

GENE-ENVIRONMENT INTERACTION IN EARLY ONSET BIPOLAR DISORDER

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ABSTRACT

MEGAN JOSEPH FREEMAN: Gene-Environment Interaction in Early Onset Bipolar Disorder

(Under the direction of Eric A. Youngstrom, PhD)

Bipolar disorder is a serious and impairing mental illness that affects up to 4% of the US population. Early onset (before age 21) suggests a more genetically driven form of the disorder and is associated with a poorer prognosis. Both genetic and environmental effects are recognized as significant contributors to the etiology of this disorder. The goal of this study was to identify gene-environment interactions (GxE) that increase or decrease the likelihood of developing early onset bipolar disorder symptomatology in late adolescence. Potential mechanisms behind the hypothesized GxE were also tested. Participants provided DNA for the sequencing of the serotonin transporter promoter polymorphism (HTTLPR), filled out questionnaires regarding retrospective family functioning and their current mood state, and completed an affective memory task measuring biased attention towards emotional stimuli. Family environment factors such as positive expressed emotion, family closeness, and conflict predicted changes in depression and/or mania symptoms. Moderated mediation models involving attention to emotion faces were not significant. There were no statistically significant gene-environment interactions, most likely due to the study being underpowered. Results from this study contribute to a growing body of knowledge regarding best practices and feasibility around gene-environment research.

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LIST OF ABBREVIATIONS

ADHD	Attention Deficit/Hyperactivity Disorder
ANOVA	Analysis of variance
BDNF	Brain-derived neurotrophic factor
COMT	Catechol-O-methyltransferase
DNA	Deoxyribonucleic acid
DRD4	Dopamine receptor D4
EE	Expressed emotion
EOBD	Early-onset bipolar disorder
FAD	Family Assessment Device
FES	Family Environment Scale
GBI	General Behavior Inventory
GxE	Gene-environment interaction
HTTLPR, 5-HTTLPR	Serotonin transporter promoter polymorphism
MAF	Minor allele frequency
MAOA	Monoamine oxidase A
PCS	Perceived Criticism Scale
POMP	Percent of maximum possible
SES	Socioeconomic status
SLC6A4	Serotonin transporter gene
SSRI	Selective serotonin reuptake inhibitor

GENE-ENVIRONMENT INTERACTION IN EARLY ONSET BIPOLAR DISORDER

Early onset bipolar disorder (EOBD) is a serious psychiatric disorder characterized by marked shifts in energy (Angst, 2009) as well as mood swings involving depression, irritability, and mania (Youngstrom, Birmaher, & Findling, 2008). Associated symptoms and behaviors include irritability, decreased need for sleep, hypersexuality, and poor judgment that in adults is characterized by risk taking behaviors such as substance abuse, overspending, and promiscuous sex. EOBD is associated with increased risk for hospitalization, psychosis, and suicide and decreases in healthy school, peer, and family functioning (Birmaher & Axelson, 2006; Geller, Bolhofner, et al., 2000; Goldstein, et al., 2005). Bipolar disorder is the seventh leading cause of years lost to disability worldwide (World Health Organization, 2008), and in the US, total economic burden has been estimated at over 150 billion dollars per year for bipolar I and bipolar II disorders alone (2009 estimate; Dilsaver, 2011).

Although it is recognized that both genetic makeup inherited from parents as well as environmental experiences contribute to risk for onset of psychiatric disorders and shape the course of these disorders (Rutter, 2009), it is only recently that we have begun to understand the ways in which this might happen. Recent work in gene-environment interaction suggests that genes and environmental experiences make not only unique contributions to mental illness, but also that they interact in complex ways to influence development and symptomatology (Caspi, et al., 2003; Etain, Henry, Bellivier, Mathieu, & Leboyer, 2008; Kim-Cohen, et al., 2006; Mill, et al., 2006; Polanczyk, et al., 2009).

The primary objective of this study is to identify a specific gene-environment interaction effect that could change risk for EOBD symptomatology. A hostile and conflictual early family environment (“expressed emotion” or EE) combined with a risk allele of a serotonin gene is hypothesized to contribute to elevated symptoms of bipolar disorder in late adolescence. Given the high rates of diagnosis (Blader & Carlson, 2007; Moreno, et al., 2007) and the suffering associated with EOBD (Geller, et al., 2001; Geller, Zimmerman, et al., 2000; Goldstein, et al., 2009; Lin, et al., 2006), highly attractive potential outcomes of this study include improved detection of factors that are associated with a heightened risk of developing mood disorders in childhood and a better understanding of how risk factors interact to produce greater or lesser levels of bipolar disorder symptomatology. On the other hand, negative findings will narrow the field of potential contributing factors to the development and maintenance of bipolar disorder in childhood and adolescence, allowing for a more streamlined approach to detection and treatment. These findings have not only public health implications, but also implications for the many families caring for an individual with bipolar disorder.

Subsequent sections of this paper will expand upon the basis for the proposed hypotheses.

EOBD Is Increasingly Diagnosed and Is Associated With Significant Functional Impairment

Bipolar disorder is proving more common than previously realized, affecting between four and six of every 100 adults in the USA based on recent epidemiological studies (Grant, et al., 2005; Judd & Akiskal, 2003; Merikangas, et al., 2007). A recent meta-analysis of epidemiological studies of pediatric bipolar disorder, however, indicated a lower overall rate

of 1.8% in youth (Van Meter, Moreira, & Youngstrom, 2011). In contrast to this report, several highly publicized reports of increasing rates in the community have elevated concerns of over-diagnosis of early-onset bipolar disorder (EOBD). Between 1996 and 2004, there was an over 400% increase in the number of children discharged from psychiatric hospitals with a diagnosis of bipolar disorder, and a nearly 300% increase for adolescents being given the diagnosis; it is now the most common discharge diagnosis of psychiatrically hospitalized youth based upon chart review studies using clinical diagnoses (Blader & Carlson, 2007). Rates of diagnosis are increasing in outpatient care as well; up to a 40-fold increase between 1994 and 2003 has been reported (Moreno, et al., 2007). Significantly, approximately 60% of adults with bipolar disorder experience the onset of their illness in childhood or adolescence (Lish, Dime-Meenan, Whybrow, Price, & Hirschfeld, 1994).

The early-onset phenotype (illness onset before age 21) may represent an especially genetically driven form of the disorder (Lin, et al., 2006; Rende, et al., 2007) and is associated with increased risk of suicide and substance abuse, above and beyond levels of risk seen in bipolar disorder in general (Lin, et al., 2006). Poor outcomes for youth have been demonstrated longitudinally in domains such as treatment response, recovery rates, and relapse rates; these outcomes are similar to those seen in adults with severe, treatment resistant bipolar disorder (Geller, et al., 2001; Geller, Zimmerman, et al., 2000). Children and adolescents with bipolar spectrum disorders utilize more inpatient services than children with depressive disorders or disruptive behavior disorders and are more likely to use multiple types of mental health services concurrently, independent of impairment and comorbidity (Mendenhall, et al., 2011). A longitudinal study of youth with bipolar disorder showed that after two and a half years, 81.5% of youth had recovered from their initial mood episode;

however, one and a half years after that, 62% had experienced another mood episode, and 30% had had more than two episodes (Birmaher, et al., 2009). Furthermore, participants had experienced symptoms during 60% of the follow-up period. A five-year follow-up of youth with bipolar disorder not otherwise specified showed that 45% converted to bipolar I or II disorder, and those that converted spent a median of only 32% of follow-up weeks with no or minimal mood symptoms (Axelson, et al., 2011).

Perhaps most concerning, nearly one-third of youth with bipolar disorder have a lifetime history of attempting suicide (Goldstein, et al., 2005), and up to 15% of individuals with bipolar disorder will go on to complete suicide at some point in their lives (Frank & Thase, 1999). In fact, early age of onset is a risk factor for subsequent suicidal behavior in adulthood (Slama, et al., 2004). Youth with bipolar disorder are also at increased risk for substance abuse, and for behavioral, academic, social, and legal problems (Birmaher, 2007). Additionally, youth with EOBBD experience greater impairment in relationships with parents, family, and peers than do youth with ADHD or typically developing youth (Geller, Bolhofner, et al., 2000).

The burden on the family of an affected individual is significant and is comparable to the burden experienced by caregivers of individuals with schizophrenia (Chadda, Singh, & Ganguly, 2007; Friedmann, et al., 1997). Eighty-nine percent of caregivers of a friend or relative with bipolar disorder report moderate or higher levels of distress as a result of the affected individual's problem behaviors (Perlick, et al., 2007). Importantly, high levels of caregiver burden result in negative effects on course and outcome for the bipolar individual, predicting depressive episodes at 7-month follow-up (Perlick, Rosenheck, Clarkin, Raue, & Sirey, 2001) and lower medication adherence by the bipolar individual (Perlick, et al., 2004).

Though a diagnosis of bipolar disorder suggests a lifelong illness that will need to be constantly monitored and managed, there is good evidence that early identification and treatment lead to better outcomes longitudinally (Berk, et al., 2007; Hirschfeld, Lewis, & Vornik, 2003; Lish, et al., 1994; c.f. Cicero, Epler, & Sher, 2009). Identifying and diverting or mitigating the course of a mood disorder beginning in childhood or adolescence could reduce the impact of the psychological, emotional, family, and financial burdens that are associated with bipolar disorder as the illness progresses.

The Importance of Genes and Environment

Although both genetic makeup (McGuffin, et al., 2003) and environment (Sameroff & Seifer, 1983) clearly make unique contributions to the etiology of mental illnesses, it is becoming evident that the interaction between the two may be of equal or greater importance (Moffitt, Caspi, & Rutter, 2006; Neiderhiser, 2001). Gene-environment interaction (GxE) is defined by the effect of an environmental stressor differing depending upon genotype. The concept of GxE is much like the classic “diathesis-stress” model of psychopathology: A pre-existing biological vulnerability to a particular disorder heightens response to an environmental stressor, “turning on” the disorder (Zuckerman, 1999). Alternately, an environmental stressor amplifies the action of some gene, resulting in psychological difficulties or changes in behavior. GxE attempts to explain the often widely different outcomes—“heterogeneous phenotypes”—that are frequently observed in human behavior despite similar genotypes or environmental experiences. A GxE model suggests that a specific genotype only confers vulnerability in the presence of certain environmental conditions, and vice versa.

The study of GxE in mental illness has been called “vital” (Craddock & Russell, 2006). Whereas in the 1980s and early 1990s most scientists dismissed GxE as uncommon and unimportant (Rutter, 2007), molecular geneticists now recognize that GxE is proving to be a pivotal mechanism in the development of many human diseases (Hunter, 2005). Indeed, scientists have been puzzled by the lack of significant findings when genome-wide association studies of complex medical and psychiatric disorders have been undertaken (Manolio, et al., 2009). This has been called “the case of the missing heritability” (Maher, 2008). Despite thorough, large-scale studies, direct effects of genes are explaining only tiny amounts of the variance in statistical models (e.g., Zeggini, et al., 2008). One of the largest findings to date is in Crohn’s disease, where a meta-analysis of three genome-wide association studies identified 32 genetic loci which explain just 10% of the overall variance in disease risk and only one fifth of the genetic risk (Barrett, et al., 2008). Though this may seem small, the magnitude dwarfs previous findings for any human disease (except age-related macular degeneration, for which several genes of large effect account for 50% of the heritability; Maller, et al., 2006). Typical findings in psychiatric genetics are tenths of a percent of the variance in risk, or odds ratios of around 1.2 (e.g., Fan & Sklar, 2008; Faraone, et al., 2005), though odds ratios between 2 and 6 can be observed (Hettema, Neale, & Kendler, 2001; Sullivan, Neale, & Kendler, 2000). The current prevailing belief is that we will never see large effects for single (candidate) genes (Clayton, 2009), and that gene-environment interplay is crucial in explaining complex human behavior such as psychiatric disorders (Narusyte, et al., 2008).

As the study of the human genome moves forward, science must focus less on simple heritability and more on the interplay of genetic vulnerabilities and environmental risk (Gray

& Hannan, 2007; Rusk & Rusk, 2007; Rutter, 2007). Experts have gone so far as to suggest that the role of genes, especially with regard to psychiatric illnesses, may be to influence the response to the environment (Insel & Collins, 2003)—in other words, that GxE is at the core of the etiology of mental illness. Some have suggested that in any instance in which there is variation in reactions to a major environmental pathogen implicated in a psychiatric disorder (such as childhood maltreatment), GxE must be involved to some extent (Gray & Hannan, 2007; Moffitt, et al., 2006). Gottlieb and Lickliter (2007) proposed that the widespread failure to replicate seen across linkage and association studies in psychiatric genetics is due to failing to consider the effect of “intervening life experiences” (p. 4; i.e., interaction with environment).

Without utilizing techniques exploring gene-environment interplay in genetics research, we cannot be certain that a failure to replicate is due to noneffect of a gene (Hoffmann, Lange, Vansteelandt, & Laird, 2009). Measuring and analyzing the impact of environmental risk factors and known pathogens will lend clarity to the debate over candidate genes and has the potential to greatly inform etiological models of mental illness.

Gene-Environment Research in EOBD is Sparse

Both genetic and environmental factors are known to influence the development of EOBD (McGuffin, et al., 2003; Pavuluri, Birmaher, & Naylor, 2005; Payne, Potash, & DePaulo, 2005). Recently, GxE has been implicated in the course of depressive symptoms in adults (e.g., Brezo, et al., 2010; Caspi, et al., 2003; Grabe, et al., 2005; Kendler, Kuhn, Vittum, Prescott, & Riley, 2005; Kilpatrick, et al., 2007; Wilhelm, et al., 2006; Zalsman, et al., 2006) and youth (e.g., Eley, Sugden, et al., 2004; Kaufman, et al., 2004; Sjöberg, et al., 2006). GxE has also been implicated in adult bipolar disorder in a small number of studies.

Savitz and colleagues (2007) found gene-environment interactions in adults with bipolar disorder: Polymorphisms in the brain derived neurotrophic factor (BDNF) gene and the apolipoprotein E gene interacted with a history of sexual abuse, resulting in impaired performance on a memory test. The authors suggest that effects on memory performance related to these gene-environment interactions may represent an endophenotype (intermediate phenotype) of bipolar disorder. In adults with major depression and bipolar disorder, Mandelli and colleagues (2007) found higher levels of depression in those with a certain form of the catechol-O-methyltransferase (COMT) gene and self-reported history of stressful life events in the past year. More recently, Vinberg and colleagues (2009) demonstrated increased cortisol response in healthy individuals with familial risk for bipolar disorder and who carried a “met” (risk) allele.

It is likely that GxE also plays a significant role in bipolar disorder symptoms in youth, though to this author’s knowledge current research remains nonexistent in this area. This study will make a significant contribution to the literature as it is the first study examining the important role of gene-environment interplay in pediatric bipolar disorder.

Role of Expressed Emotion in the Developmental Psychopathology of Bipolar Disorder

The term “expressed emotion” (EE) denotes high levels of hostility, conflict, and emotional overinvolvement within a family. EE predicts relapse in adults with many psychiatric disorders, including schizophrenia, bipolar disorder, substance use disorders, and eating disorders (Butzlaff & Hooley, 1998; Hooley & Gotlib, 2000; Rosenfarb, et al., 2001; Vaughn & Leff, 1976; Yan, Hammen, Cohen, Daley, & Henry, 2004). High EE has been found to play a strong role in the course of illness of bipolar disorder in adults, resulting in a five-fold increase in the likelihood of relapse at 9-month follow-up (Miklowitz, Goldstein,

Nuechterlein, Snyder, & Mintz, 1988). Though EE was first described in schizophrenia (Vaughn & Leff, 1976), meta-analysis has demonstrated that EE has a larger effect size for relapse in mood disorders than in schizophrenia ($r = .45$ vs $.31$, respectively; Butzlaff & Hooley, 1998).

It is important to note that EE is not always predictive of course or outcome of bipolar disorder in adults. Bipolar individuals' ratings of severity of criticism by relatives was not predictive of mood disorder symptoms at one-year follow-up in study done by Miklowitz and colleagues (2005); however, those who were more distressed by their relatives' criticism *did* experience more severe manic and depressive symptoms as well as fewer days well during the study than less-distressed participants. That is, subjective response to the criticism explained predictions of manic and depressive symptoms, rather than the severity of the criticism itself. In a small sample of remitted patients who had been on lithium prophylaxis for three years, little EE was observed, nor were probands more severely ill over the course of treatment (Priebe, Wildgrube, & Muller-Oerlinghausen, 1989). However, this sample is not likely to be representative of the general bipolar population due to the symptom-free status of the bipolar participants. Finally, individuals in a first-episode schizophrenia and major mood disorders study in Finland failed to show associations between EE and variables such as premorbid and present level of functioning, acuteness of illness onset, and symptom severity (Heikkila, et al., 2002).

Though the majority of the literature focuses on the effects of familial EE in adults, EE also has detrimental effects across the lifespan. High maternal EE measured at six months of age has been shown to predict internalizing behaviors such as anxiety and withdrawal in children at age two (St. John-Seed & Weiss, 2002). Levels of EE have predicted youth

outcomes when returning home from psychiatric hospitalization: Those returning to a low EE home were more likely to have recovered one year later, while those returning to a high EE home were more likely to continue to show mood disorder symptoms (Asarnow, Goldstein, Thompson, & Guthrie, 1993). In another study, over the course of two years during which participants received psychotherapy addressing bipolar disorder symptoms, youth with high EE relatives experienced more depressive symptoms regardless of treatment condition (family focused intervention versus treatment as usual) than youth with low EE relatives (Kim & Miklowitz, 2004). In the latter study, more frequent critical comments within the family predicted higher levels of depression and mania in probands at the two-year follow-up, particularly among those not receiving the family treatment intervention. Also in youth with bipolar disorder, those from high conflict families experienced persistent depressive symptoms despite pharmacological treatment (Townsend, Demeter, Youngstrom, Drotar, & Findling, 2007). Another study suggests, however, that among adolescents with bipolar disorder, high-EE attitudes in parents are not associated with current functioning and illness severity (Coville, Miklowitz, Taylor, & Low, 2008).

Although high familial EE is associated with a more severe course of illness in EOB (Kim & Miklowitz, 2004; Miklowitz, Biuckians, & Richards, 2006), it has yet to clearly predict onset. Additionally, despite the relevance of this construct to diathesis-stress models of mood disorder, it has never to this author's knowledge been explored within a gene-environment framework. In fact, there have been just five studies to date to the author's knowledge that have simultaneously incorporated genetic vulnerability and any kind of family stress; the target outcome in all of these was youth depression (Hammen, Brennan, Keenan-Miller, Hazel, & Najman, 2009; Laucht, et al., 2009; Rice, Harold, Shelton, &

Thapar, 2006; Sjöberg, et al., 2006; Taylor, et al., 2006). Although these studies considered family stress, they did not use the construct of expressed emotion. Furthermore, there have been no such studies in youth with bipolar disorder, making the proposed study novel in this respect. There are many reasons to suspect that GxE is at work in childhood mood disorders, and the specific serotonin gene chosen for this study has been linked to earlier age of onset bipolar disorder (Bellivier, et al., 2002).

The Serotonin Transporter Promoter Polymorphism Is a Vulnerability Marker for Mood Disorders and Is Associated With Earlier Age of Onset of Bipolar Disorder

Although clear candidate genes have yet to consistently replicate (Barnett & Smoller, 2009), there is a strong case for genetic vulnerabilities to bipolar disorder (Kato, 2007; McInnis, et al., 2003; McQueen, et al., 2005; Potash & DePaulo, 2000). Rates of concordance in monozygotic twins are as high as 79%, versus 19% in dizygotic twins (McGuffin, et al., 2003), strongly suggesting genetic pathways. A 44 base pair (bp) insertion/deletion in the promoter region of the serotonin transporter gene (*SLC6A4*) has recently garnered attention as one such potential candidate genetic polymorphism (anomaly) (Cho, et al., 2005; Furlong, et al., 1998; Lasky-Su, Faraone, Glatt, & Tsuang, 2005). The serotonin transporter is a logical candidate gene, as serotonergic drugs both effectively treat depression and could be associated with mania in mood disordered patients (Furlong, et al., 1998; Truman, et al., 2007).

Initially, two alleles of the HTTLPR were discovered – a “long” (*L*) 528 bp allele that is associated with greater transcriptional activity, and a “short” (*S*) 484 bp allele that is associated with lower transcriptional activity, subsequently lowering the biological activity of the transporter (Lesch, et al., 1996) and leading to an approximately 50% reduction in neurotransmitter availability (Pérez-Edgar, et al., 2009). Studies of the reuptake transporters

produced by varying genotypes of the HTTLPR have demonstrated that the transporters do act differently across genotypes (Fox, Hane, & Pine, 2007).

The S allele of the serotonin transporter has been linked to many interesting psychological and neurobiological correlates, such as poorer response to SSRI treatment (Serretti & Artoli, 2004), increased startle response (Armbruster, et al., 2009), mothers' lower levels of sensitive responsiveness to toddlers (Bakermans-Kranenburg & van Ijzendoorn, 2008), increased risk for infant insecure attachment (Barry, Kochanska, & Philibert, 2008), and elevated levels of brain activity in response to food stimuli (Kaurijoki, et al., 2008). Additionally, it has been implicated in neuropsychiatric outcomes such as depression (for a review see Brown & Harris, 2008), ADHD (Kopeckova, et al., 2008) and adult ADHD (Muller, et al., 2008), increased risk for depression in psychotic individuals (Contreras, et al., 2009), increased negative affect after acute tryptophan depletion (Brummett, Muller, et al., 2008; Firk & Markus, 2009), decreased hippocampal volume in depressed individuals (Frodl, et al., 2008), increased production of cortisol in response to stress in females (Gotlib, Joormann, Minor, & Hallmayer, 2008), amygdala activation (for a meta-analysis see Munafo, Brown, & Hariri, 2008) particularly in response to fearful stimuli (Hariri, et al., 2002; Lau, et al., 2009), cognitive processing of fearful stimuli (Osinsky, et al., 2009), and suicidal behavior (Neves, et al., 2008).

In 1996, Collier and colleagues (1996) first reported a main effect of the HTTLPR short allele on bipolar disorder diagnosis in adults. Approximately 30 studies to date have attempted to replicate this finding, with mixed results. Though nonreplication has been more common, meta-analyses have found a small but consistent effect for the S allele (odds ratios = 1.12 to 1.21; Cho, et al., 2005; Furlong, et al., 1998; Lasky-Su, et al., 2005).

Importantly, there is some evidence that there are additional HTTLPR alleles with varying degrees of functionality that may have confounded the results of previous studies (Nakamura, Ueno, Sano, & Tanabe, 2000). Hu et al. (2006) recently reported that among individuals of European descent the HTTLPR is functionally triallelic: There is a single nucleotide polymorphism (SNP) within the long allele, resulting in two different forms of that allele. One form of the long allele (L_A) does behave in the manner that geneticists had previously ascribed to the long allele. The other, L_G , has a SNP from adenosine (A) to guanine (G) within the allele, and is associated with lower rates of transcriptional activity, as is the S allele. Therefore, the HTTLPR has two low-activity alleles (S and L_G) and one high-activity allele (L_A). Some have suggested that the pattern of equivocal results seen in the literature for the HTTLPR is due to what have essentially been comparisons of low activity to a group that combines both low and high activity, rather than a clean comparison of low to high (Gunthert, et al., 2007; Kato, 2007). To date, no studies to this author's knowledge have compared biallelic and triallelic approaches to inform a data-based comparison of the extent to which the triallelic approach may improve classification and identification of risk.

Although there has been considerable investigation involving the serotonin transporter in adult bipolar disorder, there are no studies to this author's knowledge that examine the HTTLPR short allele or low-activity alleles in the etiology of the early-onset form of bipolar disorder. Two studies have examined the association of the HTTLPR with EOBBD diagnosis (Geller & Cook, 1999; Mick & Faraone, 2009); both found that the S allele was not associated with the diagnosis in parent-child trios. However, understanding inheritance patterns does not speak to the role of the gene in gene-environment interaction.

Importantly, *S* allele genotype has been associated with younger age of onset of bipolar disorder (Bellivier, et al., 2002; Ospina-Duque, et al., 2000; Smeraldi, Benedetti, & Zanardi, 2002), underscoring the need to investigate this polymorphism in youth. Additionally, recent research exploring cortisol stress response by HTTLPR genotype in multiple age cohorts provides strong evidence for a sensitive period in young childhood during which the experience of an endocrine stress response in those with risk genotypes may be particularly pathogenic (Mueller, et al., 2011). An interaction between HTTLPR genotype and stressful life events significantly predicted a larger cortisol response *only* in younger adults (18-31 years) and *only* when the stressful life events had occurred during the first five years of life. Interestingly, this interaction was not significant in 8-12 year olds, suggesting that the expression of the stressful life events came to full fruition later in development, perhaps during another sensitive period. Notably, while the experience of early life stress has not been defined as a specific factor in the etiology of EOBD, it has been implicated in an overall gestalt that places genetically and biologically vulnerable individuals further at risk (Miklowitz & Chang, 2008).

To date, the role of the *S*/low activity alleles in EOBD remains unclear. It may be that *S* allele genotype is a vulnerability marker for EOBD, or that youth are more affected by the serotonin transporter underactivity associated with the *S* allele, or that youth are especially vulnerable to GxE involving the *S* allele. Importantly, in an eight-year follow-up study of youth with bipolar disorder, younger age at study entry predicted more weeks ill, highlighting the importance of age of onset within the EOBD population (Geller, Tillman, Bolhofner, & Zimmerman, 2008). Placing this in context with the *S* allele genotype being

associated with younger age of onset of bipolar disorder (e.g., Bellivier, et al., 2002), it appears crucial that these relationships be further elucidated.

GxE Involving the HTTLPR Are Significant Mechanisms in the Etiology of Mood Disorders

Despite the identification of GxE as essential for understanding the etiology of mood disorders (Cho, et al., 2005), there is a scarcity of empirical literature in this area. The following is a review of GxE studies with the HTTLPR in mood disorders, the large majority of which focuses on depression. Although these results may be somewhat generalizable to bipolar disorder, it is likely that risk factors differ (Alloy, Abramson, Walshaw, & Neeren, 2006; Johnson, 2005; Wals & Verhulst, 2005). Therefore, it is imperative that GxE be more closely examined in bipolar disorder.

In 2003, in a landmark study published in *Science*, Caspi and colleagues (2003) became the first to suggest that interactions between the HTTLPR and stressful life events are related to the development of depressive symptoms. In a sample of 847 young adults who had been followed since birth, they demonstrated prospectively that stressful life events occurring between ages 21 and 26 had a greater effect on self-reported symptoms of depression in those carrying an *S* allele versus those homozygous for the *L* allele. Furthermore, stressful life events predicted the development of Major Depressive Disorder (MDD) by age 26 in *S* carriers, but not in *LL* homozygotes.

Specific to children, Kaufman and colleagues (2004) reported that among maltreated adolescents in a large longitudinal sample, those with the HTTLPR *SS* genotype and who had lower levels of social support had higher depression ratings. Similarly, Eley and colleagues (2004) found that in 377 adolescents, there was a significant interaction in females only involving high environmental risk and the HTTLPR *S* allele, corresponding to an odds ratio

of 1.85 for risk for depression in this group. Furthermore, the effect of the *S* allele was additive, such that the risk was higher for those with *SS* genotype versus *SL* genotype. It is important to note, however, that a recent study failed to find the hypothesized GxE in a large longitudinal birth cohort (Araya, et al., 2008).

Particularly relevant to the current study is the work of Hammen and colleagues (2009), who prospectively demonstrated GxE occurring in females, with chronic family stress at age 15 predicting depressive symptoms at age 20 in women who carried at least one *S* allele of the HTTLPR. Additionally, Taylor and colleagues (2006) reported that in a young adult non-clinical sample, individuals who were both homozygous for the HTTLPR *S* allele and who had experienced early family stress demonstrated significantly higher levels of depressive symptomatology than those who did not experience early adversity or who were of the *SL* or *LL* genotype. Similarly, Sjöberg and colleagues (2006) found that among females only, those who had the *SS* genotype and had been exposed to traumatic conflict within the family had higher levels of depression. Finally, Laucht and colleagues (2009) found family adversity and life stressors to be associated with depression in 19-year-olds. However, moderation was seen in the *LL* genotype only: These individuals had higher levels of depressive symptomatology as well as greater numbers of depressive and anxiety diagnoses than individuals of other genotypes. It is unclear why this study resulted in findings contrary to the rest of the literature. However, other studies done in adults examining life stress via questionnaire have found opposite results as well (Chorbov, et al., 2007; Surtees, et al., 2006; Zhang, et al., 2009).

A recent set of high-profile meta-analyses of replications of the Caspi (2003) study have cast doubt upon the HTTLPR-life stress interaction. Initially, Risch and colleagues

(2009), including noted epidemiologist Kathleen Merikangas, published a meta-analysis of a restricted subset of the replications published to date and found no effect of the HTTLPR in depression GxEs. This meta-analysis was highly publicized in the popular press and was quickly followed by a similar publication by Munafo and colleagues (2009). In 2011, Karg and colleagues (2011) published a third meta-analysis that included all replications to date with no restrictions, utilizing a different meta-analytic technique. They found a strong overall effect for increased risk of developing depression under stress in S allele carriers, and stratified the analyses by individual type of stressor as well. There were strong effects for childhood maltreatment and medical stressors and a marginal effect for stressful life events. They did not find an effect for the subsets of papers included in the Risch (2009) meta-analysis or the Munafo (2009) meta-analysis, suggesting that the latter results were due to restriction of studies rather than meta-analytic techniques used. Overall, it appears that when a broad spectrum of phenotypes and life stressors are included, there is a strong role of the HTTLPR in moderating the effect of various life stress experiences on depression-related outcomes.

The current study fits well within a broad life stressor paradigm: A specific type of stressor (family dysfunction) is of interest, and is of a more ongoing, chronic, and pervasive nature rather than the discrete or episodic nature of many “life stressors” assessed in past studies. Past studies that have looked at *family* stress have consistently found an effect for the hypothesized interaction (Hammen, et al., 2009; Sjöberg, et al., 2006; Taylor, et al., 2006). In fact, Hammen and colleagues (2009) specifically failed to find the hypothesized GxE with acute stress (i.e., the type of “life stressors” measured in most previous studies), while they

did find an effect for chronic, ongoing family stress. The prospective design of this study as well as the fairly large sample size ($N=346$) lend credibility to the findings.

Additionally, studies done in youths have consistently yielded replications, while there is more variability among replication studies with middle aged and elderly samples (Uher & McGuffin, 2008). As previously noted, there may be something about experiencing family stress during childhood or adolescence that results in a more potent effect than if the family conflict were experienced later in life (Mueller, et al., 2011). One might also speculate that the changing roles of an individual within a family across the lifespan could be related (e.g., transitioning from being a subordinate member of the family to having more power within the family dynamic; c.f. Chipman, et al., 2007).

In sum, although GxE studies in depression are relatively abundant, the role of GxE in bipolar disorder has yet to be explored to this extent. The lack of GxE studies in bipolar disorder (Wals & Verhulst, 2005) is a considerable weakness, considering their potential for informing etiologic models and prevention efforts (Uher, 2008). The situation is especially troublesome with respect to GxE involving serotonergic genes in bipolar disorder. Currently, there are just two published studies to the author's knowledge examining GxE with the HTTLPR and environmental stressors in *adults* with bipolar disorder (Mandelli, et al., 2007; Zalsman, et al., 2006), although neither study focused on bipolar disorder alone. Despite intriguing results in depression, there are no published studies to the author's knowledge that have probed GxE involving the HTTLPR and family stressors in bipolar disorder in either adults or youth —surprising, given the role of the family in youths' lives, and in the lives of adults who perhaps cannot function independently due to major mental illness. Moreover, there are no published studies to the author's knowledge that have investigated GxE models

of any kind while focusing specifically on youth with bipolar disorder. As a field, we are very much in need of studies that can help elucidate the role of GxE in models of risk for childhood mood disorders, particularly bipolar disorder.

The Importance of Mechanisms

Leading researchers in the field of GxE research now suggest that while further research into GxE is necessary, the prevailing need is no longer to identify that GxE occur (Caspi, 2009). This has been well-established and researched from a number of angles within developmental psychopathology (Caspi, et al., 2005; Kim-Cohen, et al., 2006; Mill, et al., 2006). What is needed is an understanding of the *mechanisms* underlying GxE (Hariri, 2009; Rutter, 2009), particularly mechanisms that “make sense” given the gene of interest. Some work of this nature has already begun in childhood affective disorders (Mechelli, et al., 2009).

In that vein, the current study will attempt to elucidate potential mechanisms, or mediators, underlying the hypothesized GxE effect. In keeping with recent recommendations, mechanisms that make good sense within serotonergic pathways will be explored. There is a well-established link between amygdala reactivity and the short allele of the serotonin transporter promoter polymorphism (for a meta-analysis see Munafo, et al., 2008; c.f. Shah, et al., 2009). Youth with bipolar disorder have exhibited increased amygdala activation when asked to rate faces as displaying positive or negative emotion than when asked to rate the same faces as younger or older than 35, and demonstrated greater emotional arousal than healthy control participants undergoing the same task (Pavuluri, Passarotti, Harral, & Sweeney, 2009). The amygdala is responsible for regulating a range of emotional responses (Fitzgerald, Angstadt, Jelsone, Nathan, & Phan, 2006; Phelps, 2006).

The mechanism behind vulnerability to depressive and manic symptoms in HTTLPR low-activity allele carriers may be increased amygdala response to emotional stimuli, leading to biased attention when confronted with such stimuli. This constellation of responses to emotional stimuli present in the environment that is driven by the amygdala could confer a greater sensitivity to critical and hostile family environments (i.e., EE) in youth with certain genotypes. For example, if a child or adolescent with an HTTLPR low-activity allele experiences hostility and criticism at home, he or she may attend more to those negative events than the average person (biased attention), causing him or her to have persistent, intrusive thoughts such as “Why is Mom always yelling at me?” or “Why is it always so terrible around here?” or “What did I do to make Dad mad?” It follows that depressive symptoms could result. Additionally, negative events can precipitate mania/hypomania (Alloy, et al., 2005). However, it is important to note that a study done on EE in bipolar disorder failed to find significant differences in affective reactivity to criticism, although there was a trend for participants with bipolar disorder to react more negatively to criticism than controls (Cuellar, Johnson, & Ruggero, 2009).

Significantly, the S allele of the HTTLPR has been tied to sensitivity to punishment-related (versus reward-related) processing (Blair, et al., 2008), suggesting a potential mechanism for feeding biased attention towards negative emotional stimuli. Low-allele genotype has been tied to increased limbic system reactivity in response to affectively unpleasant stimuli (Smolka, et al., 2007), as well as significant vigilance towards angry faces in adolescents (Pérez-Edgar, et al., 2009). Interestingly, in both of these studies, the effect was observed incrementally for each low-activity allele the participant carried (i.e., those with two low-activity alleles showed a more prominent effect than those with one such allele,

who in turn showed a more prominent effect than those with zero low-activity alleles). In seven-year-old children who had experienced a negative mood prime, only those homozygous for the *S* allele displayed biased memory for negative adjectives after being presented with negative and positive self-referent adjectives (Hayden, et al., 2008). Similarly, children with at least one *S* allele showed greater depressogenic attributional styles, tending to attribute negative events to internal, stable, and global factors, even though level of depression did not vary by genotype (Sheikh, et al., 2008). A recent meta-analysis across all age groups shows a significant association of medium effect size between homozygous low-activity genotypes of the HTTLPR and selective attention for negative information (Pergamin-Hight, Bakermans-Kranenburg, van Ijzendoorn, & Bar-Haim, 2012).

It follows that punishment-related processing, negative cognitive biases, and vigilance towards anger would be highly maladaptive in a high-conflict environment such as a high-EE or high-discord family, and that the effects of these maladaptive cognitive biases could increase as the number of low-activity alleles the person carried increased. However, one study revealed an attentional bias for anxious word stimuli, but not dysphoric word stimuli, in *S* carriers (Beevers, Gibb, McGeary, & Miller, 2007).

Children with certain HTTLPR genotypes have also demonstrated a lack of a protective effect, rather than the presence of a detrimental effect of biased attention. Individuals who were homozygous for the long allele (*LL*) displayed an attentional bias for positive affective pictures and avoidance of negative affective pictures, whereas this effect was not observed in *S* carriers (E. Fox, Ridgewell, & Ashwin, 2009). This is similar to a finding in the Perez-Edgar study (2009) where attention bias for happy faces was greater in adolescents with the high-activity allele genotype.

Finally, biased attention may not be limited to EE and negative aspects of the environment; adolescents may also attend more strongly to positive events in their lives and environments than negative ones and may “dwell” on these events, predisposing them to symptoms of hypomania or mania. Beevers and colleagues (2009) found that individuals with low-activity alleles of the HTTLPR (i.e., *S* and *L_G* alleles) had difficulty disengaging their attention not only from negatively-valenced emotional stimuli such as sad and fearful faces, but also from positive stimuli such as happy faces.

Although there appears to be good evidence for a link between HTTLPR genotype and biased attention for emotional stimuli, there has been less work done in this area in bipolar disorder (for a full review, see Johnson, Gruber, & Eisner, 2007). Most work that has been done is related more to reactivity to emotional stimuli than cognitive or memory biases, although one study found biased attention towards positive or negative (versus neutral) words (Elliott, et al., 2004). Nonreferred participants at increased risk for mania have shown elevated levels (versus low-risk participants) of positive emotion in response to not only positive but also negative and neutral film clips; they also showed increased irritability in response to the film clips (Gruber, Johnson, Oveis, & Keltner, 2008). Children and adults with bipolar disorder have shown increased cortical activity in response to emotional stimuli (Chang, et al., 2004; Kruger, Seminowicz, Goldapple, Kennedy, & Mayberg, 2003).

Hypotheses

The goal of this study is to identify predictors, specifically GxE, of EOBD symptomatology in late adolescence (see Figure 1). Additionally, potential mechanisms behind (mediators of) the hypothesized GxE will be tested.

Hypothesis 1. HTTLPR non- L_A -homozygote genotype will predict increased EOBD symptomatology as measured by the General Behavior Inventory (GBI; Depue, et al., 1981). The GBI has two subscales: Depression and Hypomanic/Biphasic. Outcomes will be tested using both. Additionally, exploratory sub-analyses will probe for a linear effect of low-activity allele status, i.e., does level of EOBD symptomatology increase in a linear fashion based upon genotype ($L_A L_A < \text{one } L_G \text{ or } S \text{ allele} < \text{two } L_G \text{ or } S \text{ alleles}$)?

I predict increased EOBD symptomatology in low-activity allele carriers because meta-analyses suggest there is a main effect of the HTTLPR in bipolar disorder (Cho, et al., 2005; Furlong, et al., 1998; Lasky-Su, et al., 2005). Additionally, in the previous literature, consideration of heterozygotes vs. homozygotes for the S /low activity allele has not been consistent (Uher & McGuffin, 2008); exploratory sub-analyses will contribute to our understanding of whether simply carrying a low-activity allele is sufficient to increase one's risk (e.g., Caspi, et al., 2003; Eley, Liang, et al., 2004), or whether two low-activity alleles (i.e., homozygosity) are necessary to confer risk (e.g., Kaufman, et al., 2004; Sjöberg, et al., 2006; Taylor, et al., 2006).

Hypothesis 2. Self-reported level of EE during childhood and adolescence will predict increased EOBD symptomatology. Support for this hypothesis comes from decades of research showing that increased family discord and EE are associated with increased symptomatology (Asarnow, et al., 1993; Kim & Miklowitz, 2004; Townsend, et al., 2007) and rate of relapse (Miklowitz, et al., 1988; Rosenfarb, et al., 2001) in individuals with bipolar disorder.

Hypothesis 3. The presence of a pathogenic environment (high EE) will show different effects on EOBD symptomatology across different levels of HTTLPR genotype

(e.g., high activity allele * low EE < low activity allele * high EE), and this interaction will provide a significant increment in prediction of EOBD symptomatology over genes alone or environment alone. Additionally, exploratory sub-analyses will probe for sex effects as well as a linear effect of low-activity allele status, i.e., does the effect of the gene-environment interaction vary depending upon whether individuals have one or two copies of a low-activity allele (Uher & McGuffin, 2008)?

I predict a GxE involving a conflictual family environment and HTTLPR low-activity alleles on the basis of multiple prior studies showing similar effects (Hammen, et al., 2009; Laucht, et al., 2009; Sjöberg, et al., 2006; Taylor, et al., 2006). Additionally, I plan to probe for sex effects because past research has found the hypothesized interaction in females only (Brummett, Boyle, et al., 2008; Eley, Liang, et al., 2004; Sjöberg, et al., 2006), suggesting that sex may be an important factor in this GxE equation. Finally, I will again test for incremental effects of the low-activity alleles (see Hypothesis 1) within the GxE framework.

Hypothesis 4. A measure of biased attention to emotional stimuli will significantly partially mediate the EE * HTTLPR genotype interaction with EOBD symptomatology as the dependent variable. I anticipate partial mediation, rather than full, because within a system this complex it is unlikely that biased attention to emotional stimuli would fully account for the relationship between the GxE and either depressive or manic symptomatology.

In keeping with recent recommendations by experts in GxE (Caspi, 2009; Rutter, 2008), I will explore biased attention to emotional stimuli as a mediator of the family environment-HTTLPR interaction. Prior work has demonstrated that both carriers of S/low activity HTTLPR alleles (Pérez-Edgar, et al., 2009; Smolka, et al., 2007) and individuals with bipolar disorder (Chang, et al., 2004; Elliott, et al., 2004; Gruber, et al., 2008; Kruger, et al., 2003;

Pavuluri, West, Hill, Jindal, & Sweeney, 2009); show biased attention, subjectively increased affective response, and/or related neural correlates when confronted with happy, sad, or angry stimuli (e.g., words, faces). I propose that in a hostile and critical family environment, individuals who are predisposed to orient towards and have difficulty disengaging from emotional stimuli will be at risk for increased mood disorder symptomatology.

Methods

Participants

Recruitment and demographics. Participants were recruited through the UNC Chapel Hill participant pool. Participants were 101 undergraduate students enrolled in an introductory psychology course at UNC. Students received research credit for their participation, consistent with the established guidelines and procedures for the participant pool. Although the prevalence of the HTTLPR S allele varies by ancestry (Esau, Kaur, Adonis, & Arieff, 2008), all participants will be included in the analyses.

Seventy-eight percent of the sample was female. Seventy-five percent identified as Caucasian, 13% as African American, 10% as Asian American, 6% as Hispanic or Latino, 1% as Pacific Islander, and 5% as Other (participants were permitted to check all that apply). Participants were asked about ancestry because different ancestral backgrounds may have some bearing on genetic makeup for certain polymorphisms. Again, participants were permitted to check all that apply for blood relatives back to great-grandparents (to their knowledge). Sixty-three percent reported ancestry from Europe, 33% from North America (including Native American), 17% from the British Isles, 9% from East Asia, 8% from Africa, 7% from Scandinavia, 5% from Russia, 5% from India, 4% from Central America, 3% from South America, and 1% from Australia.

Bipolar status of participants. A substantial portion of individuals with bipolar disorder will experience the onset of their illness between ages 18 and 21 (i.e., college age). In the National Comorbidity Study – Revised, the lifetime prevalence of age of onset of 17 or later for Bipolar I and Bipolar II subjects was 75% (Kessler, et al., 2005). Particularly salient is the stress associated with a transition to college during the age of risk; many individuals will get to college before their mood problems escalate and will experience exacerbation of the underlying illness as stressors increase: Up to three times as many individuals with bipolar disorder start college as finish college (Kessler, et al., 2006). Prior research suggests that significant degrees of bipolar disorder symptomatology are found within undergraduate populations (Alloy, et al., 2008; Knowles, Tai, Christensen, & Bentall, 2005) and that between 6% and 12% of undergraduates participating in psychological research would meet criteria for a bipolar spectrum disorder if given a diagnostic interview (Grandin, Alloy, & Abramson, 2007). In fact, the median age of onset of bipolar disorder is 18 (Berk, et al., 2007), which is also the modal age of an individual in the participant pool. More support comes from recent epidemiological analyses which suggest that bipolar disorder has a prevalence of 5.5%-6.2% in adolescents and young adults (age 18-24; Cicero, et al., 2009). Although diagnostic interviews were not performed, it was reasonable to assume that a substantial proportion of the sample would be experiencing significant mood symptomatology during the study.

Materials and Measures

Demographics and family history. Demographics were collected, including information regarding sex; race/ethnicity; ancestry; family socioeconomic status (SES); relationship of primary caregiver (e.g., biological parents, grandparents) and caregivers’

marital status (and if no longer together, age of participant when breakup, separation, or divorce occurred); and self-reported psychiatric illness status. Participants also provided information about family history of psychiatric illness using a brief, one page grid (Youngstrom, et al., 2009).

General Behavior Inventory (GBI). Participants filled out a General Behavior Inventory (GBI; Depue, et al., 1981), a research instrument that has demonstrated reliability and validity for assessing bipolar disorder symptomatology in youth (Danielson, Youngstrom, Findling, & Calabrese, 2003; Findling, et al., 2002; Youngstrom, Findling, Danielson, & Calabrese, 2001). The GBI was chosen because it was developed specifically to identify subsyndromal cases of mood disorder (Depue, et al., 1981) and because it has been validated extensively (Depue, et al., 1981; Klein, Depue, & Slater, 1985) and in college populations (Alloy, et al., 2008; Depue, Krauss, Spont, & Arbisi, 1989). The GBI has adequate sensitivity (.78) and high specificity (.99; Depue, et al., 1989). The GBI can either be used as a total composite score, or as two subscales: Depression and Hypomanic/Biphasic. Internal consistency when used with a non-clinical sample ranges from .90 to .96 for the total score and two subscales, with higher alphas corresponding to the total GBI score (Depue, et al., 1989; Depue, et al., 1981).

Perceived Criticism Scale (PCS). To obtain a measure of EE, participants filled out a PCS (Hooley & Teasdale, 1989), a short, valid, and reliable assessment of the degree of conflict and level of associated distress between parents and youth (Hooley & Parker, 2006). Questions such as “How critical do you think your parent is of you?” and “When your parent criticizes you, how upset do you get?” are rated on a scale from 1 (not at all critical/upset) to 10 (very critical/upset). The PCS has a three-month test-retest reliability of approximately .75

(Hooley & Teasdale, 1989). For the PCS and other family assessment measures, the participant was directed to think back on family dynamics from age eight to present. This time period was chosen because encompasses both middle childhood, of which participants were likely to have memories, as well as adolescence, a common period of family discord.

In order to obtain a measure of positive family functioning, I created several items modeled after the Perceived Criticism Scale. Questions such as “How supportive was your caregiver of you?” and “When your caregiver expressed their love for you, how loved did you feel?” were rated on a scale from 1 (not at all supported/loved) to 10 (very supported/loved). I chose to create my own items because I wanted to have an analogue to the PCS to use in models testing positive and negative family environment simultaneously. This is consistent with the work of Jay Belsky, who writes of “differential susceptibility to the environment” (Belsky & Pluess, 2009). He argues that so-called “risk” genes are actually sensitivity genes, and that we happen to only look at negative environments most of the time. In this sample, the EE Positive items showed an internal consistency coefficient of .91.

Family Assessment Device (FAD) and Family Environment Scale (FES). To assess overall family functioning, participants completed the Problem Solving, Communication, and General Functioning subscales of the FAD (range of internal consistencies of subscales = .71-.92; Epstein, Baldwin, & Bishop, 1983). They also filled out the Cohesion, Conflict, and Expressiveness subscales of the FES (range of internal consistencies of subscales = .61-.78; range of 2-month test-retest reliabilities = .54-.91; Moos & Moos, 1994). These scales have been used in other investigations of mood disorder and family environment (e.g., Belardinelli, et al., 2008; Du Rocher Schudlich, Youngstrom,

Calabrese, & Findling, 2008). For all family environment measures in this study, high scores indicate impaired family functioning.

Faces task. In order to understand mediation effects of biased attention to emotional stimuli, participants were asked to complete a memory task involving faces with affectively positive, negative, and neutral expressions. The task was modeled on the “Faces” subtest of the Children’s Memory Scale (Cohen, 1997), but was modified for length and used different faces. The faces are from the NimStim face stimulus set, a set of reliable and valid multiracial stimuli depicting a range of emotional expressions (Tottenham, et al., 2009). The set includes photos of Asian-American, African-American, European-American, and Latino-American individuals photographed under identical conditions. The NimStim set was chosen because it has been used successfully in prior GxE research with the HTTLPR (Pérez-Edgar, et al., 2009), and because the faces have been validated in a population of untrained individuals who were chosen in order to approximate average research participants (Tottenham, et al., 2009). In the latter study, Cohen’s kappas for concordance between the participant’s response and the intended facial expression ranged from .64 to .97, with the majority of kappas in the .8 to .9 range; test-retest reliability ranged from .77 to .94 (Tottenham, et al., 2009). These values suggest good reliability and validity.

Participants were shown a series of 32 happy, sad, angry, and neutral faces (approximately 4 male and 4 female of diverse ethnicities per expression) presented in random order, one face at a time. They were told “You will now see some pictures of faces, one at a time. Look at each face carefully and remember what it looks like.” After presenting a face for 1 second, the screen automatically advanced to the next stimulus. After completing the questionnaires, participants were shown 40 faces – both previously presented faces as

well as neutral distracter faces – and were asked to identify which of the faces they had seen before. Number of correctly recalled faces was recorded, along with the type of each face correctly recalled (e.g., happy, angry, neutral).

DNA collection and HTTLPR sequencing. Participants were required to provide genetic material via saliva sample. The specimens were collected using Oragene, a saliva sample collection method popular for its painlessness, simplicity, and ease of use. Oragene also captures greater quantities of DNA and has a low bacteria content compared with other oral collection methods such as cheek swab or mouthwash (Genotek, 2010). Oragene provides high-quality DNA (Rylander-Rudqvist, Hakansson, Tybring, & Wolk, 2006) and has been used previously at UNC to collect DNA from over 2,000 undergraduates (Guo & Tillman, 2009). The samples were sent to the UNC Biospecimen Processing Facility for DNA extraction under the supervision of Patricia Basta, PhD. HTTLPR genotyping was done at Duke University through the laboratory of Ahmad Hariri, PhD. Please see Appendix for sequencing details. BDNF, COMT, and DRD2 genotyping was done at the UNC Mammalian Genotyping Core under the supervision of Jason Luo, PhD.

Procedures

I posted appointment times on the Participant Pool website, and students indicated their interest in participating in the study through the site. Participants came into the lab. I obtained informed consent and answered any questions. They sat at a computer, participated in the faces task, and answered questionnaires via Qualtrics online data collection system (www.qualtrics.com). The Qualtrics system has been used by government agencies, hundreds of universities and in many dissertations involving human subjects and even disadvantaged and at risk populations, including government sponsored studies collecting data about

physical and dependency abuse for adults and youth. These are extremely confidential studies that have passed the highest level of scrutiny from human subjects committees.

Participants then provided the DNA sample via saliva collection. Participants were then debriefed and provided with contact and psychoeducational information.

Analyses

Descriptive Statistics

Data were aggregated in Predictive Analytics Software (PASW) Version 18.0 (also known as Statistical Package for the Social Sciences). Descriptive statistics quantified age, sex, race/ethnicity, ancestry, HTTLPR genotype, and level of family functioning as measured by the PCS, positive EE items, FES, and FAD. Descriptive statistics also quantified mean number of happy, sad, angry, and neutral faces recalled on the faces task, as well as percentage of participants remembering significantly more positive or negative faces than neutral faces. Analysis of variance determined whether recall of affectively laden faces differed by genotype or by family functioning status.

Missing Data Analyses

Missing data were scarce overall, likely due to the online data collection format. Among items making up the independent variables, missing data ranged from 0% to 2%. Among items making up the dependent variables, missing data ranged from 0% to 2%. Among the affective memory task (faces) items, missing data ranged from 0% to 2%. Two participants are missing data in the latter half of the protocol because the program malfunctioned and they had to terminate the data collection. All other missing data within Qualtrics appears to be due to participants either choosing not to answer a particular question or skipping a question accidentally.

Unfortunately, four participants did have to be excluded from the study entirely due to a research assistant making a labeling error on their DNA samples during two consecutive data collection sessions such that the samples could not be connected with the questionnaires. All of these participants carried at least one risk gene and two had both copies of the gene.

Regression

Differences by race/ethnicity were assessed and covaried in all models. I assessed for main effects and interactions using multiple linear regression. EE (as measured by the PCS and positive EE items), HTTLPR genotype (number of low-activity alleles), and their interaction were independent variables; level of bipolar disorder symptomatology as measured by a GBI subscale was the dependent variable. Regression diagnostics (Cook's distance, Mahalanobis distance, Student's deleted residual) were run with each regression model in order to assess for influential outliers.

Family environment variables were mean-centered, and an interaction term was created using the EE, FAD, or FES variable and dummy-coded variables for three levels of HTTLPR genotype: Zero, one, or two copies of a low activity allele. Covariates entered into the regression models first, followed by main effects, and then the interaction term. Using the procedures recommended by Preacher, Curran, and Bauer (2006), I probed interactions using an online tool available at www.quantpsy.org.

Alpha

Regression analyses consisted of main analyses examining Positive and Negative Expressed Emotion as well as Total scores for the FAD and FES. Additionally, I performed exploratory analyses of the FAD and FES subscales (e.g., FAD Problem Solving; FES Conflict). In total, 20 regression models were run. Due to the high probability of Type I error

secondary to multiple comparisons, I used an alpha of .01 for the main analyses and an alpha of .005 for the exploratory analyses. This yielded a probability of at least one Type I error of 7.7% for the main analyses (8 models at .01) and 7.0% for the exploratory analyses (12 models at .005). Since many of the analyses conducted in this paper are novel, I wanted to optimize the tradeoff between Type I and Type II error and avoid being too conservative, while preserving scientific rigor.

Moderated Mediation

I tested for moderated mediation, or “conditional indirect effects,” which occur when the strength of a mediation effect varies depending upon the level of some other variable (Preacher, Rucker, & Hayes, 2007). Biased attention to emotional stimuli during the faces task was included in the model as a mediator of the interaction between genotype and family functioning. Using procedures recommended by Preacher and colleagues (2007), I tested the moderated mediation using an SPSS macro developed by the authors (available at <http://www.afhayes.com/>).

Power

A priori power analyses were conducted with the software Power and Precision (Borenstein, Rothstein, Cohen, Schoenfeld, & Berlin, 2001). The effect size for EE on relapse in mood disorders was estimated from a meta-analysis (Butzlaff & Hooley, 1998) indicating $R^2 = .20$. This is a large effect; however, EE is not expected to be as high in a non-clinical sample, so a conservative estimate of $R^2 = .10$ for EE was used. For the HTTLPR, an odds ratio of 1.21 (Furlong, et al., 1998) was used to calculate an incremental R^2 of .0062. This is likely to be accurate or possibly an under-estimate, given that it was based on studies genotyping only two alleles for the HTTLPR. Effect size for the interaction was calculated

from a *t*-value for a similar interaction between the HTTLPR and early family stress measured in a college sample (Taylor, et al., 2006); this yielded an R^2 of .22, indicating a large effect. Because Taylor and colleagues (2006) used a different family stress construct in their study, I chose to use a more conservative estimate of a medium effect ($R^2 = .10$).

Although initial power analyses were calculated for an *N* of 500 (power approaching 1.0 for the total model), funding constraints only allowed for recruitment of 101 participants providing DNA (four of whom were mislabeled and had to be excluded; details below). Posthoc power analyses indicated that I had 75-80% power to detect EE main effects, between 5% power (for positive EE) and 30% power (for negative EE) to detect GxE involving EE, and less than 10% power to detect the direct effect of HTTLPR.

Results

Demographics & Descriptive Statistics

Participants took an average of 27.45 minutes to complete the protocol ($SD = 6.31$, range = 16 to 45 minutes). Only 1% of participants came from a family where the annual family income was less than \$20,000. Eighty percent of participants came from a family where the annual family income was at least \$60,000. Ninety-two percent of participants came from a family where the highest level of education was an associate's degree or beyond. Ninety-seven percent of participants were raised by a biological parent. Ninety-four percent of participants were raised by a primary caregiver who was married when the participant was ages 8 through 18.

Thirteen percent of participants reported a personal history of problems with depression; 1% with manic or bipolar problems; 4% with suicidality; 1% with alcohol or drugs; and 1% with psychiatric hospitalization. Two percent reported a history of physical

abuse, 6% a history of sexual abuse, and none reported neglect. Please see Table 1 for description of extended family history of mental health problems.

Genotyping. The genotyping lab was not able to determine the genotype for two samples, resulting in a 98% “call rate” or success rate, which is very good. There were 198 total alleles in the sample. Ninety were the L_A allele (.45 allele frequency), 88 were the traditional S allele (.44 allele frequency), and 20 were the L_G allele (.10 allele frequency). One individual was homozygous for the rare S_G allele, the function of which is currently undocumented but is best understood to cluster with the other low-activity alleles (S , L_G). I tested for Hardy-Weinberg equilibrium (probability of random mating in the population; Hardy, 1908; Weinberg, 1908) by adding the products of the frequencies of the alleles in their various combinations (homozygous, heterozygous, etc). The equation demonstrated that the genotypic distribution was indeed in Hardy-Weinberg equilibrium, suggesting that it is appropriate to continue analysis.

Twenty-five percent of participants did not carry a risk gene of the HTTLPR polymorphism, i.e., they carried two copies of the high-activity allele. Forty-two percent carried one low-activity allele, while 33 percent carried two copies of a low-activity allele. This is slightly higher than typical rates for European populations, but lower than typical rates for Asian populations, which is consistent with the mixed ethnic makeup of the sample. See Table 2 for genotype frequencies in this sample as well as a comparison to worldwide genotypic distribution rates. Worldwide rates are based on biallelic $LL/LS/SS$ models and not tri-allelic models, but provide a rough basis for comparison.

Questionnaire results. Participants scored an average of 18% of the maximum possible on the GBI Depression scale (min = 0%, max = 71%, median = 14%). Participants

also scored an average of 18% of the maximum possible on the GBI Hypomanic/Biphasic scale (min = 1%, max = 73%, median = 14%). Please see Table 3 for a summary of percent of maximum possible (POMP) scores for mood and family environment questionnaires.

Participants scored an average of 52% of the maximum possible on the Negative Expressed Emotion scale (min = 10%, max = 95%, median = 55%). Participants scored an average of 90% of the maximum possible on the Positive Expressed Emotion scale (min = 20%, max = 100%, median = 98%).

Participants scored an average of 36% of the maximum possible on the FAD Problem Solving scale (min = 0%, max = 94%, median = 33%). Participants scored an average of 37% of the maximum possible on the FAD Communication scale (min = 0%, max = 89%, median = 33%). Participants scored an average of 28% of the maximum possible on the FAD General Functioning scale (min = 0%, max = 100%, median = 25%). Participants scored an average of 33% of the maximum possible on the FAD Total scale (min = 0%, max = 90%, median = 31%).

Participants scored an average of 25% of the maximum possible on the FES Cohesion scale (min = 0%, max = 89%, median = 22%). Participants scored an average of 41% of the maximum possible on the FES Expressiveness scale (min = 0%, max = 89%, median = 44%). Participants scored an average of 37% of the maximum possible on the FES Conflict scale (min = 0%, max = 89%, median = 33%). Participants scored an average of 34% of the maximum possible on the FES Total scale (min = 11%, max = 81%, median = 30%).

Participants showed variable rates of success with the affective memory task. (See Table 4 for a complete listing of descriptive statistics by type of face.) Participants remembered, on average, fewest angry faces (31% mean recall), and most sad faces (44%

mean recall). ANOVA showed no significant differences between or within HTTLPR groups for memory for faces. A programming glitch resulted in the purpose of the distraction condition becoming revealed to participants, who correctly identified on average 80% of distractor faces.

Family Environment, HTTLPR, and Gene-Environment Interaction: Regressions

Covariates. Gender did not predict outcomes for any regression model, but it remained a covariate in every model. Race was not a significant predictor in any model; however, dummy codes for race were included as covariates in every model. Participant-reported history of parental depression or mania/bipolar disorder was also included in every model, and was predictive in some models.

Influential outliers. There was a group of seven influential outliers who repeatedly appeared in depression and mania models, respectively, as outliers on Mahalanobis distance (e.g., values of up to 47), Cook's distance (e.g., values of 0.54), and Student's deleted residual (e.g., values of up to 5); some were outliers on two of these in the same model. In order to better understand this group of people, I performed exploratory analyses on this subset. Univariate analyses of variance demonstrated that these individuals displayed significantly greater degrees of mood disorder symptomatology than the rest of the sample, scoring 24 points higher on the GBI Depression subscale on average and 22 points higher on the GBI Hypomanic/Biphasic subscale on average. Additionally, although these individuals did not experience significantly greater levels of negative EE, they did experience lower levels of positive EE, reporting positive EE greater than one standard deviation below the rest of the sample, on average. Finally, all of the participants reporting manic or bipolar parents were represented in this group of outliers ($n = 3$). This group was not significantly

different from the overall sample on FAD or FES Total scores, and they were representative of the sample on demographic characteristics.

It is likely that these individuals represent my target population, and that within a larger sample, they would simply denote extremes of a gradient of mood and family functioning with additional individuals along the continuum at middle points as well. In order to increase the generalizability of the results, and in order to completely represent the spectrum of mood disorder symptoms and family environment function and dysfunction reported in the sample, I decided to include these seven individuals in all regression models.

Expressed emotion questionnaire.

Negative EE and Depressive Symptoms. Participants' self-reported parental history of depression problems significantly predicted GBI depression score, $t = 2.15$, $B = 13.22$, $p = .01$. After controlling for parental depression and positive EE, higher scores on the negative EE measure significantly predicted increases in GBI depression scores, $t = 2.43$, $B = 0.61$, $p = .02$, $R^2\Delta = .05$ ($p = .02$). However, after posthoc correction, this was no longer significant. Genotype alone did not predict depression scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

Please see Table 5 for regression coefficients for all models.

Negative EE and Hypomanic/Biphasic Symptoms. After controlling for parental history and positive EE, higher scores on the negative EE measure did not significantly predict GBI Hypomanic/Biphasic score, $t = 1.62$, $B = .27$, $p = .11$. Genotype alone did not predict hypomanic/biphasic scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

Positive EE and Depressive Symptoms. After controlling for parental depression and negative EE, higher scores on the positive EE measure significantly predicted decreases in GBI depression scores, $t = -2.63$, $B = -0.94$, $p = .01$, $R^2\Delta = .06$ ($p = .01$). Genotype alone did not predict depression scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

Positive EE and Hypomanic/Biphasic Symptoms. After controlling for parental history and negative EE, higher scores on the positive EE measure significantly predicted decreases in GBI Hypomanic/Biphasic score, $t = -2.37$, $B = -0.55$, $p = .02$, $R^2\Delta = .05$ ($p = .02$). However, after posthoc correction, this was no longer significant. Genotype alone did not predict hypomanic/biphasic scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

Family Assessment Device

FAD Problem Solving and Depressive Symptoms. After controlling for parental mental health history and the other FAD subscales, FAD Problem Solving did not significantly predict lower GBI Depression scores, $t = -1.73$, $B = -.34.97$, $p = .09$. Genotype alone did not predict depression scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FAD Problem Solving and Hypomanic/Biphasic Symptoms. After accounting for control variables, FAD Problem Solving did not have a significant effect on GBI Hypomanic/Biphasic scores, $t = -1.53$, $B = -19.87$, $p = .13$. Genotype alone did not predict hypomanic/biphasic scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FAD Communication and Depressive Symptoms. After accounting for control

variables, FAD Communication did not have a significant effect on GBI Depression scores, $t = -1.26$, $B = -16.08$, $p = .21$. Genotype alone did not predict depression scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FAD Communication and Hypomanic/Biphasic Symptoms. After entering control variables, FAD Communication significantly predicted decreases in GBI Hypomanic/Biphasic scores, $t = -2.01$, $B = -25.51$, $p = .05$, $R^2\Delta = .04$ ($p = .05$). However, after posthoc correction, this was no longer significant. Genotype alone did not predict hypomanic/biphasic scores. There was a marginally significant gene-environment interaction, $t = 1.83$, $B = 15.85$, $p = .07$, $R^2\Delta = .03$ ($p = .07$). Upon probing, it appeared that the slopes were indeed different but not at a magnitude that would be clinically meaningful, suggestive of potentially spurious findings. Posthoc power analyses indicated that there was approximately 51% power to detect an interaction for this model. If the effect I found is accurate, a future study would need a sample size of 193 to have 80% power to detect a similar effect.

FAD General Functioning and Depressive Symptoms. After entry of the control variables, FAD General Functioning significantly predicted increases in GBI Depression scores, $t = 3.52$, $B = 78.51$, $p = .001$, $R^2\Delta = .10$ ($p = .001$). Genotype alone did not predict depression scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FAD General Functioning and Hypomanic/Biphasic Symptoms. After entry of the control variables, FAD General Functioning significantly predicted increases in GBI Hypomanic/Biphasic scores, $t = 4.08$, $B = 58.79$, $p < .001$, $R^2\Delta = .15$ ($p < .001$). Genotype

alone did not predict hypomanic/biphasic scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FAD Total and Depressive Symptoms. After accounting for control variables, FAD Total significantly predicted increases in GBI Depression scores, $t = 4.61$, $B = 51.94$, $p < .001$, $R^2\Delta = .18$ ($p < .001$). Genotype alone did not predict depression scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FAD Total and Hypomanic/Biphasic Symptoms. After accounting for control variables, FAD Total significantly predicted increases in GBI Hypomanic/Biphasic scores, $t = 2.95$, $B = 22.07$, $p = .004$, $R^2\Delta = .09$ ($p = .004$). Genotype alone did not predict hypomanic/biphasic scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

Family Environment Scale

FES Cohesion and Depressive Symptoms. After entering the parental mental health history and the other FES subscales, FES Cohesion did not have a significant effect on GBI Depression scores, $t = -0.61$, $B = -7.50$, $p = .54$. Genotype alone did not predict depression scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FES Cohesion and Hypomanic/Biphasic Symptoms. After accounting for control variables, FES Cohesion did not have a significant effect on GBI Hypomanic/Biphasic scores, $t = 0.59$, $B = 4.71$, $p = .56$. Genotype alone did not predict hypomanic/biphasic scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FES Conflict and Depressive Symptoms. After entry of control variables, FES Conflict significantly predicted increases in GBI Depression scores, $t = 3.85$, $B = 34.59$, $p < .001$, $R^2\Delta = .11$ ($p < .001$). Genotype alone did not predict depression scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FES Conflict and Hypomanic/Biphasic Symptoms. After entry of control variables, FES Conflict significantly predicted increases in GBI Hypomanic/Biphasic scores, $t = 2.66$, $B = 15.54$, $p < .001$, $R^2\Delta = .06$ ($p < .001$). Genotype alone did not predict hypomanic/biphasic scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FES Expressiveness and Depressive Symptoms. After accounting for control variables, FES Expressiveness significantly predicted increases in GBI Depression scores, $t = 2.35$, $B = 23.13$, $p = .02$, $R^2\Delta = .04$ ($p = .02$). However, after posthoc correction, this was no longer significant. Genotype alone did not predict depression scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FES Expressiveness and Hypomanic/Biphasic Symptoms. After entry of control variables, FES Expressiveness did not significantly predict increases in GBI Hypomanic/Biphasic scores, $t = 1.79$, $B = 11.51$, $p = .08$. Genotype alone did not predict depression scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FES Total and Depressive Symptoms. After entry of control variables, FES Total significantly predicted increases in GBI Depression scores, $t = 5.47$, $B = 58.57$, $p < .001$, $R^2\Delta$

= .24 ($p < .001$). Genotype alone did not predict depression scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

FES Total and Hypomanic/Biphasic Symptoms. After accounting for control variables, FES Total significantly predicted increases in GBI Hypomanic/Biphasic scores, $t = 5.09$, $B = 34.60$, $p < .001$, $R^2\Delta = .22$ ($p < .001$). Genotype alone did not predict hypomanic/biphasic scores. There was no significant gene-environment interaction, and adding GxE to the model did not produce a significant change in R^2 .

Moderated Mediation Models

Using Preacher et al.'s (2007) SPSS macro (<http://www.afhayes.com/>), I tested several moderated mediation models, or conditional indirect effects. The macro is built within a regression framework but does not rely on a *causal steps* strategy (e.g., Baron & Kenny, 1986; Judd & Kenny, 1981) which requires significance at each path in the model and depletes power (Preacher, et al., 2007). Rather, the macro uses the *product of coefficients* and *bootstrapping* strategies. The product of coefficients strategy uses the products of the sample regression coefficients to estimate the population quantity of the indirect effect, while bootstrapping repeatedly resamples the data as a pseudopulation to estimate the indirect effect (Preacher, et al., 2007). While the product of coefficients strategy assumes normality of the data, bootstrapping does not require that the distribution of the sample statistic meet any assumptions. For this project, given the robust statistical strategies employed by the moderated mediation macro, I tested all models as planned even given low power to detect some of the direct effects. However, I used only total or summary family environment variables to reduce the possibility of Type I error.

Results for EE, both positive and negative, suggested that there was no moderated mediation present. That is, pathways from HTTLPR through the affective memory task (biased attention to happy, sad, or angry faces) were not moderated by expressed emotion. Please see Figure 2 for an example of a moderated mediation model tested. Additional exploratory models using the FES and FAD Total scores in place of EE as the family environment variable also did not show significant moderated medication.

Notably, biased attention to sad faces significantly predicted GBI Depression scores in all models, p 's = .002 to .03, as well as GBI Hypomanic/Biphasic score in the FES Total model (p = .04). No other faces demonstrated significant prediction value.

Discussion

Bipolar disorder is lifelong diagnosis associated with significant psychosocial impairment (Birmaher & Axelson, 2006; Geller, Bolhofner, et al., 2000; Goldstein, et al., 2005). Onset often occurs in childhood or adolescence (Kessler, et al., 2005), and may be associated with the experience of acute or chronic life stressors (Grandin, et al., 2007). Experts agree that both genetic and environmental risk factors contribute to the development and maintenance of bipolar disorder (Miklowitz & Chang, 2008) and other psychopathologies, and that it is unlikely that these work in isolation (Moffitt, et al., 2006; Neiderhiser, 2001).

The goal of this study was to identify potential genetic and environmental risk factors associated with early-onset bipolar disorder symptomatology within a specific serotonergic model. Participants were 101 healthy young adults who provided answers to questionnaires regarding their current mood state, answers to questionnaires regarding retrospective recall of family environment from ages 8-18, and a saliva sample for DNA sequencing of the

serotonin transporter promoter polymorphism (HTTLPR). Additionally, they participated in an affective memory task designed to measure biased attention to emotional stimuli, a putative marker of amygdala reactivity. Regression analyses tested for main effects and gene-environment interactions (GxE), and moderated mediation analyses tested whether biased attention to emotional stimuli mediated the relationship between the GxE and the mood outcomes.

The gene chosen for this study, HTTLPR, has been the subject of some controversy recently (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Karg, et al., 2011; Risch, et al., 2009). Initially just two alleles were identified: A “low activity” *S* allele, and a “high activity” *L* allele, which lead to greater and lesser amounts of serotonin in the synapse, respectively. Individuals who are *S* carriers or who are homozygous for (have two copies of) the *S* allele demonstrate greater degrees of negative effects such as increased anxiety sensitivity (Gotlib, et al., 2008) and increased risk for depression (Brown & Harris, 2008). However, after a substantial amount of HTTLPR research had been published—some of it contradictory—it became apparent that there are actually two versions of the *L* allele, one of which mimics the activity of the *S* allele (Hu, et al., 2006). Studies that choose to genotype this variant of the *L* allele now characterize individuals as “low activity” or “high activity” allele carriers (e.g., Pérez-Edgar, et al., 2009). However, not all researchers are either aware of this development or have the resources to genotype their samples in this manner. Therefore, some studies continue to be published without separating out the low activity *L* variant, adding to the somewhat questionable reputation of this polymorphism. However, to this author’s knowledge, the literature lacks a direct comparison of biallelic and triallelic approaches, so it is unclear to what extent this may be influencing nonreplications.

Replications of both direct and GxE effects with the HTTLPR have been inconsistent. Recently, Avshalom Caspi and colleagues, the original proponents of this polymorphism as a candidate in depression GxEs, came under fire from multiple meta-analyses showing no overall effect for GxE with HTTLPR in depression (Munafo, et al., 2009; Risch, et al., 2009). Caspi et al. (2010) quickly fired back, demonstrating that when studies are conducted with rigorous methods, replication is achievable. However, when studies rely on questionnaire-based methodologies, vague definitions or brief ascertainment of stressors, and other less robust approaches, replication is unlikely (Caspi, et al., 2010).

Gene-Environment Interaction

Overall, the present study did not find significant GxE. There are several possible explanations. One possibility is that the study was underpowered. The original power analyses called for an *N* of 500; however, due to funding limitations, only 101 participants were able to give saliva samples for genotyping (overall, however, 464 participants enrolled in the study and completed questionnaires). A related possibility is that any GxE effects were too weak to be detected in a nonclinical sample. However, a previous study found the hypothesized gene-environment interaction for depressive symptoms in a young adult sample (ages 18-29) of comparable size (Taylor, et al., 2006).

Another strong possibility is that the measure of the environment was not robust enough. According to Caspi et al. (2010), nearly all subsequent replication attempts of their original finding (Caspi, et al., 2003) that have been done via interview or objective methods have yielded replications, while questionnaire-based studies have frequently failed to replicate. Though all research should strive for using the most robust and rigorous methods possible, it appears that many who undertake GxE research fall victim to the mistaken belief

that more subjects are better (the present author included). Large studies pull for questionnaire-based methodologies, while smaller studies can invest resources into observational coding- or interview-based designs, yielding more robust measures of the phenotype and the environment. Current guidelines emphasize that quality, not quantity, should dictate study design (Caspi, et al., 2010).

Negative Expressed Emotion & Conflictual Family Environment

In contrast to the study hypothesis, negative EE was not a significant predictor of depression after posthoc correction. These findings are inconsistent with a robust literature supporting the role of negative family environment in increased symptomatology (Asarnow, et al., 1993; Kim & Miklowitz, 2004; Townsend, et al., 2007) as well as increased rate of relapse (Miklowitz, et al., 1988; Rosenfarb, et al., 2001) in individuals with bipolar disorder. It is possible that this effect was just not strong enough in a nonclinical sample or that with a larger *N* this effect would have been more pronounced.

Also in contrast to the study hypothesis, negative EE was not predictive of hypomanic symptomatology at all, even before posthoc correction. Again, these findings are inconsistent with the literature connecting family environment with bipolar disorder symptomatology. Given that hypomanic symptoms are more specific to bipolar disorder than are depressive symptoms, this is most likely due to the nonclinical sample.

Although not directly measuring an EE construct, the FES Conflict scale significantly predicted greater depression and hypomanic scores. This scale measures a variety of family conflict from criticism and anger to outright hostility and aggression (Moos & Moos, 1994). Perhaps not surprisingly, it was strongly associated with depression even in this nonclinical sample. What is surprising is that it was also significantly associated with symptoms of

hypomania/mixed states. Family conflict is present in nearly all families to some degree, but it is striking that in a group of individuals not identified as at risk for psychopathology and who are currently living away from home, retrospective recall of family conflict was a strong predictor of their current mood. (It is important to note that participants rated their mood *before* they answered questions about their family.)

These findings appear to be somewhat novel, as there are scant extant findings for the FES Conflict scale as rated directly for families of bipolar youth (c.f. Belardinelli, et al., 2008). There is an existing literature for elevation of the FES Conflict scale in families where one or both parent(s) has a depressive or bipolar disorder (Chang, Blasey, Ketter, & Steiner, 2001; Ogburn, et al., 2010; Romero, DelBello, Soutullo, Stanford, & Strakowski, 2005), which is also consistent with findings in this sample—parental history of mood problems was predictive of mood symptoms in most models.

Positive EE & Positive Family Functioning

This study sought to make a contribution to the literature by exploring the role of positive expressed emotion, which has rarely been examined in bipolar disorder (c.f. Rosenfarb, et al., 2001; Simoneau, Miklowitz, & Saleem, 1998). Results indicated that greater levels of support from caregivers were associated with lower reported levels of depression symptoms. This suggests a protective effect of caregiver support during childhood and adolescence, even for youth who are not identified as at risk for psychopathology.

Additionally, the FAD General Functioning scale significantly predicted depression and hypomania scores. This scale measures a myriad of healthy family functioning characteristics such as acceptance, turning to one another in a crisis, talking about feelings, and getting along (Epstein, et al., 1983). Participants who rated their family low on such

characteristics also reported higher levels of depression and hypomania on the GBI. The implication is that healthy family functioning might protect against bipolar disorder symptomatology, which warrants further investigation. As with FES Conflict, the findings are perhaps not as surprising with depression symptoms, but are striking with hypomanic/biphasic symptoms. In a nonclinical sample, low ratings on family closeness, acceptance, and openness about emotions are associated with greater levels of mood lability, increased energy, irritability, and other symptoms of hypomania. Past research has demonstrated elevated FAD General Functioning scores in families with depressed adolescents (FAD ratings done by parents; Tamplin, Goodyer, & Herbert, 1998), although other research has failed to show significant prediction by FAD General Functioning in bipolar youth (Townsend, et al., 2007) and adults (Koyama, Akiyama, Miyake, & Kurita, 2004).

Given not only that we are seeing symptoms in a nonclinical sample but also that the lifetime prevalence of depressive disorders in teenagers is 15% (Merikangas, et al., 2010) and presumably that symptoms of depression occur in many more adolescents, the overall findings for positive family functioning present a clear area of intervention on a public health scale that would likely be easy to disseminate. Promoting family support, closeness, acceptance, and dialogue about emotions beginning in early childhood could potentially create large payoffs later in adolescence that could provide protection against mood disorder symptomatology. To the author's knowledge and based on current reviews (Gladstone & Beardslee, 2009), current family-based prevention efforts have focused on youth who have already developed symptoms of mood disorders (e.g., Miklowitz, et al., 2011) or on youth with a depressed parent (e.g., Garber, et al., 2009), rather than creating a focus on healthy

development for all children and families regardless of risk status. Some school-based models, such as the Penn Resiliency Program, have shown that a positive psychology approach produces favorable outcomes universally for school-aged children (Gillham, Brunwasser, & Freres, 2008).

Direct Effect of HTTLPR

There was no direct effect of the HTTLPR polymorphism in any model, which is roughly consistent with Mick & Faraone (2009) failing to find a direct association of the HTTLPR short allele on diagnosis in 522 individuals with pediatric bipolar I disorder. The association of the HTTLPR with bipolar disorder *symptomatology* (vs. diagnosis) has yet to be explored, and might yield different results. It is also possible that early in development, such as in pediatric populations, the effect may not yet be apparent.

However, direct effects are less likely to be found with small N 's, whereas findings are possible with small- N studies of GxE because such studies have theoretically chosen a population that has been exposed to the environmental pathogen. At “minor-allele frequencies” (MAF, i.e. least commonly occurring gene) of at least 25% and environmental exposure approaching 1.0, correlations between the predictors are substantial (Caspi, et al., 2010). In the current study, MAF was .45, but environmental exposure is more difficult to estimate. At what level of family function/dysfunction do you say that the person has experienced “family stress” in a healthy population? How do you measure that with a questionnaire or multiple questionnaires? Can you measure it validly using one informant's retrospective report?

Role of Biased Attention to Emotional Stimuli

Biased attention to emotional stimuli did not play a significant role in moderated

mediation models, contrary to the study hypotheses. Although biased attention to sad faces did predict depression in several models, biased attention otherwise did not perform as hypothesized in this study. There is strong evidence for a link between the HTTLPR and biased attention to emotional stimuli (Pergamin-Hight, et al., 2012) as well as evidence that individuals who have or who are at risk for bipolar disorder are preferentially reactive to emotional stimuli (Elliott, et al., 2004; Gruber, et al., 2008). However, although I used a validated set of emotion faces for this study that has been used in an HTTLPR/biased attention study previously, it may be that the task was not the strongest possible measure of the construct. Dot-probe tasks may yield more consistent effects (Beevers, et al., 2007; Kwang, Wells, McGeary, Swann, & Beevers, 2010; Pérez-Edgar, et al., 2009; for a meta-analysis in depression see Peckham, McHugh, & Otto, 2010).

Limitations

The primary limitation of this study is the design. I chose this sample so that I could recruit a large number of people to increase my power to detect a direct gene effect as well as the GxE effect. However, after I designed the study and began recruiting, I began working with Avshalom Caspi and Terrie Moffit who helped me to understand that my resources would have been better served conducting a smaller study that focused on interviews and/or objective measures of the environment, rather than a large study utilizing questionnaires. With a smaller sample I could have recruited participants with significant mood symptomatology and perhaps screened for high and low levels of family discord, or alternatively recruited based on exposure to the environmental pathogen. I might have chosen to recruit parent/child dyads at a sensitive period in development such early childhood, or during adolescent conflict and development of autonomy.

Another significant limitation is retrospective recall, especially by participants who may have currently been experiencing mood symptoms that could have biased their recall of past emotionally-laden experiences. Angst (2009) also suggests that simple forgetting is a significant limitation of retrospective research; individuals simply do not remember what happened a long time ago.

I have also relied on a single person's report of family environment, whereas direct (i.e., observational) measures of family environment would be most accurate. Many child clinical psychologists have had the experience of talking with a parent during an intake interview, then observing them with their child in the waiting room, and feeling as though they have met two different parents. A person's perception of their family depends on many factors, including their current mood, their personality, their perception of themselves, their position within the family, the moods and personalities of each of their family members, and how all of those factors interact. One person's report on a questionnaire cannot possibly capture such intimate and interactive dynamics.

Finally, the study was underpowered to detect direct genetic effects, which likely undermined my ability to detect effects in the moderated mediation models as well as GxE. In a study such as this one, where I did not recruit based on exposure to the environmental stressor of interest, it was indeed important to gather a large sample.

Future Directions

GxE studies that incorporate observational measures of family environment are crucial in understanding the interplay between genetic and environmental risk. Some work of this nature is already being done in other areas of developmental psychopathology. Sonuga-Barke and colleagues (2009) measured comments made by mothers during a clinical

interview and coded them for positive maternal expressed emotion. GxE were found with the HTTLPR and a dopamine gene, such that only children carrying risk genes were sensitive to EE. Lee and colleagues (2010) coded a structured mother-child interaction task and found a GxE involving a dopamine transporter gene, negative parenting, and disruptive child behavior. Van IJzendoorn and colleagues (2008) asked mothers to solve puzzles with their toddlers that were too difficult given the children's developmental level, then rated supportive presence, intrusiveness, and clarity of instruction. A GxGxE was found in mothers with the DRD4-7R and COMT val alleles who experienced more daily hassles, such that they displayed less responsiveness to their children's attachment needs.

Prospective studies that follow children over time are also needed, especially in light of recent findings suggesting that there may be sensitive periods not only for experiencing life stressors but also the expression of mood symptoms following the experience of life stress (Mueller, et al., 2011). It is important that such studies carefully measure phenotype as well as the environment. Additionally, family environment should be measured at multiple time points in order to track changes in family structure and function over time. Finally, prospective studies should not rely upon sampling at just one time point to establish genotype. It is becoming apparent that epigenetics (the study of how genes turn on and off) may tell us as much as or potentially more about genetic vulnerabilities than a person's genotype (Elia, Laracy, Allen, Nissley-Tsiopinis, & Borgmann-Winter, 2012), and studying epigenetics is a moving target. Studies such as the Pitt Mother & Child Project (Shaw, 2011) closely approximate this ideal design.

Finally, the newest wave in GxE research incorporates experimental design. Studies done not only with rats, genetically designed knockout mice, and nonhuman primates, but

also to a limited extent with humans, allow for random assignment to stress conditions and experimenter control over dosage and timing of stress (Caspi, et al., 2010). A recent experiment done with the MAOA polymorphism (the so-called “warrior gene”) married GxE research with traditional behavioral paradigms from social psychology (McDermott, Tingley, Cowden, Frazzetto, & Johnson, 2009). Participants won money by completing a vocabulary task, then an anonymous (fictional) person took away a portion of their earnings. Participants were permitted to administer hot sauce to that person as punishment. Participants played three rounds, and the experimenters manipulated whether 80% or 20% of earnings were taken across each round. Researchers found that participants with the low-activity form of the MAOA polymorphism who had 80% of their earnings taken were more likely to administer hot sauce, administered more hot sauce, and were more likely to report that they were “mad” or “angry” after the experiment versus MAOA high-activity subjects, suggesting a role for the MAOA gene in determining aggressive behavior in an experimental setting (McDermott, et al., 2009). Additional behavioral and experimental paradigms have demonstrated a role for the HTTLPR in GxE including sensitivity to financial loss during a gambling task (Crisan, et al., 2009) and HPA axis reactivity to threatening stimuli as measured by salivary cortisol after delivering a speech to a critical audience (Way & Taylor, 2010). Although every type of study has its place depending upon the type of question one is trying to answer, experimental paradigms offer the additional layer of experimenter control and may lend credence to the findings.

Conclusion

Gene-environment research is, in some ways, the future of developmental psychopathology. In the past five years, it has exploded onto the scene as both a hot topic and

a controversial one. As more funding gets diverted to projects utilizing such buzzwords as “GxE,” “polymorphism,” “endophenotype,” and “epigenetics,” it is vital that researchers, especially those who may be expanding outside the conventional scope of their training, work within a multidisciplinary team and seek consultation at all stages of research design, recruitment, data collection, genetic analysis, data analysis, and dissemination. If both the environmental stressor(s) and the phenotype are well-measured in accordance with the guidelines set forth by the current standard of practice (Caspi, et al., 2010), GxE research can be both rewarding *and* make important contributions to the literature.

Table 1

Family History of Mental Health Problems, Percentage of Sample Reporting

Mental Health Problem	Self	Parents	Siblings	Aunts/Uncles	Grandparents
Depression	12	22	7	18	12
Manic or Bipolar	1	3	3	8	1
Suicidality	4	1	2	6	2
Alcohol/Drug	1	4	1	29	11
Psychiatric Hospitalization	1	1	1	4	1

Table 2

Genotypic Frequencies and Comparison to Worldwide Distribution Rates, in percent

Sample Location	High/High [L/L] ^a	High/Low [L/S]	Low/Low [S/S]
University of North Carolina (current study)	25	42	33
African-American	54	35	11
Chinese	14	31	54
European	36	50	14
Indian	10	43	47
Israeli	33	50	18
Japanese	3	32	65
South African	61	34	5

^aWorldwide distribution rates as compiled in Esau et al. (2008); reported in bi-allelic format.

Table 3

Descriptive Statistics for Mood and Family Environment Questionnaires, scaled as Percent of Maximum Possible (POMP)

Variable	Min	Max	Mean	SD	Median
General Behavior Inventory					
Depression	0	71	18	15.5	14
Hypomanic/Biphasic	1	73	18	14.9	14
Expressed Emotion					
Positive	20	100	90	21.9	98
Negative	10	95	52	15.8	55
Family Assessment Device					
Problem Solving	0	94	36	17.6	33
Communication	0	89	37	18.5	33
General Functioning	0	100	28	20.4	25
Total	0	90	33	18.0	31
Family Environment Scale					
Cohesion	0	89	25	20.1	22
Conflict	0	89	37	24.7	33
Expressiveness	0	89	41	23.9	44
Total	11	81	34	18.4	30

Table 4

Affective Memory Task, Average Percent Correctly Recalled

Type of Face	Min	Max	Mean	SD	Median
Sad	0	100	44	20.3	50
Happy	0	88	37	23.6	38
Angry	0	100	32	20.7	25
Neutral	0	100	33	20.2	25
Distracter*	50	88	80	9.3	88

*Error in task

Table 5

Regression Coefficients

Model	<i>t</i>	<i>B</i>	<i>p</i>
Negative EE & Depressive Symptoms			
Negative EE	2.43	0.61	.02
HTTLPR	-0.14	-0.38	.89
Negative EE x HTTLPR	-0.45	-0.14	.65
Negative EE & Hypomanic/Biphasic Symptoms			
Negative EE	1.62	0.27	.11
HTTLPR	-1.27	-2.30	.21
Negative EE x HTTLPR	-0.51	-0.10	.61
Positive EE & Depressive Symptoms			
Positive EE	-2.63	-0.94	.01*
HTTLPR	-0.14	-0.38	.89
Positive EE x HTTLPR	-0.45	-0.14	.65
Positive EE & Hypomanic/Biphasic Symptoms			
Positive EE	-2.37	-0.55	.02
HTTLPR	-1.27	-2.30	.21
Positive EE x HTTLPR	-0.51	-0.10	.61
FAD Total & Depressive Symptoms			
FAD Total	4.61	51.94	<.001*
HTTLPR	-0.29	-0.79	.77
FAD Total x HTTLPR	0.53	7.54	.60
FAD Total & Hypomanic/Biphasic Symptoms			
FAD Total	2.95	22.07	.004*
HTTLPR	-1.22	-2.20	.23
FAD Total x HTTLPR	0.86	8.10	.39
FES Total & Depressive Symptoms			
FES Total	5.47	58.57	<.001*
HTTLPR	-0.23	-0.59	.82
FES Total x HTTLPR	-0.33	-4.17	.75
FES Total & Hypomanic/Biphasic Symptoms			
FES Total	5.09	34.60	<.001*
HTTLPR	-1.45	-2.40	.15
FES Total x HTTLPR	-0.97	-7.84	.34
FAD Problem Solving & Depressive Symptoms			
FAD Problem Solving	-1.73	-34.97	.09
HTTLPR	-0.22	-0.59	.82

FAD Problem Solving x HTTLPR	1.43	20.02	.16
FAD Problem Solving & Hypomanic/Biphasic Symptoms			
FAD Problem Solving	-1.53	-19.87	.13
HTTLPR	-1.16	-1.97	.25
FAD Problem Solving x HTTLPR	1.85	16.55	.07
FAD Communication & Depressive Symptoms			
FAD Communication	-1.26	-16.08	.21
HTTLPR	-0.22	-0.59	.82
FAD Communication x HTTLPR	1.27	17.10	.21
FAD Communication & Hypomanic/Biphasic Symptoms			
FAD Communication	-2.01	-25.51	.05
HTTLPR	-1.16	-1.97	.25
FAD Communication x HTTLPR	1.83	15.85	.07
FAD General Functioning & Depressive Symptoms			
FAD General Functioning	3.52	78.51	.001**
HTTLPR	-0.22	-0.59	.82
FAD General Functioning x HTTLPR	-0.07	-0.91	.94
FAD General Functioning & Hypomanic/Biph. Symptoms			
FAD General Functioning	4.08	58.79	<.001**
HTTLPR	-1.16	-1.97	.25
FAD General Functioning x HTTLPR	0.27	2.18	.78
FES Cohesion & Depressive Symptoms			
FES Cohesion	-0.61	-7.50	.54
HTTLPR	0.50	1.37	.62
FES Cohesion x HTTLPR	1.38	19.65	.17
FES Cohesion & Hypomanic/Biphasic Symptoms			
FES Cohesion	0.59	4.71	.56
HTTLPR	-1.10	-1.97	.27
FES Cohesion x HTTLPR	-0.06	-0.56	.95
FES Conflict & Depressive Symptoms			
FES Conflict	3.85	34.59	<.001**
HTTLPR	0.50	1.37	.62
FES Conflict x HTTLPR	0.85	8.23	.40
FES Conflict & Hypomanic/Biphasic Symptoms			
FES Conflict	2.66	15.54	<.001**
HTTLPR	-1.10	-1.97	.27
FES Conflict x HTTLPR	-0.36	-2.30	.72
FES Expressiveness & Depressive Symptoms			
FES Expressiveness	2.35	23.13	.02
HTTLPR	0.50	1.37	.62

FES Expressiveness x HTTLPR	-0.52	-5.48	.60
FES Expressiveness & Hypomanic/Biphasic Symptoms			
FES Expressiveness	1.79	11.51	.08
HTTLPR	-1.10	-1.97	.27
FES Expressiveness x HTTLPR	-1.19	-8.09	.24

Note. $\alpha = .01$ for main analyses, $\alpha = .005$ for exploratory analyses.

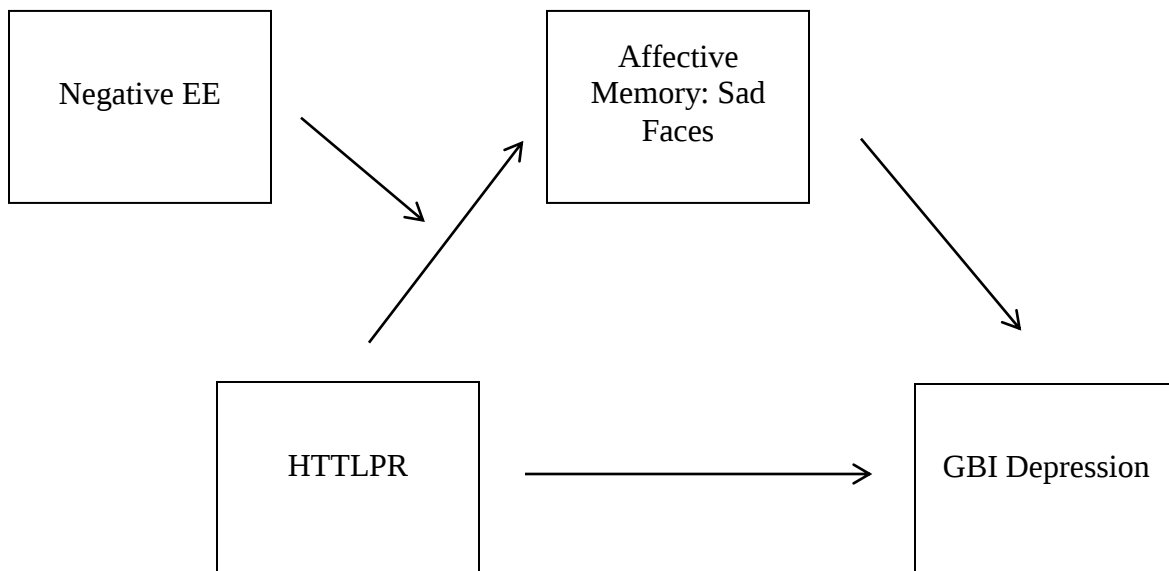
* Significant at .01

** Significant at .005

Figure 1. Overview of Hypotheses 1-3

		Status of Expressed Emotion within Family	
		Low EE	High EE
HTTLPR Genotype	Homozygous for High-Activity Allele	Rate at which individuals would develop EOBD symptomatology without contributions from HTTLPR or family environment (presumed low)	Effect of environment alone (Hypothesis 2)
	Carrying at Least One Low-Activity Allele	Effect of gene alone (Hypothesis 1)	Joint effect of genes and environment on EOBD symptomatology (Hypothesis 3)

Figure 2. Moderated mediation model example: Negative EE moderates mediation of relationship between gene and depression by memory for sad faces



APPENDIX

5-HTTLPR Sequencing Technique

Primer sequences for 5-HTTLPR are described by Gelernter et al. (1997), the forward primer having the sequence (5'- ATGCCAGCACCTAACCCCTAATGT-3') and the reverse (5'- GGACCGCAAGGTGGGCGGGA-3'). PCR was conducted using the following cycling conditions: initial 15- min denaturing step at 95°C, followed by 35 cycles of 94°C for 30 sec, 66°C for 30 sec and 72°C for 40 sec, and a final extension phase of 72°C for 15 min. Reactions were performed in 10X reaction Buffer IV (ABgene), 1.5mM MgCl₂, 50ng of genomic DNA, 5pmols of each primer, 0.3mM dNTPs and 1 unit of Native *Taq* (Promega). PCR products were subsequently digested by MspI restriction enzyme for 4 hours at 37°C. The digestion products were separated on a 3% agarose gel (MultiABgarose, ABgene) supplemented with Ethidium bromide (0.03%, BDH) and visualised by ultraviolet transillumination.

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