The Serotonin Transporter Polymorphism (5-HTTLPR) and potential interactions with adverse life events leading to Major Depressive Disorder: An approach addressing inconsistencies in the literature

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Dedication

This thesis is dedicated to my parents, Lee and Lucy Schissel.

Abstract

Serotonin (5-HT) has a demonstrated influence on several aspects of the human experience including personality, behavior, and psychopathology. One aspect of the 5-HT system that has received considerable attention in research concerns individual differences in the 5-HT transporter promoter region polymorphism (5-HTTLPR) and their associations with behavior. A well-researched component of the 5-HTTLPR literature focuses on the potential interactions between 5-HTTLPR genotype and adverse life events on the development of Major Depressive Disorder (MDD). The validity of this interaction, however, has been questioned due to inconsistent findings. The current project is an attempt to replicate the 5-HTTLPR by environment interaction in a large epidemiological sample, while also examining some of the proposed reasons for inconsistent findings. Results consistently suggest stressful life events (SLE) are related to the development of MDD; however, main effects of 5-HTTLPR and the interactions between 5-HTTLPR variants and SLE on the development of MDD are non-significant. Potential reasons for these negative findings, as well as limitations of this current project, are discussed.

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Chapter 1: Introduction and Background

Many studies have examined the role of the monoaminergic neurotransmitter, serotonin (5-HT), on personality, behavior, and psychopathology. As the field of genomics advances, researchers have considered genetic influences over functional levels of 5-HT. Moreover, over the past decade, increasing focus has been on potential interactions between 5-HT related genotypes and environmental factors, and how these interactions may influence the development of psychopathology. Specifically, Caspi and colleagues (2003) published findings indicating that individual differences in a polymorphism in the promoter region of the gene that codes for the serotonin transporter (5-HTTLPR) interacted with adverse life events and contributed to the development of Major Depressive Disorder (MDD). This seminal work has given rise to a considerable number of research projects and publications where researchers have attempted to replicate, and build upon, this gene by environment interaction. However, findings on the whole have been inconsistent, and several other researchers have been unable to replicate this finding from Caspi et al. (2003). These difficulties with replication have led many to speculate as to why these inconsistencies exist, leading to considerable controversy. This project is an attempt to replicate the original Caspi et al. (2003) findings, as well as to investigate some of the potential reasons for the discrepancies in the literature on the whole.

Brief background summary of serotonin's influence in various areas of human emotional and behavioral experiences

A consistent finding has been that lower levels of 5-HT, as measured through several different techniques, are associated with higher levels of behavioral impulsivity, negative affect, impulsive aggression, and lower levels of social cooperativeness. For instance, Knutson et al. (1998) manipulated 5-HT through the administration of a selective serotonin reuptake inhibitor (SSRI) over the course of four weeks in healthy participants. SSRI administration would be expected to increase serotonin levels over time. When compared with controls, who did not receive SSRIs, the medicated participants reported lower levels of impulsive aggression, irritability, and negative affect. The medicated participants also demonstrated higher levels of social cooperativeness when asked to complete a group task. Another study also investigated social cooperativeness and SSRI administration by examining SSRI administration in healthy individuals playing a competitive game. Compared with unmedicated controls, those receiving SSRIs were considered less submissive, but also more cooperative (Tse & Bond, 2002). Consequently, increased 5-HT appears to be related to both decreases in passivity and increases in cooperation in these experimental instances.

Additionally, similar results are evident when 5-HT levels are experimentally manipulated through acute tryptophan depletion. Tryptophan is a precursor to 5-HT, and reductions in tryptophan result in decreases in the brain's ability to synthesize 5-HT. Some studies have examined individual differences in aggression levels following tryptophan depletion. Individuals with higher baseline levels of aggression become even

more aggressive and argumentative following tryptophan depletion. Such changes are not seen in those with lower overall levels of aggressions (Bjork, Dougherty, Moeller, & Swann, 2000; Cleare & Bond, 1995).

Two reviews (Young & Leyton, 2002; Carver & Miller, 2006) also examine the literature suggesting that tryptophan depletion, along with the resulting decline in functional 5-HT levels, leads to increases in interpersonal aggression, while tryptophan supplementation generally increases social cooperativeness. Conclusions suggest manipulations resulting in increases in 5-HT function result in decreases in impulsive aggression as well as increases in social cooperativeness and social potency or dominance.

While higher levels of 5-HT appear to improve functionality, in terms of increasing social cooperativeness and decreasing negative affect, high 5-HT also appears related to increases in symptoms of Obsessive-Compulsive Disorder. Although this finding may seem counter-intuitive, some authors (e.g., Fineberg et al., 1997) have argued that this relationship reflects decreases in impulsivity with increased 5-HT levels. As discussed above, higher levels of 5-HT are associated with lower levels of aggressive impulsivity, and some have argued this relationship may then explain the over-controlled nature of those with Obsessive-Compulsive Disorder.

In contrast, decreases in 5-HT neurotransmission appear related to increases in Major Depressive Disorder (MDD). An early focus of this research examined the relative amounts of the 5-HT metabolite 5-Hydroxyindoleacetic acid (5-HIAA) in cerebral spinal fluid of depressed patients. The presumption has been that lower CSF 5-HIAA is

associated with lower levels of 5-HT in neurons (Mann 1999). Several studies during the 1970s and 1980s found lower levels of CSF 5-HIAA in depressed patients, particularly depressed patients who had attempted suicide (Mann 1998).

Lucki (1998) contends lower levels of 5-HT in those with MDD are largely consistent with the nature of symptoms of MDD. Given serotonin's influence on appetite, sleep, activity levels, sexual functioning, and cognitive functioning, it makes logical sense that alterations in 5-HT functioning could lead to abnormalities in these specific areas. Lucki (1998) also reviews the various aspects of serotonergic dysfunction that are evident in MDD, including diminished levels of tryptophan in the brain, decreases in 5-HT synthesis or release, and dysfunction in 5-HT receptors. Moreover, when 5-HT reuptake is altered through treatment with SSRIs, depressive symptoms are typically reduced (for a meta-analysis on SSRI efficacy, see Anderson, 2000).

Several decades of research have implicated 5-HT in MDD, where lower functional levels of 5-HT are associated with MDD (e.g., Mann 1999; Mann 1998; Lucki 1998). This conclusion has been supported through research in various methodological areas, including apparent reductions in CSF 5-HIAA in depressed patients, reduced plasma concentrations of tryptophan in depressed patients, and the clinical efficacy of anti-depressants targeting the 5-HT system (Owens & Numeroff, 1994).

The Serotonin Transporter Polymorphism (5-HTTLPR)

One portion of the 5-HT system that has received attention for its potential influence on aspects of personality and psychopathology is the serotonin transporter (5-

HTT). 5-HTT is a protein found in the membranes of pre-synaptic neurons. 5-HTT is responsible for taking synaptic 5-HT back into the pre-synaptic neuron, thereby ending its potential for activity in the synaptic cleft (Seretti, Calati, Mandelli, & De Ronchi, 2006). 5-HTT synthesis is coded by the gene SLC6A4 located on chromosome 17. One way that 5-HTT transcriptional activity is controlled is through a repetitive sequence on the SLC6A4-linked polymorphism (5-HTTLPR). Individuals vary in terms of whether they possess a short sequence versus a long sequence. One copy of the sequence is transmitted from each parent. These short and long versions influence 5-HTT function (Canli & Lesch, 2007), such that those with the short (S) variant experience apparent decreases in the 5-HTT expression and activity compared with those who have the long (L) variant (Seretti, et al., 2006). Therefore, importantly, the behavioral differences conferred by these different polymorphisms exist, because it is believed the S variant leads to comparably lower 5-HT activity. Please see Figure 1 for a graphic representation of the long and short variants and 5-HTT function.

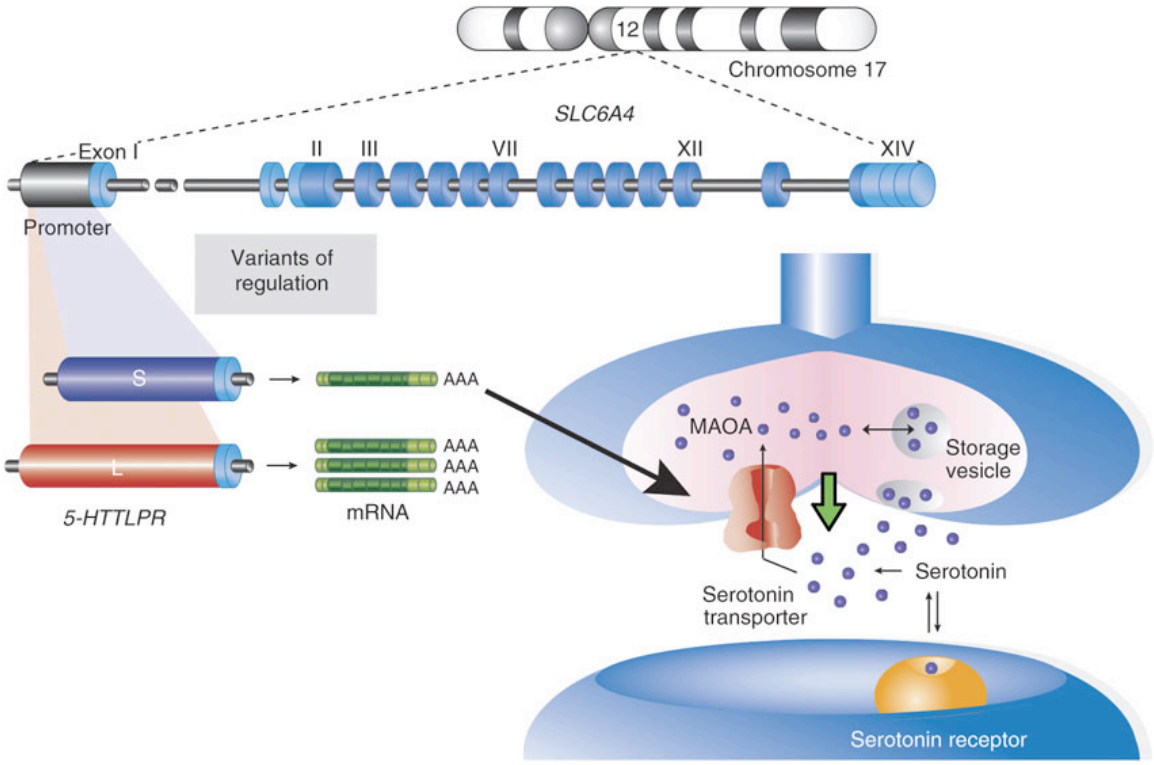

Figure 1. Graphical representation of 5-HTTLPR on serotonergic function from Canli and Lesch, 2007. The short variant is in purple, and leads to significantly less 5-HTT, which results in greater synaptic concentrations of 5-HT compared with the long variant (red). The short variant has shown to be related to anxiety and affective difficulties (Canli & Lesch, 2007).

Although individual differences in 5-HTTLPR represent only one way of examining 5-HT activity, group differences between S and L carriers have been found over several biological and psychological measures. One brain region that has received considerable attention is the amygdala due to its apparent role in stress reactivity and MDD (Whalen, Shin, Somerville, McLean, & Lim 2002). For instance, when comparing brain function, differing levels of amygdala activation in response to environmental stimuli are observed as a function of the 5-HTTLPR genotype. Heinz and colleagues (2005) found that healthy individuals (e.g., non-depressed) who were S carriers exhibited

greater amygdala activation when viewing aversive visual stimuli. Healthy (e.g., non-depressed) S carriers were also found to have greater amygdala activation when faced with threatening environmental stimuli by Hariri et al. (2005).

More recently, Kruschwitz et al. (2014) examined 5-HTTLPR and its influence on both neuroticism and resting state functional connectivity of the amygdala and fusiform gyrus. These authors found a correlation between neuroticism and 5-HTTLPR status, as well as altered amygdala and fusiform gyrus activity when observing negative faces. Neuroticism and both amygdala and fusiform gyrus activity were higher in S carriers.

Munafo, Brown, and Hariri (2008) performed a meta-analysis of 14 studies that examined 5-HTTLPR and amygdala activation. This meta-analysis supported the overall finding that S carriers exhibit increased amygdala activation in response to negative environmental stimuli. Specifically, these authors suggested 10% of the phenotypic differences in amygdala reactivity appeared related to genotypic status. However, a subsequent meta-analysis, published in 2013, contends this figure is an overestimation, and likely the result of publication bias. Specifically, Murphy and colleagues (2013) performed a meta-analysis, which included 34 samples. These authors found a small, but consistent, effect of 5-HTTLPR variants on amygdala activation, where S carriers showed greater activation. The authors of this study report that 5-HTTLPR status may account for approximately 1% of the phenotypic differences in amygdala activation (Murphy et al., 2013). Furthermore, Bastiaansen and colleagues (2014) found no relationship between in both a replication study and a meta-analysis including unpublished data. The authors conclude the relationship between 5-HTTLPR and

amygdala activation is likely smaller than previously indicated, or perhaps does not exist at all.

Some researchers have also focused on the relationship between 5-HTTLPR status and anxiety and neuroticism. These findings have been mixed. Gonda and colleagues (2009) examined healthy females and found that S carriers had higher scores on measures related to negative emotionality, including anxiety and depression symptoms, though subclinical. Clarke, Flint, Attwood, and Munafo (2010) performed a meta-analysis looking at the main effect of 5-HTTLPR variants on MDD. This group found a small, but significant, effect of 5-HTTLPR on MDD, where S carriers appeared to have greater risk of developing the disorder. However, other groups (e.g., Middledorp et al., 2007; Vinberg, Mellerup, Andersen, Bennike, & Kessing, 2009) have not found a main effect of 5-HTTLPR status on neuroticism or negative affect.

In terms of risks associated with the L allele, Glenn (2011) suggests there are psychophysiological and neurological findings that suggest L homozygotes may be more prone to psychopathy compared with S carriers. Specifically, Glenn (2011) reviews literature suggesting L homozygotes are more likely to show decreased amygdala reactivity to negative stimuli, reduced error processing in the prefrontal cortex, reduced resting heart rate, and reduced skin conductance during fear conditioning. These findings are also evident in psychopathy (Glenn 2011).

Consequently, there are various avenues of 5-HT research suggesting that, overall, lower levels of 5-HT as conferred by the S variant of 5-HTTLPR are related to negative affect and neuroticism (e.g., Carver & Miller, 2006), while it has also been

suggested that L homozygotes may be more prone to the development of psychopathy (Glenn 2011). Furthermore, S carriers may experience lower levels of overall 5-HT, and subsequently greater amygdala reactivity in response to stressful stimuli (e.g., Murphy et al., 2013) and increases in negative affect (e.g., Gonda et al., 2009). However, these findings are inconsistent in the literature with several groups being unable to find an association between 5-HTTLPR and affect (e.g., Middledorp et al., 2007).

Chapter 2: Interactions between 5-HTTLPR variants and Stressful Life Events (SLE) on the development of Major Depressive Disorder (MDD)

In addition to the main effect of 5-HTTLPR genotype on aspects of emotion, aggression, and neural function, researchers have also focused on an interaction between 5-HTTLPR status and environmental stressors, and how this relationship may influence internalizing psychopathology, particularly MDD. Caspi and colleagues (2003) investigated this relationship between 5-HTTLPR status and stressful life events (SLE) on the development of MDD in young adults. At the age of 26 years, participants (*N*=847) were assessed for SLE occurring within the previous five years using a life events calendar interview. SLE included difficulties ranging from employment, finances, housing, health, and relationships. These subjects were also assessed for MDD within the past year through structured diagnostic interviews. Findings indicated that when exposed to life stressors between the ages of 21 and 26 years, individuals with the S variant of 5-HTTLPR (including both S/S and S/L individuals) were more likely to receive an MDD diagnosis, whereas such a relationship was not seen in the L/L individuals. In an effort to examine the potential reasons behind the gene by environment interaction, Caspi et al. (2010) discuss the possibility that having an S allele makes it more likely that individuals will have higher levels of neuroticism, as supported by Ball et al. (1997) and Lesch et al. (1996). Then, when this heightened neuroticism is coupled with adverse life circumstances, the interaction results in the development of MDD.

The Caspi et al. (2003) gene by environment finding led to several subsequent papers that further examined this interaction. Some researchers have found evidence to support Caspi et al. (2003) and suggest that S carriers are more likely to experience MDD in the face of adverse life events (e.g., Daniele et al., 2011; Vervilla et al., 2007; Kendler et al., 2005). However, others have not been able to replicate these findings (e.g., Munafo, Durrant, Lewis, & Flint, 2009; Risch et al., 2009) or have only found this relationship to exist in certain groups, such as females (e.g., Åslund et al., 2009). Additionally, other authors have reported the opposite pattern, where the L allele confers great risk for MDD when exposed to SLE (Chipman et al., 2007; Luacht et al., 2009).

Controversies and Inconsistencies in the 5-HTTLPR Gene by Environment Interactions Research

Munafo and colleagues (2009) carried out a meta-analysis of studies examining the relationship between SLE and the 5-HTTLPR genotype. This group identified 14 studies that examined the potential relationship between SLE and 5-HTTLPR genotype with MDD as an outcome measure. The results of this meta-analysis suggest that very few studies have replicated the original finding of Caspi et al. (2003). Additionally, they argued that the SLE by 5-HTLLPR literature is rife with studies suffering from low power, and consequently, the occasional positive findings are likely due to chance.

Similarly, Risch et al. (2009) also performed a meta-analysis including 14 studies investigating SLE by 5-HTTLPR interactions and the development of MDD. Like Munafo et al. (2009), Risch et al. (2009) found no evidence of an interaction between

SLE and 5-HTTLPR genotype on MDD, but did identify a main effect of SLE on the development of MDD.

Many responses to Risch et al. (2009) have been published, including several letters to the editors of the *Journal for the American Medical Association (JAMA),* where the results were initially published, as well as in other publications. In a response published by the *Archives of General Psychiatry*, Michael Rutter (2009) raised several criticisms regarding the analytic approach taken by Risch et al. (2009). Rutter argued that the inclusion and exclusion of the studies was inconsistent and not representative of the literature in its entirety. He also raised questions about the generalizability of their findings, given different studies used different measures and methods for obtaining data. Although Risch et al. (2009) did attempt to recode SLE data in order to gain some consistency, given the significant heterogeneity in the data across studies Rutter argues this method may reduce statistical power (2009). Furthermore, Rutter criticizes Risch et al.'s (2009) lack of comprehensiveness, saying that to truly appreciate and understand the gene by environment interactions, data from the animal literature, as well as data regarding medication response in humans, should be considered. Therefore, Rutter believes considerable areas of research supporting the 5-HTTLPR gene by environment interactions were not given appropriate consideration in Risch et al. (2009) (Rutter, 2009).

Palla, Higgens, Wareham, and Sharp (2010) also raise methodological issues with Risch et al. (2009) and with meta-analyses in general. These authors argue that meta-analyses are problematic when investigating gene by environment interactions. Similar

to concerns raised by Rutter (2009), Palla et al. (2010) suggest that meta-analyses in this area of research are highly problematic because of the lack of consistency in measures across studies. In terms of inconsistent measures, the definition of SLE is ill-defined, and, depending on the specific study, can vary greatly from childhood maltreatment (e.g., Kaufman et al., 2006) to chronic disease or unemployment (e.g., Grabe et al., 2005). Furthermore, the measures of MDD also vary greatly. Some studies rely on questionnaire methods while others use fully structured or semi-structured interviews (Risch et al., 2009). Palla et al. (2010) are also highly critical of the meta-analytic approach in gene by environment research due to the heterogeneity in both study design and statistical analytic methods used, and the bias in both the publication of only positive findings as well as bias in terms of what studies are selected for inclusion. While these are often issues for meta-analytic approaches in general, Palla et al. (2010) argue they are even greater hurdles for gene by environment interaction research, given that the focus is on interactions. These authors contend these interactions involve greater inconsistency in the statistical analytic approaches, as well as inconsistency in how these interactions are reported compared with meta-analyses that focus on main effects. These authors subsequently conclude that meta-analyses focusing on gene by environment interactions are not likely to "provide meaningful quantitative conclusions," and time and energy should instead be spend establishing consortia in order to establish more homogeneity in the data (Palla et al., 2010).

Karg, Burmeister, Shedden, and Sen (2011) performed a more inclusive meta-analysis using a different method of combining data, which they argue is more

appropriate than the methodology used in meta-analyses elsewhere (e.g., Munafo, et al., 2009; Risch et al., 2009). These authors used a statistical method to combine *p*-values across studies as opposed to using raw data (as was done in Risch et al., 2009). The authors suggest this method addresses issues with the methodological heterogeneity in the different studies including their meta-analysis. Karg et al. (2011) performed a meta-analysis including 54 studies examining this gene by environment interaction. Moreover, this group also used the 14 studies specifically reviewed by Risch et al. (2009) separately to evaluate their analytic procedures.

When all 54 studies were included and this different data extraction method was used, Karg et al. (2011) found evidence to support an interaction between SLE and 5-HTTLPR genotype on the development of MDD. Specifically, the authors contend this relationship is particularly robust when childhood maltreatment is used as the SLE. However, when only using the 14 studies from Risch et al. (2009), this group also failed to find a gene by environment interaction leading to MDD. However, Karg et al. (2011) report that when more studies are included, and a statistical technique involving the combination of *p*-values is used opposed to the combination of raw data, the gene by environment interaction is found. Additionally, these authors argue childhood maltreatment is a specific form of SLE that leads to significant gene by environment findings (Karg et al., 2011).

In another attempt to discount the results of Munafo et al. (2009) and Risch et al. (2009), Caspi and colleagues (2010) criticize the use of paper/pencil self-report measures to quantify adverse life events. They believe in-depth, face-to-face interviews are

preferable for collecting valid data. Furthermore, as argued by others described above (e.g., Rutter, 2009; Karg et al., 2011) the authors note what is meant by "life stressors" is tremendously heterogeneous. Different studies define SLE in drastically different ways, and may use the term to include several non-specific life experiences. Consequently, Caspi et al. (2010) believe the best, most consistent data are found when a very specific and narrow definition of life stressor is used, and that too much variability is introduced when the net is cast too widely. Even more specifically, Karg et al. (2011) suggest childhood maltreatment may provide the most robust results when examining this gene by environment interaction.

In addition to the methodological differences in data collection, and the heterogeneity in the definition of SLE, several other publications have suggested various reasons for the inconsistency in findings within the 5-HTTLPR gene by environment interaction research. Uher and colleagues (2011) found an interaction between childhood maltreatment and 5-HTTLPR status on "persistent" MDD, but not for single episodes. Using a longitudinal approach, this group found the S allele conferred greater risk for the development of persistent MDD (having a diagnosis of MDD a minimum of two times out of four assessments) when coupled with childhood maltreatment. However, another group failed to find any gene by environment interaction involving SLE and 5-HTTLPR variants on persistent MDD (Fisher et al., 2012). It is potentially notable, however, that this second publication used SLE focusing on serious illness of self, serious illness or death of loved ones, financial problems, among other environmental stressors (from

Brugha, Bebbington, Tennant, & Hurry, 1985), but did not use any SLE variables involving childhood maltreatment in their analyses.

Duncan and Keller (2011) raise several valid criticisms of the gene by environment interaction research in psychiatry. In their review, they contend the positive findings in the literature are likely the result of publication bias. To support this contention, the authors note that 96% of novel gene by environment findings reported in the literature were positive. However, when replications were examined, only 27% of these were positive (Duncan & Keller, 2011). Additionally, they point out that articles where replication occurs have significantly smaller sample sizes than those failing to replicate. For instance, they report the median sample size in studies replicating gene by environment interactions was 154, while the median sample size of reports not supporting the gene by environment findings was 377. They argue if the gene by environment findings are valid, larger samples should be *more* likely to replicate interactions. They also criticize the Caspi et al. (2010) argument that larger studies have poorer measures of SLE and MDD. Duncan and Keller (2011) insist that even if this argument were true, it would not explain why there are more failures to replicate in the literature on the whole, or why studies with negative findings alone always appear to have the largest sample sizes. Consequently, Duncan and Keller (2011) believe the majority of the positive gene by environment findings are the result of publication bias.

This controversy clearly indicates significant problems with the current understanding of gene by environment interactions, particularly in reference to 5-HTTLPR and SLE, and their influences on the development of MDD. As discussed

above, several inconsistencies have been found in the published literature; however, whether these inconsistencies simply reflect a poverty in data quality (as suggested by Caspi et al., 2010; Uher & McGuffin, 2010) or important information contradicting the validity of the 5-HTTLPR and SLE interactions (as proposed by Risch et al., 2009; Munafo et al., 2009; Duncan & Keller, 2011) remains to be seen.

Relative Independence or Dependence of Stressful Life Events (SLE) and Genetic Background

A potential complication in the gene by environment interaction research comes from considering the relative dependence or independence of adverse life events, where individuals with certain genetic backgrounds may be more likely to experience certain stressful life events (SLE). For instance, a child may have a particularly difficult temperament due, at least in part to his or her genetic background. Then, if he or she is expelled from school because of these difficulties, it is likely his or her genes are influencing this specific environmental stressor (Scarr & McCartney, 1983). This relationship between genetic background and environmental stressors may complicate the investigation of gene by environment interactions since these two factors are apparently not entirely independent of one another. The potential interdependence between genes and environment has also not been considered when investigating the relationship between 5-HTTLPR and SLE. Furthermore, since approximately one-third of the relationship between SLE and MDD is due to common genes (Kendler et al., 1999), it

may be possible the relationship between SLE and MDD may over-estimate any potential gene by environment interaction (Boardman, Alexander, & Stallings, 2011).

Summary

One relatively common way of investigating the influence of 5-HT on several phenotypes is through examining the 5-HTTLPR genotype, where different polymorphisms appear to confer different levels of risk for certain kinds of psychopathology. Several studies have suggested that S carriers experience greater activity in the amygdala when presented with negative stimuli, as well as tendencies towards greater negative affect, although these findings are inconsistent. In addition to these main effects of 5-HTTLPR, another area of research has grown around the concept that 5-HTTLPR may predispose some individuals to develop MDD when confronted with adverse life events. As discussed above, these gene by environment findings are also inconsistent, and have led to great debate within the field. Several potential reasons for these discrepant findings have been posited, including the quality of data acquisition (in-person interviews versus paper/pencil questionnaires) and the nature of which adverse life events may be more likely to lead to MDD in S carriers. Despite these many arguments presented to explain the discrepant gene by environment findings, questions remain as to why such inconsistency exists. In the analyses below, some of the potential reasons for these inconsistencies will be investigated.

Chapter 3: Current Project

With the current project, the relationship between the 5-HTTLPR genotype and SLE on MDD will be investigated in a large epidemiological sample of individuals who contributed various sources of data on history of SLE in the context of a longitudinal study. With the large, longitudinal dataset obtained and maintained by the Minnesota Center for Twin and Family Research (MCTFR), interactions between the 5-HTTLPR variants and SLE can be examined in detail. The dataset is larger than many of the previously published reports, including the original Caspi et al. (2003) publication. Furthermore, this dataset includes several high quality interview data measures. As Caspi et al. (2010) suggested, brief self-report forms assessing SLE may be insufficient. Moreover, this large dataset is longitudinal, and thus the relationship between 5-HTTLPR variants and adverse life events can be evaluated over the course of several years (between 6 and 9 years) as opposed to one or two time points that span shorter durations, which may be important given the contention of Uher et al. (2011) that gene by environment interactions may only be seen in "persistent" MDD.

Specific aim 1a: Replication

The first goal of these analyses is an attempt to replicate the original gene by environment interaction found by Caspi et al. (2003) in a sample of individuals from the Minnesota Center for Twin and Family Research (MCTFR). This sample of individuals was evaluated between the ages of 17 and 25 years. During this time, the participants'

total stressful life events (SLE) were measured through the Life Events Interview (LEI: Billig, Herschberger, Iacono, & McGue, 1996). The occurrence of Major Depressive Disorder (MDD) was also determined during this period. In an effort to replicate Caspi et al. (2003), it will be investigated whether S carriers have higher rates of MDD at age 25 years compared with L/L individuals when exposed to higher levels of SLE between the ages of 17 and 25 years, while the different genotypes will not differ in MDD rates at low levels of SLE. Consistent with the compelling arguments of Duncan & Keller (2011), and the analyses of Risch et al., (2009) and Munafo et al., (2009), it is hypothesized that no significant interaction between genotype and SLE (when SLE are broadly defined) on the development of MDD will be found. Thus, it is anticipated that Caspi et al. (2003) will not be replicated.

Specific Aim 1b: Childhood Maltreatment

Within the context of this overarching analysis, childhood maltreatment may be isolated as a specific kind of SLE, which may be examined in additional analyses, since some authors have suggested this form of SLE may be most likely to lead to a gene by environment interaction (e.g., Karg et al., 2011). Lifetime experiences with childhood maltreatment through the age of 18 years, as measured through the Childhood Experience Questionnaire (CEQ: e.g., Bornovalova, et al., 2012) will be used. If Karg et al. (2011) is correct about the influence of childhood maltreatment on this gene by environment interaction for MDD, it would be hypothesized that a significant gene by environment interaction on the occurrence of MDD will be found. However, it is difficult to ascertain

why different "kinds" of stressful life events would impact differences in genetic risk for MDD. Consequently, despite the contention of Karg et al. (2011), it is hypothesized that no significant interaction between 5-HTTLPR variants and childhood maltreatment on the occurrence of MDD will be found.

Specific Aim 1c: Persistent MDD

As these data are longitudinal, "persistent" MDD, as described by Uher et al. (2011) can also be assessed. Persistent MDD will be defined as the occurrence of MDD at two or more time points through assessments at the approximate ages of 17, 20, and 25 years. SLE will be measured using the Life Events Interview (LEI) data through the age 25 assessment. It is hypothesized that S carriers will be more susceptible to persistent MDD when facing higher levels of SLE compared with L/L participants, but rates of MDD will not differ between genotypes at low SLE levels. It is important to note that within the persistent MDD analyses, SLE and the diagnosis of MDD are concurrent.

Specific Aim 2

Since genes and environment are not completely independent of one another, one's genetic background may influence the kinds of environmental stressors one experiences. Adverse life events taken from the Life Events Interview (LEI) discussed above were studied by Bemmels, Burt, Legrand, Iacono, and McGue (2008). These authors created three scales: independent life events (SLE likely to not be influenced by the participant, e.g., being mugged or robbed), dependent adverse life events (SLE that

may be influenced by the participant, e.g., spending time in jail), and familial adverse life events (experienced by a member of the family). Please see Appendix C for the events considered dependent and independent from this publication. Results indicated additive genetic effects contributed significantly to the dependent events, while nonshared environmental effects contributed most to the independent life events. The largest contributor to the familial life events involved shared environmental effects. These results suggest genetic background may have considerable impact on certain kinds of life events.

In adolescence and young adulthood, it is estimated that approximately 37% of the variance in the development of MDD is due to genetic factors (Sullivan, Neal, & Kendler, 2000) and approximately one-third of the relationship between SLE and MDD is due to the common genes (Kendler, et al., 1999). Consequently, it would make sense that the relationship between dependent SLE and MDD may over-estimate any potential gene by environment interaction (Boardman et al., 2011). The goal of Specific Aim 2 is to investigate whether independent forms of SLE interact with the 5-HTTLPR genotype in influencing the development of MDD differently than the dependent forms of SLE. It is hypothesized that dependent SLE will show a significant gene by environment interaction on the development of MDD, while independent SLE will not show a significant interaction. If dependent SLE interact with 5-HTTLPR and independent SLE do not, it may suggest studies using dependent SLE in gene by environment research may be over-estimating the gene by environment interaction because the presence of dependent SLE and MDD may simply be coming from the same underlying genes.

Specific Aim 3: Negative Emotionality and 5-HTTLPR Genotype

As discussed above, there is a potential relationship between 5-HTTLPR and negative affect (e.g., Ogilvie et al., 1996; Kurschwitz et al., 2014). Specifically it will be investigated whether S carriers have significantly higher negative affect compared with L/L individuals, and whether negative affect, when coupled with SLE may lead to MDD. Data from the Multidimensional Personality Questionnaire (MPQ: Tellegen, 1982) include the scale Negative Emotionality (NEM), which is comprised of subscales including stress reactivity, alienation, and aggression. It will be investigated whether 5-HTTLPR genotype is related to NEM at age 17, and whether NEM at age 17 influences the development of MDD by age 25 years. It is hypothesized that S carriers will be more likely to experience higher levels of NEM, which will influence the frequency with which they experience MDD.

Chapter 4: Methods

Sample

Participants are individuals enrolled in research with the Minnesota Center for Twin and Family Research (MCTFR: Iacono, McGue, & Krueger, 2006), a longitudinal epidemiological series of studies examining twins born in Minnesota, as well as their siblings and parents. The MCTFR data have been collected with an emphasis on psychological adjustment in twins and family members, with a special interest in externalizing psychopathology and substance use disorders (Iacono et al., 2006). The MCTFR began with two cohorts, twins aged 11 or 17 years when initially enrolled. Participants were identified through public birth records between 1972 and 1978 in the case of the 17-year-old cohort and between 1978 and 1982 in the case of the 11-year-old cohort. More than 90% of the same sex male and female twins born during these respective time periods were located for inclusion in this research project. Individuals were excluded if either twin had a serious physical or cognitive disability that would prevent them from engaging in the assessments or if they lived farther than one day's drive from the labs in Minneapolis. A total of 78% of eligible families agreed to participate, and the sample (98% Caucasian) was representative of the overall racial make-up of Minnesota during the years of recruitment. After initial participation in this project, twins were invited back in approximate 3-year intervals for testing.

The current study uses a subset of this dataset. The total dataset examined 1,517 twins at intake in the 11-year-old cohort and 1,252 twins at intake for the 17-year-old

cohort. However, not all of these participants provided DNA samples, and subsequently fewer individuals are used in these current analyses. Individuals were included in this current project if they contributed DNA, have MDD data from the Structured Clinical Interview for the DSM-III R (SCID) (Spitzer et al., 1990) at the three time points discussed below, and had completed the Life Events Interview (LEI) at the three time points discussed below. This method of inclusion yielded a total of 740 participants from the younger cohort and 729 participants from the older cohort, totaling 1,469 individuals (53% of the 2,769 who completed an intake assessment). These 1,469 participants in the current analyses did not differ in terms of reported SLE at age 25 assessment from those without genotyping data (who were therefore excluded) in either the younger cohort (included: M= 6.35, SD= 3.31; excluded: M=6.67, SD=3.75) ($t(1311) = -1.599$, $p = 0.110$) or the older cohort (included: M= 6.69, SD=3.27; excluded 6.28, SD=3.08) ($t(1112) = 1.964$, $p = 0.050$). The excluded participants in the neither the younger (χ^2 (1, N=1206) = 0.932, $p = 0.334$) nor the older cohort (χ^2 (1, N=1108) = 0.001, p=0.979) differed significantly in rates of MDD from those included in the study.

To increase the overall N, the 11 and 17-year-old cohorts were combined for analyses. The data from three time points was used, when the participants were an average of 17.81 years, 21.07 years, and 24.94 years. These data points are labeled Age 17 Assessment, Age 21 Assessment, and Age 25 Assessment respectively. In order to use both cohorts at these ages, data from follow-up 2, 3, and 4 were used from the younger cohort and data from intake, follow-up 1, and follow-up 2 were used from the older cohort. Table 1 summarizes the number of individuals with genotyping data, MDD

data, and LEI data by cohort and follow-up. Table 2 displays rates of MDD, total SLE, independent SLE, and dependent SLE by gender and genotype.

Table 1

Participant Characteristics

Assessment	Younger Cohort	Older Cohort	Total
Total			
N	740	729	1469
Number male	362	325	687
Age 17			
Mean Age (SD)	18.13 (0.70)	17.47 (0.46)	17.79 (0.67)
Age 21			
Mean Age (SD)	21.43 (0.78)	20.66 (0.55)	21.03 (0.78)
Age 25			
Mean Age (SD)	25.22 (0.67)	24.65 (0.89)	24.92 (0.84)

Note. Participants from each cohort, by assessment, including mean age and standard deviations.

Table 2

Number (and percentages) of participants with MDD at Age 25 Assessment, SLE, ISLE, and DSLE at Age 25 Assessment by gender and genotype.

	Female			Male		
	S/S	S/L	L/L	S/S	S/L	L/L
MDD	20 12.7%	58 15.8%	31 12.1%	11 8.4%	25 7.7%	17 7.4%
SLE	12.37 (4.82)	12.62 (5.13)	11.83 (5.22)	13.10 (5.75)	13.04 (6.27)	12.28 (5.71)
ISLE	1.69 (2.76)	1.81 (2.53)	1.69 (2.71)	1.53 (2.31)	1.90 (3.34)	1.21 (2.21)
DSLE	2.59 (3.60)	3.11 (7.58)	2.52 (2.76)	2.99 (4.95)	2.84 (3.99)	2.66 (4.14)

Note. Mean (SD) Stressful Life Events (SLE), Independent Stressful Life Events (ISLE), and Dependent Stressful Life Events (DSLE) by gender and genotype.

Measures

The presence or absence of MDD was determined using an altered version of the Structured Clinical Interview for the DSM-III R (SCID) (Spitzer et al., 1990), using criteria from the DSM-III R. The interviewers had psychology backgrounds and underwent considerable training in diagnostic interviewing. Consensus meetings were held with a minimum of two graduate students or individuals with advanced training in the area of psychological assessment, and tapes of the interviews were reviewed. A sample of 600 study participants were selected and yielded a kappa reliability of .78

(Wilson, Vaidyanathan, Miller, McGue, & Iacono, 2014). Persistent MDD, taken from Uher et al. (2011), is defined as individuals meeting criteria for MDD at two or more time points between the ages of 17 and 25 years.

The 17-year-old cohort had lifetime prevalence of MDD measured at their intake and follow-up 1, at approximate ages 17 and 21. Cases of MDD occurring in this cohort between intake and follow-up 1 were extrapolated by identifying those reporting MDD episodes at follow-up 1 that did not report episodes at intake. At follow-up 2, at approximate age 25, this cohort reported new cases of MDD occurring since follow-up 1.

In terms of the 11-year-old cohort, these participants were asked to report symptoms of MDD that occurred prior to intake. Each subsequent follow-up assessed MDD as it had occurred over the previous three years.

The SLE data include the Life Events Interview (LEI: Billig, Herschberger, Iacono, & McGue, 1996). This in-person interview was completed by participants at each assessment and asks about a broad range of potential adverse life events. The definition of SLE in this interview is variable and includes several life events that may differ greatly in their perceived intensity (e.g., having a fight with a close friend versus the death of a loved one). A total of 45 questions were used from each visit. For a list of SLE questions used in these analyses, please see Appendix A. At intake, participants were asked whether these incidents had occurred during their lifetimes, and at each subsequent follow-up they were asked to report incidents having occurred in the previous three years. Total number of SLE was summed across the questions asked, with 45 being the maximum number of SLE that could be endorsed at each time point. This variable

was then divided into quartiles, and four groups were created indicating minimal, mild, moderate, or high incidence of SLE (e.g., Risch et al., 2009). For the ranges, means, and standard deviations for each of these four groups, please see Table 3.

Table 3

The range of SLE, means, and standard deviations in each of the four LEI groups.

Group	*N*	Range of SLE	Mean (SD)
Group 1	364	0.00 – 8.00	6.31 (1.68)
Group 2	467	9.00 – 12.00	10.58 (1.14)
Group 3	342	13.00 – 16.00	14.28 (1.12)
Group 4	296	17.00 – 45.00	20.63 (3.98)

The other method used to measure SLE is from the Childhood Experience Questionnaire (CEQ: e.g., Bornovalova et al., 2012). This self-report questionnaire was given after the participants reached adulthood and asked them to think back upon their childhood and potential abusive or neglectful incidents they may have experienced through the age of 18 years. These data were collected at follow-up 4 (approximate age 24.99 years) for the 11-year-old cohort. In terms of the 17-year-old cohort, these data were collected from female participants only at follow-up 3 (approximate age 29). Consequently, only the younger cohort is included in these analyses. For a list of the SLE data included in this self-report questionnaire, please see Appendix B. The total number of events was summed. As with the SLE from the LEI, 4 groups were created

indicating whether the participants had experienced 0 (N = 197), 1 (N = 243), 2 (N = 108), or 3 or more (N = 232) negative life events described in this questionnaire. This method yielded four levels of SLE, which, again, is similar to the approach taken in Risch et al. (2009).

Negative emotionality (NEM) was derived from the Multidimensional Personality Questionnaire (MPQ: Tellegen 1982). NEM is comprised of three subscales, including aggression, alienation, and stress reactivity. NEM data from females and males in both the younger and older cohorts at the Age 17 Assessment were included in these analyses. It was investigated whether NEM at the Age 17 Assessment was related to the presence of MDD at the Age 25 Assessment, and whether NEM at the Age 17 Assessment interacted with SLE on the presence of MDD at age 25.

Statistical Analyses

Generalized Estimating Equations (GEE) were used to determine the level of risk for MDD associated with each factor, which included gender, 5-HTTLPR status (coded 0 for L/L individuals, 1 for L/S individuals, and 2 for S/S individuals), cohort (either the younger or older cohort), and the number of SLE coded on a 4 point scale, as well as interactions between these variables. GEE was used because this analytic method allows for the interrelatedness of subjects (twins) while extending the general linear model (Hardin & Hilbe 2013). The sample used in these current analyses had 66.3% monozygotic twins. In terms of the model, an unstructured working correlation matrix was used, and statistical significance was determined using the p-value from the Wald

type 3 statistic. Since the outcome variable was dichotomous (depressed versus not depressed) a binary logistic regression model was used. Analyses were completed using SPSS version 20 for Macintosh computers.

No assumptions regarding additivity or dominance of 5-HTTLPR effects were made beforehand. Analyses were performed for both the additive and dominance models, where the additive model compared the three 5-HTTLPR variants (S/S, S/L, and L/L) and the dominance model focused on the presence of the S allele by comparing S/S and S/L individuals to L/L individuals. Like Caspi et al. (2003), an additive model was used where the genotype was coded 0, 1, or 2, representing the number of S alleles each individual has. The dominance model compares L homozygotes to S carriers, and these participants are coded as either 0 or 1, where 1 indicates the presence of at least one S allele.

Chapter 5: Results

The sample was comprised of 19.7% S/S, 47.2% S/L, and 33.1% L/L individuals, which does not deviate from the Hardy-Weinberg equilibrium (χ^2 (2) = 1.4339, $p > 0.50$). Results of an ANOVA suggest the L homozygotes had significantly lower rates of SLE compared with the heterozygotes ($F(2, 1466) = 4.165$, $p=0.016$). No other significant differences regarding SLE between the genotypic groups were noted.

Findings, which generally do not confirm the studies hypotheses, are organized by aim.

Specific Aim 1a: Replication

First, in an attempt to replicate Caspi et al. (2003), the relationship between SLE and 5-HTTLPR on the presence of MDD at age 25 was investigated with both additive and dominance models. SLE was measured through adding the total LEI events reported between the 17 and 25 year assessments. The presence of MDD, likewise, was measured between the 17 and 25 year assessments. Gender and cohort were also included in this model. For a summary of the average LEI reported at each visit, as well as the percentage of participants with MDD at age 25, please see Table 2.

Additive Analyses

Results indicate a significant main effect of SLE on the presence of MDD at age 25 years, where greater SLE was associated with higher rates of MDD. There was no significant main effect of cohort, gender, or 5-HTTLPR on the presence of MDD, nor

were any interactions between these variables significant in their influence over MDD.

For a summary of these results, please see Table 4 and Figure 2.

Table 4

Results of Generalized Estimating Equations (GEE) examining gender, cohort, LEI, and 5-HTTLPR on the presence of MDD at age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	0.439 (1)	.508
	Cohort	3.830 (1)	.050
	5-HTTLPR	0.002 (1)	.961
	SLE	20.377 (1)	.000
	Gender*5-HTTLPR	0.008 (1)	.927
	Gender*SLE	0.000 (1)	.988
	5-HTTLPR*SLE	0.084 (1)	.772
	5-HTTLPR*SLE*Gender	0.000 (1)	.995

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, SLE (as measured by LEI reported between the ages of 17 and 25) and 5-HTTLPR (additive) on the presence of MDD between the ages of 17 and 25 years.

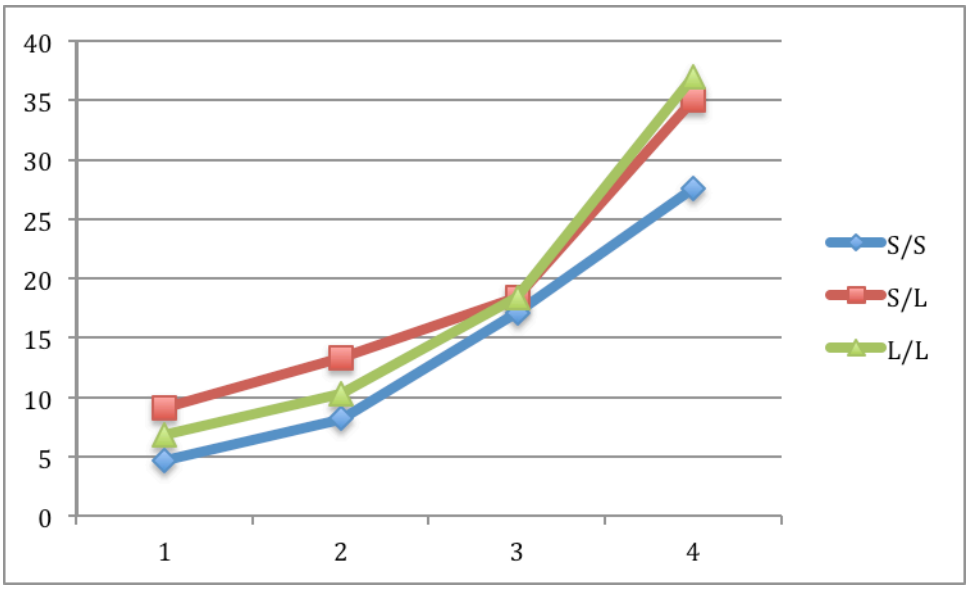

Figure 2. Percentage of participants with Major Depressive Disorder (MDD) by genotype and SLE (LEI) group (comprised of quartiles).

Dominance Analyses

The dominance analyses were much the same as the above-discussed additive analyses. There was a significant main effect of SLE. No significant main effects for gender, cohort, or 5-HTTLPR were significant. No interactions were significant. For a summary of these results, please see Table 5.

Table 5

Results of Generalized Estimating Equations (GEE) examining gender, cohort, LEI, and 5-HTTLPR on the presence of MDD at age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	0.001 (1)	.970
	Cohort	3.572 (1)	.059
	5-HTTLPR	0.704 (1)	.401
	SLE	16.166 (1)	.000
	Gender*5-HTTLPR	0.555 (1)	.456
	Gender*SLE	0.144 (1)	.705
	5-HTTLPR*SLE	0.478 (1)	.490
	5-HTTLPR*SLE*Gender	0.250 (1)	.617

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, SLE (as measured by LEI reported between the ages of 17 and 25) and 5-HTTLPR (dominance) on the presence of MDD between the ages of 17 and 25 years.

Specific Aim 1b: Childhood Maltreatment

The next set of analyses focus on childhood maltreatment as a specific form of SLE. In this case, instances of abuse and neglect were recorded by use of the Childhood Experience Questionnaire (CEQ). The CEQ was administered to members of the 11-year-old cohort at the age 25 assessment. Using this retrospective questionnaire, the participants were asked to report on their experiences through the age of 18 years. MDD was measured again as occurring between the ages of 17 and 25 years.

Additive Analyses

Results indicate no significant main effects of SLE, as measured by the CEQ, gender, cohort, or 5-HTTLPR on the presence of MDD at the age of 25 years. No interactions were significant. Please see Table 6 and Figure 3 for a summary of these results.

Table 6

Results of Generalized Estimating Equations (GEE) examining gender, cohort, CEQ, and 5-HTTLPR on the presence of MDD at age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	0.900 (1)	.343
	5-HTTLPR	0.007 (1)	.932
	SLE	3.124 (1)	.077
	Gender*5-HTTLPR	0.011 (1)	.916
	Gender*SLE	0.017 (1)	.895
	5-HTTLPR*SLE	0.034 (1)	.854
	5-HTTLPR*SLE*Gender	0.001 (1)	.978

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, SLE (as measured by Childhood Experience Questionnaire – through age 18 years) and 5-HTTLPR (additive) on the presence of MDD between the ages of 17 and 25 years. These analyses were completed only in the younger cohort because the CEQ was only administered to females in the older cohort.

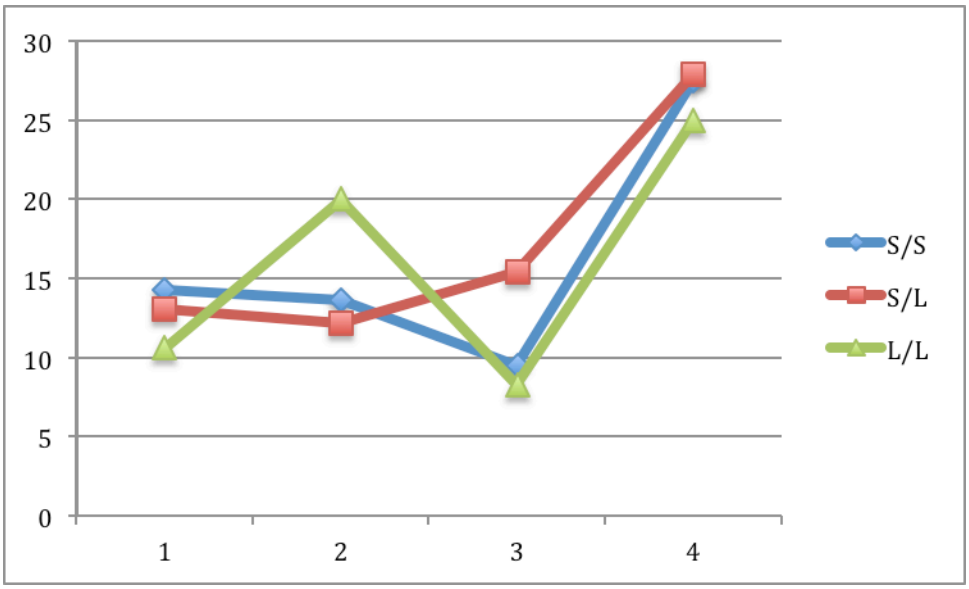

Figure 3. Percentage of participants with Major Depressive Disorder (MDD) by genotype and SLE (CEQ) group (comprised of quartiles).

Dominance Analyses

When comparing S carriers to L homozygotes via dominance analyses, again no main effects were significant. No interactions were significant, as was the case with the above discussed additive analyses. Please see Table 7 for a summary of these results.

Table 7

Results of Generalized Estimating Equations (GEE) examining gender, cohort, CEQ, and 5-HTTLPR on the presence of MDD at age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	1.545 (1)	.214
	5-HTTLPR	0.150 (1)	.698
	SLE	2.177 (1)	.140
	Gender*5-HTTLPR	0.159 (1)	.690
	Gender*SLE	0.166 (1)	.684
	5-HTTLPR*SLE	0.150 (1)	.699
	5-HTTLPR*SLE*Gender	0.128 (1)	.721

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, SLE (as measured by Childhood Experience Questionnaire – through age 18 years) and 5-HTTLPR (dominance) on the presence of MDD between the ages of 17 and 25 years. These analyses were completed only in the younger cohort because the CEQ was only administered to females in the older cohort.

Specific Aim 1c: Persistent MDD

Persistent MDD, for these analyses, was defined as the presence of MDD at two or more time points measured at the 17, 21, and 25 year assessments.

Additive Analyses

The results indicate a significant main effect of SLE on the development of persistent MDD, where those exposed to higher numbers of SLE were more likely to experience persistent MDD. No main effects of cohort, gender, or 5-HTTLPR were significant. There was also a significant interaction between 5-HTTLPR and SLE on the presence of persistent MDD, where S/S individuals at higher rates of SLE actually

showed lower rates of MDD compared with S/L and L/L individuals, which is contrary to expectation. Please see Table 8 and Figure 4 for a summary of these results.

Table 8

Results of Generalized Estimating Equations (GEE) examining gender, cohort, LEI, and 5-HTTLPR on persistent MDD by age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	0.049 (1)	.825
	Cohort	1.369 (1)	.242
	5-HTTLPR	2.652 (1)	.103
	SLE	34.270 (1)	.000
	Gender*5-HTTLPR	0.012 (1)	.914
	Gender*SLE	2.170 (1)	.141
	5-HTTLPR*SLE	4.117 (1)	.042
	5-HTTLPR*SLE*Gender	0.522 (1)	.470

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, SLE (as measured by LEI reported between the ages of 17 and 25) and 5-HTTLPR (additive) on the presence of persistent MDD between the ages of 17 and 25 years.

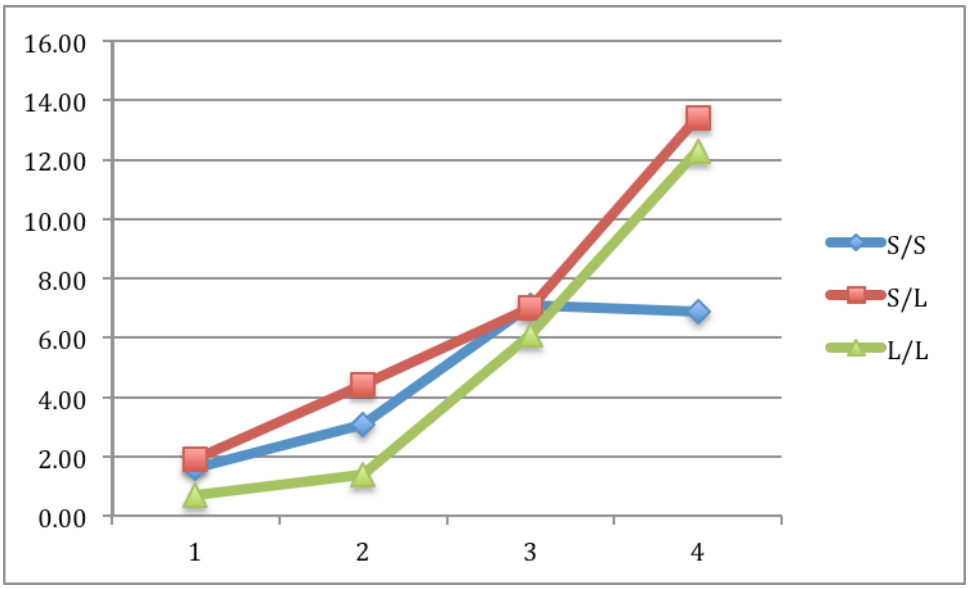

Figure 4. Percentage of participants with persistent Major Depressive Disorder (MDD) by genotype and SLE (LEI) group (comprised of quartiles).

Dominance Analyses

When comparing S carriers to L homozygotes via dominance analyses, there was a significant main effect of SLE on the experience of MDD, where higher rates of SLE were related to higher rates of persistent MDD. There was also a significant interaction of gender by SLE on the presence of MDD, where females exposed to higher rates of SLE were more likely to develop persistent MDD. Again, there was also a significant interaction between 5-HTTLPR and SLE where S/S individuals showed lower rates of persistent MDD at higher rates of SLE compared with S/L and L/L individuals. All other main effects and interactions were non-significant. Please see Table 9 for a summary of these results.

Table 9

Results of Generalized Estimating Equations (GEE) examining gender, cohort, LEI, and 5-HTTLPR on persistent MDD by age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	1.649 (1)	.199
	Cohort	1.200 (1)	.273
	5-HTTLPR	3.912 (1)	.048
	SLE	25.284 (1)	.000
	Gender*5-HTTLPR	1.255 (1)	.263
	Gender*SLE	2.595 (1)	.038
	5-HTTLPR*SLE	4.365 (1)	.037
	5-HTTLPR*SLE*Gender	2.377 (1)	.123

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, SLE (as measured by LEI reported between the ages of 17 and 25) and 5-HTTLPR (dominance) on the presence of persistent MDD between the ages of 17 and 25 years.

Specific Aim 2: Independent versus Dependent SLE

The next set of analyses focused on potential differences in the gene by environment analyses when using independent versus dependent SLE. First, independent SLE (those events unlikely to be influenced by the individual) were considered. Analyses focused on exposure to independent SLE between the 17 and 25 year assessments, as well as the presence of MDD during the same time frame.

Additive Analyses on Independent SLE

Additive analyses indicated a significant main effect of independent SLE on the development of MDD, where those with higher rates of independent SLE were more

likely to experience MDD. All other main effects and interactions were non-significant.

Please see Table 10 and Figure 5 for summaries of these results.

Table 10

Results of Generalized Estimating Equations (GEE) examining gender, cohort, independent LEI, and 5-HTTLPR on the presence of MDD at age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	0.320 (1)	.572
	Cohort	1.567 (1)	.211
	5-HTTLPR	0.024 (1)	.877
	SLE	8.140 (1)	.004
	Gender*5-HTTLPR	1.401 (1)	.236
	Gender*SLE	0.258 (1)	.611
	5-HTTLPR*SLE	0.234 (1)	.629
	5-HTTLPR*SLE*Gender	1.512 (1)	.219

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, independent SLE (as measured by LEI reported between the ages of 17 and 25) and 5-HTTLPR (additive) on the presence of MDD between the ages of 17 and 25 years.

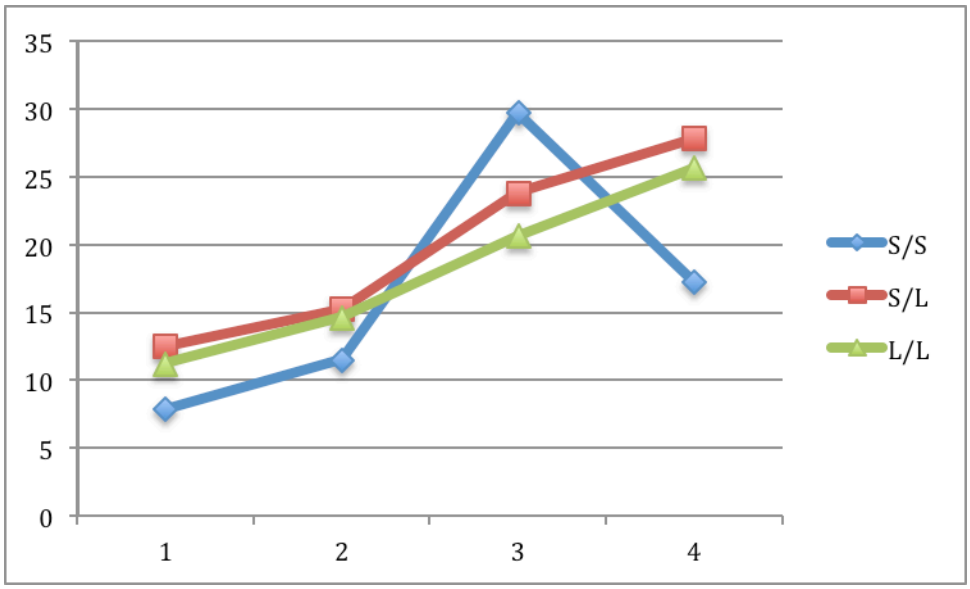

Figure 5. Percentage of participants with Major Depressive Disorder (MDD) by genotype and independent SLE (LEI) group (comprised of quartiles).

Dominance Analyses on Independent SLE

Next, L homozygotes were compared with S carriers. As with the additive analyses, there was a significant main effect of independent SLE, where increases in independent SLE were related to higher rates of MDD. No other main effects or interactions were significant. For a summary of these results, see Table 11.

Table 11

Results of Generalized Estimating Equations (GEE) examining gender, cohort, independent LEI, and 5-HTTLPR on the presence of MDD at age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	0.066 (1)	.797
	Cohort	1.469 (1)	.266
	5-HTTLPR	1.219 (1)	.270
	SLE	7.552 (1)	.006
	Gender*5-HTTLPR	3.506 (1)	.061
	Gender*SLE	1.189 (1)	.276
	5-HTTLPR*SLE	0.659 (1)	.417
	5-HTTLPR*SLE*Gender	3.174 (1)	.075

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, independent SLE (as measured by LEI reported between the ages of 17 and 25) and 5-HTTLPR (dominance) on the presence of MDD between the ages of 17 and 25 years.

Additive Analyses on Dependent SLE

Dependent SLE (those events that could be influenced or "selected" by individuals) were examined next. Dependent SLE emerged as a significant predictor of MDD. Those exposed to higher levels of dependent SLE were more likely to develop MDD. There was no main effect of 5-HTTLPR and no significant interaction between 5-HTTLPR and dependent SLE on the development of MDD. For a summary of these results, please see Table 12 and Figure 6.

Table 12

Results of Generalized Estimating Equations (GEE) examining gender, cohort, dependent LEI, and 5-HTTLPR on the presence of MDD at age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	1.995 (1)	.158
	Cohort	4.694 (1)	.030
	5-HTTLPR	0.091 (1)	.763
	SLE	6.373 (1)	.012
	Gender*5-HTTLPR	0.531 (1)	.466
	Gender*SLE	0.665 (1)	.415
	5-HTTLPR*SLE	0.217 (1)	.641
	5-HTTLPR*SLE*Gender	1.358 (1)	.244

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, dependent SLE (as measured by LEI reported between the ages of 17 and 25) and 5-HTTLPR (additive) on the presence of MDD between the ages of 17 and 25 years.

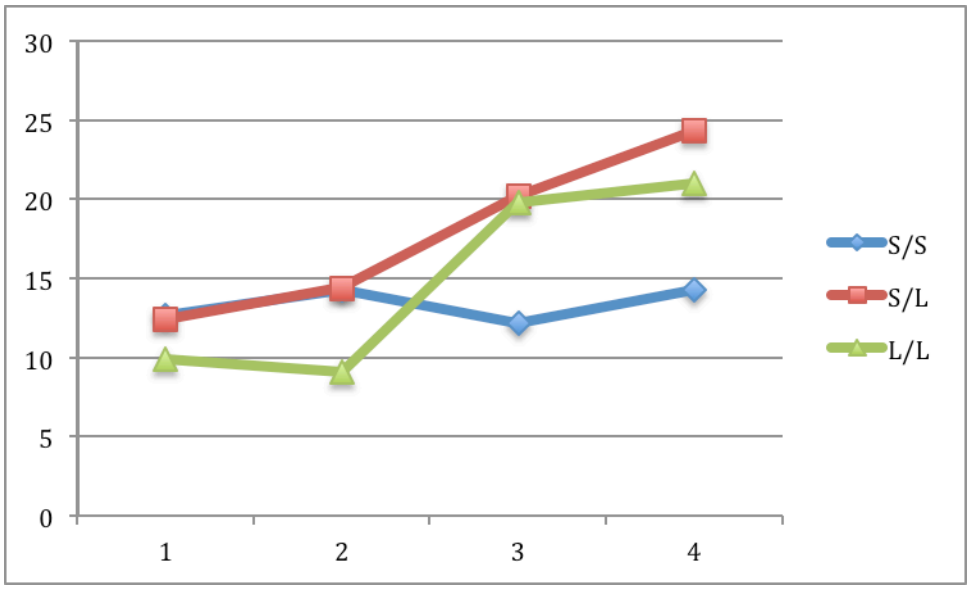

Figure 6. Percentage of participants with Major Depressive Disorder (MDD) by genotype and dependent SLE (LEI) group (comprised of quartiles).

Dominance Analyses on Dependent SLE

Main effects of cohort and SLE on the development of MDD were seen. Again, no main effect of genotype or an interaction between 5-HTTLPR and SLE were significant. For a summary of these results, please see Table 13.

Table 13

Results of Generalized Estimating Equations (GEE) examining gender, cohort, dependent LEI, and 5-HTTLPR on the presence of MDD at age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	0.452 (1)	.502
	Cohort	4.592 (1)	.032
	5-HTTLPR	0.780 (1)	.377
	SLE	4.531 (1)	.033
	Gender*5-HTTLPR	0.004 (1)	.951
	Gender*SLE	0.090 (1)	.764
	5-HTTLPR*SLE	0.205 (1)	.650
	5-HTTLPR*SLE*Gender	0.198 (1)	.656

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, dependent SLE (as measured by LEI reported between the ages of 17 and 25) and 5-HTTLPR (dominance) on the presence of MDD between the ages of 17 and 25 years.

Specific Aim 3: NEM, 5-HTTLPR, SLE, and MDD

The influence of 5-HTTLPR and Negative Emotionality (NEM) on the presence of MDD was investigated using the additive and dominance models. For the means and standard deviations of NEM, and its facets (stress reactivity, alienation, and aggression) please see Table 14. Levels of NEM did not vary across the three 5-HTTLPR variants, (F(2, 1381)=0.409, p=.664). When males and females were examined separately, NEM still did not vary across the three 5-HTTLPR variants, (F(2, 640)=0.979, p=.376) for males and (F(2, 738)=2.580, p=.076) for females. Similar results were found for the three facets of NEM, including stress reactivity (F(2, 1398)=1.251, p=.286), alienation (F(2, 1399)=1.999, p=.136) and aggression (F(2, 1398)=2.671, p=.070).

Table 14

Mean (standard deviation) of Negative Emotionality (NEM), and its subscales Stress Reactivity, Alienation, and Aggression for each genotypic status.

	N	NEM	Stress Reactivity	Alienation	Aggression
S/S	276	89.23 (14.56)	43.22 (9.56)	35.40 (9.03)	37.83 (10.11)
S/L	657	89.43 (14.25)	43.63 (9.41)	35.35 (8.55)	37.27 (9.19)
L/L	451	88.66 (13.42)	42.75 (8.64)	34.40 (7.89)	38.62 (9.85)

Additive Analyses

It was further investigated whether NEM at age 17 was associated with MDD at age 25, and whether NEM interacted with SLE or 5-HTTLPR status on the presence of MDD at age 25, while continuing to investigate the potential effects of gender and cohort. Cohort and gender emerged as significant indicators of MDD. NEM was not associated with the presence of MDD at age 25, nor were any interactions between NEM and SLE or 5-HTTLPR significant. Please see Table 15 for these results.

Table 15

Results of Generalized Estimating Equations (GEE) examining gender, cohort, NEM, LEI, and 5-HTTLPR on the presence of MDD at age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	11.439 (1)	.001
	Cohort	7.526 (1)	.006
	5-HTTLPR	0.869 (1)	.351
	NEM	1.926 (1)	.165
	SLE	0.513 (1)	.474
	NEM*SLE	0.251 (1)	.616
	NEM*5-HTTLPR	0.899 (1)	.343
	NEM*5-HTTLPR*SLE	0.004 (1)	.952

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, NEM, SLE (as measured by LEI reported between the ages of 17 and 25) and 5-HTTLPR (additive) on the presence of MDD between the ages of 17 and 25 years in participants.

Dominance Analyses

The dominance analyses were much the same as the additive analyses. Again cohort was a significant predictor of the presence of MDD at age 25 years. Gender also emerged as a significant predictor of MDD at age 25 years. NEM was not significantly associated with the presence of MDD at age 25, nor were any interactions between NEM and either SLE or 5-HTTLPR significant. Please see Table 16 for these results.

Table 16

Results of Generalized Estimating Equations (GEE) examining gender, cohort, NEM, LEI, and 5-HTTLPR on the presence of MDD at age 25

Phenotype	Predictor	Wald Chi-Square (df)	*p*-value
MDD	Gender	11.062 (1)	.001
	Cohort	7.451 (1)	.006
	5-HTTLPR	0.106 (1)	.745
	NEM	1.089 (1)	.297
	SLE	0.588 (1)	.443
	NEM*SLE	0.224 (1)	.636
	NEM*5-HTTLPR	0.060 (1)	.807
	NEM*5-HTTLPR*SLE	0.005 (1)	.944

Note. Results of Generalized Estimating Equations (GEE) examining the effects of gender, cohort, NEM, SLE (as measured by LEI reported between the ages of 17 and 25) and 5-HTTLPR (dominance) on the development of presence of MDD between the ages of 17 and 25 years in participants.

Chapter 6: Discussion

The purpose of this project was to evaluate the interaction of 5-HTTLPR and stressful life events (SLE) on the presence of Major Depressive Disorder (MDD) in an epidemiological sample that as studied longitudinally. First, an attempt at replicating Caspi et al. (2003) was made by investigating the potential interaction between 5-HTTLPR variants and SLE on the presence of MDD at the age of 25. While SLE showed a significant influence on the presence of MDD, the main effect of 5-HTTLPR and the interaction between SLE and 5-HTTLPR were non-significant. Consequently, this current study was unable to replicate the original findings of Caspi et al. (2003).

In addition to the replication of Caspi, some potential arguments for the broad inconsistencies in the literature were examined. First, childhood maltreatment was examined as a specific form of SLE that may interact with 5-HTTLPR variants (e.g., Karg et al., 2011) to produce MDD. There were no significant main effects childhood maltreatment, 5-HTTLPR status, gender, or cohort on the presence of MDD at age 25. There were also no significant interactions between these variables on MDD, suggesting that childhood maltreatment is not a unique form of SLE more likely to elicit a gene by environment interaction.

Specific aim 1c focused on persistent MDD (e.g., two or more instances of MDD across three visits). Again, there was a main effect for SLE influencing the presence of persistent MDD by the age of 25, and in the additive analysis, there was also an interaction between 5-HTTLPR and SLE on the presence of MDD. However, this

interaction was found where S/S individuals were significantly less likely to exhibit MDD in response to high levels of stress compared with S/L and L/L individuals. The dominance analysis also revealed a gender by SLE interaction where females exposed to higher rates of SLE were more likely to develop MDD.

In terms of whether findings might vary depending on the nature of life events experienced, both independent and dependent SLE appeared to have influenced the presence of MDD, with no difference between the two. Facets of negative emotionality (NEM) were also examined. Neither NEM nor interactions between NEM and SLE or 5-HTTLPR significantly predicted the presence of MDD.

These findings would appear to contradict those presented and discussed in Caspi et al. (2003) and Caspi et al. (2010). No significant interactions between 5-HTTLPR variants and SLE appeared to have influenced the presence of MDD in the predicted way where S carriers were more likely to exhibit MDD in response to higher levels of stress compared with L/L individuals. As Caspi et al. (2010) suggested, one potential reason for lack of replication may be the use of paper/pencil questionnaires as opposed to in-person interviews. They argue that interviews are more appropriate because they allow for the interviewer to clarify the information collected, and they believe paper/pencil questionnaires offer inconsistent and "idiosyncratic" results (Monroe 2008; Dohrenwend 2006). The SLE data used in these analyses from the LEI were part of in-depth in-person interviews and still no relationship between 5-HTTLPR and SLE was found for MDD. Despite Karg et al.'s (2011) suggestion that childhood maltreatment may be an especially important form of SLE in this gene by environment interaction research, childhood

maltreatment did not emerge as a form of SLE that interacted with 5-HTTLPR to contribute to the presence of MDD.

Although these results do not support the findings of Caspi et al. (2003), findings from this current project are consistent with several publications. In a meta-analysis, Risch et al. (2009), found SLE to be a significant predictor of MDD, but also did not find an interaction between 5-HTTLPR and SLE on MDD. Furthermore, these current findings support the many articles that have criticized the 5-HTTLPR and SLE interaction literature. For instance, Duncan and Keller (2011) have suggested this area of research is heavily influenced by publication bias, which is supported by numerous attempts at replication, which have not been successful.

In addition to the potential influences of publication bias, other differences between the current project and the original Caspi et al. (2003) may have influenced differential findings. The original Caspi paper was not based on twins, whereas the analyses in this current project involve a sample of twins. Additionally, Caspi et al. (2003) focused on stress between the ages of 21 and 26 years, and included MDD that developed between ages 25 and 26. The current study used stress and MDD occurring concurrently between the ages of 17 and 25 years. With stress and MDD occurring concurrently, it is possible people with MDD reported more stress during this period.

While no evidence for gene by environment interactions were found in this current project, the findings do support the well-established relationship between life stress and depression that has been reported for decades (e.g., Hammen 2005). The main effect of SLE on the presence of MDD was clearly significant, supporting the idea that

greater exposure to stressors increases an individual's risk of experiencing MDD. Even when separating out dependent and independent SLE, both remained significant predictors of MDD, suggesting that regardless of the kind of SLE present, there is still a significant relationship between SLE and MDD. From a clinical standpoint, it suggests that psychotherapeutic interventions for those experiencing stress may decrease future instances of MDD. Improving social support for those experiencing stress may also decrease subsequent psychopathology. For instance, some work has suggested that improving living conditions and support for children exposed to stress may decrease problematic emotional responses later in development (e.g., Gunnar & Quevedo, 2007).

Potential Reasons for Non-Replication

Although the findings of this current study do not support the gene by environment interaction elucidated by Caspi et al. (2003), there are several reasons why such inconsistencies in data analyses may be seen. Some of these further reasons for discrepancies within the literature are examined in this section.

Methodological Differences between the Current Study and Caspi et al. (2003)

As mentioned briefly above, the timing of the stress and MDD reports differ between the current study and the original Caspi publication. The original Caspi et al., (2003) had individuals report their stress between the ages of 21 and 26 years of age. Then, they examined MDD occurring between the ages of 25 and 26 years. The current study examines SLE and MDD between the ages of 17 and 25 years. The differences in

timing could account for differences in findings. Additionally, the original Caspi et al. (2003) sample did not include twins, and the current study's sample did.

Development

The timing of early life stress can play an important role in the development of pathology. Young children, during certain periods, when exposed to childhood maltreatment or parental deprivation appear to experience differential levels of risk for developing behavioral problems. For instance, Gunnar et al. (2007) found that children adopted after the age of 24 months were more likely to exhibit behavioral difficulties compared with children adopted into families prior to this specific age. Additionally, in a retrospective study, Andersen et al. (2008) found that young adult women who had a history of sexual abuse between the ages of 3 and 5 years and again between 11 and 13 years had smaller hippocampal volumes, while those who experienced sexual abuse between the ages of 14 and 16 years had smaller frontal cortical volume.

In terms of MDD, one study suggests children losing a parent prior to the age of 9 years are more likely to develop MDD than children losing a parent after the age of 9 (Agid et al., 1999). Another study suggests political traumatization occurring prior to the age of 12 years is more likely to result in MDD compared with similar trauma after the age of 12, which is more likely to result in Post-Traumatic Stress Disorder (Maercker et al., 2004). As suggested by the ages of the participants in Agid et al. (1999) and Maercker et al. (2004), pubertal maturation may mark a particularly vulnerable time period for environmental stressors and the impact of such stressors on later development.

During puberty, the hypothalamic pituitary adrenal (HPA) axis, which is involved in stress reactivity, is undergoing maturational changes and this time period may see additional vulnerabilities to stress, compared with other developmental stages (Heim & Binder, 2012).

Taken together, these studies suggest stress during certain early time frames may have greater or lesser impacts on pathological psychological or biological development. Consequently, it is fathomable that the timing of certain stressors may also influence the main effects of 5-HTTLPR on MDD, and thus also the interactions that were examined (Heim & Binder, 2012). The current study involves participants at the ages of 11 and 17 years at intake, and examines the influences of stress at the later ages between 20 and 25, which is similar to the age ranges studied by Caspi et al. (2003). The current study, as well as Caspi et al. (2003), does not take into account previous SLE that may have happened during developmentally sensitive periods, such as during pubertal maturation. While the current study overlaps with the time frame studied by Caspi et al. (2003), developmental differences could contribute to the discrepancies seen in the literature on the whole. Therefore, work could be done examining genetics and stress during different developmentally sensitive periods.

5-HTTLPR and rs25531

Another potential complication in 5-HTT research involves the SNP, rs25531. Numerous recent studies have included the SNP, rs25531, in 5-HTTLPR analyses. With rs25531, individuals can either have A and/or G alleles. The A allele, is far more

frequent and is associated with higher serotonergic expression. On the other hand, the relatively rare G allele is associated with lower serotonergic expression. When considering the 5-HTTLPR in conjunction with rs25531, the G allele renders the L allele functionally equivalent to the S allele. Therefore, in studies where rs25531 genotypes are acquired, individuals with the G allele are typically grouped with the S carriers (Hu, et al., 2005). Wendland, and colleagues (2006) reported the following allelic frequencies: S_A (43.5%), S_G (0.25%), L_A (50.5%), and L_G (6.5%). These reported rates appear similar to several other studies reporting these frequencies in Caucasians (e.g., Beevers et al., 2011; Quaak, van Schzyck, Postma, Wagena, & van Schooten, 2011).

Some articles have reported that allelic differences in rs25531 lead to certain functional differences. For instance, G carriers, when compared with A carriers, exhibit differential attentional biases (Fox, Ridgewell, & Ashwin, 2009; Kwang, Wells, McGeary, Swann, & Beevers, 2010), have greater amygdala response to emotional stimuli (Dannlowski et al., 2007), show greater error related activation in the nucleus accumbens (Holmes, Bogdan, & Pizzagalli, 2010), and experience irritable bowel syndrome more frequently (Kohen, et al., 2009). Also G carriers may experience treatment resistant depression at higher rates than A carriers (Bonvicini et al., 2010).

While some studies suggest rs25531 is an important SNP to consider when investigating 5-HTTLPR, several other studies fail to find differences between G and A carriers in relation to several phenotypes, including Alzheimer's Disease (Polito et al., 2011), depression (Antypa & van der Does, 2010; Coventry et al., 2010; Wray et al., 2009), depression in survivors of traumatic brain injury (Chan et al., 2008), suicide

(Coventry et al., 2010; Segal et al., 2009), depression in survivors of stroke (Kohen et al., 2008; Mak, Kong, Mak, Sharma, & Ho 2013), depression in individuals with Parkinson's disease (Zhang et al., 2009), cognitive deficiencies in schizophrenics (Konneker et al., 2010), premenstrual dysphoric disorder (Magnay et al., 2010), bipolar disorder (Pinto et al., 2011), alcohol and nicotine consumption (Rasmussen et al., 2009), borderline personality disorder (Tadic et al., 2010), and obsessive-compulsive disorder (Wendland et al., 2007). Furthermore, since G carriers are relatively rare, some researchers have explicitly said that including the rs25531 in their 5-HTTLPR data analyses failed to change their results (e.g., Beitchman et al., 2006; Sonuga-Barke et al., 2011; Antypa & van der Does, 2010). Due to the relatively low rates of G carriers, and the inconsistency of these findings, L_G / L_A status was not considered for this current project, although it is conceivable that individual variation in the rs25531 may play a part in the inconsistencies with 5-HTTLPR by SLE interaction literature. However, given the relatively small number of L_G individuals, its influence would likely be minimal.

Presence of SSRI Medication

Another factor that may contribute to discrepancies in findings involves the use of selective serotonin reuptake inhibitors (SSRIs). According to the Centers for Disease Control (CDC) 11% of Americans took SSRI medication in 2011 and between 1988 and 2008, there was a 400% increase in the use of SSRI medications amongst Americans. Since this medication targets the 5-HTT system, it is plausible that the presence of SSRI medication may make the influence of 5-HTTLPR on the presence of MDD harder to

detect. Consequently, it may also make gene by environment interactions more difficult to find. For instance, Scheid and colleagues (2007) initially did not find an interaction between 5-HTTLPR and SLE on the presence of MDD. However, when they eliminated the 13% of their sample that was taking psychotropic medication, they were able to find this gene by environment effect. Since the popularity of SSRI medication has grown exponentially since 1988, many more individuals are on this medication, which could complicate the results of more recent gene by environment studies.

Nature of SLE

Although this current project looked at different sorts of SLE (e.g., dependent versus independent SLE), still others may be responsible for the gene by environment interaction. Vrshek-Schallhorn and colleagues (2014) have suggested that interpersonal SLE and family stress are specific forms of SLE that may interact with 5-HTTLPR and lead to MDD. These authors argue that interpersonal SLE represent social loss and might be more likely to evoke a depressive response. Their findings suggest S carriers, when compared with L/L homozygotes were more likely to experience MDD in the presence of interpersonal SLE.

Therefore, while the current study fails to demonstrate a gene by environment interaction on the presence of MDD, it does not rule out the importance of serotonin in pathogenesis of MDD. Moreover, these results do not indicate the gene by environment findings are false. This study, instead, illustrates the difficulties in finding significant gene by environment interactions due to several inconsistent and heterogenous factors in

data. For instance, several factors including the timing of SLE, rs25531 status, and the ever increasing use of SSRI medications may all influence data analyses used to examine, replicate, and expand upon the original findings in Caspi et al. (2003).

Limitations

Future research in the area of 5-HTTLPR by SLE interaction research would benefit from taking into account potential differences in developmental time periods. As discussed above, different developmental periods may confer differing levels of risk to environmental stressors (Heim & Binder, 2012). Additionally, future studies can continue to examine the type of stressor. In the current project, the childhood maltreatment information came from a retrospective questionnaire, which can be plagued by problems associated with memory. Concurrent measures of childhood maltreatment may be more likely to elicit gene by environmental findings.

Statistical power, on the whole, is another issue plaguing much of the gene by environment interaction research. While the sample in this current study was relatively large, several other publications have relied on samples in the low hundreds. Considering Murphy and colleagues indicate only about 1% of the variance in amygdala reactivity is related to 5-HTTLPR status, any interaction 5-HTTLPR may have with SLE on behavioral phenotypes is potentially smaller.

While the longitudinal data collected and maintained by the MCTFR allow for several complex analyses, there are limitations within this design. For instance, there are two different cohorts that are combined for analyses. The CEQ was only used in analyses

including the younger cohort, as the older cohort males did not complete this questionnaire.

Despite the limitations of the current study, it provides support for the role of SLE in the presence of MDD. Like many other publications, the results suggest some skepticism regarding any potential 5-HTTLPR by SLE interaction on MDD, although several potential reasons for these inconsistencies in this literature have been proposed and discussed.

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Appendix A.

Stressful Life Events from the Life Events Interview (LEI).

1. Have you moved?
2. Have you moved more than 50 miles?
3. Have you had a serious problem with a friend?
4. Were you unable to see, or lost, your friend?
5. Have you had a close friend seriously ill or injured?
6. Have you had a friend die?
7. Have you had a pet die?
8. Have you had a relative die?
9. Have you had a widowed parent remarry?
10. Has your family experienced money problems?
11. Have your parents divorced?
12. Have you had a divorced parent remarry?
13. Have you (or a partner) worried about being pregnant?
14. Have you (or a partner) been pregnant?
15. Have you (or a partner) given birth?
16. Have you (or a partner) has a miscarriage or abortion?
17. Have you (or a partner) given a child up for adoption?
18. Have you been separated from your spouse?
19. Has your spouse been unfaithful to you?
20. Have you been unfaithful to your spouse?

21. Have you gotten divorced?

22. Has your former spouse remarried?

23. Have you been widowed?

24. As an adult, have you received welfare or aid?

25. As an adult, have you had welfare or aid cut off?

26. Have you lost a job?

27. Have you been unemployed for a period of 6 months or more?

28. Have you had trouble with alcohol or drugs?

29. Have any members of your family had trouble with alcohol or drugs?

30. Have you had traffic citations?

31. Have you had trouble with the police?

32. Have you gone to court?

33. Have you been adjudicated as a juvenile?

34. Have you had a family member arrested?

35. Have you had a family member sent to jail?

36. Has any family member had a serious illness?

37. Has any family member had a serious injury?

38. Have you seen a serious accident?

39. Have you been a driver in a serious accident?

40. Has a family member attempted suicide?

41. Has a family member completed suicide?

42. Have you received any psychiatric hospitalizations?

43. Have you been robbed or mugged?

44. Has a member of your family been a victim of violence?

45. Have you experienced sexual harassment?

Appendix B.

Stressful Life Events from the Childhood Events Questionnaire (CEQ). Please note, not all of these questions were asked at each time point.

1. Before the age of 9 years, did your parents ever leave you alone?
2. Did your parents ever forget to do important things for you?
3. Did your parents ever act so badly you were embarrassed?
4. Did your parents call you inappropriate names?
5. Did your parents torment you with scary things (like snakes or ghosts)?
6. Did your parents threaten to leave you?
7. Did your parents ever confine you to a dark, small place?
8. Did your parents ever threaten to hurt you or something you cared about?
9. Did your parents ever destroy anything of yours, including a pet?
10. Were you spanked?
11. Were you spanked so hard it left marks?
12. Were you hit with objects?
13. Were you hurt with a weapon?
14. Were you hurt in other ways?
15. Did anyone ask you to do something sexual?
16. Did someone expose his/herself to you?
17. Did anyone have you expose yourself?
18. Were you ever forced to engage in sexual acts?
19. Were your sexual parts ever fondled?

20. Did anyone ask you to fondle his/her sexual parts?
21. Did anyone put his/her hands inside of you or perform sexual acts with his/her mouth?
22. Did anyone force you to put your hands inside of them or do sexual things to him/her with your mouth?
23. Did anyone attempt to force you into intercourse?
24. Did you ever experience sexual assault involving force, physical restraint, or threats of physical violence?

Appendix C.

Independent and dependent adverse life events from Bemmel, Burt, Legrand, Iacono, and McGue (2008).

Event Class: Independent

4 items

1. Have you had a close friend move away so you couldn't see them any more?
2. Was a close friend of yours ever seriously ill or hurt?
3. Has a close friend of yours died?
4. Were you ever mugged or robbed?

Event Class: Dependent

9 items

1. Have you ever had a serious problem with a close friend?
2. Have you and a romantic partner ever broken up?
3. Have you or your romantic partner become pregnant?
4. Have you or your romantic partner given birth to a child?
5. Did you or your romantic partner ever have an abortion?
6. Have you ever gotten into trouble because of your use of drugs or alcohol?

7. Have you ever been in trouble with the police (for traffic violations or any other reason)?
8. Have you ever had to go to court?
9. Were you ever sent to a juvenile detention center (jail)?