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Examining the Relationship Among Genes, Attention Bias to Emotion, Depression in Youth

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Examining the relationship among genes, attention bias to emotion, and depression in youth

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Abstract

The investigation of biologically initiated pathways to psychological disorder is critical to advance our understanding of mental illness. Research has suggested that attention bias to emotion may be an intermediate trait for depression associated with biologically plausible candidate genes, such as the serotonin transporter (5-HTTLPR) and catechol-o-methyl-transferase (COMT) genes, yet there have been mixed findings in regards to the precise direction of effects. The experience of recent stressful life events (SLEs) may be an important, yet currently unstudied, moderator of the relationship between genes and attention bias as SLEs have been associated with both gene expression and attention to emotion. Additionally, although attention biases to emotion have been studied as a possible intermediate trait associated with depression, no study has examined whether attention biases within the context of measured genetic risk lead to increased risk for clinical depressive episodes over time. Therefore, this research investigated both whether SLEs moderate the link between genetic risk (5-HTTLPR and COMT) and attention bias to emotion and whether 5-HTTLPR and COMT moderated the relationship between attention biases to emotional faces and clinical depression onset prospectively across 18 months within a large community sample of youth (n= 467). Analyses revealed a differential effect of

gene. Youth who were homozygous for the low expressing allele of 5-HTTLPR

(S/S) and had experienced more recent SLEs within the last three months

demonstrated preferential attention toward negative emotional faces (angry and

sad). However, youth who were homozygous for the high expressing COMT

genotype (Val/Val) and had experienced more recent SLEs showed attentional

avoidance of positive facial expressions (happy). Additionally, youth who

avoided negative emotion (i.e., anger) and were homozygous for the S allele of

the 5-HTTLPR gene were at greater risk for prospective depressive episode onset.

Increased risk for depression onset was specific to the 5-HTTLPR gene and was

not found when examining moderation by COMT. These findings highlight the

importance of examining risk for depression across multiple levels of analysis,

such as combined genetic, environmental, and cognitive risk, and is the first study

to demonstrate clear evidence of attention biases to emotion functioning as an

intermediate trait predicting depression.

Acknowledgments

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Introduction

Depression in children and adolescents is a serious and debilitating disorder (Birmaher et al., 1996). Around two percent of children and five to eight percent of adolescents are diagnosed with depression each year (Lewinsohn et al., 1994). Most individuals experience their first depressive episode in adolescence (Costello et al., 2003; Kim-Cohen et al., 2003), and adolescent-onset depression substantially increases risk for continuity and recurrence of depression into adulthood (Rutter et al., 2006). Youth diagnosed with major depression are at risk for developing other emotional and behavioral problems in adulthood, such as criminality, substance use disorders, and social difficulties (Fombonne et al., 2001; Knapp et al., 2002; Rao et al., 1993; Weissman et al., 1999), as well as committing suicide (Shaffer et al. 1996) which is the third leading cause of death among 15-to 24-year-olds (Center for Disease Control and Prevention, 2010). Understanding more about the pathophysiology and risk mechanisms for youth depression could greatly assist in the prevention of such serious mental health problems among youth and later on in life.

Cognitive theories of depression theorize that information processing of emotional cues may contribute to depression (Beck, 2008; Gotlib & Krasnoperova,

1998). Specifically, biased attention to emotional stimuli has been linked to depression in both adult (Mathews & MacLeod, 2005) and youth samples (Gibb, Beevers, & McGeary, 2013). However, it is unknown whether such biases in attention function as a cause, correlate, or consequence of depression as none of the prior research in adults or youth has utilized a longitudinal design to examine clinical onset of depressive episodes. Studies with adults have shown that information processing biases are modifiable (Hallion & Ruscio, 2011). Therefore, knowing whether attention biases function as a risk factor among youth is critical in order to better inform prevention of a depressive episode during highly vulnerable periods of development.

This research involved two studies that take a developmental psychobiological approach to elucidate how the integration of genetic and information processing biases to emotion affect the developmental trajectory and onset of youth depression (Cicchetti, 2006). This approach allowed for a greater understanding of the developmental pathways through which biological and cognitive vulnerabilities may influence depression and can advance knowledge on pathways related to genetic markers of intermediate phenotypes, or traits, contributing to depression among youth (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Gottesman & Gould, 2003). No study has simultaneously examined associations among genetics, information processing, and clinical depression among youth. Therefore, this research examined biased information processing (attention to emotional stimuli) as one potential intermediate trait that may be associated with the development of depression.

The relationship between attention biases to emotional stimuli and various theoretically relevant genes associated with attention to and processing of emotion may contribute to the onset of depression over time. Therefore, this research aimed to explore whether biased attention to emotional stimuli is an intermediate trait for depression onset through two studies. The first study (Chapter 1) examined the direct relationship between biologically plausible candidate genes, including the serotonin transporter gene promoter polymorphism (5-HTTLPR) and catechol-o-methyl-transferase gene (COMT). Although studies have found a direct relationship between 5-HTTLPR and attention bias to emotion, there have been mixed findings in regards to the precise direction of effects, particularly within the youth literature. Additionally, the experience of recent stressful life events (SLEs) may be an important, yet currently unstudied, moderator of the relationship between genes and attention bias as SLEs have been associated with both gene expression and attention to emotion. Therefore, the first study investigated whether SLEs moderate the link between genetic risk (5-HTTLPR and COMT) and attention bias to emotion within a large community sample of youth. It was hypothesized that there will be a gene x environment interaction (GxE) whereby youth who are homozygous for the S allele (S/S) and who have experienced a greater amount of recent SLEs, will show biased attention toward negative emotional faces (i.e., sad and angry faces). The association between COMT genotype and attention bias to emotion was also explored.

Furthermore, the investigation of biologically initiated pathways to psychological disorder is critical to advance our understanding of mental illness. Although attention bias to emotion has been studied as a possible intermediate trait

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associated with depression, no study has examined whether attention biases within the context of measured genetic risk lead to increased risk for clinical depressive episodes over time. Therefore, the second study (Chapter 2) investigated whether genetic risk as indexed by 5-HTTLPR and COMT gene, moderated the relationship between attention biases to emotional faces and clinical depression onset prospectively across 18 months in a large (n= 428) community sample of youth. It was hypothesized that youth S/S carriers of the 5-HTTLPR polymorphism who avoided negative emotional faces (sad or angry faces) would be at increased risk for an onset of clinical depression over time. An exploratory aim of the current study was to examine whether the relationship between attention biases to emotion and depression would be moderated by youth's COMT genotype or whether moderation would be specific to 5-HTTLPR genotype.

Overall, this research aimed to make an innovative contribution to the field of translational research by examining both the relationship between biased attention to emotional stimuli and theoretically relevant candidate genes along with how these factors may contribute onset of depression longitudinally among youth.

Chapter 1

Examining the relationship between genes and biased attention to emotional faces:

The moderating effect of stressful life events

Introduction

Investigating the role of specific, biologically plausible candidate genes in the

development of depression is an important step to advance the understanding of both

etiology and possible mechanisms of intervention. However, research has had little

success in identifying a direct link between candidate genes and depression, most

likely due to the complex etiology of the disorder (Caspi, Hariri, Holmes, Uher, &

Moffitt, 2010). Intermediate traits are thought to be more proximal to disorder

(Gottesman & Gould, 2003) and part of developmental pathways to disorder initiated

by candidate genes (Caspi et al., 2010; Gibb, Beevers, & McGeary, 2013). Biased

processing of emotional information has been identified as a possible intermediate

trait for depression among adults (Hasler, Drevets, Manji, & Charney, 2004) and

youth (Gibb et al., 2013) at a measured genetic risk (e.g., serotonin transporter gene

promoter polymorphism, or 5-HTTLPR). However, research findings have been mixed with some studies showing individuals at high genetic risk to display preferential attention toward both positive and negative emotions (e.g., Beevers, Wells, Ellis, & McGeary, 2009), and other studies finding preferential attention specifically for negative emotion (e.g., Perez-Edgar et al., 2010). Given mixed findings within the literature, investigating the role of moderators shown to influence gene expression, such as environmental stress (e.g., Chaouloff, Berton, & Mormède, 1999), is an important next step. Therefore, the present study aimed to clarify the relationship between biological plausible candidate genes and biased processing of emotional faces by examining the role of stressful life events (SLEs) as a possible moderator.

Attention Biases to Emotion

Cognitive theories of depression posit that information processing of socially imbued affective cues, such as emotional facial expressions, may contribute to the development, maintenance, and recurrence of depression (Beck, 2008; Gotlib & Krasnoperova, 1998). Indeed, there is a large body of studies with adults demonstrating that currently depressed adults exhibit an attention and memory bias for negative material (see Mathews & MacLeod, 2005 for a review). Additionally, there is evidence supporting cognitive theories of depression among youth (Abela & Hankin, 2008). The dot probe task is frequently used to measure aspects of cognitive mechanisms that are thought to function outside of one's awareness (e.g., encoding, attention) as this task is considered to be less susceptible to reporting biases as compared to self-report measures (Gotlib & Neubauer, 2000). Attentional bias for

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negatively valenced material has been observed among adults who are currently

depressed and dysphoric (Beevers & Carver, 2003; Gotlib, Kasch, et al., 2004;

Joormann & Gotlib, 2007) or have a history of remitted depression (Joormann &

Gotlib, 2007). Additionally, two studies have found attentional bias for negative

emotional faces in youth at-risk for depression (e.g., offspring of depressed mothers)

(Gibb, Benas, Grassia, & McGeary, 2009; Joormann, Talbot, & Gotlib, 2007) as well

as currently and remitted clinically depressed youth (Hankin, Gibb, Abela, & Flory,

2010). Given the strong evidence that attentional bias for negative emotional material

is linked to depression in adults and youth, it is important to understand the

underlying mechanisms, such as genetic risk factors, associated with attention bias.

Genetic Risk and Attention Biases to Emotion

It is important to consider the appropriate genetic methodology to examine

attention bias to emotion as a possible intermediate trait. There are two typical

approaches, including the theory-free examination of numerous single-nucleotide

polymorphisms (SNPs) used in genome-wide association studies (GWAS) or a

theory-based selection of candidate genes approach. A major disadvantage of a

theory-free GWAS approach includes the need for sample sizes that often prohibit the

careful measurement of the phenotype examined. Alternatively, thoughtful selection

of only a few genes theorized to be associated with a specific, carefully measured

intermediate trait (i.e., attention bias to emotion) will help to minimize the possibility

of Type I errors that can arise when conducting a number of statistical tests among a

large data set of genes (Moffitt, Caspi, & Rutter, 2006; van den Oord & Sullivan,

2003). Accordingly, the present study chose to examine whether two biologically

plausible candidate genes, the serotonin transporter gene promotor polymorphism (5-HTTLPR) and the catechol-o-methyl-transferase (COMT) gene, were associated with attention bias to emotion.

5-HTTLPR. Within the extant literature, 5-HTTLPR has been one of the most studied genes associated with risk for depression within the context of gene by environment interactions (GxE) (e.g., Caspi et al., 2003; Hankin, Jenness, Abela, & Smolen, 2011; Jenness et al., 2011; see meta-analysis by Karg, Burmeister, Shedden, & Sen, 2011) and attention biases to negative emotion (see Pergamin-Hight, Bakermans-Kranenburg, van IJzendoorn, & Bar-Haim, 2012 for a meta-analysis). Research has shown that the serotonin transporter (5-HTT) is a protein critical to the regulation of serotonin function in the brain because it terminates the action of serotonin in the synapse via reuptake. This is a well-studied protein that has a functional number of tandem repeats (VNTR) polymorphism in the promoter region. The variant site of the serotonin transporter gene is commonly known as 5-HTTLPR. Variants of 5-HTTLPR are the long allele (L), consisting of 16 copies of an approximately 22 base pair (bp) repeat unit, and a short allele (S), comprised of 14 copies (for a review see Hariri & Holmes, 2006). The S allele is associated with decreased transcriptional efficiency when compared to the L allele. The decreased transcriptional efficiency associated with the S allele results in approximately 50% less serotonin being recaptured in the pre-synaptic neuron when compared to the L allele (Lesch et al., 1996).

Research with adults has suggested that 5-HTTLPR may be a biological marker for the biased processing of emotional stimuli (Beevers, Gibb, McGeary, &

Miller, 2007; Beevers et al., 2009). For example, variations in 5-HTTLPR expression appear to affect neural circuits associated with the processing of negatively valenced emotionally stimuli with adults (Canli et al., 2005; Hariri et al., 2002, 2005; Heinz et al., 2004, 2007; Pezawas et al., 2005). These studies demonstrated that S carriers have greater amygdala activation and reduced functional communication between the prefrontal cortex and limbic system when viewing negatively valenced pictures (Heinz et al., 2004), processing negative words (Canli et al., 2005) or undefined task conditions (Heinz et al., 2007), and matching fearful and angry faces (Hariri et al., 2002, 2005; Pezawas et al., 2005). In sum, 5-HTTLPR has been linked with dysfunctional neural processing of negative emotional information when utilizing diverse measurement types suggesting that it could serve as a promising candidate gene of interest related to the biased attention to negative emotional stimuli.

More recently, researchers have begun to investigate how variants of 5-HTTLPR impact attention to emotional cues behaviorally with adults. However, findings have been mixed with some studies showing that both psychiatric inpatients (Beevers et al., 2007) and healthy adults (Beevers et al., 2011; Beevers, Pacheco, Clasen, McGeary, & Schnyer, 2010; Beevers et al., 2009) possessing one or two copies of the S allele allocated more attentional resources broadly to any type of emotional face (e.g., happy, sad, fearful versus neutral) as compared to those homozygous for the L allele. However, a recent meta-analysis found that those homozygous for the S allele showed an attention bias specifically to negative stimuli (e.g., sad and fearful, valenced faces, words, or pictures) (Pergamin-Hight et al., 2012). Although, this meta-analysis suggests that 5-HTTLPR is a biological marker

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for biased processing of negative emotional stimuli, questions remain regarding the finding from several studies showing a broader bias to both positive and negative emotional stimuli.

COMT. In addition to 5-HTTLPR, the present study sought to provide a preliminary examination of the associations between attention biases to emotional stimuli and the catechol-o-methyl-transferase (COMT) gene, which is associated with dopamine and norepinephrine neurotransmission (Opmeer, Kortekaas, & Aleman, 2010). The monoamine hypothesis suggests that depression is caused by a disturbance in monoamine (serotonin, dopamine, norepinephrine) neurotransmission (Ruhé, Mason, & Schene, 2007). However, there have been mixed findings when directly examining the relationship between depression and COMT with some studies finding no association and others finding a relationship between COMT and depression onset (see Antypa, Drago, & Serretti, 2013 for a review). Therefore, similar to 5-HTTLPR, it has been suggested that COMT may be more closely associated with basic and homogenous processes related to depression, such as attention and other cognitive processes (e.g., Mier, Kirsch, & Meyer-Lindenberg, 2009).

The majority of studies examining the influence of COMT have focused on the Val108/158Met polymorphism, which is involved in catabolizing dopamine and norepinephrine. Val homozygotes catabolize dopamine at up to four times the rate of COMT Met carriers, which leads to Val homozygotes performing worse on tasks that involve prefrontal cortex function (PFC) (Camara et al., 2010; Egan et al., 2001). However, a meta-analysis examining brain imagining data found differential neural activation across COMT variants with Val allele carriers showing impaired

performance in cognitive paradigms (i.e., encoding and memory) while Met allele carries had less efficient processing during emotionally valenced tasks (i.e., viewing valenced pictures) (Mier et al., 2009). As proposed by Mier and colleagues (2009), these findings suggest that COMT variants' relationship with PFC functioning may demonstrate an inverted U-shaped curve with either extreme in dopamine and norepinephrine levels conferring risk for inefficient neural processing of information. Although important for our overall understanding of how COMT function impacts neural processing of information, none of the studies included in this meta-analysis examined the association between COMT variants and tasks measuring attention biases to emotional faces, which is a task that involves cognitive, or attentional, control within the context of emotional stimuli. Given previously established research demonstrating the relationship between attention biases and depression, it will be important to examine whether COMT variants are related to biased attention to emotional information in order to better understand the possible genetic contribution to this risk factor.

Attention Biases to Emotion as an Intermediate Trait among Youth

Very few studies have investigated how 5-HTTLPR is related to processing of affective cues in youth samples, and no study has examined whether COMT is associated with attention biases to emotion among either adults or youth. Of the limited research examining 5-HTTLPR and attention biases among youth, findings have been mixed: two studies found 5-HTTLPR variants to be associated with attention biases toward negative emotional faces (angry faces in Perez-Edgar et al., 2010; fearful faces in Thomason et al., 2010), while others showed biases away from

sad (Gibb et al., 2009) and angry (Gibb et al., 2011) faces. Discrepancies within the child literature may be accounted for by methodological and participant differences. For example, neither Perez-Edgar et al. (2010; examined angry and happy faces) nor Thomason et al. (2010; examined angry and fearful faces) included sad faces within their stimuli set. Additionally, both studies finding 5-HTTLPR to be associated with preferential attention toward negative emotional faces (Perez-Edgar et al., 2010; Thomason et al., 2010) utilized community samples of youth that were not pre-selected for depression risk. However, Gibb and colleagues examined 5-HTTLPR and attention biases for sad, angry, and happy faces among children at-risk for depression (i.e., children of depressed mothers) as well as included mothers' depressive symptoms (Gibb et al., 2009) and mothers' expressed criticism about their child (Gibb et al., 2011) as moderators to the association between 5-HTTLPR and attentional avoidance of negative emotion. Overall, it appears that sample characteristics, including risk for psychopathology and the type of stimuli used, may have an influence over the types of biases observed within the context of genetic risk.

Examining attention biases as an intermediate trait related to depression is of particular importance among children and adolescents. Most individuals experience their first onset of depression during adolescence (Hankin et al., 1998) and adolescent-onset depression has been shown to substantially increase the risk for recurrence of depression in adulthood (Rutter, Kim-Cohen, & Maughan, 2006). Given that biases in attention to emotional stimuli have been theorized (Gotlib & Krasnoperova, 1998; Mogg & Bradley, 2005) and studied (Beevers et al., 2007, 2009) as a possible intermediate trait of depression primarily among adults, it is important to

clarify the genetic correlates to attention biases among youth as well as the specific direction of this relationship.

Examining Stressful Life Events as a Moderator

Research shows that stressful life events (SLEs) are associated with increased risk for depression (e.g., Hammen, 2005) and investigators have theorized that recent, discrete SLEs (within approximately 3-6 months) are more closely related to risk for depression compared to chronic stress (see Monroe & Reid, 2008 for a review). Given that 5-HTTLPR variants have been shown to predict a broad bias to any emotion as well as attention toward and away from negative emotional stimuli, it is possible that other, yet unstudied, factors influence the types of attention biases observed within the context of genetic risk. As cognitive models of depression posit that SLEs trigger cognitive biases, such as preferential attention toward negative emotion (Gotlib & Krasnoperova, 1998), SLEs could function as a potential moderator to the relationship between genetic risk and attention biases to emotion.

Within the animal literature, it has been well established that stress alters serotonin synthesis and release (Chaouloff et al., 1999) in the brain. In particular, acute stress has been shown to influence serotonin neurotransmission in mice and rats (Amat, Matus-Amat, Watkins, & Maier, 1998; Keeney et al., 2006). Among humans, a recent meta-analysis demonstrated that individuals homozygous for the S allele of 5-HTTLPR show increased cortisol secretion in response to a laboratory stressor (Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2012). Additionally, there is initial evidence showing that S/S carriers demonstrate decreased inhibition when processing negatively valenced pictures after an acute laboratory stressor in a small

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sample of adults (Markus & De Raedt, 2010). These findings are suggestive that acute stress affects attentional processes differentially based on 5-HTTLPR genotype status.

Stress has also been shown to affect dopaminergic function in animals (Arnsten & Goldman-Rakic, 1998; Murphy, Arnsten, Goldman-Rakic, & Roth, 1996) and a study in mice found variations in COMT enzyme activity to be related to differential stress responses among mice (Papaleo et al., 2008). There are also initial findings suggesting that COMT gene variants are associated with cortisol release during a laboratory stressor among children (Armbruster et al., 2012). Although, to our knowledge, there is no research examining the relationship among COMT, attention biases to emotion, and stress, these findings indicate stress may also play a critical role in how COMT genotype relates to attentional biases. Therefore, the current study sought to examine whether recent, discrete SLEs (events within the last 3 months) functioned as a moderator of 5-HTTLPR and COMT genetic risk to predict attention biases among youth.

Current Study

The current study sought to uniquely contribute to research on attentional biases to emotion (sad, happy, and angry) as a potential intermediate trait influenced by theoretically motivated candidate genes (5-HTTLPR and COMT) among youth. Given the mixed findings in the extant literature, particularly among studies examining the role of 5-HTTLPR, SLEs within the last three months were investigated as a possible moderator of the relationship between genotype and attention biases to emotion. As a recent meta-analysis (Pergamin-Hight et al., 2012)

showed 5-HTTLPR to be associated with biased attention toward negative stimuli, it is hypothesized that there will be a GxE whereby youth who are homozygous for the S allele (S/S) and who have experienced a greater amount of recent SLEs, will show biased attention toward negative emotional faces (i.e., sad and angry faces). Additionally, due to the lack of research investigating the relationship between COMT and attention biases to emotion, exploratory analyses will be conducted to examine the association between COMT and attention biases to emotion, along with whether SLEs moderate this relationship.

Method

Participants

Participants included 467 children and adolescents who were recruited from suburban and urban school districts in Colorado and New Jersey. A brief screening was conducted with parents to determine eligibility of their child. Youth had to currently be in 3rd, 6th, or 9th grade. They were excluded if they had a severe learning or psychiatric problem (e.g., autism, psychosis) that was likely to interfere with completion of an extensive laboratory protocol. The sample was approximately evenly divided by sex, of mixed ethnic origin representative of their geographic region, and ranged in age from 7 to 16 years old (see Table 1.2). Parents of youth were primarily mothers (91%). Median annual parental income was $90,000 and 18% of the youth received free/reduced lunch at school.

Procedures

Each eligible parent and youth visited the laboratory to complete the dot-probe task, DNA collection via saliva, and questionnaire data with youth and parents

about their child, in that order. Parents provided informed written consent for their

participation and for their child and youth provided written assent. Trained and

supervised graduate students, staff and undergraduate research assistants administered

the measures. All procedures were approved by the Institutional Review Board at

both sites. Youth and parents were reimbursed for their participation. All youth and

parents were given referral forms with lists of various affordable psychological

services and community mental health centers in the area.

Measures

Attentional biases. Youth's attentional biases for facial displays of emotion

were assessed using a modified dot-probe task (MacLeod, Mathews, & Tata, 1986)

administered using E-Prime. Stimuli for the dot-probe task consisted of pairs of

facial expressions that contained one affective (angry, sad, or happy), and one neutral

photograph from the same actor taken from a standardized stimulus set (Tottenham et

al., 2009). Photographs from each actor (16 men and 16 women) were used to create

sad–neutral, happy-neutral, and angry–neutral stimulus pairs (96 pairs).

Each stimulus pair was presented in random order over the course of two blocks,

with a rest in between blocks, for a total of 192 trials. Each trial began with a blank

computer display with only a white fixation cross in the middle of the screen for

1,000 milliseconds (ms). Then, a pair of pictures was presented for 1,000ms, followed

by a dot where one of the prior pictures had been (either the affective or neutral

picture) that was presented for 1000ms. Youth were instructed to indicate as quickly

as possible the location of the dot (left vs. right side of the screen) using the computer

keyboard ("z" labeled "left"; "/" labeled "right"). The computer recorded the

accuracy and response time for each response. Consistent with prior research (Gotlib, Kasch, et al., 2004), trials with response errors were excluded as were trials with response times less than 150ms or greater than 1,500ms. Error rates were quite low (less than 1.5%), and a small portion (1.8%) were excluded for being out of response time range. Of the 467 children who completed the dot-probe task, 416 had completed genotype data for 5-HTTLPR and 456 had completed genotype data for COMT. The final samples within each gene did not differ from the total sample on age, gender, or ethnicity/race ($ps > .13$).

Mean attention bias scores (Mogg, Bradley, & Williams, 1995) were then calculated separately for each affective stimulus type (angry, sad, or happy face) by subtracting the mean response time for cases in which the probe replaced the affective face from mean response times for cases in which the probe replaced the neutral face. Bias scores greater than zero represent preferential attention toward the affective face, whereas bias scores less than zero indicate attentional avoidance of the affective face. Given previous research indicating 5-HTTLPR to be associated with biases to negative emotion overall (Pergamin-Hight et al., 2012), a composite negative emotion bias variable was created by summing bias scores for both sad and angry faces to represent attention biases to negative emotion; positive emotion bias refers to bias scores calculated for happy facial emotion trials.

Stressful Life Events (SLEs). The Adolescent Life Events Questionnaire (ALEQ) (Hankin & Abramson, 2002) consists of 37 items that assess the number of SLEs occurring within the past 3 months. The ALEQ assesses a broad range of negative life events that typically occur among youth, including school, friendship, romantic, and

family events. Respondents indicated whether or not the event occurred within the past 3 months and is scored by summing the number of events endorsed. Both the child (ALEQ-C) and parent (ALEQ-P) reported on the child's exposure to stressors by indicating whether or not a stressor occurred within the last 3 months. ALEQ-C and ALEQ-P were given at the baseline assessment. ALEQ-C and ALEQ-P scores were moderately correlated ($r(466)= .23$, $p < .001$), so they were standardized and averaged together to form an overall score. Scores for ALEQ-C ranged from 0 to 37 ($M = 16.46$, $SD = 7.83$), and ALEQ-P scores ranged from 0 to 37 ($M = 15.53$, $SD = 7.41$). The ALEQ demonstrated good validity in past research (Hankin, 2008a, 2008b; Hankin, Stone, & Ann Wright, 2010). In addition, validity of the ALEQ is supported by significant correlations with objective ratings of episodic stressors ($r = .44$, $p < .001$) from a contextual stress interview (Karen D. Rudolph & Flynn, 2007). In sum, the ALEQ possesses strong psychometric properties and provides reasonably objective, reliable, valid assessment of stressors among youth.

Genotyping. Saliva samples were obtained from all study participants with Oragene™ (DNA Genotek, Ontario, Canada) collection kits, and DNA was extracted using standard salting-out and solvent precipitation methods. The method for 5-HTTLPR and SNP rs25531 (n=416) is detailed in Whisman, Richardson, & Smolen (2011). The rs25531 SNP genotypes (L_A vs. L_G) were obtained by incubating the PCR products with MspI (Wendland, Martin, Kruse, Lesch, & Murphy, 2006). Two groups of participants were formed based on their 5-HTTLPR genotyping: youth homozygous for the lower expressing S or L_G alleles (i.e., S/S) and those heterozygous or homozygous for the higher expressing L_A allele (i.e., SL/LL).

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Genotyping of Val158Met rs4680 in *COMT* (n= 456) are outlined in Haberstick &

Smolen, (2004). Three groups of participants were formed based on their COMT

genotyping: children homozygous for the higher expressing Val allele (i.e., Val/Val),

those heterozygous (i.e., Val/Met), and those homozygous for the low expressing Met

allele (i.e., Met/Met). The successful call-rate for the overall project was 97.5% for 5-

HTTLPR and 96.3% for COMT. All of the genotypes were in Hardy-Weinberg

Equilibrium.

Results

Preliminary Analyses

Means and standard deviations for all primary variables (Table 1.1) and

descriptive statistics (Table 1.2) overall and separated by genotype for 5-HTTLPR

and COMT are presented. There were no significant differences among genotypes for

age (ps> .28), race (ps> .17), or gender (ps> .09).

Data Analytic Plan

Multiple regression analyses were used to test G (5-HTTLPR or COMT) x E

(SLEs) as a predictor of attention bias to positive (happy) and negative (angry, sad)

emotional faces using the SPSS macro PROCESS (Hayes, 2013). SLE scores, as

measured by the ALEQ, were centered prior to analyses. All main effects and

interactions were entered simultaneously and unstandardized regression coefficients

are reported (Hayes, 2013) for each set of analyses.

Post hoc analyses of significant interactions were conducted (Aiken & West,

1991; Holmbeck, 2002). New product terms were computed at different levels (i.e.,

genotype groups) of the moderator variable. Separate regressions were conducted that

included each of these product terms. This enables examination of the significance of simple sloes at different levels of genotype.

5-HTTLPR. There was no significant gene-environment correlation (rGE) between 5-HTTLPR and SLEs (r(415) = -.09, p= .07). Multiple regression analyses revealed a significant interaction between SLEs and 5-HTTLPR predicting attention bias to negative emotion (Table 1.3). Analyses revealed a significant interaction between SLEs and 5-HTTLPR predicting attention bias to negative emotion (Table 1.3)[1]. This GxE effect is shown in Figure 1.1 with SLEs depicted at 1 SD above and below the mean. Post-hoc analyses showed a significant slope for those with the S/S (b = 3.91, SE = 1.30, t = 3.01, p =.003) genotype indicating that youth homozygous for the S allele exhibited biases toward negative emotion when experiencing high as compared to low levels of SLEs. The slope for the LL/SL genotype group was not significant (b = -.14, SE = .69, t = -.20, p = .84).

COMT. There was no significant rGE between COMT and SLEs (r(453) = -.009, p= .85). Multiple regression analyses revealed a significant main effect of gene, which should be interpreted in light of a significant interaction between SLEs and COMT predicting attention bias to positive emotion (Table 1.4)[1]. There was no significant main effect of SLEs (Table 1.4). The GxE effect is shown in Figure 1.2 with SLEs depicted at 1 SD above and below the mean. Post-hoc analyses showed a significant slope for those with the Val/Val genotype (b = -1.43, SE = .66, t = -2.16, p

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[1] Regression coefficients were virtually the same when controlling for youth self-reported baseline anxiety and depression symptoms as measured by the Multidimensional Anxiety Scale for Children (MASC; March, Sullivan, & Parker, 1999) and the Children's Depression Inventory (CDI; Kovacs, 1981).

=.03) indicating that youth homozygous for the Val allele experienced greater avoidance of positive emotion when experiencing high as compared to low levels of SLEs. The slope for the Met/Met ($b = .82$, SE = .73, t = 1.13, $p = .26$) and Val/Met ($b = -.31$, SE = .41, t = -.75, $p = .45$) genotype groups were not significant. No significant main effects or interactions were found when examining attention biases to negative emotion (Table 1.4).

Discussion

The primary aim of this study was to investigate the role of particular theoretically specified and biologically plausible candidate genes, specifically 5-HTTLPR and COMT genotype, in youths' attention to emotion as well as whether the relationship between genotype and attention biases was moderated by recent stressful life events (SLEs). Results supported the hypothesized interaction, demonstrating that youth at high genetic risk (S/S genotype) who also experienced higher levels of recent SLEs showed attentional biases toward negative emotional faces. Additionally, this study sought to explore whether COMT genotype was associated with biased attention to emotion within the context of experiencing recent SLEs. This study provides initial support for GxE effect with COMT Val/Val carriers showing attentional avoidance to positive emotion in those with recent high levels of SLE exposure. Overall, findings supported the previously untested notion that stress plays a critical role in understanding the relationship between genetic risk and attention biases to emotion.

The current study provided a unique perspective on the mixed findings seen within the 5-HTTLPR and attention bias literature. Although the majority of studies

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have found 5-HTTLPR genetic risk to be associated with attention biases toward negatively valenced material, as evidenced by a recent meta-analysis (Pergamin-Hight et al., 2012), there have been several studies both within the child and adult literature that have found either attentional avoidance of negative emotion (Gibb et al., 2011, 2009) or a broad attention bias toward any emotion (Beevers et al., 2007, 2009). The current study demonstrated the importance of considering exposure to environmental stress, particularly recent SLEs, as a moderator of the 5-HTTLPR and attention bias relationship. Although little is known about the precise mechanisms behind this relationship, it is possible that exposure to recent SLEs for those at high genetic risk (S/S carriers) may prime individuals to attend more to negative information within their environment. S/S carriers of 5-HTTLPR have been shown to experience greater cortisol reactivity in response to laboratory stressors (see Miller et al., 2012 for a meta-analysis) as well as show increased risk for depression when exposed to greater environmental stress (see Karg et al., 2011 for a meta-analysis). Indeed, it has been theorized that S/S carriers of 5-HTTLPR are more sensitive to their environment and therefore more susceptible to risky outcomes, such as depression, when exposed to greater environmental stress as well as benefit more from a positive or nurturing environment (i.e., differential susceptibility; Belsky & Pluess, 2009; Caspi et al., 2010). Although the present study did not measure positive environmental exposure, it may be informative for future research to include measures that capture both positive and negative environmental factors to determine whether the relationship between genes and attention biases functions within a differential susceptibility framework. Additionally, the present study's results are

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suggestive that attention biases may be a more proximal risk factor associated with both 5-HTTLPR genetic risk and stress, potentially functioning as an intermediate trait between genetic risk and disorder. Future research incorporating measures of depression will be necessary in order to determine the specific relationship among attention, stress, 5-HTTLPR, and depression.

This study also sought to examine whether COMT genotype interacted with recent SLEs to predict attention biases among youth. Interestingly, findings show a differential effect between 5-HTTLPR and COMT, with COMT Val/Val carriers showing attentional avoidance of happy faces in youth with higher levels of recent SLEs. This is the first study to examine whether COMT genotype is related to attention biases to emotion, particularly within the context of SLEs, so it is important to interpret these findings carefully. Still, it is possible to speculate as well as look within the broader COMT literature to understand this initial finding. One possibility is that the specificity for avoidance of positive emotion reflects Val/Val carriers' sensitivity to rewarding stimuli, particularly when exposed to a greater number of SLEs. There is considerable evidence showing happy faces function as socially rewarding starting in infancy (e.g., Tronick, Als, Adamson, Wise, & Brazelton, 1979) and activate reward neural circuitry (Phillips et al., 1998). The association between COMT and avoidance of rewarding stimuli could relate to dopamine's role in reward processing that has been linked to midbrain structures and the ventral striatum (Wise, 2002). Indeed, a neuroimaging study demonstrated abnormal reward processing among Val/Val participants as compared to Met/Met carriers (Camara et al., 2010). Additionally, avoidance of rewarding stimuli (i.e., anhedonia) is a key diagnostic

feature of depression and depressed adolescents show dysfunctions in reward neural circuitry (see Forbes & Dahl, 2012 for a review), which suggests that processing of happy faces may be a relevant factor to consider within the context of risk for depression.

Of note, the finding that Val/Val carriers are more prone to exhibit biased attention to positive emotion is somewhat contrary to a recent meta-analysis examining COMT and prefrontal neural activation during executive cognition and emotion based tasks. This meta-analysis demonstrated Val allele carriers to have decreased neural efficiency (increased activation) during cognitive tasks whereas Met allele carriers showed this pattern of neural activation during emotion based tasks (Mier et al., 2009). It is important to note that the emotion tasks examined in this meta-analysis did not include attention dot-probe tasks and did not examine behavioral performance. Given that the dot-probe task involves both cognitive aspects (attentional control) along with emotion processing, it is unclear whether direct comparisons can be drawn.

Overall, the differential findings between COMT and 5-HTTLPR variants predicting attention biases to emotion represent an intriguing step toward a better understanding of possible intermediate traits for depression. It is possible that differential pathways exist to depression within an equifinality framework or that each GxE may be part of a specific and separate pathway to disorder. Further research is needed to examine whether these associations predict future onset for disorder, and whether there is specificity to predict certain disorders depending on the risk factors observed.

There were several conceptual and methodological strengths of this study. The majority of previous research has investigated genetic risk for attention biases to emotion among adult samples, with limited studies investigating these processes among youth. Given the majority of first onsets of depression occur during adolescence (Hankin et al., 1998), the lack of research within youth samples is a notable gap in the literature. Additionally, none of the previous investigations among youth or adults considered SLEs as a possible moderator to the association between genetic risk and attention bias. Therefore, this is the first study to examine SLEs as a moderator to the relationship between 5-HTTLPR or COMT and biased attention to emotion. This is also the first study within either the adult or youth literature to investigate COMT genotype in association with attention biases to emotion. The differential findings between 5-HTTLPR and COMT provide an interesting avenue for future research on the developmental pathway to disorder. Methodological strengths include utilizing a large community sample of youth that allowed for greater ability to detect effects, use of a sample representative of the geographic area of recruitment, and employing a less biased measure of cognitive bias (dot-probe task). These strengths speak to the novel contribution this study provides to the broader literature on understanding intermediate traits for depression.

Limitations of the study provide avenues for future research. Firstly, although participants' reported SLEs occurring in the three months prior to the laboratory visit, this study is still considered cross-sectional and causal inferences cannot be made. It is possible there is a transactional relationship between stress and attention biases, such that biased attention to emotion contributes to increased experience of SLEs

(i.e., stress generation; Hammen, 1991). Future research utilizing multiple assessments of attention bias is needed to examine this question. The self-report measure of SLEs is a well validated, reliable measure that assesses a broad range of SLEs typically experienced by youth. Still, use of a stress interview to evaluate SLEs and their impact would provide a more thorough and objective measure of life stress. As previously stated, the current study did not examine whether the GxE predicted onset of psychological disorders or symptoms, which will be an important next step to determine whether 5-HTTLPR, COMT, and attention biases contribute to the developmental pathway to depression onset among youth. Finally, although the current study's measure of attention bias is more objective compared to self-reported cognitive biases, there are other, more precise measurement tools available to investigate information processing biases. For example, eye-tracking methodology provides real time assessment of attention biases at various interval lengths, which allows for examination of the time-course of attention biases as opposed to studying bias at one given point in time (i.e., 1000ms).

In sum, those who experienced high levels of recent SLEs and were at high genetic risk for 5-HTTLPR (S/S) and COMT (Val/Val) were found to exhibit biased attention to emotion. These GxE findings were differentiated by gene whereby 5-HTTLPR predicted attention toward negative facial expressions (sad and angry faces) whereas COMT predicted avoidance of positive facial expressions (happy faces). These findings suggest that the experience of stress plays a role in the relationship between genetic risk and attentional biases, which has implications for research examining intermediate traits for depression among youth.

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Chapter 2

Genetic and attentional risk factors predicting depressive episodes prospectively

among youth

Introduction

Rates of depression increase markedly across adolescence (Hankin et al., 1998)

and depression onset in adolescence predicts emotional and behavioral difficulties

into adulthood (Rutter, Kim-Cohen, & Maughan, 2006). Therefore, it is of particular

importance to investigate possible risk mechanisms for youth depression, particularly

within an integrated psychobiological framework in order to identify pathways to

disorder (Hankin, 2012). The study of attention biases are rooted in cognitive theories

of depression, which posit that information processing of affective cues, especially

those of social importance like emotional faces, may contribute to the development,

maintenance, and recurrence of depression (Beck, 2008; Gotlib & Krasnoperova,

1998). Additionally, there is a growing body of literature suggesting an important role

of certain biologically plausible candidate genes that may contribute to the

relationship between attention biases and depression (see Gibb, Beevers, & McGeary, 2013 for a review). However, previous studies in both adults and youth have either been cross-sectional or assessed symptoms rather than clinical levels of depression, so little is known about whether attention biases to emotion function as a cause, correlate, or consequence of clinical depression. The current study aims to fill this notable gap in the literature by examining attention biases to emotional faces and theoretically identified candidate genes as prospective predictors of depression onset among youth.

Attention Biases to Emotion as a Cognitive Risk Factor

Many studies have demonstrated that currently depressed adults exhibit cognitive biases for negative material (e.g., Mathews & MacLeod, 2005), and there is evidence supporting similar cognitive theories of depression among youth (e.g., Gibb et al., 2013). However, much of the research has utilized self-report measures of cognitive biases, which lend themselves to reporting biases due to mood or personality characteristics. Many of the theorized underlying cognitive risk mechanisms (e.g., encoding, attention, memory) are thought to function outside of one's awareness and are best measured by information processing tasks that are less susceptible to reporting biases (Gotlib & Neubauer, 2000), such as the dot-probe task (c.f., MacLeod, Mathews, & Tata, 1986) utilized by researchers to investigate attention biases to emotional stimuli. Several cross-sectional studies in adults have utilized similar attention tasks and shown those diagnosed with current or past major depression demonstrate attentional biases toward negative facial expressions (Gotlib, Kasch, et al., 2004; Gotlib, Krasnoperova, Yue, & Joormann, 2004; Joormann &

Gotlib, 2007). However fewer studies have examined the relationship between depression and attention biases in youth, with the pattern of findings being less clear as compared to the adult literature.

In the youth depression literature, there have been mixed findings regarding the type of attention biases found, with some studies showing attentional avoidance of negative emotion and others finding attention toward negative emotion. Within this literature, there have been a few cross-sectional studies examining attention biases to emotion among currently depressed (Hankin, Gibb, Abela, & Flory, 2010) and at-risk youth (e.g., youth whose mothers have a history of depression) in an experimentally induced negative mood state (Joormann, Talbot, & Gotlib, 2007). Similar to the adult literature, both studies found at-risk and depressed youth demonstrated biased attention toward negative emotion as compared to control participants. Although these studies provide valuable insight into possible risk mechanisms associated with youth depression, the lack of a prospective design precludes drawing causal inferences regarding the role of attention biases in the development of depression.

Attention Biases Predicting Longitudinal Change in Depression

Building upon this line of work, (Gibb and colleagues (2009) used a longitudinal design to examine the interaction among genetic risk (serotonin transporter gene promotor polymorphism, 5-HTTLPR), attention biases to emotion, and mothers' symptoms of depression in predicting child depressive symptoms over time. Interestingly, this study found a relationship between increases in child depressive symptoms over time and mothers' symptoms of depression among children at high genetic risk who *avoided* negative emotion. The different patterns in attentional bias

among studies in the adult and child literature may stem from the examination of attention biases while in a current negative mood state (Hankin, Gibb, et al., 2010; Joormann & Gotlib, 2007) as opposed to predicting future increases in depression (Gibb et al., 2009). For example, it is possible that avoidance of negative emotion acts as a contributing risk factor for depression whereas biases toward negative emotion arise as a consequence of being in a currently depressed mood state.

Cognitive theorists posit that biased attention to emotional material, especially when conveying negative emotion, may increase the risk of experiencing clinical depression. However, while only one study among youth looked at prospective increases in depression symptoms over time, no study, child or adult focused, has examined whether attention biases lead to increased risk for clinical depressive episodes over time. Predicting clinical depression as measured by a structured diagnostic interview is a notable gap in the literature given the impairment, distress, and other interpersonal and health risks associated with clinical levels of depression (Fombonne, Wostear, Cooper, Harrington, & Rutter, 2001; Rao, Weissman, Martin, & Hammond, 1993).

Genetic Risk, Attention Biases, and Depression

Within the cognitive model of depression framework it is theorized that information-processing biases, including attention biases, must be activated in order to increase risk for depression. The ability to effectively process and regulate emotion may be an important activator in the pathway from attention biases to depression. As noted earlier, there is a growing body of literature suggesting the need to investigate the role of candidate genes known to influence neurotransmitters involved in the

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processing and regulation of emotion when studying attention biases to emotion and depression (see Gibb et al., 2013 for a review). Two candidate genes of interest within the literature that are biologically relevant to both attention biases to emotion and depression are a functional polymorphism (5-HTTLPR) in the serotonin transporter gene (*SLC6A4*) and the catechol-O-methyltransferase (COMT, rs4680) gene.

5-HTTLPR. There is a large body of research within the neuroimaging and emotional reactivity literatures showing 5-HTTLPR to play a key role in the processing of and reactivity to emotional stimuli (e.g., Munafò, Brown, & Hariri, 2008; Pergamin-Hight, Bakermans-Kraneburg, Van Ijzendoorn, & Bar-Haim 2012). The serotonin transporter (5-HTT) is a protein critical to the regulation of serotonin function in the brain because it terminates the action of serotonin in the synapse via reuptake. This is a well-studied protein that has a functional number of tandem repeats (VNTR) polymorphism in the promoter region referred to as 5-HTTLPR. Variants of 5-HTTLPR are the long allele (L), consisting of 16 copies of an approximately 22 base pair (bp) repeat unit, and a short allele (S), comprised of 14 copies (for a review, see Hariri & Holmes, 2006). The S allele is associated with decreased transcriptional efficiency resulting in approximately 50% less serotonin being recaptured in the pre-synaptic neuron when compared to the L allele (Lesch et al., 1996).

Specifically, studies show that the S allele of the serotonin transporter gene is associated with increased amygdala activation and reduced connectivity between regions of the prefrontal cortex and amygdala when viewing negative stimuli (Heinz

et al., 2004) as well as greater cortisol reactivity to a laboratory stressor (Gotlib, Joormann, Minor, & Hallmayer, 2008). Additionally, research has shown that the serotonin transporter gene moderates the relationship between stress and depression (see Karg et al., 2011 for a meta-analysis). Given that 5-HTTLPR has been implicated in emotional processing and reactivity, the current research posits that this gene may act as an important moderator to the relationship between attention biases to emotion and depression onset.

     COMT. Although 5-HTTLPR is one of the most studied genes in relation to both attention biases and depression, research in related fields has suggested the possible importance of other candidate genes. In particular, the COMT enzyme is involved in dopamine degradation primarily in the PFC (Frank, Doll, Oas-Terpstra, & Moreno, 2009) and is encoded for by the COMT gene (22q11.2) (Grossman, Emanuel, & Budarf, 1992). Dopamine has been thought to play an important role in underlying processes associated with depression, such as emotion regulation (Ashby, Isen, & others, 1999), reward processing (Wise, 2002) and attention (Nieoullon, 2002). COMT contains a functional single nucleotide polymorphism (SNP), rs4680, which codes for the methionine (Met) and valine (Val) alleles. The Val allele has four times the enzymatic activity compared to the Met allele (Lachman et al., 1996). Research in animals and humans have found those homozygous for the Val allele to perform worse on tasks requiring PFC involvement, such as tasks examining attentional flexibility (Egan et al., 2001), reward processing (Camara et al., 2010), and stress reactivity (Papaleo et al., 2008). However, a meta-analysis by Mier et al. (2009) demonstrated that those homozygous for either the Val or Met alleles (Val/Val

or Met/Met carriers) show a differential impact on cognitive and emotional function. Specifically, Val/Val carriers demonstrated less efficient PFC function in the context of cognitive paradigms (i.e., encoding and memory) while Met allele carries had less efficient processing during emotionally valenced tasks (i.e., viewing valenced pictures). Therefore, Val/Val and Met/Met carriers both experience an inhibition of PFC functioning due to their impact on dopamine levels. Given the initial evidence that COMT may be implicated in processes related to attention biases and depression, the current study also aims to explore the possible role COMT may play in the relationship between attention biases to emotion and depression.

Current Study

The current study aimed to investigate whether genetic risk (5-HTTLPR and COMT) moderates the relationship between attention biases to emotional faces and clinical depression onset prospectively across 18 months in a large (n= 428) community sample of youth. Given 5-HTTLPR was found to interact with avoidance of negative emotion to predict increases in depressive symptoms (Gibb et al., 2009), we hypothesized that youth at highest genetic risk of the 5-HTTLPR polymorphism (S/S carriers) who avoided negative emotional faces (sad or angry faces) would be at increased risk for an onset of clinical depression over time. An exploratory aim of the current study was to examine whether the relationship between attention biases to emotion and depression would be moderated by youth's COMT genotype or whether moderation would be specific to 5-HTTLPR genotype. Importantly, this study was designed to fill several notable gaps within the attention bias and depression literature including, 1) examining clinical depression onset via diagnostic interview rather than

self-reported depression symptomatology, 2) utilizing prospective measurement in order to establish a temporal precedence between attention biases and the development of depression, and 3) studying the relationship among attention biases, genetics risk, and depression in a sample of youth, which provides a developmentally significant time frame to identify risk factors for depression onset (Rutter et al., 2006).

Method

Participants

Participants included 428 children and adolescents who were recruited from suburban and urban school districts in Colorado and New Jersey. A brief screening was conducted with parents to determine eligibility of their child. Youth had to currently be in 3rd, 6th, or 9th grade. They were excluded if they had a severe learning or psychiatric problem (e.g., autism, psychosis) that was likely to interfere with completion of an extensive laboratory protocol. The sample was approximately evenly divided by sex, was of mixed ethnic origin representative of their geographic region, and ranged in age from 7 to 16 years old (see Table 2.2). Parents of youth were primarily mothers (91%). Median annual parental income was $90,000 and 16% of the youth received free/reduced lunch at school.

Procedures

Each eligible parent and youth visited the laboratory for the baseline assessment and 18-month follow-up assessments. Parents provided informed written consent for their own and their child's participation, and youth provided written assent at each in-person laboratory visit. The initial baseline assessment consisted of

youth completing the dot-probe task, collecting youth DNA via saliva collection, and diagnostic interviewing with youth and parents about their child, in that order. Follow up assessment conducting diagnostic interviews consisted of calling families 6 and 12 months after the baseline visit. Youth and their parents returned to the laboratory for the 18-month follow-up assessment where updated diagnostic interviewing took place with youth and parents about their child. The retention rate from baseline to 18-month follow-up was 89%. Trained and supervised graduate students, staff and undergraduate research assistants conducted the in-person and phone follow-ups. All procedures were approved by the Institutional Review Board at both sites. Youth and parents were reimbursed for their participation at baseline and all subsequent follow-ups. All youth and parents were given referral forms with lists of various affordable psychological services and community mental health centers in the area, regardless of diagnostic status.

Measures

  Diagnostic Status. Trained interviewers administered the Mood Disorders and Psychosis subsections of the well-validated Schedule for Affective Disorders and Schizophrenia for School Age Children (K-SADS-PL; Kaufman et al., 1997) to youth and parents about their child to assess for current and past episodes of depression and mania at baseline and each follow-up. No youth was diagnosed with a bipolar spectrum disorder or psychosis. Interviewers then utilized both youth report and parent report about youth to determine youth diagnostic status using best estimate diagnostic procedures. Interviewers and graduate students were trained by Ph.D. level, licensed psychologists to conduct the diagnostic interviews. Additionally, 20%

of interviews were randomly selected to conduct reliability analyses, and inter-rater reliability showed kappa was .91. Furthermore, all interviews containing a subthreshold or threshold criterion symptom of depression were reviewed for accuracy by another graduate student and disagreements were resolved by consensus. Youth participants were included in the Depression Onset group if they met DSM-IV criteria for a clinically significant depressive episode (i.e., Major Depressive Disorder or Depressive Disorder- Not Otherwise Specified) between the baseline and 18-month follow-up assessments or were currently depressed at the 18-month follow-up assessment. Approximately 16% of youth (n= 70) experienced an onset of clinical depression over 18 months. The No Depression Onset group (n= 358) consisted of youth who had not experienced an episode of clinical depression between the baseline and 18-month follow-up assessment. Rates of depressive episode onset found in the sample are similar to those found in other studies utilizing community samples of youth (Avenevoli, Knight, Kessler, & Merikangas, 2008; Lewinsohn, Hops, Roberts, Seeley, & Andrews, 1993).

   Attentional biases. Youth's attentional biases for facial displays of emotion were assessed using a modified dot-probe task (MacLeod et al., 1986) administered using E-Prime. Stimuli for the dot-probe task consisted of pairs of facial expressions that contained one affective (angry, sad, or happy), and one neutral photograph from the same actor taken from a standardized stimulus set (Tottenham et al., 2009). Photographs from each actor (16 men and 16 women) were used to create sad–neutral, happy-neutral, and angry–neutral stimulus pairs (96 pairs).

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Each stimulus pair was presented in random order over the course of two blocks, with a rest in between blocks, for a total of 192 trials. Each trial began with a blank computer display with only a white fixation cross in the middle of the screen for 1,000 milliseconds (ms). Then, a pair of pictures was presented for 1,000ms, followed by a dot where one of the prior pictures had been (either the affective or neutral picture) that was presented for 1000ms. Youth were instructed to indicate as quickly as possible the location of the dot (left vs. right side of the screen) using the computer keyboard ("z" labeled "left"; "/" labeled "right"). The computer recorded the accuracy and response time for each response. Consistent with prior research (Gotlib, Kasch, et al., 2004), trials with response errors were excluded as were trials with response times less than 150ms or greater than 1,500ms. Error rates were quite low (less than 1.5%), and a small portion (1.8%) were excluded for being out of response time range. Of the 428 children who completed the dot-probe task and follow-up depression interviews, 378 had completed genotype data for 5-HTTLPR and 415 had completed genotype data for COMT. The final samples within each gene did not differ from the total sample on age, gender, or ethnicity/race ($ps > .13$).

Mean attention bias scores (Mogg et al., 1995) were then calculated separately for each affective stimulus type (angry, sad, or happy face) by subtracting the mean response time for cases in which the probe replaced the affective face from mean response times for cases in which the probe replaced the neutral face. Positive bias scores represent preferential attention toward the affective face; negative scores indicate attentional avoidance of the affective face.

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Genotyping. Saliva samples were obtained from all study participants with Oragene™ (DNA Genotek, Ontario, Canada) collection kits, and DNA was extracted using standard salting-out and solvent precipitation methods. The method for 5-HTTLPR and SNP rs25531 (n=378) is detailed in Whisman, Richardson, and Smolen (2011). The rs25531 SNP genotypes (L_A vs. L_G) were obtained by incubating the PCR products with MspI (Wendland et al., 2006). Two groups of participants were formed based on their 5-HTTLPR genotyping: youth homozygous for the lower expressing S or L_G alleles (i.e., SS) and those heterozygous or homozygous for the higher expressing L_A allele (i.e., SL/LL). Genotyping of Val158Met rs4680 in *COMT* (n= 415) are outlined in (Haberstick and Smolen (2004). Three groups of participants were formed based on their COMT genotyping: children homozygous for the higher expressing Val allele (i.e., Val/Val), those heterozygous (i.e., Val/Met), and those homozygous for the low expressing Met allele (i.e., Met/Met). The successful call-rate for the overall project was 97.5% for 5-HTTLPR and 96.3% for COMT. All of the genotypes were in Hardy-Weinberg Equilibrium.

Results

Preliminary Analyses

Means and standard deviations for all primary variables separated by genotype for 5-HTTLPR and COMT (Table 2.1) and descriptive statistics separated by diagnostic status (Table 2.2) are presented. There were no significant differences among genotypes for age (ps> .18), race (ps> .26), or gender (ps> .27). There were no depression group differences in ethnicity or race (ps> .16; Table 2.2). Consistent with epidemiological studies of youth depression (e.g., Avenevoli et al., 2008), the

Depression Onset group consisted of older youth and slightly more females than males (see Table 2.2).

Data Analytic Plan

Logistic regression analyses were used to test the Gene (5-HTTLPR or COMT) x Attention Bias (sad, angry, or happy) interaction as a predictor of depression group status (Depression Onset coded 1 and No Depression Onset coded 0) across 18 months using the SPSS macro PROCESS (Hayes, 2013). Attention bias scores were centered prior to analyses. All main effects and interactions were entered simultaneously and unstandardized regression coefficients are reported (Hayes, 2013) for each set of analyses.

Post hoc analyses of significant interactions were conducted (Aiken & West, 1991; Holmbeck, 2002). New product terms were computed at different levels (i.e., genotype groups) of the moderator variable. Separate regressions were conducted that included each of these product terms. This enables examination of the significance of simple sloes at different levels of genotype.

5-HTTLPR. There were no significant gene-environment correlations (rGEs) between 5-HTTLPR and attention biases to any emotion (*ps*> .07). Logistic regression analyses revealed a significant interaction between 5-HTTLPR and attention bias to angry emotion predicting depression group status across 18 months (Table 2.3)[2]. This effect is shown in Figure 2.1 across the range of attention bias

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[2] Results were virtually the same controlling for baseline and lifetime depression diagnoses with the K-SADS and baseline symptoms of anxiety symptoms as measured by the Multidimensional Anxiety Scale for Children (MASC; March, Sullivan, & Parker, 1999).

scores seen in our sample. Post-hoc analyses showed a significant slope for those with the S/S genotype (b = -1.43, SE = .66, t = -2.16, p =.03) indicating that youth homozygous for the S allele were more likely to experience an onset of clinical depression when avoiding compared to attending to angry facial expressions. The slope for the SL/LL genotype group (b = .82, SE = .73, t = 1.13, p = .26) was not significant. No significant main effects or interactions were found when predicting depression group status with attention biases to sad or happy faces (Table 2.3).

COMT. There were no significant rGEs between COMT and attention biases to sad or angry emotion (ps> .30). However there was a significant rGE between COMT and attention bias to happy faces (p=.003). Analyses showed that there was not a main effect of COMT or attention bias to any emotion and attention biases did not interact with COMT to predict depression group status across 18 months (Table 2.4).

Discussion

Despite considerable research within the depression literature on information processing biases and genetic risk factors, no individual study has examined whether attention biases to emotion within the context of genetic risk predicts the onset of clinical depression, in either adults or youth. The current study aimed to bridge this notable gap by investigating whether biased attention to emotional faces was moderated by two biologically plausible genes (5-HTTLPR, COMT) to predict clinical depression over time. Results supported hypotheses and demonstrated that youth who avoided negative emotion (i.e., anger) and were homozygous for the S allele of the 5-HTTLPR gene predicted prospective depressive episode onset among

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youth. Increased risk for depression onset was specific to the 5-HTTLPR gene and was not found when examining moderation by COMT. These findings are partially consistent with a previous study by Gibb and colleagues (2009) that demonstrated avoidance of sad faces was moderated by 5-HTTLPR genotype to predict increases in depression symptoms over time among a sample of youth at-risk for depression. In sum, the current study provided an important next step in the field by predicting onset of disorder using rigorous methodologies (objective measure of biases, gold-standard assessment of depression, prospective design) within a psychobiological framework of risk during a developmentally significant time frame for onset of depression diagnosis.

The current study is a unique and exciting contribution to the depression literature in several ways. Primarily, it is the first study to directly examine the prospective risk for depression conferred by attention biases to emotion and genetic risk. This is an important advancement as attention bias to emotion has been theorized to function as an intermediate trait associated with 5-HTTLPR to predict depression, but no previous research has tested this model within a single investigation. Additionally, previous research findings have been mixed with cross-sectional studies of attention biases within clinically depressed samples of adults and youth finding attentional biases toward negative emotional stimuli (Hankin, Gibb, et al., 2010; Joormann & Gotlib, 2007) and one longitudinal examination among at-risk youth finding attentional avoidance of negative emotion to be predictive of increases in depression symptoms (Gibb et al., 2009). It is possible that the paucity of prospective designs, diagnostic interview data, and integration among risk factors across multiple

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levels of analysis may have contributed to the lack of cohesion among findings in the attention bias and depression literature. The methodologically rigorous design of the current study addressed these concerns in order to provide clarity among mixed findings.

Although there is little empirical evidence examining the mood and interpersonal consequences of repeated patterns of preferential attention versus avoidance over time, it is possible that these patterns may emerge differentially in the trajectory of depression. Evidence suggests that individuals in a current negative mood exhibit mood congruent preferential attention to negative emotion (e.g., Hankin, Gibb, et al., 2010; Joormann & Gotlib, 2007) whereas findings from the current study and Gibb et al., (2009) show attentional avoidance of negative emotion to be a predictor of future risk for depression symptoms on clinical onset. This leads to the question: Why would avoidance of negative emotion function as a risk factor for depression? One possibility is that attentional avoidance of negative emotion, particularly an emotion that confers interpersonal threat such as anger, may be associated with maladaptive coping responses to interpersonal stress. Research shows that interpersonal difficulties and subsequent maladaptive coping (i.e., ineffective problem solving, disengagement coping) are prospectively related to depression in adolescence (Flynn & Rudolph, 2010; Rudolph, 2009). It is possible that the transactional processes between interpersonal stress and attentional avoidance to negative emotion are contributing to depression onset, whereas mood congruent attention biases, such as preferentially attending to sad faces, are observed once the individual is currently experiencing a depressive episode. It will be important for future research to

investigate both the time course of attention biases from risk to onset of depression as well as the possible ecological consequences of attentional avoidance of negative emotion in order to better understand the relationship between attention biases and depression.

Furthermore, the current study's findings highlight the importance of examining risk for depression across multiple levels of analysis, such as combined genetic and cognitive risk. Our results suggest that attentional avoidance alone did not predict future onsets of depression; rather, only youth who exhibited both attentional avoidance and were at high genetic risk (i.e., SS carriers of 5-HTTLPR) were found to be at greater risk for depression onset across 18 months. Given that the S allele of 5-HTTLPR has been associated with emotional reactivity (Gotlib et al., 2008; Munafò et al., 2008), it is possible that those who are both more reactive to their environment and engage in maladaptive coping strategies, such as avoidance of strong negative emotions, are at highest risk for experiencing an episode of depression over time. Additionally, these findings support the need for further research examining the efficacy of attention bias modification in the treatment of youth depression. Previous research has shown mixed findings for the efficacy of attention bias modification in depressed adults (Hallion & Ruscio, 2011). The current study's findings suggest that the inclusion of certain related factors, such as emotion regulation deficits, may reveal moderators to treatment success. It will also be informative for future research to examine behavioral and other biological indicators (e.g., cortisol reactivity) of emotion regulation in conjunction with attention biases to emotion and genetic risk to predict depression onset among youth.

Although exploratory, we hypothesized COMT would interact with attention bias to emotion to predict depression onset given studies implicating COMT in processes related to attention biases and depression (Egan et al., 2001; Papaleo et al., 2008). Importantly our findings were specific to 5-HTTLPR as no main effects or interactions were found with COMT predicting depression onset. This finding is consistent with a recent review demonstrating less robust effect sizes when examining COMT's relationship to both depression diagnosis and behavioral correlates of depression, such as startle response and emotion identification as compared to the association between COMT and neurological functioning (Antypa et al., 2013). Although these findings do not directly mirror the current study's methodology, they are suggestive that COMT is more sensitively studied through neuroimaging rather than behavioral techniques. Future studies integrating brain imaging, genetics, behavioral tasks and diagnostic interviewing techniques within a prospective design would help to clarify the specificity of findings within the attention bias, candidate gene, and depression literature.

Several features of the current study provided a methodologically rigorous and novel approach to examining the relationship among attention biases, genes, and depression in youth. Primarily, this is the first study to examine the prospective impact of attention biases to emotion on the development of depression among either adults or youth. Additionally, the present study employed a multi-informant, well-validated diagnostic interview to evaluate depression diagnoses using best estimate procedures over 18 months. Previous research has either utilized cross-sectional or symptom level data, which did not allow for examination of attention biases as a

predictive risk factor for clinical depression. The current study investigated multiple theoretically based and biologically plausible candidate genes rather than an atheoretical GWAS methodology or a single candidate gene design. A theoretical approach to gene selection allows for a more sensitive and powerful test of genetic effects. Another strength is the use of a large community sample of youth as it provided a powerful test and more generalizable results compared to clinically selected samples (Cohen & Cohen, 1984). Finally, we utilized a more objective measure of cognitive bias that is less prone to reporting biases. Overall, the use of multi-informant, interview based depression assessment, longitudinal design, theoretically chosen candidate genes, and a generalizable youth sample lends confidence the present study's findings.

Despite considerable strengths, limitations of the current study provide avenues for future research. Although depression diagnoses were assessed over time, attention biases were assessed at one time point. Future research should consider assessment of attention biases at multiple time points to allow for examination of stability versus change and how patterns of biases may impact risk for depression. Multiple assessments of attention biases would also allow for examination of whether patterns in biases change pre- to post-depression onset. Additionally, the current study did not assess for possible moderators or mediators to the gene x attention bias interaction predicting depression. Given research shows stress to play an important role in the relationship between 5-HTTLPR and depression (Karg et al., 2011), it may be important to investigate the impact of stressors occurring prior to the onset of depression. It is possible that the type of bias (e.g., type of emotion, avoidance versus

preferential attention) associated with depression onset may change if the individual has experienced recent stressors and/or chronically high levels of stress. Finally, although the current study's measure of attention bias is more objective compared to self-report data, there are other, more precise measurement tools available to investigate information processing biases. For example, eye-tracking methodology allows for real time assessment of attention biases at various interval lengths, which allows for examination of whether the time-course of attention biases is related to depression onset.

In sum, the current study demonstrated that youth who showed attentional avoidance of angry faces and were homozygous for the S allele of 5-HTTLPR were at higher risk for experiencing an episode of clinical depression over time. This effect was specific to 5-HTTLPR and not seen when examining COMT variants. These findings suggest that avoidance of anger, especially among youth at a measured genetic risk, play a role in the development of depression and may inform future depression intervention research involving attention biases to emotion.

Conclusion

This research aimed to take a psychobiological approach to elucidating how the relationship between genetic risk (5-HTTLPR and COMT) and attention bias to emotion is related to the onset of youth depression. The first aim of this research was to examine the direct relationship between genetic risk and attention bias to emotion with findings demonstrating a differential effect of gene. Youth who were homozygous for the low expressing allele of 5-HTTLPR (S/S) and had experienced more recent SLEs within the last three months demonstrated preferential attention toward negative emotional faces (angry and sad). However, youth who were homozygous for the high expressing COMT genotype (Val/Val) and had experienced more recent SLEs showed attentional avoidance of positive facial expressions (happy). These findings are suggestive that different pathways may exist either both predicting depression onset (i.e., equifinality) or that divergent pathways to disorder may exist initiated by specific genes and attention biases.

Given these findings, a second goal of this research was to examine the full pathway from gene to attention bias to depression onset (Chapter 2). However, instead of a mediation model that much of the literature proposes, the results seen in

Chapter 2 demonstrated an interaction effect whereby youth who avoided negative emotion (i.e., anger) and were homozygous for the S allele of the 5-HTTLPR gene were at greater risk for prospective depressive episode onset. This increased risk for depression onset was specific to the 5-HTTLPR gene and was not found when examining moderation by COMT.

Although the findings from Chapter 2 were somewhat unexpected based on the results from Chapter 1 showing a direct correlation between genetic risk and attention biases within the context of increased stress exposure, it is important to note that Chapter 1 and previous research examining 5-HTTLPR and attention bias to emotion (Pergamin-Hight et al., 2012) have not included depression onset as an outcome. Furthermore, results seen in Chapter 2 are somewhat consistent with the single study that has examined attention bias and genetic risk predicting depression symptom increases longitudinally among youth. Specifically, Gibb and colleagues (2009) found that youth of depressed mothers who avoided sad faces and were S carriers of 5-HTTLPR predicted increases in child depressive symptoms over time. Although Gibb and colleagues did not examine clinical onset, it suggests that attention bias to emotion moderates the relationship between genetic risk and depression as opposed to mediates this relationship.

Additionally, it is notable that it was the avoidance of anger that interacted with 5-HTTLPR to predict depression onset, yet 5-HTTLPR genotype status predicted attention toward negative emotion (both sad and angry faces) within the context of greater stressful life events in Chapter 1. Furthermore, this research did not find a relationship among COMT genotype, attention bias to emotion and depression

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onset, whereas COMT was related to avoidance of happy faces within the context of greater stressful life events. These findings may appear contradictory; however, it is possible that attention bias to emotion may operate differently when considering risk for clinical depression onset as opposed to examining the relationship between genes and attention bias among a community sample of youth. Additionally, the role of stressful life events was not examined in relation to attention bias to emotion and onset of depression. It is also important to note that both studies were focused primarily on depression as opposed to other clinical disorders, so it is conceivable that the gene-attention bias relationships seen in Chapter 1 are part of pathways to other psychological disorders, such as anxiety disorders or externalizing psychopathology. Future research that examines clinical onset of various forms of internalizing and externalizing psychopathology is needed in order to fully understand the relationship among genes, attention bias to emotion, and psychopathology among youth.

In sum, this research made an innovative contribution to the field of translational research by examining both the relationship between biased attention to emotional stimuli and theoretically relevant candidate genes along with how these factors may contribute onset of depression longitudinally among youth. This research may also have important clinical applications given the burgeoning field of utilizing attention retraining tasks in the treatment of depression and other internalizing psychopathology.

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Table 1.1

Descriptive statistics overall and by 5-HTTLPR and COMT genotypes.

| | | 5-HTTLPR | | | COMT | | |
| | Full Sample | LL/SL | S/S | | Met/Met | Val/Met | Val/Val |
Variable	Mean(SD)	Mean(SD)	Mean(SD)		Mean(SD)	Mean(SD)	Mean(SD)
Negative Bias	-13.19(74.18)	-6.50(74.46)	-29.58(72.87)		-9.89(63.59)	-12.51(79.68)	-16.24(74.56)
Positive Bias	-12.52(52.59)	-8.97(53.90)	-19.15(48.09)		-18.23(46.60)	-15.29(51.67)	.55(55.74)
ALEQ	16.00(5.98)	15.66(5.99)	14.65(6.22)		15.72(5.98)	15.44(6.04)	15.47(6.28)

Note: ALEQ=Adolescent Life Events Questionnaire combined parent and child report; ALEQ scores were computed by averaging the number of parent and child reported life events in the previous 3 months and ranged from 1.50 to 31. Negative Bias combined sad and angry attention bias scores and Positive Bias score included happy attention bias scores.

Table 1.2
Demographic Characteristics by Genotype

	Full Sample	5-HTTLPR (n= 416)		COMT (n= 456)		
	(n=467)	LL/SL	SS	Met/Met	Val/Met	Val/Val
Age (Mean, SD)	11.91(2.30)	11.90(2.28)	12.19(2.39)	12.07(2.12)	11.96(2.40)	11.95(2.36)
Girls	59%	58%	58%	54%	36%	46%
Boys	41%	42%	42%	46%	64%	54%
Ethnicity						
Hispanic	8%	9%	6%	8%	6%	11%
Non-Hispanic	92%	91%	94%	92%	94%	89%
Race						
Caucasian	69%	69%	62%	72%	67%	68%
African American	12%	10%	16%	11%	13%	13%
Asian/P. Islander	7%	11%	16%	12%	12%	12%
Other/Mixed Race	7%	8%	6%	5%	8%	7%

Table 1.3

Prediction of Attention Biases to Negative and Position Emotional Faces from 5-HTTLPR and Stress Life Events (SLEs)

Negative Emotion (Angry/Sad)

Predictor	b (SE b)	t	p
5-HTTLPR	-18.59(4.16)	-2.23	.03
SLEs	-.14(.69)	-.20	.84
5-HTTLPR x SLEs	4.05(1.47)	2.75	.006

Positive Emotion (Happy)

Predictor	b (SE b)	t	p
5-HTTLPR	-9.93(6.07)	-1.64	.10
SLEs	-.37(.42)	-.88	.38
5-HTTLPR x SLEs	-.1464(1.0)	-.15	.88

Table 1.4

Prediction of Attention Biases to Negative and Position Emotional Faces from COMT and Stress Life Events (SLEs)

Negative Emotion (Angry/Sad)

Predictor	*b (SE b)*	*t*	*p*
COMT	-3.13(4.50)	-.69	.49
SLEs	.92(.94)	.98	.33
COMT x SLEs	.005(.74)	.006	.99

Positive Emotion (Happy)

Predictor	*b (SE b)*	*t*	*p*
COMT	9.69(3.35)	2.89	.004
SLEs	.73(.66)	1.11	.27
COMT x SLEs	-1.07(.55)	-1.94	.05

Table 2.1

Descriptive statistics overall and by 5-HTTLPR and COMT genotypes

Variable	Full Sample (n= 428) Mean(SD)	5-HTTLPR (n=378) LL/SL Mean(SD)	S/S Mean(SD)	COMT (n= 415) Met/Met Mean(SD)	Val/Met Mean(SD)	Val/Val Mean(SD)
Angry Bias	-8.67(52.83)	-16.75(50.71)	-4.73(54.14)	-6.36(45.16)	-7.18(54.02)	-13.36(56.04)
Happy Bias	-11.24(52.46)	-16.81(49.69)	-8.72(54.30)	-18.31(46.01)	-14.12(52.01)	1.80(56.85)
Sad Bias	-3.301(52.27)	-9.59(46.95)	.19(53.68)	-5.16(50.03)	-2.01(54.74)	-1.07(50.22)

Table 2.2
Demographics by Depressive Episode Onset from Baseline to 18-Month Follow-up

	No Depressive Episode (n=358)	Depressive Episode (n= 70)	Total Sample (n=428)
Age (Mean (SD))***	11.77(2.28)	12.96(2.07)	11.97(2.28)
Gender[†]			
Girls	57%	69%	59%
Boys	43%	31%[†]	41%
Ethnicity			
Hispanic	8%	4%	8%
Non-Hispanic	92%	96%	93%
Race			
Caucasian	68%	72%	69%
African American	12%	10%	12%
Asian/P. Islander	13%	6%	12%
Other	7%	12%	7%

Note. Significant depression group differences indicated by ***$p<.001$ and [†]$p< .06$

Table 2.3

Prediction of depressive episode onset across 18 Months from 5-HTTLPR and Attention Biases to Emotional Faces (Sad, Angry, Happy)

Sad Faces

Predictor	b (SE b)	z	p
5-HTTLPR	.18(.34)	.52	.60
Sad Bias	-.003(.003)	-.93	.35
5-HTTLPR x Sad Bias	.01(.008)	1.39	.16

Angry Faces

Predictor	b (SE b)	z	p
5-HTTLPR	-.08(.39)	-.20	.84
Angry Bias	.004(.003)	1.32	.19
5-HTTLPR x Angry Bias	-.02(.007)	-2.46	.01

Happy Faces

Predictor	b (SE b)	z	p
5-HTTLPR	.15(.34)	.43	.67
Happy Bias	-.001(.003)	-.34	.73
5-HTTLPR x Happy Bias	.008(.007)	1.26	.21

Table 2.4

Prediction of depressive episode onset across 18 Months from COMT and Attention Biases to Emotional Faces (Sad, Angry, Happy)

Sad Faces

Predictor	*b (SE b)*	*z*	*p*
COMT	-.25(.18)	-1.38	.17
Sad Bias	.001(.004)	.12	.90
COMT x Sad Bias	-.001(.004)	-.18	.85

Angry Faces

Predictor	*b (SE b)*	*z*	*p*
COMT	-.25(.18)	-1.38	.17
Angry Bias	-.003(.005)	-.65	.51
COMT x Angry Bias	.003(.004)	.87	.38

Happy Faces

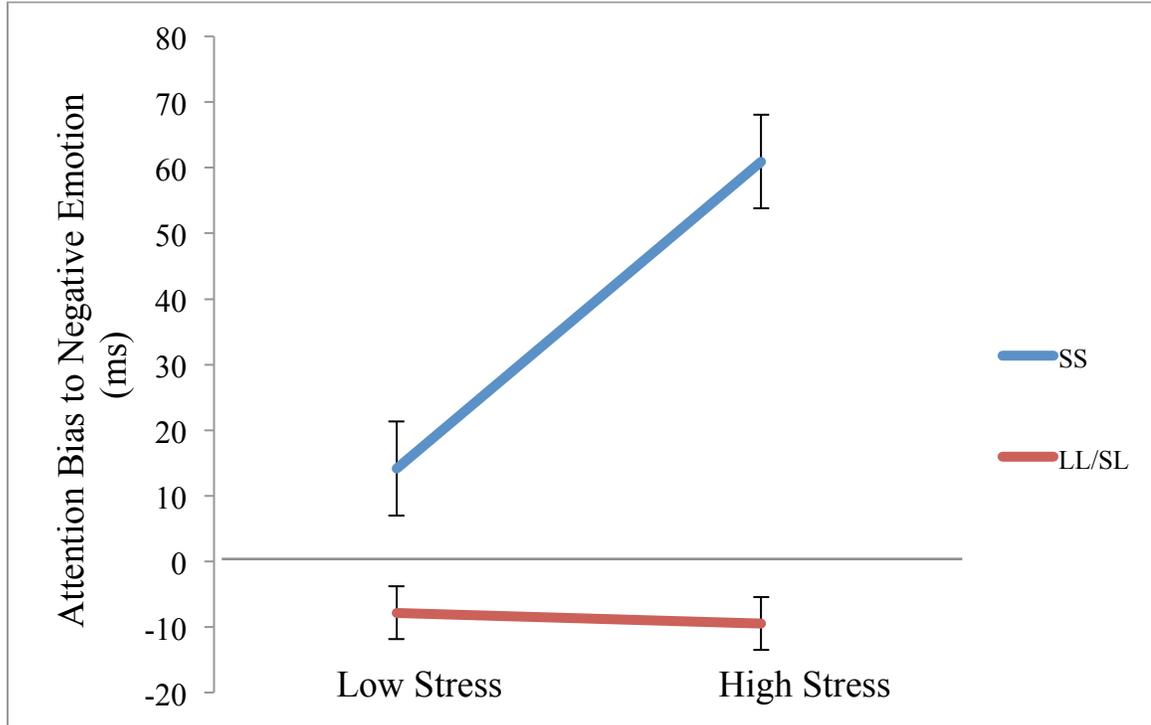| Predictor	*b (SE b)*	*z*	*p*
COMT	-.25(.18)	-1.38	.17
Happy Bias	.003(.005)	.56	.57
COMT x Happy Bias	-.002(.004)	-.65	.51

Figure 1.1 Interaction between 5-HTTLPR and stressful life events (SLEs) predicting attention bias to negative emotion (i.e., sad and angry emotional faces) with SLEs depicted at 1 SD above (High Stress) and below (Low Stress) the mean.
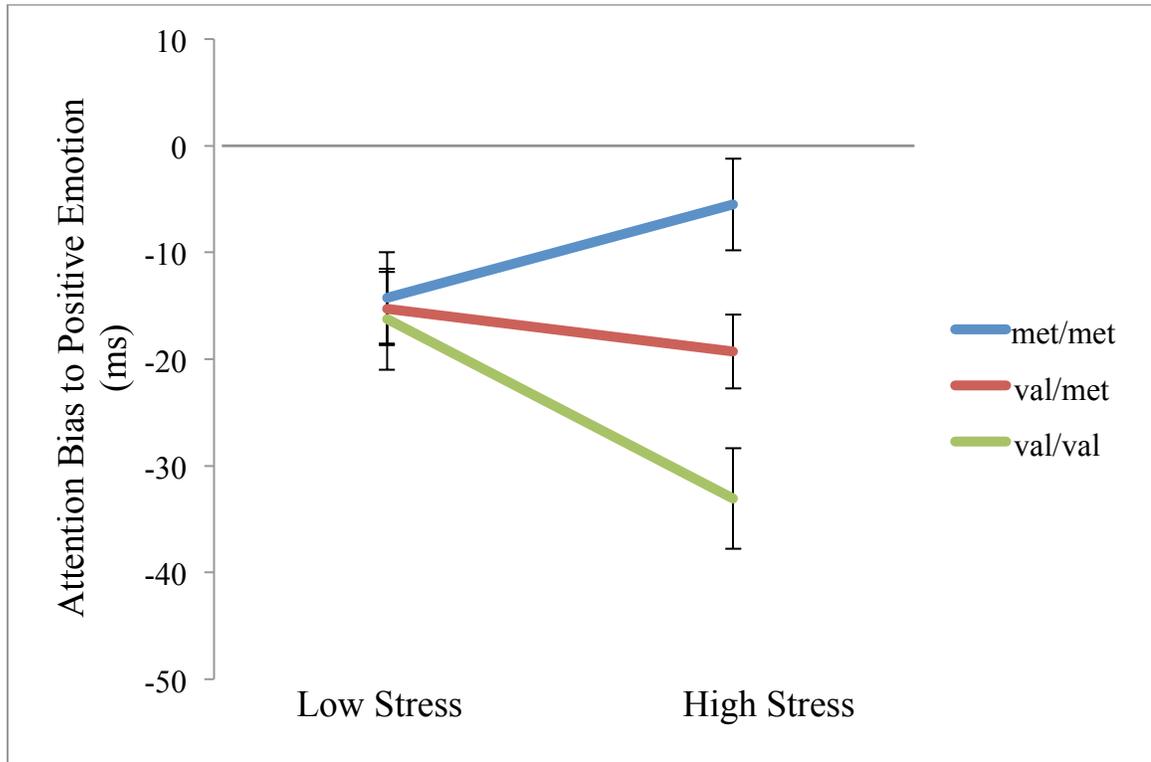
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Figure 1.2. Interaction between COMT and stressful life events (SLEs) predicting attention bias away from positive emotion (i.e., happy faces) with SLEs depicted at 1 SD above (High Stress) and below (Low Stress) the mean.
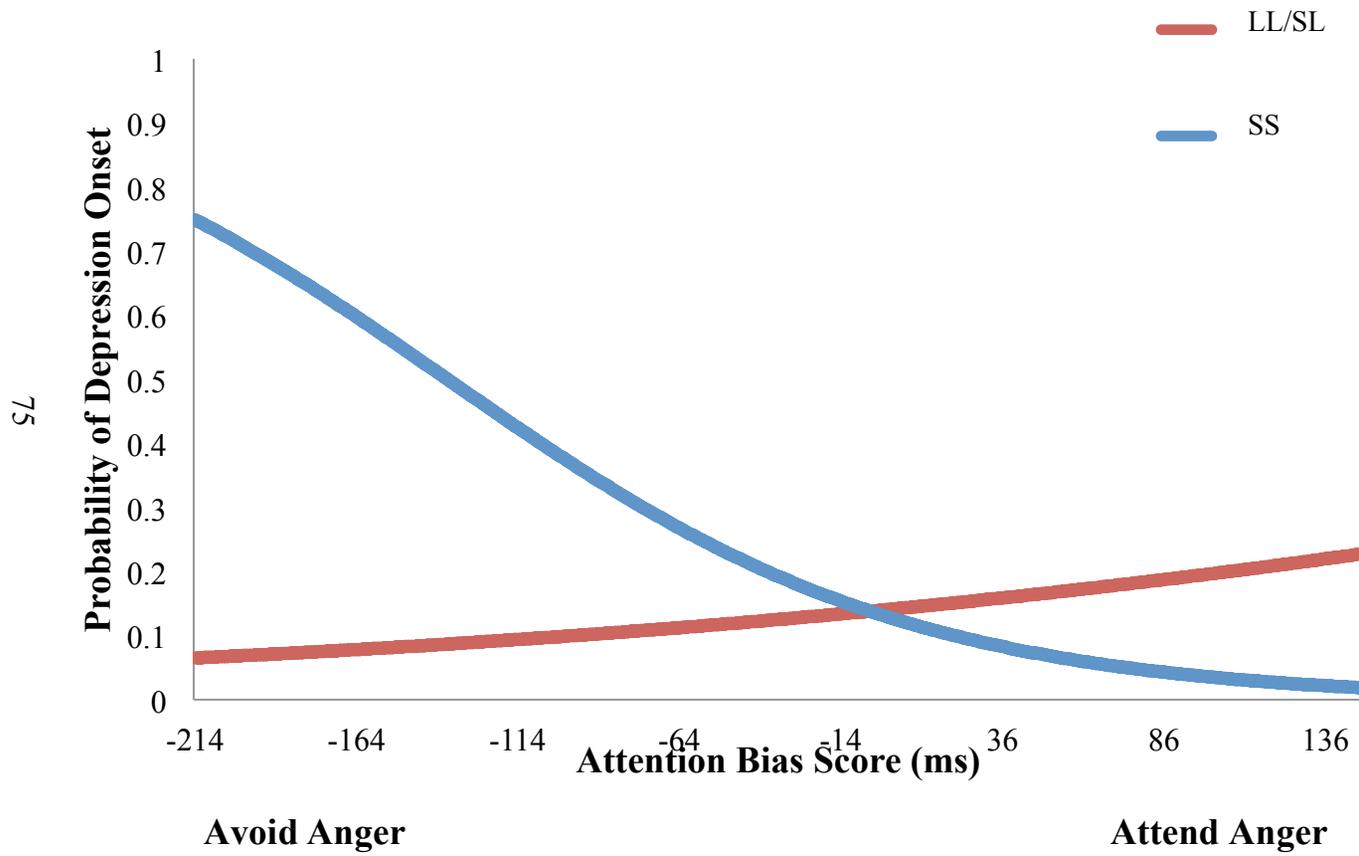
Figure 2.1. Interaction between 5-HTTLPR and bias away from angry faces predicting depression onset across 18 months using observed attention bias scores within the study's sample.