing Data. Prior to testing hypotheses, preliminary analyses were conducted to assess differences related to missing data. In these analyses, several demographic characteristics in this study's sample (n = 77) were compared to all other participants not included in this sample due to missing data. Results of these tests revealed no relation between missing data and any of the demographic variables assessed, including: ASD-Risk Status, χ^2 (1, n = 153) = 1.25, p = .26, Race/Ethnicity: χ^2 (4, n = 160) = 2.07, p = .72, Gender, χ^2 (1, n = 160) = 2.28, p = .13, and Maternal Education, χ^2 (1, n = 149) = 5.48, p = .24.

Within the study sample (n = 77), 22 participants were missing empathy outcome data at 24 months (n = 11) or 30 months (n = 11). In addition, 15 participants were missing parent-child interaction data at 15 months (n = 9) or 18 months (n = 6). To retain participants with missing data, individual data points (i.e., 15 or 18 months, 24 or 30 months) were used in lieu of the mean Affective Mutuality, Emotional Supportiveness, and Empathy scores. There were no differences when the scores of participants with missing data were compared to mean scores of participants with data at both ages in Affective Mutuality, t(75) = .61, p = .54, Emotional Supportiveness, t(75) = 1.43, p = .16, or Empathy scores, t(75) = .59, p = .56.

OXTR Genotypes. The distributions of the rs53576 and rs2254298 genotypes were tested for Hardy-Weinberg equilibrium using the calculator provided by the Online Encyclopedia for Genetic Epidemiology studies website (http://www.oege.org/software/hwe-mr-calc.shtml). Genotype distributions for both SNPs were consistent with Hardy-

Weinberg equilibrium (rs53576: χ^2 (2, n = 71) = .00, p = .96; rs2254298: χ^2 (2, n = 75) = 1.64, p = .20). Table 4 reports genotype frequencies and mean Empathy scores for the sample. Due to the expected low frequencies of AA genotypes for both SNPs, participants with AA genotypes were combined with the AG groups for analyses of each SNP.

There were a total of 71 participants for analyses involving rs53576 and 75 participants for those involving rs2254298. Genotypes for both SNPs were dummy coded as 0 or 1, with GG considered the reference group (concordant with hypotheses). In the final sample, there was no association between participants' genotypes on rs53576 (GG vs. AG/AA) and rs2254298 (GG vs. AG/AA), χ^2 (1, n = 69) = .34, p = .56. The relation between ASD Risk Status and Genotype was also assessed. There was no relation between ASD Risk Status and Genotype for rs53576, χ^2 (1, n = 71) = .74, p = .39; however, there was a relation between these variables for rs2254298, χ^2 (1, n = 75) = 3.97, p < .05, with more high-risk siblings than expected in the GG group.

Parent-Child Interaction. There were no age differences in Affective Mutuality, t(61) = -1.54, p = .13 (15-month M = 4.31, SD = .88; 18-month M = 4.52, SD = .96), or Emotional Supportiveness, t(61) = -.51, p = .61 (15-month M = 5.05, SD = .82; 18-month M = 5.11, SD = .94). In addition, the parent-child interaction variables were correlated between ages (Affective Mutuality r(61) = .33, p < .01; Emotional Supportiveness r(61) = .52, p < .01). The mean of the two ages were consequently considered appropriate to use for analyses as planned. Prior to calculating the means of the 15- and 18-month ratings for each participant, the Affective Mutuality and Emotional Supportiveness ratings were mean-centered within each age. The final Affective

Mutuality and Emotional Supportiveness scores were highly correlated, $r(76) = .76$, $p < .01$; however, they were examined separately as they are theoretically distinct (i.e., dyadic vs. parenting variables). Examination of skew and kurtosis for the final Affective Mutuality and Emotional Supportiveness scores indicated relatively normal distributions (Affective Mutuality: *Skew* = .01, *SE* = .27, *Kurtosis* = -.30, *SE* = .54; Emotional Supportiveness: *Skew* = -.50, *SE* = .27, *Kurtosis* = -.29, *SE* = .54).

There were no differences between parent-child interaction scores based on Genotype for rs53576 (Affective Mutuality, $t(69) = -.28$, $p = .78$; Emotional Supportiveness, $t(69) = -.59$, $p = .55$) or for rs2254298 (Affective Mutuality, $t(73) = .63$, $p = .53$; Emotional Supportiveness, $t(73) = 1.14$, $p = .26$). In addition, there were no differences between parent-child interaction scores based on Gender (Affective Mutuality, $t(75) = .28$, $p = .78$; Emotional Supportiveness, $t(75) = 1.12$, $p = .26$) or ASD Risk Status (Affective Mutuality, $t(75) = -.09$, $p = .93$; Emotional Supportiveness, $t(75) = -.03$, $p = .98$).

Empathy. There was a significant age difference between 24- and 30-month Empathy ratings, $t(54) = -2.35$, $p = .02$ (24-month $M = 2.89$, $SD = 1.46$; 30-month $M = 3.45$, $SD = 1.71$). In addition, Empathy was correlated between ages, $r(54) = .38$, $p < .01$. The raw Empathy ratings were standardized within each age to convert them into comparable scales. A mean of these two standardized scores was calculated to create a single Empathy score in subsequent analyses. Examination of skew and kurtosis for the final Empathy scores indicated an approximately normal distribution (Empathy: *Skew* = .29, *SE* = .27, *Kurtosis* = -.31, *SE* = .54).

There were differences in Empathy scores based on Gender, $t(75) = -2.00$, $p = .05$, with females showing higher levels of empathy than males (Female $M = .21$, $SD = .83$; Male $M = -.19$, $SD = .89$). In addition, there were differences in Empathy scores based on ASD Risk Status, $t(75) = 1.98$, $p = .05$, with low-risk siblings showing higher levels of empathy than high-risk siblings (Low-Risk M (z-score) = .22, $SD = .71$; High-Risk M (z-score) = -.18, $SD = .96$). Thus, Gender and ASD Risk Status were included as covariates in analyses for Hypotheses 1-3.

Results for Hypotheses 1 – 3

Model Building. Hypotheses 1 through 3 were assessed through two multiple regressions. Data were examined separately for each OXTR SNP (rs53576 and rs2254298). The first model predicted Empathy with rs53576 Genotype, Affective Mutuality, Emotional Supportiveness, the interaction between rs53576 Genotype and Affective Mutuality (rs53576 x Affective Mutuality), the interaction between rs53576 Genotype and Emotional Supportiveness (rs53576 x Emotional Supportiveness), as well as Gender and ASD Risk Status. This model significantly predicted Empathy, $R^2 = .25$, $F(7, 63) = 2.99$, $p < .01$, with the overall model explaining 25% of the variability in Empathy (see Table 5 for individual estimates). Emotional Supportiveness and rs53576 x Emotional Supportiveness were not significant predictors of empathy, so they were removed from the model. The removal of these non-significant predictors did not have a significant effect on model fit, F *change* $(2, 63) = .74$, $p = .48$, R^2 *change* $= .02$; thus, the more parsimonious model was retained. This new model (see Table 5 for individual estimates) which included rs53576 Genotype, Affective Mutuality, rs53576 x Affective

Mutuality, as well as Gender and ASD Risk Status significantly predicted Empathy, R^2 = .23, $F(5, 65) = 3.92$, $p < .01$, explaining 23% of the variability in Empathy.

The second model predicted Empathy with rs2254298 Genotype, Affective Mutuality, Emotional Supportiveness, the interaction between rs2254298 Genotype and Affective Mutuality (rs2254298 x Affective Mutuality), the interaction between rs2254298 Genotype and Emotional Supportiveness (rs2254298 x Emotional Supportiveness), as well as Gender and ASD Risk Status. This model significantly predicted Empathy, $R^2 = .19$, $F(7, 67) = 2.12$, $p < .05$, with the overall model explaining 19% of the variability in Empathy (see Table 6 for individual estimates). In this model, only Gender and Affective Mutuality were significant predictors of Empathy. As a next step, Emotional Supportiveness, rs2254298 x Emotional Supportiveness, and ASD Risk Status were removed from the model to assess the influence of rs2254298 x Affective Mutuality without these non-significant predictors. The inclusion of the non-significant predictors did not improve model fit, *F change* (2, 67) = .62, $p = .61$, R^2 *change* = .02; therefore, the more parsimonious model was retained. This new model (see Table 6 for individual estimates), which included rs2254298 Genotype, Affective Mutuality, rs2254298 x Affective Mutuality, and Gender, significantly predicted Empathy, $R^2 = .16$, $F(4, 70) = 3.44$, $p = .01$, explaining 16% of the variability in Empathy. Results for the individual estimates did not change meaningfully for either model when using a stepwise approach.

To assess for differential associations between predictors and Empathy based on ASD Risk Status, three interaction terms (Status x Affective Mutuality, Status x Emotional Supportiveness, and Status x Genotype) were calculated and entered into each

of the original regression models. Individual estimates for these interactions terms were not significant in the model for rs53576 (Status x Affective Mutuality, $t(60) = .89$, $p = .38$; Status x Emotional Supportiveness, $t(60) = -.28$, $p = .78$; Status x Genotype, $t(60) = -.41$, $p = .68$) or for rs2254298 (Status x Affective Mutuality, $t(64) = .02$, $p = .98$; Status x Emotional Supportiveness, $t(64) = .63$, $p = ..53$; Status x Genotype, $t(64) = -.91$, $p = .37$). Thus, there was no evidence that predictors of empathy differed by ASD risk group.

Hypothesis 1: OXTR Genotype to Empathy. The first hypothesis, which predicted that children with at least one A allele on rs53576 and rs2254298 would have lower empathy scores in the third year of life than children homozygous for the G allele was not supported. For rs53576, Genotype did not significantly predict Empathy, $\beta = .18$, $t(65) = 1.65$, $p = .10$. For rs2254298, Genotype did not predict Empathy, $\beta = -.13$, $t(70) = -1.15$, $p = .25$.

Hypothesis 2: Parent-Child Interaction to Empathy. The second hypothesis, which predicted that children of dyads with higher affective mutuality and of parents with higher emotional supportiveness in the second year of life would have higher empathy scores in the third year of life, was partially supported. Affective Mutuality predicted Empathy in the final models for each SNP (rs53576: $\beta = .45$, $t(65) = 2.93$, $p < .01$; rs2254298: $\beta = .31$, $t(70) = 2.65$, $p = .05$). Conversely, Emotional Supportiveness did not predict Empathy in the original models for either SNP (rs53576: $\beta = -.27$, $t(65) = -1.21$, $p = .23$; rs2254298: $\beta = -.16$, $t(67) = -.77$, $p = .45$).

Hypothesis 3: OXTR x Parent-Child Interaction to Empathy. The third hypothesis, which predicted that OXTR genotype would moderate the relation between parent-child interaction variables and later empathy scores, was also partially supported.

For rs53576, Genotype moderated the relation between Affective Mutuality and Empathy, β = -.36, $t(65)$ = -2.32, p = .02 (see Figure 2), although in an opposite direction than initially expected. Children with the GG genotype appeared to be more susceptible to early dyadic interaction quality. On average, they had lower empathy scores than children with at least one A allele in the context of lower affective mutuality, but not in the context of higher affective mutuality. Contrary to expectations, for rs53576, Genotype did not moderate the relation between Emotional Supportiveness and Empathy, β = .17, $t(65)$ = .74, p = .46.

For rs2254298, Genotype did not significantly moderate the relation between either parent-child interaction variable and Empathy (Genotype x Affective Mutuality: β = -.23, $t(70)$ = -1.94, $p < .06$; Genotype x Emotional Supportiveness: β = -.01, $t(67)$ = -.07, p = .94,); however, the interaction between rs2254298 Genotype and Affective Mutuality approached significance in the final model. Children with the GG genotype tended to have higher empathy scores than children with the AG/AA genotypes in the context of higher affective mutuality, but not in the context of lower affective mutuality.

To follow-up, I asked whether the significant interaction between rs53576 and Affective Mutuality supported the dual-risk or differential susceptibility model. The interaction partially fulfilled the criteria presented in Belsky et al. (2007) for differential susceptibility (see Figure 2). In favor of this model, there was a crossover effect (i.e., the regression lines crossed) and the slope of the GG genotype, or susceptible, group was greater than zero and greater than the slope of the AG/AA genotype, or nonsusceptible, group. The other criterion, however, was not clearly fulfilled, as the susceptible group means included the worst, but not the best outcomes. As such, the interaction between

rs53576 and Affective Mutuality may be more indicative of dual-risk than differential susceptibility.

Hypothesis 4: OXTR Genotype to ASD Symptom Severity

Preliminary Analyses. ADOS severity scores ranged from 1 to 8, with scores of 4 or above corresponding to clinically significant levels of ASD symptomatology. Skew and kurtosis values for the ADOS severity scores were acceptable (ADOS severity: *Skew* = .67, *SE* = .35, *Kurtosis* = -.62, *SE* = .70). Of the 45 children included in the sample, 18 received clinically elevated severity scores. There was no significant difference in ADOS severity scores based on Gender, $t(43) = -1.26$, $p = .21$ (Male ($n = 30$): $M = 3.33$, $SD = 2.23$; Female ($n = 15$): $M = 2.53$, $SD = 1.41$).

Results. Hypothesis 4 explored the relation between variations in OXTR genotypes and ASD symptom severity in the high-risk group. Two linear regressions were conducted to assess for differences in children's ADOS severity scores based on Genotype. The Genotype groupings from the primary analyses were retained for these secondary analyses. Results from these analyses should be considered preliminary due to the relatively low sample size. No difference in ASD Symptom Severity was found for either SNP (rs53576: $R^2 = .01$, $F(1, 38) = .42$, $p = .52$; rs2254298, $R^2 = .01$, $F(1, 43) = .51$, $p = .48$). Descriptive information for these analyses is presented in Table 7.

CHAPTER 4: DISCUSSION

This study investigated the influence of two oxytocin receptor gene variants and early parent-child interactions on individual differences in empathy in young children at high- and low-risk for an ASD. Findings indicate that children of parent-child dyads who were characterized by higher levels of affective synchrony in the second year of life demonstrated higher levels of empathic behavior in response to parental distress in the third year of life; moreover, children with the GG genotype, as opposed to children with at least one A allele, on rs53576 appeared to be more susceptible to the influence of early dyadic interaction quality. By contrast, parents' levels of emotional supportiveness, which is defined by parental sensitivity, positive regard toward their child, and respect for their child's autonomy, did not predict later empathy. Preliminary analyses suggested no relation between either of the OXTR variants and ASD symptom severity. This may be the first study to investigate possible contributors to individual differences in empathy in children at risk for an ASD, and appears to be the first to investigate the relation between OXTR and ASD symptom severity in this high-risk group.

The Importance of Early Social Interactions for Empathy Development

Findings from the current study extend the literature on predictors of empathy, which has primarily focused on typically developing children. As expected, children of parent-child dyads with higher affective mutuality had higher levels of later empathy. This dyadic variable is theoretically consistent with the concepts of affective synchrony and mutually responsive orientation proposed by Feldman (2007) and Kochanska (2002), respectively. This study expands the literature by reproducing findings from typically developing samples to a sample that included children at high-risk for an ASD. In

Kochanska's typically developing samples, children of dyads characterized by high levels of shared positive affect and high maternal responsitivity in the first and second years of life, demonstrated better later conscience development, including higher levels of empathy (Kochanska et al., 1999; Kochanska et al., 2005; Kochanska & Murray, 2000). Similarly, in Feldman's typically developing sample, children of dyads demonstrating higher levels of affective synchrony and mutual influence in the first year of life had higher levels of empathy in childhood and adolescence (Feldman, 2007). Together, these findings suggest that these early social interactions are an important context for learning to empathize with others.

Interestingly, while dyadic affective mutuality predicted later differences in children's empathic behaviors in this study, contrary to hypotheses, specific parenting behaviors did not. This is inconsistent with some previous studies, which have found that parenting behaviors, including warmth and responsivity, were related to individual differences in children's empathy (Kochanska et al., 1999; Robinson et al., 1994; Zhou et al., 2002). One issue that may have affected the lack of findings in this area is the context of the interaction. It may be that the free play measure used in this study was ideal for capturing dyadic synchrony, but not as well suited to measuring parenting variables, such as sensitivity. Rather, as argued in Fenning and Baker (in press), more challenging tasks, such as a parent-child problem solving task, may more accurately measure parental sensitivity.

The lack of association for specific parenting behaviors and empathy may also be due to sample-related differences or the timing of the age assessments. The present study did not find significant differences in the strength of associations between ASD risk

groups (i.e., lack of significant interactions between status and predictors); however, there may be a small, but meaningful difference in the degree to which parenting behaviors influence children with atypical social development. Research by van IJzendoorn et al. (2007) suggests that there may be differences in the susceptibility of children with ASDs to parenting influences, at least in regard to the relation between parental sensitivity and attachment security. These parenting behaviors may also have less of an influence, particularly with an at-risk sample, during the second year of life, when the child is a more active participant in interactions. An indirect relationship may exist, with parents with higher levels of warmth and responsiveness during interactions with their infants in the first year of life having more positive and synchronous interactions with their toddler in the second year of life, which, in turn, may influence the child's empathy development.

In sum, the overall quality of early parent-child interactions, which is influenced by both the parent and the child, rather than individual parenting behaviors, was most closely linked to empathy in this sample. However, the influence of these early interactions appears to depend, in part, on genetic factors.

The Role of the Oxytocin Receptor Gene in Empathy

The current study did not find a direct effect of OXTR on empathy; however, as expected, the rs53576 genotype moderated the relation between early parent-child interaction quality and later empathy, as evidenced by an interaction effect. These results support the importance of investigating the moderating role of common genetic variants, rather than focusing solely on direct genotype effects on complex phenotypic outcomes (i.e., empathy).

The rs53576 genotype associated with greater susceptibility to early parent-child interactions was the opposite of that predicted; it was the GG, rather than the AG/AA, group that was more susceptible to the influence of early parent-child interaction quality. It is unclear why children with at least one A allele were less at risk for poorer empathy outcomes in this study. Previous studies have found associations between the A allele of rs53576 and less optimal outcomes, such as lower empathy (Rodrigues et al., 2009), lower self-reported sociality (Tost et al., 2010), and autism (Liu et al., 2010; Wu et al., 2005). In contrast, a recent study by Sturge-Apple, Cicchetti, Davies, and Suor (2012) found evidence for increased susceptibility to interparental conflict on parental sensitivity among mothers with the GG genotype on rs53576. Consequently, it may be important for future studies to examine this OXTR variant in a moderating role, rather than examining its direct effect on phenotypic outcomes.

The interaction found in this study was assessed for consistency with the differential susceptibility hypothesis. While it satisfied some of the criteria proposed by Belsky et al. (2007), including a crossover effect, as well as a greater slope for the susceptible than the nonsusceptible group, the means for the susceptible group (GG) did not include the lowest *and* the highest empathy scores (see Figure 2). Thus, although the GG genotype group appeared to be more susceptible to early interaction quality, it could also be argued that the interaction in the current study provides clearer evidence for a dual-risk model, which posits that some individuals, based on genotype, are more at risk for adverse outcomes based on their environmental circumstances (Belsky et al., 2007). Specifically, a GG genotype on rs53576, in combination with lower dyadic affective

mutuality in the second year of life, put a child at highest risk for a less optimal empathy outcome.

Evidence is accumulating suggesting that variations in OXTR are associated with individual differences in social cognition abilities and social behaviors, but the specific mechanisms through which this occurs are not well understood. Some studies have found a relation between OXTR variants and structure and function in areas of the brain associated with social functioning, including the amygdala and hypothalamus (Furman, Chen, & Gotlib, 2011; Inoue et al., 2010; Tost et al., 2010). Although more research is needed, it is likely that OXTR, through its influence on oxytocin receptors, affects neurological functioning, which, in combination with other factors, affects behavior.

Interestingly, this study and several previous studies of OXTR have found relations between SNPs in the intron 3 area of the gene, the functions of which are not well understood (e.g., Rodrigues et al., 2009; Tost et al., 2010; Wu et al., 2005). OXTR spans ~19 kb and is made up of three introns and four exons (Inoue et al., 1994). The third intron spans a relatively large area of the gene (12 kb), and is surrounded by coding regions in exons 3 and 4 (Lerer et al., 2008; Inoue et al. 1994). It is possible that SNPs in intron 3 of OXTR do not have an important function, but rather are in linkage disequilibrium with other parts of the gene, or other genes, with more influential functions (Lerer et al., 2008). Conversely, it is also possible that this part of the gene has important functions that are not yet understood. A functional study of OXTR revealed that the third intron may be involved in transcriptional suppression or downregulation of the gene (Mizumoto, Kimura, & Ivell, 1997). This suggests a possible function of

OXTR's intron 3 that may be related to phenotypic outcomes, however, it is an area in need of additional research.

The Role of the Oxytocin Receptor Gene in ASDs

The current study also explored the relation between OXTR variants and ASD symptom severity. Results did not support a direct relation between OXTR and severity of ASD symptomatology. This study was unique in that it measured autism symptoms in a continuous manner, using a calibrated severity score from a well-established observational instrument (Gotham et al., 2009), and examined a sample of young children at high-risk for an ASD. These findings should be considered preliminary though, given the relatively small sample size in this analysis (n = 45).

Although there was no direct relation between the selected OXTR variants and ASD severity, there was an overrepresentation of high-risk siblings, compared to low-risk siblings, in the rs2254298 GG genotype group. This finding is somewhat counterintuitive, as several previous studies have found evidence for overtransmission of the A allele of this SNP in children with autism (e.g., Liu et al., 2010; Wu et al., 2005). Jacob et al. (2007), however, found evidence for overtransmission of the G allele on rs2254298 in a sample of Caucasian children with autism, suggesting possible sample-related differences. In addition, there have been no other investigations in high-risk siblings in particular.

Findings from previous research into the potential link between OXTR and ASDs are inconsistent. While some investigators have found evidence for overtransmission of particular alleles for some OXTR SNPs (Lerer et al., 2007; Liu et al., 2010; Wu et al., 2005), others have found either no association (Tansey et al., 2010) or differential

associations (Jacob et al., 2007). It may be that rather than being directly associated with a phenotype as broad and complex as ASD diagnosis or symptomatology, OXTR exerts its effects on more specific social behaviors, such as empathy. Specifically, some OXTR genotypes may put children at risk for adverse outcomes, such as deficits in empathy, which, in combination with a multitude of other genetic and environmental factors, lead to clinically significant levels of ASD symptomatology (Geschwind, 2011).

Autism is considered a complex genetic disorder, and recent work has focused on both rare and common genetic variants in the etiology of the disorder. Geschwind (e.g., 2011) argues that there are likely multiple biological pathways to ASD symptomatology. Several recent studies have found that rare, often non-heritable mutations, termed copy number variants, account for a minority of autism cases (e.g., Levy et al., 2011; Sebat et al., 2007). These are much more common in simplex, or sporadic, autism cases (i.e., one family member affected) than multiplex cases (i.e., families with multiple affected members). It may be that for multiplex cases (e.g., high-risk siblings who go on to have an ASD), a complex array of common genetic "risk" variants, each with small effect sizes, interact with other factors to produce the specific behavioral outcomes indicative of ASDs (Abrahams & Geschwind, 2008; Geschwind, 2011). It will be important to investigate OXTR in combination with potential genetic and environmental interacting factors to determine whether there is a moderating effect of OXTR on specific autism symptoms.

Scientific and Clinical Implications

Findings from the current study contribute to the literature on empathy across different developmental contexts. This study provides compelling evidence that an

OXTR variant (rs53576) plays a moderating role in the relation between early parent-child interaction and empathic behavior in a sample of children at varying risk for an ASD. This advances our understanding of the factors that play a role in the development of this important ability. Findings also suggest that the processes that influence empathy in typically developing children may be analogous to those that influence empathy in children at heightened risk for deficits in this area.

The importance of these findings also extends to the autism literature, as they improve our understanding of an important deficit associated with the disorder. Given the lack of findings with OXTR and ASD symptom severity in this high-risk sample, the therapeutic use of oxytocin is not indicated at this time; however, oxytocin administration may eventually prove to be a useful tool for improving outcomes in children at risk for empathy deficits (Green & Hollander, 2010; Hollander et al., 2007). Furthermore, interventions focused on facilitating more affectively synchronous parent-child interactions early in life may have a positive impact on children at risk for empathy deficits.

Limitations and Future Directions

Although the current study provides novel contributions to the literature, the findings should be considered in light of its limitations. First, this study's sample size was relatively small for a study of its kind, although it was sufficient to find some significant effects. However, a larger sample would allow for investigation of a broader group of OXTR SNPs and haplotypes, as well as examination of individuals homozygous for minor alleles. Second, it would be beneficial to examine the role of parenting behaviors during the first year of life in relation to later parent-child interaction quality and empathy

in children at risk for an ASD. A more quantitative, micro-coding approach, such as a coding system similar to that utilized by Feldman (e.g., 2007), may be beneficial. Third, it will be important to re-examine these children as they reach three years of age to determine whether ASD diagnosis, rather than ASD risk status, is a moderator of the associations found in this study. Fourth, further investigation of additional factors that may mediate the association between OXTR and empathic behavior is necessary. Although evidence suggesting a relation between OXTR and empathy is building, the mechanisms (e.g., neural functioning) that mediate this association are still not clear. Finally, additional factors that may contribute to empathy development, such as the functioning of the mirror neuron system, should be further examined, in clinical and at-risk samples, as well as in typically developing children.

Table 1.

Sample demographics by ASD risk group

Demographic variable	Total n (%)	Low-Risk n (%)	High-Risk n (%)
ASD-risk group	77	31 (40%)	46 (59%)
Gender			
Male	45 (58%)	14 (45%)	30 (65%)
Female	33 (42%)	17 (55%)	16 (35%)
Ethnicity			
White/Caucasian	28 (36%)	10 (32%)	18 (39%)
African-American	2 (3%)	1 (3%)	1 (2%)
Hispanic/Latino	35 (45%)	15 (48%)	20 (44%)
Asian/Asian-American	2 (3%)	1 (3%)	1 (2%)
Mixed Ethnicity/Other	10 (13%)	4 (13%)	6 (13%)
Maternal Education			
High school	4 (5%)	1 (3%)	3 (7%)
Some college	3 (4%)	1 (3%)	2 (4%)
2-year college	12 (16%)	5 (16%)	7 (15%)
4-year college	20 (26%)	8 (26%)	12 (26%)
Advanced/Professional degree	38 (49%)	16 (52%)	22 (48%)
Mean (SD) MSEL developmental scores*			
Early Learning Composite (Standard score)	93.7 (19.5)	103.0 (15.1)	87.6 (19.8)
Visual Reception (T-score)	50.3 (15.4)	56.1 (11.9)	46.6 (16.3)
Fine Motor (T-score)	43.9 (12.1)	50.0 (10.5)	40.0 (11.5)
Receptive Language (T-score)	44.5 (12.0)	49.7 (9.4)	41.0 (12.4)
Expressive Language (T-score)	47.1 (10.6)	50.0 (8.5)	45.2 (11.4)

* Developmental scores were obtained from the Mullen Scales of Early Learning (MSEL; Mullen, 1995), which is a normed standardized developmental measure for children from birth to 68 months of age. The majority (n = 62) of MSEL scores are from the 36-month administration. Results from the 24-month MSEL administration were used for the remaining participants (n = 14), due to missing data at 36 months (n = 3), or not yet reaching 36 months (n = 11). One participant was excluded from these analyses due to missing data at both ages.

Table 2.

Sample demographics for ASD symptom severity analyses

Demographic variable	*n* (%)
Gender	
Male	30 (67%)
Female	15 (33%)
Ethnicity	
White/Caucasian	18 (40%)
African-American	1 (2%)
Hispanic/Latino	19 (42%)
Asian/Asian-American	1 (2%)
Mixed Ethnicity/Other	6 (13%)
Maternal Education	
High school	3 (7%)
Some college	2 (4%)
2-year college	7 (16%)
4-year college	12 (27%)
Advanced/Professional degree	21 (47%)
Measure	**Mean (SD)**
Mean (SD) MSEL developmental scores	
Early Learning Composite (Standard score)	87.6 (20.0)
Visual Reception (T-score)	46.5 (16.5)
Fine Motor (T-score)	40.0 (11.7)
Receptive Language (T-score)	40.8 (12.5)
Expressive Language (T-score)	43.4 (11.5)
ADOS severity score	3.1 (2.0)

Table 3.

Descriptions of Parent-Child Interaction ratings

Measure (1-7 scales)	Descriptor
Affective Mutuality	Assesses the availability and mutuality of emotion between the child and parent and how secure the child appears to feel with the parent. Reflects the degree of shared positive affect and affective synchrony within the dyad.
Emotional Supportiveness	A composite score that reflects the degree of warmth and acceptance, responsiveness to the child's needs, and balanced maternal involvement with a respect for the child's desires and emerging independence. This score will be calculated from the mean of the three below rating scales.
Parental Sensitivity	The defining characteristic is that it is child-centered. Sensitive parents are tuned in to their child and manifest awareness of their child's needs, moods, interests, and capabilities. They allow this awareness to guide their behavior with their child.
Respect for Autonomy	Reflects the degree to which the parent acts in a way that recognizes and respects the validity of the child's individuality, motives, and perspectives in the play session.
Positive Regard	Rates parents' positive feelings toward their child expressed during interaction with him/her. Positive regard can be demonstrated when parents display warmth, acceptance, and respect for their child.

Table 4.

Genotype information for rs53576 and rs2254298

	Genotype		
		rs53576	
	AA	AG	GG
n	6	29	36
Empathy *M (SD)*	-.03 (1.25)	.19 (.74)	-.10 (.94)
	AG/AA		GG
n	35		36
Empathy *M (SD)*	.15 (.83)		-.10 (.94)
		rs2254298	
	AA	AG	GG
n	3	16	56
Empathy *M (SD)*	-.90 (.42)	-.06 (.78)	.00 (.89)
	AG/AA		GG
n	19		56
Empathy *M (SD)*	-.19 (.79)		.00 (.89)

Table 5.

Prediction of Empathy by rs53576 Genotype and Parent-Child Interaction (n = 71)

Variable	*t*	*p*	*β*
Original Model			
Gender	-1.94*	.06	-.22
ASD Risk Status	-2.03*	.05	-.23
Genotype (rs53576)	1.70	.09	.19
Affective Mutuality (AM)	2.87**	<.01	.66
Emotional Supportiveness (ES)	-1.21	.23	-.27
Genotype x AM	-2.13*	.04	-.49
Genotype x ES	.74	.46	.17
Final Model			
Gender	-2.03*	.05	-.23
ASD-Risk Status	-2.03*	.05	-.23
Genotype (rs53576)	1.65	.10	.18
Affective Mutuality (AM)	2.93**	<.01	.45
Genotype x AM	-2.32*	.02	-.36

* Significant at p < .05 level

** Significant at p < .01 level

Table 6.

Prediction of Empathy by rs2254298 Genotype and Parent-Child Interaction (n = 75)

Variable	*t*	*p*	*β*
Original Model			
Gender	-1.95	.06	-.22
ASD Risk Status	-.99	.33	-.12
Genotype (rs2254298)	-1.40	.17	-.16
Affective Mutuality (AM)	2.13*	.04	.42
Emotional Supportiveness (ES)	-.77	.45	-.16
Genotype x AM	-1.06	.29	-.18
Genotype x ES	-.07	.94	-.01
Final Model			
Gender	-2.40*	.02	-.26
Genotype (rs2254298)	-1.15	.25	-.13
Affective Mutuality (AM)	2.65*	.01	.31
Genotype x AM	-1.94	.06	-.23

* Significant at $p < .05$ level

Table 7.

Mean ADOS Severity scores (and SDs) by OXTR Genotype

Grouping	Genotype (*n*)		
		rs53576	
	AA (3)	AG (18)	GG (19)
Original Groupings	1.67 (.58)	3.00 (1.75)	3.21 (2.20)
	AG/AA (21)		GG (19)
Final Groupings	2.81 (1.69)		3.21 (2.20)
		rs2254298	
	AA (2)	AG (*5*)	GG (38)
Original Groupings	4.00 (2.83)	3.4 (2.51)	2.97 (1.97)
	AG/AA (7)		GG (38)
Final Groupings	3.57 (2.37)		2.97 (1.97)

Figure 1. Proposed Model

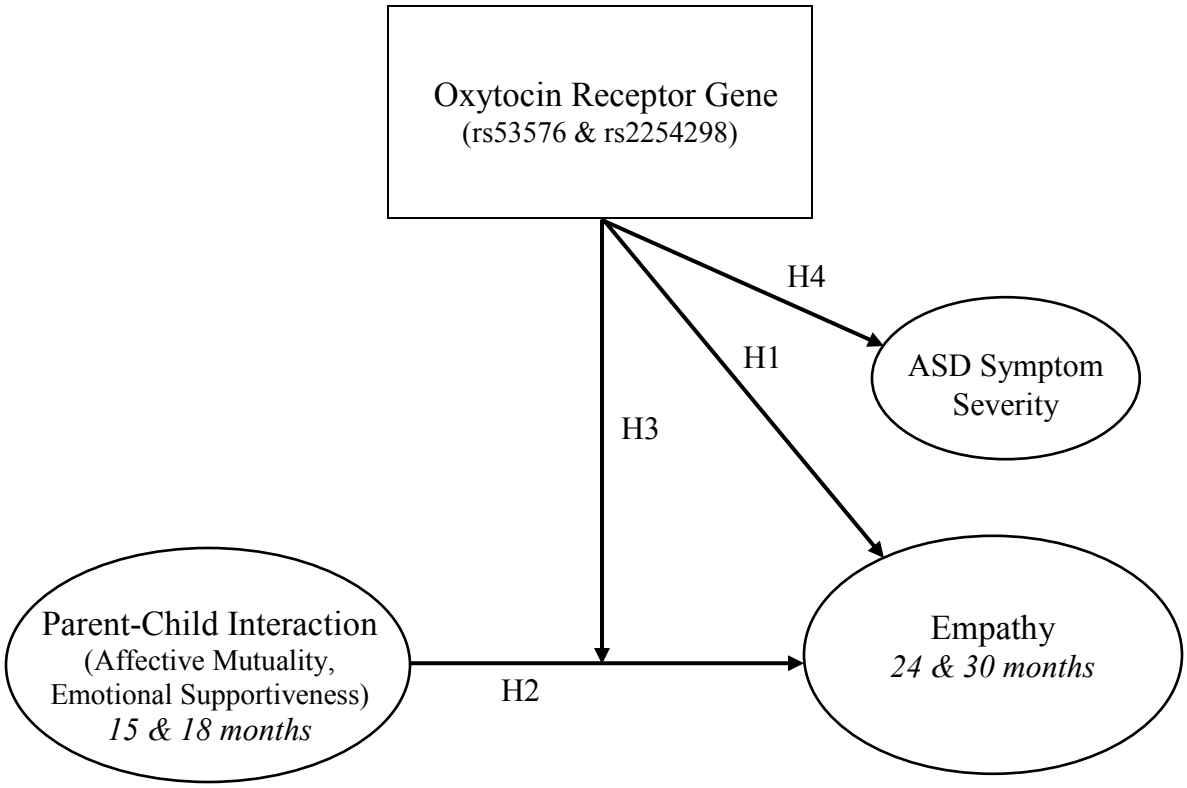
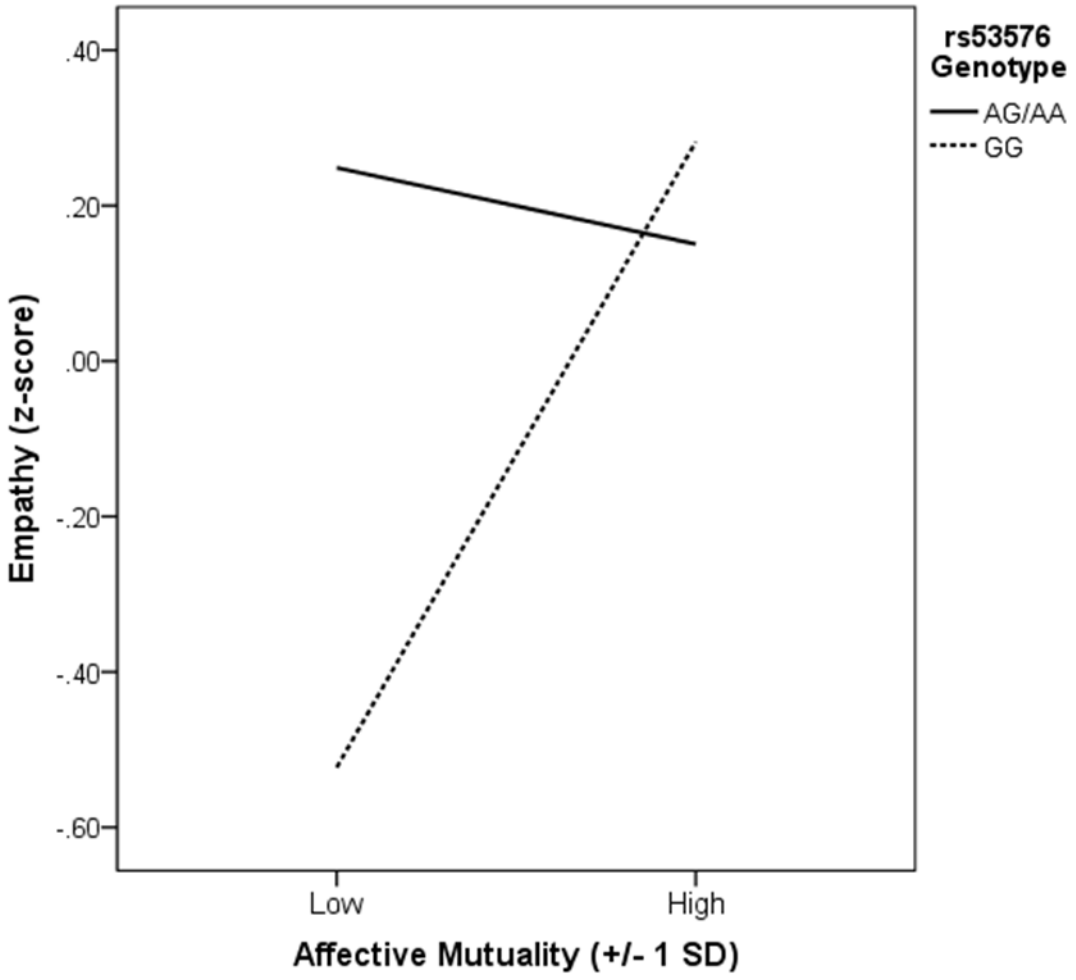


Figure 2. Empathy by rs53576 Genotype x Affective Mutuality



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