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Genome-Wide Association Study on the Sleep Symptom of Post Traumatic Stress Disorder

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This is to certify that the doctoral dissertation by

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Abstract

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MS, Walden University, 2007

BA, University of Hawaii, 1993

Dissertation Submitted in Partial Fulfillment

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Abstract

Posttraumatic stress disorder (PTSD) is a psychiatric condition that presents with 3 main symptoms—re-experiencing, avoidance/numbing, and hyper arousal—after an individual experiences a traumatic event. Recent evidence suggests a potential genetic basis for PTSD and a sub symptom of hyper arousal, sleep, as a potential pathway for PTSD development, but no study has identified candidate genes associated with specific symptoms such as sleep difficulty. Based on a conceptual framework in which specific genes are associated with the onset of PTSD, this study used a genome-wide association study (GWAS) method with a case control study design to compare the genomes of individuals with and without PTSD. A secondary GWAS dataset from a study on alcohol dependence in European and African Americans was obtained from the National Center for Biotechnology Information. PTSD cases and controls were analyzed using PLINK software. Signals from 2 single nucleotide polymorphisms (SNPs), which have not been previously associated with PTSD, exceeded the established genome-wide threshold: SNP rs13160949 on chromosome 5 ($p = 7.33x10^{-9}$, OR: 1.565) and SNP rs2283877 on chromosome 22 ($p = 2.55x10^{-8}$, OR: 1.748). Neither SNP, though, maintained genome-wide significance following corrected tests for multiple testing, population stratification, and false discovery, so the planned analysis for possible associations with PTSD by symptom category then by the sub symptom of sleep could not be completed. The results of this study suggest that PTSD may be the result of polygenic SNPs with weak effects, which supports a recent study indicating the disease may be highly polygenic. Positive social change implications include bringing attention to the clinical and research community that PTSD may involve complex polygenic factors in need of further study.

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Dedication

I dedicate this dissertation to my husband, Jim, who endured countless hours of my work and challenges I can't even put into words. His patience and consistent love helped me get this far. To my dear son, Noah, who gave up time with Mommy on many days, I dedicate this to you. To my friends and family who supported and prayed for me through this process -- Thank You. Your support meant more to me than I can express. And especially to Jesus for His divine help and grace in keeping me going and getting me through a very challenging process.

Acknowledgments

I would like to acknowledge and thank Dr. Shen for helping me to complete my dissertation. The process has been a long journey for me, and Dr. Shen believed I could make it; for that I am very grateful.

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Chapter 1: Introduction to the Study

Posttraumatic stress disorder (PTSD) is a psychiatric condition that develops in some individuals following a traumatic event. Prevalence rates indicate that only a portion of individuals (3.6%) develop PTSD during a 12-month period despite a majority of individuals (80%) experiencing a traumatic event (Frans, Rimmo, Aberg, & Fredrikson, 2005; National Comorbidity Survey, 2007; Yehuda, Koenen, Galea, & Flory, 2011). Some individuals may be more susceptible due to psychological factors, which may be influenced by the genetic makeup of the individual. Feodorova and Sarafian (2012) and Koenen (2007) have suggested that genetic risk factors should be considered when variance is greater than 30%. A 37-41% variance has been found in PTSD heritability studies (Bailey et al., 2010; Goenjian et al., 2012).

Human genome epidemiology (HuGE) is a field of epidemiology that uses epidemiological approaches to study variations in disease associated with genetics (Khoury, Little, & Burke, 2004). In this dissertation, I proposed a human genome epidemiology approach to discover possible genetic risk factors associated with posttraumatic stress disorder. Using a case control study design and a genome-wide association study method (GWAS), the genome of individuals with PTSD was compared to that of controls. A database containing genomic data for individuals with PTSD was requested from the National Center for Biotechnology Information (NCBI). The database that contained the PTSD dataset was from an original study, phs000425.v1.p1, "Alcohol Dependence GWAS in European and African Americans" (Gelertner & Kranzler, 2010). In this dissertation I focused on the sub symptom of sleep that is part of the hyper arousal

symptom of PTSD. Initial analysis consisted of a multistep process that examined the entire genome (PTSD cases vs. controls). Any gene/genetic allele that reached significance was further analyzed to determine whether an association existed between the genetic allele and the main symptoms of PTSD, re-experiencing/intrusion, avoidance/number, and hyper arousal, including the sub symptom of sleep. A study by Bailey et al. (2010) examined heritability of PTSD and the symptoms associated with PTSD and found support for both the heritability of PTSD (0.37) and the symptoms of PTSD (re-experiencing 0.75, avoidance and hyper arousal 0.39. As mentioned, sleep is part of the hyper arousal symptoms. There have been four GWAS published on PTSD; however, none of these studies examined the symptoms of PTSD, and none focused specifically on the symptom of sleep (Guffanti et al., 2013; Logue et al., 2012; Solovieff et al., 2014; Xie et al., 2013).

Recommendations from the literature support further study of the link between PTSD and sleep as a potential pathway for PTSD development (Krakow, Germain, et al., 2001; Krakow, Hollifield, et al., 2001; Walker & van der Helm, 2009; Yetkin, Aydin, & Ozgen, 2010). Sleep difficulties in the form of insomnia, poor sleep quality, and nightmares are reported by 70-91% of individuals diagnosed with PTSD (American Psychiatric Association [APA], 2000; Maher, Rego, & Asnis, 2006). Studies that focused on treating sleep problems have resulted in improvements in sleep functioning, symptoms of posttraumatic stress disorder, and cognitive functioning, particularly long-term verbal memory (Krakow, Hollifield, et al., 2001; Raboni, Suschecki, & Tufik, 2009; Ulmer, Edinger, & Calhoun, 2011). Walker and van der Helm's (2009) "sleep to remember

sleep to forget" hypothesis suggests that sleep is not a symptom of PTSD but rather a causal factor for its development. Stickgold (2002) has suggested that PTSD develops as a function of neurotransmitter failure or disruption preventing normal integration of memory processes. This memory processing occurs during sleep; "posttraumatic re-enactments in the form of nightmares or dreaming are understood as a psychological correlate of pathological changes to normal brain functioning during rapid eye movement (REM) sleep" (Wittman, 2007, p. 4).

One of the completed GWAS lends support for further study of PTSD and sleep difficulty (Logue et al., 2012). Logue et al. (2012) found SNP rs8042149 in the retinoid-related orphan receptor alpha gene (RORα) associated with PTSD. The RORα gene is thought to be involved in circadian rhythm functioning and in the circadian clock (Akashi & Takumi, 2005). Additionally, there is one candidate gene, catechol-o-methyltransferase (COMT), that has been studied in both PTSD and sleep research (Goel, Banks, Lin, Mignot, & Dinges, 2001; Kolassa, Kolassa, Ertl, Papassotiropoulos, & DeQuervain, 2010).

Positive social change implications of this research include bringing attention to the genetic risk factors associated with PTSD, a disorder for which there has been little attention devoted to genetic risk factors compared to other disorders such as major depression or schizophrenia and highlighting methodological concerns related to the GWAS method (Cornelius, Nugent, Amstadter, & Koenen., 2010). Prior to background information on PTSD being provided, some basic concepts surrounding genetics and genomic research are reviewed to provide understanding of the dissertation. *Genomics* is

the study of individual gene functioning as well as genetic functioning in the context of the larger genome (Guttmacher, 2002, as cited by Khoury et al., 2004). Peyser and Burns (2004) suggested,

> Different genetic backgrounds may cause genetic susceptibility to the same disease. Genes that confer a high risk may be present in only a subset of affected persons. The trait may be caused by different genetic loci acting together, or a predisposing gene may only manifest in the presence of a particular environmental exposure. (p. 39)

Other terms used in genetics are *genotype* and *phenotype*. *Genotype* is the nonobservable information contained in a gene and has to be identified through genetic tests; this is the genetic makeup of an individual that is contained in alleles ("Examples of Genotype & Phenotype," 2013). *Alleles* are "pairs or series of genes on a chromosome that determines the heredity characteristic…an example of an allele is a gene that determines eye color" ("Allele," 2013, ¶1). *Phenotype* is the notable difference that can be identified from the interaction between the environment and the gene (Peyser & Burns, 2004). Examples of phenotypes are observable differences that can be seen in hair and eye color ("Examples," 2013). In the literature and in this dissertation, the term *candidate gene studies* refer to individual genes or genotype, though specific references may be made to phenotype. Another relevant term is *single nucleotide polymorphisms* (SNPs), which are variations that occur at one nucleotide; for example, an A may be replaced with a C in a genetic sequence (Ellsworth & O'Donnell, 2004). These basic concepts should assist the reader in understanding this dissertation.

Background Information

Posttraumatic stress develops in some individuals following an experience in which their life was in danger or they witnessed a traumatic event that left them feeling an "intense fear, helplessness, and horror" (APA, 2000; Frans, et al., 2005; Pietzral, Goldstein, Southwick, & Grant, 2011; U.S. Department of Veterans Affairs: National Center for PTSD, 2007). The symptoms associated with PTSD are re-experiencing/intrusion, avoidance/numbing, and hyper arousal, with sleep difficulty being part of the hyper arousal symptoms. Diagnostic criteria for each of these symptoms are provided in the Appendix (APA, 2000).

The literature on PTSD is extensive and has focused on trauma exposure, gene candidate studies, gene expression studies, biological mechanisms, environmental and social risk factors, and, most recently, GWA studies (Guffanti et al., 2013; Logue et al., 2012; Koenen, Amstadter, & Nugent, 2009; Kolassa, Kolassa, et al., 2010; Morey et al., 2011; Schmidt, Holsboer, & Rein, 2011; Solovieff et al., 2014; Uddin et al., 2011; Xie et al., 2013; Yehuda et al., 2011). These studies have primarily focused on the disorder of PTSD, with very few candidate gene studies focusing on the individual symptoms associated with PTSD; no GWAS have studied PTSD symptoms, and none have focused on the role sleep may play in the development of PTSD.

Candidate genes associated with PTSD have included the following: "G" alleles of rs12944712 of the corticotrophin-releasing hormone (CRH) receptor 1 gene, the solute carrier family 6 (neurotransmitter transporter) member 3 (SLC6A3) dopamine active transporter 1 (DAT1) allele; the SLC5A3 locus (DAT1 or DAT) 9R allele, the dopamine

receptor D2 (DRD2) region of the dopamine system with the 957 C>T polymorphism specific to the "C" allele, the variable number tandem repeat (VNTR) polymorphism on exon III of the DRD4 gene, long DRD4 variants (seven-repeat ones), and the dystrobrevin binding protein (DTNBP1) SNP rs9370822 allele (Amstadter et al., 2011; Change et al., 2012; Dragon & Onisczenko, 2009; Segman et al., 2002; Voisey et al., 2010; Voisey et al., 2009). Other genes associated with PTSD were the FK506 binding protein (FKBP5), the glucocorticoid receptor, the serotonin transporter linked gene polymorphic region (5HTTLPR, the "L" allele, rs16965628 with a "GG" genotype, allele frequency "s/s" carriers at region 7q11-1-q12) and in Intron 2 (VNTR), the 12 allele (Hauer et al., 2011; Lee et al., 2005; Morey et al., 2011; Sarapas et al., 2011; Sayin et al., 2010). Ressler et al. (2011) found SNP rs2267735 part of the adenylate cyclase activating polypeptide 1 (pituitary) receptor 1 (ADCYAP1R1) predicted PTSD in females only. Lee et al. (2011) found that the "CC" genotype of rs2267735 in women was associated with total PTSD symptoms and hyper arousal symptoms. A study related to variance found the "t" allele of tryptophan hydroxylase 1 (TPH1), SNP rs2108977 accounted for 3% of the variance while the "s" allele of the TPH2, SNP rs11178997 accounted for 4% of the variance in PTSD (Goenjian et al., 2012).

While these studies have advanced the understanding of potential genetic risk factors, other studies have found no association or mixed results (Bailey et al., 2010; Mellman et al., 2009; Morgan et al., 2003**;** Pivac, Kozaric-Kovacic, & Muck-Seler, 2006; Sah et al., 2009). No associations were found between allele, genotype, or frequency for the brain-derived neurotrophic factor (BDNF) gene Val66Met polymorphism or plasma

dopamine betahydroxylase (DBH) and 5-HT (Lee et al., 2006; Pivac et al., 2006). Mellman et al. (2009) found no association with the 5HTTLPR, functional genotypes high expression (HE) or low expression (LE). Two genes associated with the dopaminergic systems DRD2 and DAT were also not associated with PTSD (Bailey et al., 2010; Pivac et al., 2006).

Mixed results have been found in the gene associated with resiliency that may confer a protective factor, Neuropeptide Y. Morgan et al. (2003) found a negative association between Neuropeptide Y receptors (NYP), the number of traumatic life-threatening events, and PTSD symptoms, suggesting that exposure to trauma rather than PTSD symptoms was related to lower NPY levels; Sah et al. (2009) found that PTSD cases had lower levels of NPY compared to healthy controls and suggested that higher levels of CSF NPY may serve as a protective factor in some individuals. Limitations noted in candidate gene studies have included small sample sizes, methodology problems, trauma exposure and severity, co-occurring disorders associated with PTSD, and the disorder itself in terms of its complexity (Cornelius et al., 2010; Norrholm & Ressler, 2009). Koenen (2007) indicated that identifying which specific genes to study presents challenges to researchers, as associations may be missed due to incorrectly studying irrelevant genes.

There were no published GWA studies of PTSD prior to 2012; recommendations from the literature on PTSD indicated that a genome-wide association study was needed to fill this gap in the literature (Cornelius et al., 2010; Feodorova & Sarafian, 2012; Norrholm & Ressler, 2009). Since 2012, four genome-wide association studies have

been published examining PTSD, each finding novel genes (SNPs rs363276 in SLC18A2, RORα, Tolliod-Like 1 gene [TLL1], SNP rs10170218 in ACO68718.1) not previously identified in the literature (Guffanti et al., 2013; Logue et al., 2012; Solovieff et al., 2014; Xie et al., 2013).

Problem Statement

While the recent GWAS have found new genetic alleles, none of the studies examined the potential relationship between genetic alleles and the symptom of sleep. The GWAS study by Logue et al. (2012) lends support for further study of PTSD and sleep. The RORα gene identified by Logue et al. is a gene thought to be involved in circadian rhythm functioning and in the circadian clock (Akashi & Takumi, 2005). This finding is intriguing, as sleep disturbance is noted as a symptom associated with PTSD in the form of insomnia, poor sleep quality, and nightmares. The literature suggests an intimate relationship between sleep and PTSD, with sleep problems identified as a "core feature" of PTSD (Germain, Buysse, Shear, Fayyad, & Austin, 2004; Krakow, German, et al., 2001; Krakow, Hollifield, et al., 2001; Yetkin et al., 2010, p. 309).

Boscarino, Kirschner, Hoffman, and Ehrlich (2013) found that the best predictors of PTSD were a high lifetime trauma exposure, a history of major depression, and a history of reported sleep problems ($p < 0.001$). Recommendations from the literature also support further study of this link between PTSD and sleep as a potential pathway for PTSD development (Krakow, Germain, et al., 2001; Krakow, Hollifield, et al., 2001; Walker & van der Helm, 2009; Yetkin et al., 2010). Studies that have focused on treating sleep problems have resulted in improvements in sleep functioning, symptoms of

posttraumatic stress disorder, and cognitive functioning, particularly long-term verbal memory (Krakow, Germain, et al., 2001; Krakow, Hollifield, et al., 2001; Raboni et al., 2009; Ulmer et al., 2011). Walker and van der Helm's (2009) "sleep to remember sleep to forget" hypothesis suggests that sleep is a causal factor for the development of PTSD. Sleep dysfunction is viewed as a primary determinant for PTSD development and was the theoretical framework used for this research, with an explanation provided in the theoretical framework section (Walker & van der Helm, 2009).

Purpose of the Study

This purpose of this dissertation study was to examine the genomes of individuals with PTSD and controls to discover whether there are genomic regions associated with PTSD, specifically concerning the symptom of sleep. The independent variable was PTSD status (i.e., PTSD case population vs. non-PTSD control population). The dependent variables were the symptoms associated with PTSD (re-experiencing/intrusion, avoidance/numbing, hyper arousal, and specifically the sub symptom of sleep). The analysis examined the genome of individuals with and without PTSD with a second analysis planned with the main symptoms of PTSD and the variable of sleep for any alleles that exceeded the genome wide association threshold. Confounding variables identified in the literature included the following: BMI, age, gender, race/ethnicity, use of anxiolytics, antidepressants, socioeconomic status, type and intensity of trauma, education, rural/urban, co-occurring disorders such as major depressive disorder, drug dependence, substance abuse, Axis 2 disorders, and age of traumatic exposure (Babson et al., 2011; Frans et al., 2005; Krakow, Germain, et al.,

2001; Krakow, Hollifield, et al., 2001). Confounding variables that were included were the variables included in the database (i.e., age, gender, race, stimulant dependence, sedative dependence, tobacco dependence, and substance abuse).

Research Questions

The genome-wide method is not a hypothesis-driven method but rather a discovery-driven method. This method is similar to casting a wide fishing net and seeing what is found. Therefore, specific hypotheses are not provided; however, the following research questions were considered:

- Research Question 1: Are there significant genetic alleles associated with PTSD?
- Research Question 2: Is there a significant association between genetic allele(s) and the symptoms of PTSD?
- Research Question 3: Is there a significant association between genetic allele(s) and the symptom of sleep? Is either the COMT or RORα, both genes that have been identified in sleep and PTSD, associated with sleep?

Walker & van der Helm Theoretical Foundation

The foundation of this dissertation was a novel conceptual framework by Walker and van der Helm (2009) "sleep to remember sleep to forget" because of its focus on sleep as the mechanism for PTSD development. Walker and van der Helm suggested that sleep dysfunction is not just a symptom associated with PTSD, but may be a possible pathway for the development of PTSD. They suggested that PTSD develops as a result of a failure in processing emotionally laden content due to impaired sleep (Walker & van

der Helm, 2009). Lee and Douglass (2010) indicated that although the "function of sleep is unknown, decades of research strongly implicates that sleep has a vital role in the central nervous system (CNS) restoration, memory consolidation, and affect regulation" (p. 403). Walker (2009) indicated that sleep is involved in different processes related to memory, cognition, brain plasticity, and psychiatric disorders. *Brain plasticity* refers to changes that occur in the brain's structure and function based on exposure to new, different, or complex experiences (Kolb & Gibb, 2011; Kolb, Gibb, & Robinson, 2003). Brain plasticity occurs throughout one's lifetime and can be impacted by epigenetics, hormones, stress, experience, drugs, anti-inflammatory medications, diet, disease, and brain injury (Kolb & Gibb, 2011; Kolb et al., 2003). Dang-Vu, Desseilles, Peigneux, and Maquet (2006) suggested that sleep plays a role in brain plasticity by impacting learning and memory.

Walker (2009) suggested that sleep, particularly REM sleep, may impact the emotional response of memory. Walker (2009) indicated that sleep is important to both encode and form lasting memories. *Declarative memory* is a type of memory involved in the recall of facts and concepts (Martin, 1993). This type of memory is thought to be affected by stressful events, with sleep enhancing declarative memory processing (Golier, Harvey, Legge, & Yehuda, 2006; Walker, 2009). Memories are thought to be accessed during the sleep cycle (Walker, 2009). Walker indicated that declarative memory consolidation may occur during short-wave sleep and takes place in the hippocampus region of the brain. Walker suggested that sleep deprivation may impact one's memory by magnifying negative emotional content while decreasing positive emotional content

(Zohar, Tzischinsky, Epstein, & Lavie, 2005, as cited by Walker, 2009). For a more detailed discussion of the "sleep to forget and sleep to remember" framework, see Chapter 2 (Walker & van der Helm, 2009, p. 741).

Conceptual Framework

There have been no studies to date that have focused on sleep difficulties and PTSD in the context of potential genetic vulnerabilities; however, COMT and SNP rs8042149 in the RORα gene have been associated with PTSD (Kolassa, Ertl, et al., 2010; Kolassa, Kolassa, et al., 2010; Landolt, 2008; Logue et al., 2012). The RORα gene is thought to be a gene involved in circadian rhythm functioning (Akashi & Takumi, 2005). The RORα gene was found to be important in regulating *Bmal1* transcription factor in mice (Akashi & Takumi, 2005). When the RORα did not function properly in promoting *Bmal1*, the circadian rhythm did not function properly; the RORα is considered a "component of the core circadian clock"… and "acts as to promote *Bmal1* transcription, thereby maintaining a robust circadian rhythm" (Akashi & Takumi, 2005, p. 441). The RORα gene may be involved in chronic inflammatory processes such as heart disease or arthritis (Delerive et al., 2001); Boscarino (2008) found that individuals with higher PTSD scores had a 20% increased risk of mortality from heart disease.

The literature suggests an intimate relationship between sleep and PTSD; sleep-disordered breathing (SDB) or sleep movement disorders (SMD) have been reported in 77% of sexual assault survivors (Krakow, Hollifield, et al., 2001). Kobayashi, Boarts, and Delahanty (2007) indicated that a frequent complaint of individuals diagnosed with PTSD is sleep disturbances. Kobayashi et al. found that individuals diagnosed with

PTSD compared to individuals without PTSD spent more time in Stage 1 sleep, had less slow-wave sleep, and exhibited an increase in REM density. Other sleep difficulties have included difficulty with sleep onset, sleep maintenance, and nightmares, which have been significantly related to severity of PTSD symptoms (Babson et al., 2011). Germain et al. (2004) examined subjects with PTSD who also had untreated sleep disturbances and found that as sleep problems increased, overall PTSD symptoms increased as well. Increased sleep disturbances were unrelated to gender, age, type of trauma, length of PTSD, or other co-occurring psychiatric conditions (Germain et al., 2004). Krakow, Germain, et al. (2001) found that sleep quality and PTSD both predicted sleep disordered breathing (SDB) and sleep movement disorders (SMD); further analysis after controlling for other factors indicated that only PTSD predicted SDB or SMD and SDB combined. They recommend further consideration of a potential relationship between sleep quality, sleep disorders, and PTSD (Krakow, Germain, et al., 2001). Genetic risk factors have been identified for sleep disorders including restless leg syndrome, insomnias, and obstructive sleep apnea syndrome (Dauvilliers & Tafti, 2008). Tafti (2009) recommended a GWAS to examine normal sleep and sleep disorders.

Deductive and inductive reasoning was used to develop this research idea. According to deductive reasoning, if sleep is a primary risk factor for the development of PTSD and genes are associated with both PTSD and sleep, then studying the genome may result in identifying a common genetic risk factor for sleep and PTSD. According to inductive reasoning, treating nightmares decreases nightmare frequency and/or improved sleep and PTSD symptoms, suggesting that treatment for sleep problems results in

improvement in PTSD (Krakow, Germain, et al., 2001; Krakow, Hollifield, et al., 2001). Refer to Chapter 2 for a more detailed discussion of the literature review. Figure 1 provides a flow chart for the reader to conceptualize the variables in this study.

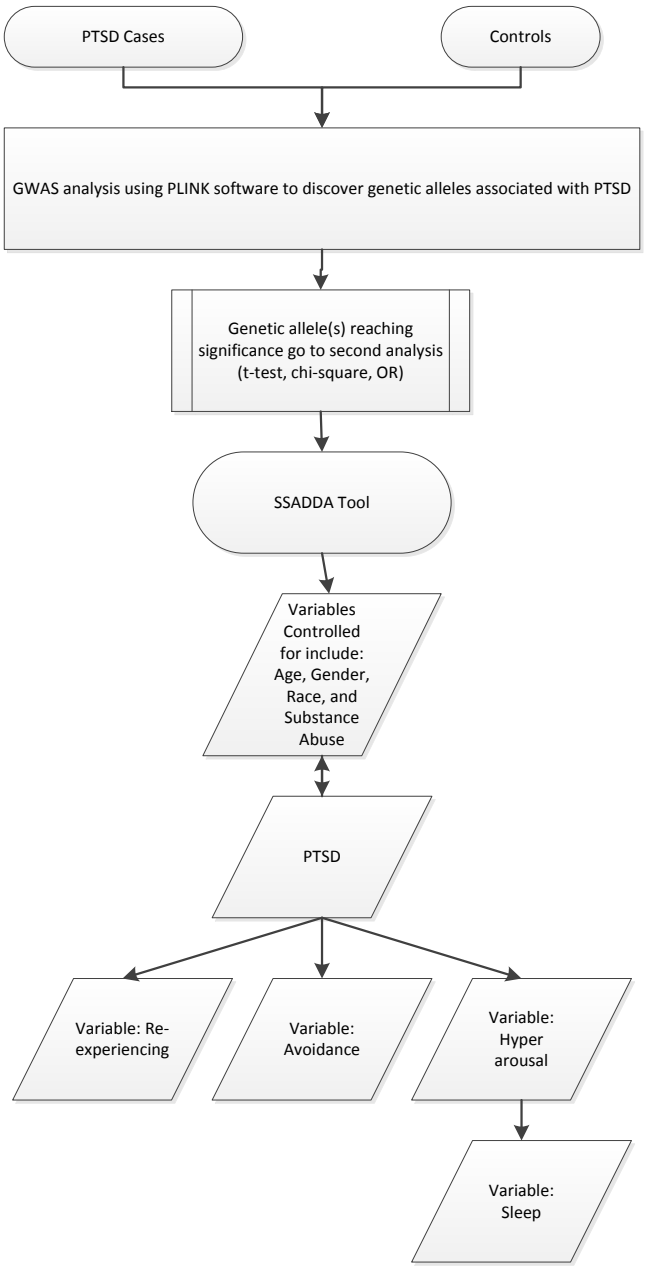

Figure 1. Conceptual flowchart of PTSD variables.

Nature of the Study

The GWA approach has been considered "a completely untapped avenue for future research in measured genes and PTSD" (Cornelius et al., 2010, p. 8). The National Center for Biotechnology Information's (NCBI) had a de-identified PTSD dataset collected as part of another GWAS study. I applied to use the genome-wide association database which was available through the Database of Genotypes and Phenotypes (dbGAP) website (Gelertner & Krazler, 2010). The original study collected genomic data on the symptoms of PTSD and was completed as part of a larger dataset on alcohol dependence in European and African Americans. Quantitative data consisted of genomic data on PTSD from this database. Molecular data were collected using whole-genome typing with the Illumina chip HumanOmni1_Quad_v1-0_B (dbGAP, n.d.). The variables collected from the dbGaP included the following: gender, age, race, sedative dependence, tobacco dependence, stimulant dependence, and substance use (Gelertner & Krazler, 2010). The dbGaP database used de-identified data from the National Library of Medicine's National Center for Biotechnology Information (NCBI). NCBI indicates that in some instances, dbGAP provides individual-level participant data for the purpose of follow up. A request was made to access individual-level data for the purpose of completing the study.

The use of this database requires a doctoral level individual to be the principal investigator (PI) along with the signing official (SO) to be able to access the database. The signing official for my use of this database was required to be someone from Walden University, either the head of the department or a designee from Walden. Dr. Shen

agreed to be the PI for the purpose of obtaining the database; however, I completed the research project. Both the PI and the SO cosigned the data access request. The data access request form, SF424 (R&R) required both the PI and SO to have accounts through the National Institutes of Health (NIH) and eRA Commons.

Spencer, Zhan, Donnelly, and Marchini (2009) reviewed design issues related to genome-wide association studies. They suggested that a GWA research design has to account for the type of chip used to complete the study and an adequate sample size to detect an effect. They reviewed several different types of chips that can be used and suggested that the number of cases to controls be between 1,257 and 2,653 to obtain power from 0.635 to 0.821 (Spencer et al., 2009). The Illu610 k chip requires 2,212 cases and 2,212 controls to obtain a power of 0.818, "assuming a disease causing allele with a relative risk of 1.5, a minor allele of at least 0.05, and a *p* value threshold of $p < 5x10^{-7}$" (Spencer et al., 2009, p. 9). Zondervan and Cardon (2007) suggested that a sample size of 1,000 cases and controls is needed to detect an odds ratio of ~ 1.5 with 80% power. Three of the four genome-wide association studies have had smaller sample sizes: Logue et al. (2012, 295 cases of PTSD), Xie et al. (2013, 300 cases of PTSD), and Guffanti et al. (2013; 94 cases of PTSD). The fourth study by Solovieff et al. (2014) had a larger number of PTSD cases ($n = 845$). Prior to obtaining the database the website information indicated the database contained 500 PTSD cases and 491 controls. The entire sample from the NCBI database was used. The independent variable was PTSD status (PTSD case population vs. non-PTSD control population). The dependent

variables were the symptoms of PTSD: re-experiencing/intrusion, avoidance/numbing, hyper arousal, and sleep); refer to Chapter 3 for additional information.

Confounding variables included those variables included as part of the database: age, gender, race/ethnicity, sedative dependence, tobacco dependence, stimulant dependence, and substance use. I acknowledge that other variables in the literature were not included in this study, including BMI, use of anxiolytics, use of antidepressants, socioeconomic status, type and intensity of trauma, education, rural/urban, co-occurring disorders such as major depressive disorder, Axis 2 disorders, and age of traumatic exposure, and this is noted in the limitations of the study (Babson et al., 2011; Frans et al., 2005; Krakow, Germain, et al., 2001; Krakow, Hollifield, et al., 2001).

Demographics for participants were included. The genomic data were analyzed initially using PLINK software to identify any area of the genome that reached genome-wide significance. Statistical measures included frequency, means, and odds ratio of genetic alleles for PTSD cases and controls. Groups were analyzed to ensure that they did not differ using Hardy-Weinberg equilibrium. Any gene that reached genome-wide significance moved to the second phase of analysis to be further analyzed with the dependent variables. This was done using SPSS and included comparison of *t* test and chi square. Odds ratio was calculated, as appropriate.

Definitions

The db GAP database identified individuals with PTSD using the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA, n.d.). The PTSD section of the SSADDA was used to collect data on 12 different types of traumatic experiences:

direct combat in a war; seriously physically attacked or assaulted; physically abused as a child; seriously neglected as a child; raped; sexually molested or assaulted; threatened with a weapon; held captive or kidnapped; witnessed someone being badly injured or killed; involved in a flood, fire, or other natural disaster; involved in a life-threatening accident; suffered a great shock because one of these events happened to someone close to you; and other (Xie et al., 2009).

The SSADDA tool uses a number of questions that identify symptoms associated with PTSD (Pierucci-Lagha et al., 2005; SSADDA, n.d.). Participants responded with "yes" or "no" when answering the questions. These questions operationalized PTSD symptoms. Examples of questions are noted below:

Re-Experiencing/Intrusions

O2 Did memories, visions, thoughts, or feelings about (EVENT) <u>often</u> keep coming to your mind even though you didn't want them to?

O4 Did you ever suddenly act or feel as if the (EVENT) was happening again? This may include flashbacks, or hallucinations, even if they occur when you are just waking up.

Avoidance/Numbing

O8 Did you ever try to avoid thinking about or having feelings about (EVENT) and find that you couldn't?

O9 Did you ever avoid activities, places, or people that reminded you of (EVENT)?

O12 During that period of time, did you feel more cut off, distant, or separated from people than before the (EVENT) happened?

O13 Were there times when you believed you had lost the ability to experience emotions that you had before (EVENT) happened? For example, did you feel you couldn't have loving feelings or anything like that?

O14 Were there times when you felt that there was no point in planning for the future—that you might not have a rewarding career; a happy family; or a long, good life?

Hyper Arousal

06 Did things that remind you of the (EVENT) make you sweat, tense up, breathe hard, tremble, or respond in some other physical way?

O19 Were there times when unexpected noise, movement, or touch startled you more than before (EVENT)?

O20 Were you more watchful or extremely aware of things around you? For example, were you more aware of certain sounds, smells, or sights?

The SSADDA has questions specific to sleep and memory.

Sleep

O3 Did you have unpleasant dreams again and again about (EVENT)?

O16 Did you have more trouble falling asleep or staying asleep than before (EVENT)?

Memory

O10 Did you find that you sometimes could not remember important things about (EVENT)?

The SSADDA collected information related to alcohol and drug usage while experiencing symptoms. These questions were identified as the questions that would be used in the second phase of analysis to control for alcohol and drug abuse in this population. Specifically, on *Question O21* Participants responded "yes—clean" were included in the response.

Alcohol and Drug-Related Questions

O21 You have told me about things such as reliving the event through dreams, memories, or feelings; avoiding things that reminded you of the event; and problems with sleep, mood, or thinking. Did these experiences last longer than 1 month? Participants responded "Yes —clean were included in the response.

Additionally, participants responded with a "no" on the two questions below related to alcohol and drug use.

Clustering at onset.

A. Around the time you first had these very intense feelings, were you having experiences from three or more boxes found on this (ALCOHOL/COCAINE/ OPIATES/OTHER DRUG) sheet?

Heavy use when not clustering

B. Around the time you first had these very intense feelings, were you (drinking heavily, using DRUGS) daily or almost daily?

Assumptions

Cooley, Clark, and Page (2011) identified a number of problems associated with data accuracy, including imputation errors when data are entered, differing algorithms being used, genotyping error rates due to duplication, and phenotype misclassification. Errors can also occur at the point of diagnosis (sensitivity and specificity; Cooley et al., 2011). This dissertation involved an assumption that the NCBI genetic database accurately diagnosed PTSD and that initial data collection techniques were completed accurately. Bias and confounding may be minimized in association studies according to Botto and Khoury (2004), as recall bias is minimized and genotypes are stable over time. Refer to Chapter 3 for additional discussion of the nature of the study, specific research questions, hypotheses, and research objectives.

Scope and Delimitations

The SSADDA is a tool developed to study genetic association involving cocaine and opioid dependence (Pierucci-Lagha et al., 2005). The SSADDA tool was originally developed from the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA); a free copy of the tool is available at http://genetics.bumc.bu.edu/ssadda/ (Boston University, n.d.). "The SSADDA provides extensive coverage of the physical, psychological, social, and psychiatric manifestations of cocaine and opioid abuse and dependence in addition to a number of related Axis I and Axis II disorders" (Samet, Waxman, Hatzenberger, & Hasin, 2007, p. 28).

Pierucci –Lagha et al. (2005) examined inter rater reliability and test-retest reliability for the SSADDA using 293 participants in two sub studies. For the PTSD section of the tool, they found inter rater reliability rates to be fair (k = 0.59, 95% CI 0.40-0.77; Y = 0.73) and test retest reliability to be k = 0.76 (95% CI 0.53-0.99), Y = 0.88. PTSD was the only diagnosis whose prevalence rates increased between the test and retest period (p = 0.041; Pierucci-Lagha et al., 2005). The SSADDA tool has been used in studies related to genes and PTSD; Xie et al. (2009) studied interactive effects of PTSD, childhood adversity, adult trauma, gene (5 HTTLPR region), and gene environment interactions. Douglas et al. (2010) used the SSADDA to evaluate adverse childhood experiences (ACE) as risk factors for substance abuse. Ford et al. (2009) used the SSADDA to evaluate comorbid psychiatric and substance disorders treatment use in the context of several groups diagnosed with cocaine dependence. Thavichachart et al. (2009) used the SSADDA to identify the presence of chronic PTSD following the December 2004 tsunami off the Andaman coast in Thailand. The SSADDA has been used to study attention deficit hyperactivity disorder, substance abuse disorders, in genome-wide association studies on substance abuse disorders, and in a genome-wide association study on PTSD (Arias et al., 2008; Gelernter et al., 2006; Xie et al., 2013).

Walker and van der Helm's (2009) theory reviewed under the theoretical framework was chosen as the framework for this research because of its focus on sleep as the mechanism for PTSD development. There are several other theories that were not chosen as the theoretical framework; these theories, which suggest that PTSD develops as a result of different experiences, are briefly reviewed. *Stress theory* (*residual or stress*

evaporation) suggests that PTSD develops from the conflicts of war (Donovan, Padin-Rivera, Dowd, & Blake, 1996). *Residual stress theory* indicates that exposure to combat is a sufficient traumatic experience to explain the development of PTSD in veterans (Donovan et al., 1996). *Stress evaporation theory* focuses on experiences that occur prior to war as the main predictor of individuals being more vulnerable to PTSD or chronic PTSD following a combat-related experience (Donovan et al., 1996).

Trauma theory indicates that when individuals experience a significant threat or perceived threat to their lives, they experience biological, psychological, and social changes that may be helpful to them at the time but can have long term effects if not adequately treated (Bills, 2003). Frans et al. (2005) suggests that PTSD develops from fear conditioning and may be a mechanism for the differences noted between genders. Genetic factors or neurobiological differences that occur in the neurotransmitters dopamine and serotonin and in thrombocyte monoaminooxidase activity may play a role in fear conditioning (Garpenstrand, Annas, Ekblom, Oreland, & Fredrikson, 2001, as cited by Frans et al., 2005). One or several of these theories may be implicated in the etiology of PTSD. These theories have been provided for reference only, but none of these theories implicate a symptom of PTSD as a pathway for its development and therefore were not chosen for the theoretical framework.

In 1999, the United States Preventative Services Task Force developed criteria for evaluating internal validity for individual case control studies (National Center for Biotechnology Information U.S. National Library of Medicine [NCBINLM], n.d.). The

criteria indicated a study has good internal validity if it includes: valid selection of cases, exclusion criteria applied equally to cases and controls, an 80% response rate, the same measurements applied to cases and controls, and addresses confounding variables (NCBINLM, n.d.). These criteria evaluated internal validity.

Considerations that focused on external validity included: selection of cases and controls, the country participants were selected from (in this case, cases and controls were African Americans and Europeans), eligibility and exclusion criteria, severity of illness, race, and measurement tool outcomes (Rothwell, 2005). Prevalence rates of PTSD differ by race. Lifetime prevalence of PTSD in European countries (Belgium, France, Germany, Italy, Netherlands, and Spain) was 1.1% (95% CI 1.0-1.3) to 1.9% (95% CI 1.7-2.1) (Alonso et al., 2004; Darves-Bornoz et al., 2008). Alonso et al. (2004) indicated prevalence rates for PTSD in Europeans over the past twelve months was 0.9% (95% CI 0.7-1.1). African Americans had lifetime prevalence rates of 8.7% compared to Whites whose rates were 7.0-7.4%; African Americans were also less likely to seek treatment for PTSD (Roberts, Gilman, Breslau, Breslau, & Koenen, 2011). Boscarino, Kirschner, Hoffman, and Ehrlich (2013) used the New York PTSD Risk Score [NYPRS], core psychosocial measures, and genetic alleles (COMT-rs4680; FKBP5-rs9470080; CHRNA5-rs16969968; CRHR1-rs110402) in a prediction model of PTSD and found by adding genetic allele's sensitivity increased to 100% and specificity increased to 95.6%. Long-term prevalence rates in the population studied by Boscarino et al. (2013) was 14.3% (95% CI – 11.1-11.8) and current PTSD was 10.7% (95% CI – 7.9-14.1). These prevalence

rates are provided for comparison purposes for this research study. The results from this dissertation should not be generalized beyond the population under study.

Significance

The implications for positive social change include understanding the genetic determinants of PTSD, which may lead to expanded pharmacological avenues for exploration, prevention, early intervention, and more effective treatment for individuals who develop PTSD (Koenen, 2007). This dissertation brought attention to the research community of the genetic risk factors associated with PTSD, an area that has not received as much attention as other more prominent mental health conditions such as depression or schizophrenia and identified methodological concerns with the GWAS method (Cornelius, Nugent, Amstadter, & Koenen, 2010). With many veterans returning to the United States following the recent war, the importance of effective PTSD diagnosis and treatment is in the forefront of public health. A study of the whole genome may identify additional correlations previously undiscovered, which could generate additional research areas or topics for future research (Cornelius et al., 2010). Additionally, this research contributed to the framework for GWA studies on PTSD.

Summary

The goal of this research was to study the genomes of individuals with PTSD and controls to identify whether genetic determinants that may be associated with PTSD and specifically to examine how sleep may be involved with the development of PTSD. Zondervan and Cardon (2007) indicated that identifying genetic variants, even those "that result in a low increased relative risk of a common condition may still have major public

health importance in terms of the number of people affected" (p. 2,492). Chapter 2 follows with a comprehensive review of the literature focusing on the research surrounding the genetic alleles and candidate gene studies of PTSD, the literature related to the symptoms of PTSD, the non-genetic research related to sleep difficulty and PTSD, and the literature on GWAS to date. Additional evidence for confounding variables, moderator, and predictor variables is included in the review.

Chapter 1 began with the introduction followed by the problem statement and purpose of the study. The research questions and theoretical framework were reviewed, followed by the nature of the study, definitions, scope, delimitations, and limitations. The chapter concluded with a review of the significance of the study and a summary of the research idea. Chapter 2 provides a comprehensive review of the literature from 2001 to the present. Additional evidence for confounding, moderator, and predictor variables is included in the review. Chapter 3 focuses on the research design, including information about the database, sample size, power, and genome-wide association study method. Chapter 4 focuses on the database used, variables, methods, and a comprehensive data analysis. The research questions are also answered in this chapter. Chapter 5 contains an interpretation of the findings of the genome-wide study in the context of the literature reviewed in Chapter 2 and the theory used for the research study. Limitations of the study as well as recommendations for further research are included in Chapter 5. The chapter ends by highlighting social change implications and the conclusion to the research.

Chapter 2: Literature Review

Introduction

Recommendations from the literature on PTSD identified that a genome-wide association study was needed to fill a gap in the literature (Cornelius et al., 2010; Feodorova & Sarafian, 2012; Norrholm & Ressler, 2009). Although GWAS had been completed, there had been no GWAS to discover whether an association existed between genetic alleles and sleep difficulty. The purpose of this study in the initial analysis phase was to discover whether a new genetic risk factor could be identified in individuals with PTSD or whether results from prior GWAS could be replicated in a different sample. For any gene that reached genome-wide significance in the initial analysis, a second phase of analysis was planned that examined the genetic allele(s) and the symptoms associated with PTSD and the specific sub symptom of sleep.

Prevalence rates indicate that only a portion of individuals develop PTSD, suggesting a genetic component to the disorder (Koenen, 2007; Yehuda et al., 2011). A study by Bailey et al. (2010) examined heritability of PTSD and the symptoms associated with PTSD and found support for heritability for B, C, and D cluster symptoms. Heritability of PTSD was 0.37. Bailey found PTSD symptom clusters had heritability of 0.75 for B symptoms (re-experiencing/intrusion) and 0.39 for both C symptoms (avoidance/numbing) and D symptoms (hyper arousal). Sleep is considered part of the D cluster of symptoms. Recommendations from the literature supported further study of the potential link between PTSD and sleep as a potential pathway for PTSD development (Krakow, German, et al., 2001; Krakow, Hollifield, et al., 2001; Walker & van der Helm,

2009; Yetkin et al., 2010). Sleep difficulties (in the form of insomnia, poor sleep quality, and nightmares) are reported by 70-91% of individuals diagnosed with PTSD (APA, 2000; Maher et al., 2006). Studies focused on treating sleep problems have resulted in improvements in sleep functioning, symptoms of posttraumatic stress disorder, and cognitive functioning (particularly long-term verbal memory; Krakow, Germain, et al., 2001; Krakow, Hollifield, et al., 2001; Raboni et al., 2009; Ulmer et al., 2011).

Walker and van der Helm's (2009) "sleep to remember sleep to forget" hypothesis suggests that sleep is not a symptom of PTSD but rather a causal factor for its development. Stickgold (2002) has suggested that PTSD develops as a function of neurotransmitter failure or disruption preventing normal integration of memory processes. This memory processing occurs during sleep; "posttraumatic re-enactments (in the form of nightmares or dreaming) are understood as a psychological correlate of pathological changes to normal brain functioning during (REM) sleep" (Wittman, 2007, p. 4). Four genome-wide association studies on PTSD have been published, with one study lending support for further study of PTSD and sleep difficulty (Guffanti et al., 2013; Logue et al., 2012; Solovieff et al., 2014; Xie et al., 2013). Logue et al. (2012) found SNP rs8042149 in the retinoid-related orphan receptor alpha gene (RORα) associated with PTSD. The RORα gene is thought to be involved in circadian rhythm functioning and in the circadian clock (Akashi & Takumi, 2005).

Past genetic research on PTSD has focused on individual candidate gene studies; these studies have found a number of individual genes associated with PTSD (Amstadter et al., 2011; Change et al., 2012; Dragon & Onisczenko, 2009; Goenjian et al., 2012;

Hauer et al., 2011; Lee et al., 2005; Morey et al., 2011; Ressler et al., 2011; Sarapas et al., 2011; Segman et al., 2002; Voisey et al., 2010). While these studies have advanced the understanding of potential genetic risk factors, other studies have found no association or mixed results (Bailey et al., 2010; Mellman et al., 2009; Morgan et al., 2003; Pivac et al., 2006; Sah et al., 2009).

Chapter 2 begins with a review of the literature search strategy followed by the theoretical foundation that provides the basis for the study. The next section contains a review of how researchers have approached the topic in the past, including collection techniques, measurement tools, and common statistical techniques, followed by a review of the genetic alleles identified in the literature (i.e., glucocorticoid, dopamine, gluteramеric, and serotonin systems). The literature review continues with a review of the few studies that have examined genetic alleles and the symptoms associated with PTSD. The genetic allele COMT has been implicated in both sleep and PTSD is reviewed, leading into the next section that provides the non-genetic literature showing the relationship of sleep and PTSD. The chapter concludes with literature on the GWAS method and a review of the current GWAS studies with a transition to Chapter 3.

Literature Search Strategy

The literature review involved using the Academic Search Premier database with a focus on scholarly, peer-reviewed journals (articles 2001-present) with word search terms *PTSD*, *genes*, and *genetics*. One hundred fourteen articles were reviewed from this search. Other keyword terms, *PTSD*, *genes* or *genetics*, and *sleep*, were also used. This search yielded only two results. *PTSD* and *epigenetics* yielded 22 results. Key terms

PTSD + epidemiology + sleep disorder yielded 501 results. Additional Academic Search Premier searches were done to review *PTSD+allele* and *PTSD+single nucleotide polymorphism"*, which yielded an additional 74 results that were reviewed. Google Scholar was searched with the terms *GWAS*, "*PTSD*, and *epidemiology*. Results yielded a total of 284 additional articles. Additional literature search terms included *PTSD*, *gene*, and each symptom of PTSD (*re-experiencing* [two articles], *hyper arousal* [three articles], and *avoidance* [three articles]). There were no articles found related to *GWAS* and PTSD symptoms. There were no articles related to *GWAS*, *PTSD*, and *sleep*. Reference articles used by other researchers were further investigated for their relevance. I subscribed to the National Center for Biotechnology Information (NCBI) at the U.S. National Library of Medicine (NLM) to obtain the most recent articles published related to posttraumatic stress disorders and genes.

The literature related to potential genes associated with PTSD includes candidate gene association studies, single nucleotide polymorphs (SNP) studies, haplotype studies, linkage studies, GWAS, and twin studies. Twin studies were not included in this literature. Gene expression studies (epigenetic studies) were excluded in this review unless the gene studied was directly associated or no association was found with PTSD and the gene was not acting as a modifier or had an interaction affect. Studies outside of the United States were included.

Theoretical Foundation—Walker's "Sleep to Forget, Sleep to Remember" (SFSR)

Various theories were reviewed in the introduction; however, none of the theories reviewed identified a symptom or symptoms of PTSD as a possible pathway. The fear

conditioning theory was considered for this dissertation; however, the focus of the theory is related to the structure(s) in the brain (amygdala) involved in fear and not believed to be related to a specific symptom of PTSD. Walker's (2009) "sleep to forget sleep to remember" (SFSR) hypothesis focuses on the specific symptom of sleep and therefore was chosen. Walker (2009) suggested that sleep is involved in the processes the brain uses to initially store events as memories (referred to as *encoding*) and in the process used to maintain those memories (known as *memory consolidation*). Sleep deprivation may play a role in memory, particularly for memories with emotional content (Walker, 2009). Walker stated,

> the impact of sleep loss on basic emotional regulation and perception has received limited research attention … the absence of research is striking considering that nearly all psychiatric and neurological mood disorders express co-occurring abnormalities of sleep, suggesting an intimate relationship between sleep and emotion. (Walker & van der Helm, 2009, p. 738)

Walker (2009) suggested that the inability to discharge negative, emotionally laden memories combined with the inability to properly retain positive or neutral memories may be an avenue of study for emotional problems that have co-occurring sleep issues. Walker and van der Helm (2009) suggested that this framework may be specifically relevant to major depression disorder and PTSD, both known to have co-occurring sleep problems (i.e., insomnia or nightmares) associated with them. Figure 2 indicates how this framework might be applied.

This “sleep to forget sleep to remember” hypothesis by Walker (2009) is a new model related to sleep and emotion that is being developed and has not been widely applied in the literature. In an unpublished study reviewed in Walker, sleep deprivation and retention of positive, negative, and neutral words were studied. Walker found that sleep deprivation (38 hours total) resulted in 60% retention in memories. In those that were sleep deprived, neutral and positive words were not retained; however, negative words were not affected by sleep deprivation. Walker suggested that people “sleep to forget” and also to remember based on the observation that initially memories may contain significant emotion associated with them but over time, as memories are recalled, there is less emotion associated with the memory (p. 189). This emotion acts like a “blanket” around a memory that is removed over time with only the factual information retained, with REM sleep being the conduit for this process (p. 189). In another study by Walker (2011), the amygdala of individuals was less reactive in response to emotional stimuli following a night’s sleep compared to individuals who did not sleep.

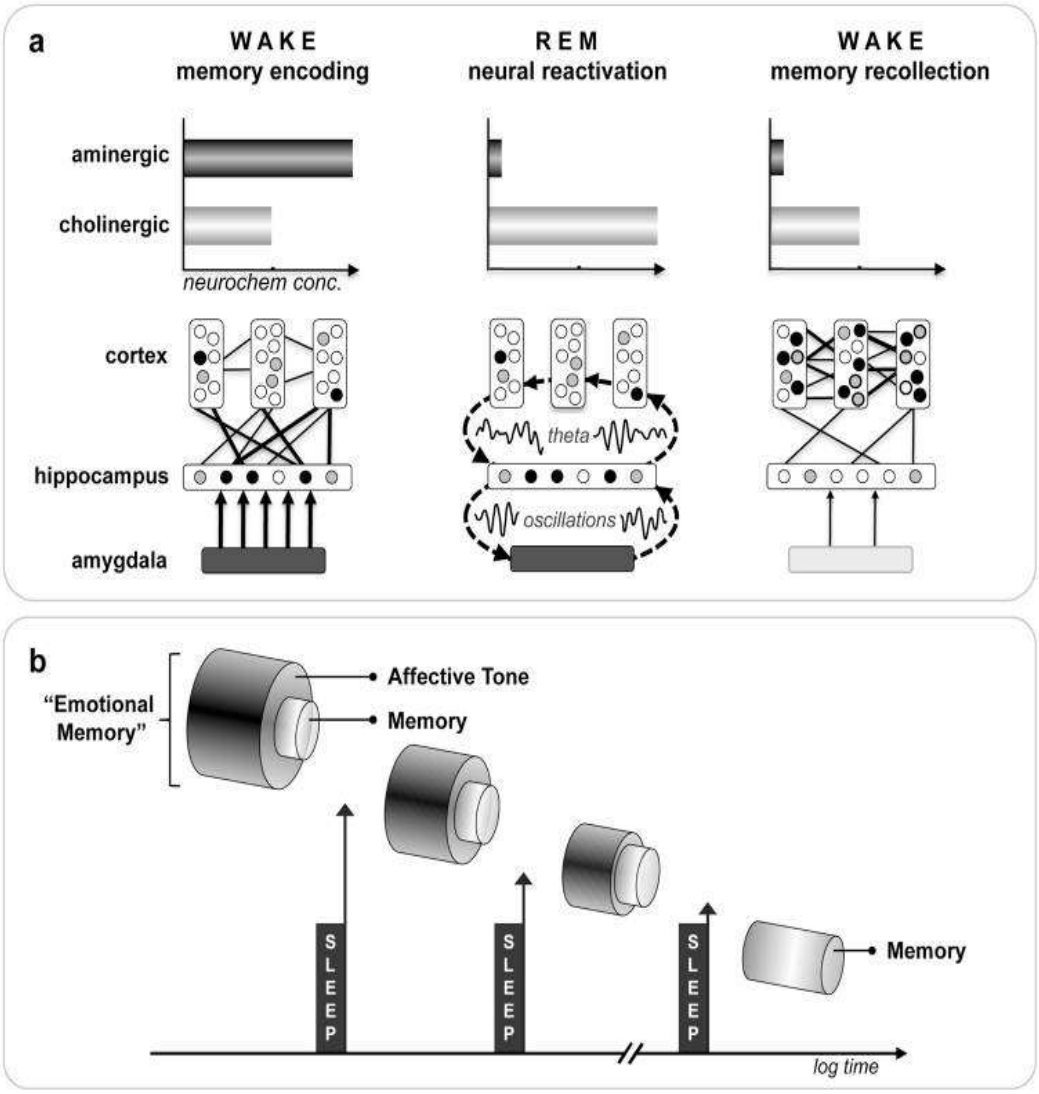

Figure 2. Sleep to forget sleep to remember (SFSR) framework.

The sleep to forget and sleep to remember (SFSR) model of emotional memory processing: (a) Neural dynamics. Waking formation of an episodic emotional memory involves the coordinated encoding of hippocampal-bound information within cortical modules, facilitated by the amygdala, and modulated by high concentrations of aminergic neurochemistry. During subsequent REM sleep, these

same neural structures are reactivated, the coordination of which is made possible by synchronous theta oscillations throughout these networks, supporting the ability to reprocess previously learned emotional experiences. However, this reactivation occurs in a neurochemical milieu devoid of aminergic modulation and dominated by cholinergic neurochemistry. As a consequence, emotional memory reprocessing can achieve, on the one hand, a depotentiation of the affective tone initially associated with the event(s) at encoding, while on the other, a simultaneous and progressive neocortical consolidation of the information. The latter process of developing stronger cortico-cortical connections additionally supports integration into previous acquired autobiographical experiences, further aiding the assimilation of the affective event(s) in the context of past knowledge, the conscious expression of which may contribute to the experience of dreaming. Cross-connectivity between structures is represented by number and thickness of lines. Circles within cortical and hippocampal structures represent information nodes; shade reflects extent of connectivity: strong (filled), moderate (grey), and weak (clear). Color fill of amygdala and arrow thickness represents magnitude of co-activation with and influence on the hippocampus. (b) Conceptual outcome. Through multiple iterations of this REM mechanism across the night, and/or across multiple nights, the long-term consequence of such sleep-dependent reprocessing would allow for the strengthening and retention of salient information previously tagged as emotional at the time of learning. However, recall no longer maintains an

> affective, aminergic charge, allowing for post-sleep recollection with minimal autonomic reactivity (unlike encoding), thereby preventing a state of chronic anxiety. (Walker & van der Helm, 2009, p. 742. Reprint permission obtained November 19, 2012, from Mr. Walker)

Past Research Approaches to PTSD

Researchers have approached the study of genetic risk factors for PTSD primarily through studies of genetic alleles or candidate gene studies. Collection methods of genetic material for candidate gene studies have been through saliva and blood samples (Kolassa, Ertl, et al., 2010; Kolassa, Kolassa, et al., 2010; Sayin et al., 2010). Methods of identifying genes have included genotyping, genotyping and functional MRI imaging (fMRI), and polymerase chain reaction (Lee et al., 2005; Lee et al., 2006; Mellman et al., 2009; Morey et al., 2011; Sah et al., 2009; Segman et al., 2002; Voisey et al., 2009). Walden University does not allow for original data collection of biological material; therefore, a database was sought that had already completed the collection and genotyping of the genomic data.

Measurement tools used in the literature to examine PTSD have included the Clinician Administered PTSD Scale (CAPS), the Post Traumatic Stress Diagnostic Scale, the PTSD Checklist (PCL), the Mississippi Scale for combat-related PTSD, the PTSD-F and PTSD-C, the Structured Clinical Interview for DSM-III-R (SCID), the Korean version of the SCID, the UCLA PTSD Reaction Index, , the Davidson Trauma Scale™, and the Semi Structured Assessment and Drug Dependence and Alcoholism (Blake et al., 1995; Chang et al., 2012; Davidson, 1997; Douglas et al., 2010; Dragan & Oniszczenko,

2009; Foa, 1996; Foa, Cashman, Jaycox & Perry, 1997; Ford et al., 2009; Goenjian et al., 2012; Lee et al., 2005; Keane, Caddelle, & Taylor, 1998; Kolassa, Ertl, et al., 2010; Kolassa, Kolassa, et al., 2010; Mellman et al., 2009; Morey et al., 2011; Pivac et al., 2006; Pivac et al., 2007; Pynoos, Rodriguez, Steinberg, Stuber, & Frederick, 1998; Sah et al., 2009; Sarapas et al., 2011; Sayin et al., 2010; SSAADDA, n.d.; Spitzer, Williams, Gibbon, & First, 1992; Steinberg, Brymer, Decker, & Pynoos, 1998; Weathers, 1993; Weathers, Litz, Huska, & Keane, 1994; Voisey et al., 2009; Xie et al., 2009; Xie et al., 2013).

Statistical procedures in the literature consist of many different approaches. Candidate gene studies consistently ensure that groups are in Hardy Weinberg equilibrium (Chang et al., 2012; Dragan & Oniszczenko, 2009; Hauer et al., 2011; Lee et al., 2005; Lee et al., 2006; Morey et al., 2011; Pivac et al., 2007; Sarapas et al., 2011; Segman et al., 2002; Voisey et al., 2009). Bonferri correction was used to account for multiple testing (Sarapas et al., 2011; Xie et al., 2010). The Nyholt correction was used by Morey et al. (2011). Other statistical tools used have included the following: ANOVA, the two-way ANOVA, the MANCOVA logistic regression, chi square, *t* test, and x^2 (Dragan & Oniszczenko, 2009; Kolassa, Kolassa, et al., 2010; Lee et al., 2005; Lee et al., 2006; Martel et al., 2012; Morgan et al.; 2003; Pivac et al., 2006; Pivac et al., 2007; Ressler et al., 2011; Sarapas et al., 2011; Sah et al., 2009; Segman et al., 2002; Voisey et al., 2009;Voisey et al., 2010; Xie et al., 2010). Less used tools included the Mantel-Haenszel test, odds ratio (OR), population attributable fractions, attributable risks, and general linear modeling (Morey et al., 2011; Voisey et al., 2009).

Studies examining sleep and PTSD have used different measurement instruments including in-home polysomnography and actigraphic recording (Germain et al., 2004; Klein, Koren, Arnon, & Lavie, 2003; van Liempt, Westenberg, Arends, & Vermetten, 2011). Measurement tools have included the Pittsburg Sleep Quality Index (PSQI), the UCLA PTSD Reactive Index, the Impact Event Scale, and the Patient Checklist (PCL) for PTSD (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989; Gellis, Gehrman, Mavandadi, & Oslin, 2010; Germain et al., 2004; Horowitz, Wilner, & Alvarez, 1979; Langston, Davis, & Swopes, 2010; Lewis, Creamer, & Failla, 2009; Pynoos et al., 1998; Weathers, 1993; Weathers et al., 1994). Consistent with PTSD studies, statistical techniques used in studying sleep and PTSD have included the *t* test, ANOVA, MANOVA, logistical regression, and meta analyses (Gellis et al., 2010; Klein et al., 2003; Liempt et al., 2010; Lewis et al., 2009).

GWA studies have been used to examine a number of physical health conditions (diabetes, obesity, Crohn's disease, ulcerative colitis, multiple sclerosis, HDL, and several other medical conditions) as well as behavioral health conditions such as schizophrenia and bipolar disorder (Visscher, Brown, McCarthy, & Yang, 2012). The four GWA studies on PTSD have all used PLINK software and logistic regression (Guffanti et al., 2013; Logue et al., 2012; Solovieff et al., 2014; Xie et al., 2013). Chapter 3 contains further description of the measurement tools and statistical techniques.

Candidate Gene/Genetic Allele Literature

Information in this section addresses *Research Question 1* and reviews the candidate gene literature on PTSD.

Genetic Alleles of the Serotonin Transporter Region

Mellman et al. (2009) examined the HT2A and the SLC6A4 in the 5HTTLPR (serotonin transporter region) in an African American population and found that being female with a traumatic sexual experience and depression were significantly associated with a diagnosis of PTSD; however, no association was found with the SLC6A4 gene. Mellman et al. indicated that although the sample size had sufficient power to detect for a general, allelelic, or recessive model, it did not have sufficient power to detect for a dominant model, which may be why an association within the SLC6A4 in the 5HTTLPR region was not found. Goenjian et al. (2012) examined the SLC6A4 in earthquake survivors and also found no association. These results contradict a study by Thakur, Joober, and Brunet (2009) that found an association with "*ll"* homozygotes individuals with the SLC6A4 gene in post-motor vehicle accident victims. At both 1 month and 12 months, "*ll"* homozygotes had a greater percentage of PTSD development; 82% at 1 month for acute PTSD and 55% at 12 months for chronic PTSD (Thakur et al., 2009). At the 12-month time period, the "*ll"* homozygote for the PTSD group had an odds ratio of 4.8 compared to the "*ss"* and "*sl"* genotype. Thaker et al. indicated that the "*s"* allele has been shown to be a risk factor in other studies and should be evaluated to determine if it is a risk factor or a protective factor.

Morey et al. (2011) examined the SLC6A4 gene in trauma exposed PTSD cases and trauma exposed controls. Functional MRI (fMRI) recorded cases and controls as they were engaging in tasks related to working memory (viewing three faces over a specific period of time) while experiencing combat scenes (negative emotion), non-

combat scenes, or scrambled pictures, which were controls (Morey et al., 2011). Cases and controls were then engaged in a retrieval phase of one of the faces while brain activity was compared (Morey et al., 2011). Morey et al. found certain SNPs activated areas of the brain when the pictures were viewed when comparing cases to controls. Specifically, SNP rs16965628 with a "GG" genotype in the SLC6A4 activated the ventrolateral PFC in PTSD patients viewing combat scenes with a delay in working memory. When examined by race, the SNP rs1696528 in African Americans with PTSD was significant compared to controls but this was not observed in European Americans (Morey et al., 2011). Morey et al. suggested that this SNP may be a moderator variable. Morey et al. examined the amygdala focusing on the biallelic, "S" allele carriers (*s/s* or *l/l*) and found the left region of the amygdala for "S" allele carriers to be more active in PTSD cases compared to controls when viewing combat distracter scenes. There was no association noted for the "*ll"* genotype.

Mellman et al. (2009) found that the "G" allele in the 5HTR2A region of the serotonin transporter predicted PTSD. Another area of the serotonin transporter promoter gene, the polymorphism region (SERTPR) located at 17q11-1-q12 was examined by Lee et al. (2005). Significant differences were not found in genotypes (*s/s* or *l/l*) between cases and controls. Allele frequencies (*s or l*) and "s/s" carriers were significantly different compared to "*s/l" + "l/l"*. Lee et al. suggested that the "*s"* allele and the "s/*s"* genotype may be involved and increase susceptibility of PTSD.

Goenjian et al. (2012) examined two isomophisms in 200 Caucasian, Armenia Spitak earthquake survivors from 12 multigenerational families thought to be involved

with the serotogonergic system: Tryptophan Hydroxylase 1 (TPH1) on chromosome 11 and TPH2 on chromosome 12. A significant association was found with PTSD and the ”t” allele of TPH1, SNP rs2108977 accounting for 3% of the variance (Goenjian et al., 2012). Additionally, the “s” allele of the TPH2, SNP rs11178997 was associated with PTSD symptoms accounting for 4% of the variance (Goenjian et al., 2012).

Limitations noted in these studies included: small sample sizes, low power, population-stratification bias, recall bias, lack of controlling for potentiating confounding variables such as sex, depression, sexual assault, gender, or for trauma exposure in controls leading to the possibility that some controls had the genetic vulnerability but did not have the trauma exposure to trigger possible development of PTSD, and results could not be generalized beyond the study population (Goenjian et al., 2012; Lee et al., 2005; Mellman et al., 2009; Morey et al., 2011; Thaker et al., 2009).

Brain-Derived Neurotrophic Factor (BDNF)

Lee et al. (2006) examined brain derived neurotrophic factor (BDNF) gene Val66Met polymorphism. The BDNF gene is involved in the hypothalamic-pituitary-adrenal axis area of the brain and thought to be important in modulating stress (Lee et al., 2006). The Val66Met polymorph was grouped in two categories: Met66 allele (Met/Met and Met/Val) or the Val/Val genotype. Lee et al. compared the genotype and allele frequencies of 107 PTSD cases and 161 controls in a Korean population. No association was found for the Met66 or the Val/Val genotype. Limitations of this study included: small sample size, possibility of population stratification bias, other variants of the BDNF gene not studied may have contributed to PTSD, and exposure to trauma was not a

variable controlled for in the study; therefore controls may have carried a genotype or allele frequency making any association difficult to detect (Lee et al., 2006).

Glucocorticoid Receptor Pathway

van Zuiden et al. (2012) examined pre-existing vulnerability factors for PTSD in 448 male, Dutch Armed Forces soldiers deployed to Afghanistan prior to and 6 months after deployment. Vulnerability factors included pre-deployment expression of GR target genes (FK Binding Protein 5, GILZ, and SGK1), cortisol levels, and childhood trauma. Of the 448 soldiers, 35 developed PTSD symptoms following return from deployment with 413 soldiers serving as the control group. Cases had significant predeployment and post deployment PTSD symptoms compared to the control group. Deployment increased PTSD symptoms in cases but not in controls. Interaction effects were noted for cases with self-reported symptoms of depression, anxiety, and sleep disturbances compared to controls. After deployment, independent associations were found with PTSD and the following variables: pre-deployment glucocorticoid receptor number, low FKBP5 mRNA expression, high GILZ mRNA expression, and childhood trauma. PTSD and SNP associations were not able to be evaluated due to the small sample size.

Sarapas et al. (2011) examined survivors of 9/11 with and without PTSD. The sample consisted of 20 cases with lifetime PTSD compared to 20 controls matched by similar exposure (high, low, or indirect), age, gender, and race. Signal transducer and activator of transcription 5B (STAT5B) and Nuclear Factor I/A, two genes in the glucocorticoid receptor, reduced expression in PTSD cases. Sarapas et al. further studied four SNPs in the FKBP5 region: rs3800373, rs9296158, rs1360780, and rs9470080 and

found “homozygosity for any of 4 PTSD risk-related polymorphisms at FKBP5 predicted FKBP5 expression, which mediated indirect effects of genotype on plasma cortisol and PTSD severity” (p. 101). Xie et al. (2010) studied these same four SNPS in the FKBP5 region in 1,143 European Americans (EA) and 1,284 African Americans (AA) and found no association in the FKBP5 region. An association was found when the 4 SNPs and childhood adversity were entered into logistic regression; SNP rs9470080 moderated the effect of childhood adversity only and risk for PTSD in the AA population. Following, Bonferroni correction, 3 SNPs (rs3800373, rs9296158, and rs9470080) remained significant with SNP rs9470080 having a continued moderator effect for PTSD risk when there was exposure to childhood adversity (Xie et al., 2010). No effect was noted for the EA population.

Additional analyses focused on risk of PTSD with childhood adversity and the FKBP5 region; rs9470080 polymorph was statistically significant (Xie et al., 2010). Interestingly, with rs9470080 in the AA population, individuals with the “TT” genotypes were less likely to develop PTSD compared to the “CC” or “CT” genotypes. However, when individuals that had “TT” genotypes and had also experienced childhood adversity their risk for developing PTSD was greatest among the three groups (though not significant). This was only noted in the AA population and not found in the EA population. Xie et al. (2010) studied opioid, cocaine, and alcohol phenotype effects on the FKBP5 genotypes and childhood adversity as they relate to PTSD development. Only alcohol dependence was associated with risk for PTSD onset in the presence of the rs9470080 SNP and childhood adversity for both the AA and EA populations. For EAs,

FKBP5 modified the effect of alcohol and cocaine on PTSD risk. When modifiers were removed from the model, no interaction was noted for FKBP5 and childhood adversity and risk of PTSD among the EA population. Xie et al. suggested possible reasons for the results of their study that found an effect in one population and not the other may be due to other variants interacting with SNPs in linkage disequilibrium, differences in allele frequencies that occur among different populations, different gene expressions, which may be related to other genetic factors, protective factors that were not identified in the present study, or interactions with other genes that were not identified in this study.

Hauer et al. (2011) examined another section of the glucocorticoid receptor the Bc/l polymorphism. Groups studied included: a homozygous carrier group, a second group that consisted of heterozygous Bc/l *G carrier, and non-carriers of Bc/I *G. Participants included 126 patients undergoing cardiac surgery followed by intensive care treatment. Homozygous carriers had higher PTSD scores one week after surgery compared to the heterozygous and non-carrier group and remained higher but was not significant 6 months later. Hauer et al. also found homozygous Bc/I *G carriers experienced more traumatic memories from ICU than the heterozygous and non-carrier group and had lower quality of life scores. The limitation of Hauer et al.'s study was its low power. Other limitations noted in studies related to the glucocorticoid receptor pathway included low statistical power due to the study design, small sample size, lack of classification of PTSD using DSM-IV criteria, recall bias of childhood adversity, and the type of adversity was not exhaustive (van Zuiden et al., 2012; Xie et al., 2010).

Gastrin-Releasing Peptide Receptor (GRP) and Stathmin 1 (STMN 1)

Martel et al. (2012) studied s*tathim* and *gastrin-releasing peptide receptor (GRPR, "fear memory-related genes")* expression in mouse brains (p. 1). Martel et al. studied the effect these genes had on fear extinction. They initially used a technique to evoke a response. Martel et al. found that if the same environment was present; a conditioned response can still occur despite efforts at extinguishing fear. Martel et al. (2012) examined two different mice lines (knock out [KO] and wild type) along with controls. In one mice line (GRPR), when neuronal activity was shifted from the prefrontal cortex (PFC) to the amygdala fear increased and extinction decreased (it took longer for fear to extinguish). When the neuronal activity was shifted from the amygdala to the prefrontal cortex, the opposite was noted in stathmin mice, fear decreased and extinction increased (fear extinguished more rapidly). Mice with GRPR exhibited freezing when the cue occurred. GRPR mice froze 42% of the time, while KO mice froze 62% of the time. Martel et al. studied the two genotypes, GRPR and strathmin separately. They found GRPR KO mice froze more often than the GRPR wild type. Fifteen days after the initial testing, GRPR KO mice still exhibited more freezing than the WT mice. Mice with the stathmin genotype both knockout (KO) and wild type (WT) were similar in their freezing without significant differences noted. A two way ANOVA found a difference between the two stathmin mice with KO mice freezing less often than WT mice. Martel et al. noted this was related to a cue but not specific to context and suggested that additional research is needed.

Dopamine Transporter System (SLC6A3 (DAT 1) and Dopamine Transport

Segman et al. (2002) studied SLC6A3 also known as dopamine active transporter (DAT1) in 102 Jewish Ashkenazi or non-Ashkenazi individuals with PTSD. Control subjects (*n* = 104) were recruited from a hospital emergency department and exposed to a traumatic event but did not develop PTSD. Segman et al. compared cases and controls on the SLC6A3 allele frequencies (9 repeat/9R and 10 repeat/10R) and the genotype frequencies and found that there were more 9 repeat alleles in cases compared to controls (43% compared to 30.5%). Cases with the 9 repeat allele had an odds ratio of *OR* = 1.72 (95% CI = 1.12-2.62). Genotype distribution was also significantly different with the "9-9" allele having a greater distribution (20.43%) in PTSD cases compared to controls (9.47 %); an increased odds ratio, 2.45, was also noted for the "9-9" allele (95%, CI = 0.98-6.52). Segman et al. suggested that the variable number of tandem repeats (VNTR) polymorphism may be how the dopamine transporter (DAT) gene is expressed through either linkage disequilibrium or mRNA transcription. Results indicated that the DAT region was associated with an increased risk of PTSD that was not related to confounding or bias from population stratification (Segman et al., 2002). Segman et al. suggested that a portion of the population may possess a risk for developing PTSD but trauma exposure is required for PTSD to develop. Segman et al. suggested that there may be alleles, without the environmental trigger, that provide protective effects.

Chang et al. (2012) examined the SLC5A3 (DAT1) in 62 PTSD cases and 258 controls from a Detroit neighborhood. Alleles were identified at: 3R, 7R, 8R, 9R, 10R, and 11R. The greatest frequencies were found at the 10R (75.42%) and 9R (17.7%) for

all 362 participants. When age, gender, socioeconomic status, race, smoking, number of traumatic events, and lifetime depression were controlled for in 9R and 10R, odds ratio for individuals with PTSD with the SLC6A3 genotype 9R allele were similar to Segman et al. (2002) OR of 1.72 compared to 1.98 (Chang et al., 2012).

Pivac et al. (2006) examined dopamine beta-hydroxylase (DBH) in 93 Croatian war veterans hospitalized in an inpatient psychiatric setting diagnosed with chronic posttraumatic stress disorder (PTSD). Controls were 124 healthy males matched for age, gender, smoking, and other demographics. Significant differences were found between cases and controls in cortisol levels, thyroid levels, and lipid levels (higher cholesterol, lower HDL or good cholesterol, and higher triglyceride levels). Results indicated plasma DBH and 5-HT was not significant between cases and controls.

Voisey et al. (2009) examined three polymorphs: SNP 957C>T, the deletion polymorphism -141delC in the DRD2 gene, and a SNP, *Taq* 1A, rs1800497 in 127 PTSD cases and 228 controls. Voisey et al. found a significant association in the DRD2 region with the 957C>T polymorphism specific to the "C" allele. The frequency of the "C" allele in PTSD patients was 51.7% compared to controls (42.5%). The OR of having PTSD with the "C" allele was 1.45. Voisey et al. found that individuals with PTSD were two times more likely to have the "CC" genotype compared to the "TT" genotype than controls. The population attributable risk found 14% susceptibility for PTSD related to the "C" allele with the "CC" genotype (Voisey et al., 2009). No association was found for the *Taq1A* or the *-141delC* polymorphism (Voisey et al., 2009). Limitations included:

population stratification, a limited sample size, and *p* values that were not adjusted for multiple testing (Voisey et al., 2009).

Dragan and Oniszczenko (2009) examined the dopamine receptor D4 exon III polymorphism (DRD4 gene) in 107 individuals exposed to massive flooding. Groups were divided into low and high trauma exposure along with the number of their traumatic experiences (zero to six). Dragan and Oniszczenko found that individuals with the long DRD4 variants (7R) had more intense PTSD symptoms (scored higher on scales measuring PTSD). Significant correlations were also found between age and PTSD score intensity, number of previous traumas and PTSD score intensity, and gender and the intrusion/arousal (I/A) PTSD subscale (Dragan & Oniszczenko, 2009). When examining main effects from the DRD4 genotype, type of trauma, and PTSD intensity while controlling for covariates (age, gender, and previous traumas) Dragan and Oniszczenko found a main effect of genotype with the avoidance/numbing (AN) subscale. Limitations included: a small sample size, low power, and potential confounding variables such as alcohol use or tobacco use that were not controlled for in the study (Dragan & Oniszczenko, 2009). The authors also indicate the measurement tools they used were not tools used in other research studies.

Glutameric System

Voisey et al. (2010) studied the dysbindin gene, DTNBP1 SNP rs9370822 in 250 cases and controls in several psychiatric disorders including PTSD cases (127), opiate dependents cases (120), nicotine dependent cases (147), and alcohol dependent cases (231). The DTNBP1 SNP rs9370822 allele was associated with PTSD, *OR* = 1.74

(Voisey et al., 2010). This same allele association was noted for nicotine and opiate dependence (Voisey et al., 2010). No association was found for alcohol dependence (Voisey et al., 2010). Genotype associations (AA, AC, and CC) of DTNBP1 SNP rs9370822 differed among psychiatric disorders (Voisey et al., 2010). Genotypes with "CC" had an OR of 3.14 for PTSD, compared to AC, *OR* = 1.93 and AA, *OR* = 1.00 (Voisey et al., 2010). Voisey et al. noted sample size was a limitation to their study.

Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP)

Ressler et al. (2011) examined 44 single nucleotide polymorphisms (SNPs) of the PACAP (14 SNPs from ADCYAP1) and PAC1 (30 SNPs from ADCYAP1R1) region from 798 individuals (503 females and 295 males) who had experienced severe trauma. Ressler et al. found one significant association in SNP rs2267735, part of the ADCYAP1R1 receptor for females only. The association was significant after correcting for multiple testing. Ressler et al. replicated their findings in a second population of 439 participants (260 females and 179 males). They found SNP rs2267735 was associated with females in the second population. When both populations were combined the association remained significant in females only.

Monamine Oxidase (MAO) Intron 13 Polymorphism

Pivac et al. (2007) studied monamine oxidase intron (MAO) 13 polymorphism (A to G change) in 386 Croatian, Caucasian, male participants. Cases consisted of 106 war exposed combat veterans with current and chronic PTSD both with and without psychotic symptoms, 41 war exposed combat veterans without PTSD, and 242 healthy controls. A two way ANOVA found no significant effect for genotype, though a significant effect

was found for nonsmoking, psychotic PTSD veterans with the "A" allele. This group had higher platelet MAO activity than veterans without PTSD and "A" allele carriers, PTSD without psychosis and "A" allele carriers, and healthy controls with the "A" allele. Pivac et al. suggested that the effect was related to the diagnosis of PTSD with psychosis rather than the "A" allele. No effect was found for the "G" allele.

Neuropeptide Y (NPY)

Morgan et al. (2003) examined neuropeptide Y in two studies (A and B). Study A examined if trauma alone or PTSD impacted NPY levels. Participants included 8 healthy combat veterans without PTSD as the control group, 18 combat veterans with PTSD, and 8 healthy controls. ANOVA compared baseline plasma NPY levels of the three groups; the three groups differed significantly at baseline. Combat controls and PTSD participants had similar baseline plasma levels while healthy controls had plasma levels that were 30% lower (Morgan et al., 2003).

Study B consisted of 41 active military personnel that were involved in a special forces training course. A negative association was found between the number of traumatic life-threatening events and baseline NYP levels. No other associations were found. Morgan et al. suggested that exposure to trauma rather than PTSD symptoms were related to lower NPY levels. Lower NPY levels are thought to impact the flight/fight response experienced during extreme stress and may play some role in the development of PTSD (Morgan et al., 2003).

Sah et al. (2009) examined NPY levels from 10 men with combat related chronic PTSD and 13 male controls. Sah et al. found that PTSD cases had lower levels of CSF

NPY compared to healthy controls. Sah et al. suggested that higher levels of CSF NPY may serve as a protective factor promoting resilience in some individuals. The procedure for collecting CSF NPY samples involved a lumbar puncture procedure; the authors indicate the procedure itself could have been a difficult experience for some individuals however, if the procedure alone accounted for the lower levels observed, a similar level of CSF would have been found in controls. Sah et al. indicated healthy controls were not exposed to extreme trauma therefore additional studies are needed.

PTSD Symptoms

This section addresses *Research Question 2* and provides the candidate genes studies that have examined PTSD symptoms.

Corticotropin-Releasing Hormone Receptor (CRHR1)

Amstadter et al. (2011) studied eight SNPs from the corticotrophin-releasing hormone (CRH) receptor 1 gene. PTSD symptom frequency was collected at 3, 12, and 18 month intervals during a hospital stay; one SNP, rs12944712 was associated with PTSD symptoms. This SNP, rs12944712, was further analyzed and found acute PTSD symptoms increased with the "G" alleles. Amstadter et al. found that the more "G" alleles an individual had, the more rapidly PTSD symptoms decreased over time. The authors suggested the decline in the "G" allele may be related to higher PTSD symptoms that then sharply decline. A protective factor was found for rs1294412, minor allele "A", for the level of symptoms that were collected at the acute hospital visit, which was not believed to be related to population stratification. Acute PTSD symptoms were more prominent in females. Violent injury was associated with a longer time frame of PTSD

symptoms. Limitations included: the small sample size, inadequate power, attrition rates, results cannot be generalized, and population stratification (Amstadter et al., 2011).

Thoeringer et al. (2012) studied the CRH receptor in mice. They used fear conditioning to examine fear memories and hyper arousal in knock out and wild type mice. The CRH receptor did not function properly allowing Thoeringer et al. to subject mice to an electric foot shock creating trauma memories. Thoeringer et al. indicated this allowed the researchers to examine "associative learning accounting for intrusive memories and avoidance behavior, whereas non-associative (or stress related learning) results in hyper arousal" (Siegmund &Wotjak, 2006, as cited by Thoeringer et al., 2012, p. 788). Thoeringer et al. found CRH impacted consolidation of memories in the first week but did not impact the retention of later recall of fear memories. CHRHC1 impacted remote memories. KO mice that did not have CHRHC1 had less fear one month later. The authors suggest blocking CHRC1 through pharmacological measures may impact the development of PTSD (Thoeringer et al., 2012).

Dopamine System

Bailey et al. (2010) examined the DRD2 and DAT gene in 200 Armenian earthquake survivors as well as cluster symptoms of PTSD (B-intrusion, C-avoidance/number, and D-hyper arousal). There were 39 participants that had the DRD2/A1+ allele; no association was found between the DRD2/A1+ allele and PTSD, total PTSD-RI score, B symptoms, or C symptoms. D symptoms were not heritable based on analysis and were not considered for additional testing. There were a total of 86 participants with the DAT/9 repeat allele; no association was found for the DAT/9 repeat

allele and PTSD, total PTSD-RI score, B symptoms, or C symptoms. Bailey et al. controlled for alcohol dependency for DAT participants and found no association. Limitations noted included: lack of generalizability beyond the Armenian participants, the sample size may have been too small to detect a small effect, and the results may only apply to chronic PTSD as the earthquake occurred in 1988 and acute symptoms may not have been present (Bailey et al., 2010).

WFS1 Gene

Kesner et al. (2009) examined the WFS1 gene in mice, specifically the glycoprotein Wolframin. Several rats were housed together and then exposed to a predator scent (cases) compared to rats that were not exposed to the scent. The rats freezing time and startle response (following exposure to a loud noise) were monitored and considered part of a hyper arousal response. Cases were separated into PTSD-like symptoms and non-PTSD symptoms and treated either with citalopram or a saline solution. Controls were also divided into two control groups, one treated with citalopram and the other with saline solution. Researchers were blinded in the study. PTSD like behaviors developed in 16% of the rats, exhibiting symptoms from all three clusters above an established baseline. PTSD like symptoms reduced after administering citalopram to PTSD-rats; Kesner et al. (2009) suggest the Wolframin gene may be a biomarker for PTSD.

Hyper Arousal and Avoidance Symptoms: Serotonin Transporter System Region

Sayin et al. (2010) conducted a prospective study with 77 Turkish individuals that suffered a trauma that focused on the 5 HTTLPR gene polymorphic region and Intron 2

(variable number of tandem repeats, VNTR). The genotype that occurred most often in the 5 HTTLP region was “SL” (60%), followed by “LL” (24.4%) and “SS” was 15.6%. The “L” allele was three times (3:1) more likely to be present than the “S” allele. In the VNTR polymorphism, the 12.12 repeat allele occurred most frequently (58.9%), the 10.10 allele occurred 22.2% of the time and the frequency of the 12.10 was 18.9%. The 12 allele was also three times (3:1) more likely to occur compared to the 10 allele. Current PTSD was found in 23.3% of individuals and 50.0% had lifetime PTSD symptoms. Further analysis only included lifetime PTSD individuals due to a small sample size with only current PTSD symptoms. No association was found for severity of PTSD and gender, occupational status, marital status, psychiatric history, or the 5HTTLPR and VNTR regions. Both alleles with “higher activity", the “L” and the 12 allele impacted symptoms associated with PTSD. The “L” allele impacted hyper arousal symptoms with individuals having milder or less hyper arousal symptoms while the 12 allele impacted the severity of avoidance symptoms. Individuals with the “S” allele had more severe PTSD but this was not observed when other variables were controlled. The “L” allele continued to be significant in its effect when other variables were controlled. Weaknesses noted for this study included the small sample size and the severity and type of trauma, which was physical trauma and considered "mild" (Sayin et al., 2010, p. 75).

Hyper Arousal Symptoms Only: Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP)

Ressler et al. (2011) analyzed PTSD symptom clusters and genotype. The “CC” genotype of rs2267735 in women was associated with total PTSD symptoms and hyper

arousal symptoms. When other variables were controlled (childhood trauma, adult trauma, age, and race), the "CC" genotype was associated with hyper arousal symptoms compared to "G" carriers. Ressler et al. further examined fear and startle response (hyper arousal symptoms) related to gender, genotype, and PTSD. Females with PTSD and the "CC" genotype (rather than G genotype) of rs2267735 were less able to tell the difference between a conditioned stimulus and a safety stimulus and exhibited a greater startle response in the dark than in the light compared to men. Ressler et al. suggested that this SNP may be implicated in why gender differences are noted between males and females in their PTSD symptoms.

Re-Experiencing Symptoms Only: Apolipoprotein E Isoforms

Freeman, Roca, Guggenheim, Kimbrell, and Griffen (2005) studied apolipoprotein (Apo) E alleles (apoE2, apoE3, and apoE4) in 54 Caucasian, combat related PTSD veterans. Individuals with one allele (apoE4) were compared with other subjects; no association was found (Freeman et al., 2005). An association was found for individuals with the apoE2 allele and the re-experiencing symptom of PTSD (Freeman et al., 2005). Individuals with this allele scored lower on memory scores (Freeman et al., 2005). Limitations of this study included the small sample size and high psychiatric comorbidity (Freeman et al., 2005). Olsen, Agam, Davis, and Raber (2012) also examined the ApoE isoforms (apoE2, apoE3, and apoE4) in targeted and wild type mice and found mice with apoE2 acquired and retrieved a fear response but were not able to extinguish the fear response. Wild type mice with apoE3 and apoE4 were able to extinguish the fear response.

PTSD and Sleep (Nongenetic Literature)

This section reviews the literature for *Research Question 3*. The literature focuses on one candidate gene that has been identified in PTSD and also in genetic literature related to sleep. The remaining literature review focuses on the nongenetic literature establishing the relationship between PTSD and sleep related concerns. The chapter concludes with the GWAS completed to date.

Catechol-o-methyltransferase (COMT)

Kolassa, Kolassa, et al. (2010) examined different genotypes (Val/Val, Met/Met, and Val/Met) of the catechol-*o*-methyltransferase (COMT) encoded on chromosome 22q11.1-q11.2 with polymorphism at codon 158 and the interaction of traumatic load (defined as the number of different traumatic experiences not the frequency of witnessed or experienced events). A total of 424 non-related Rwandese refugees participated. Results included dose-response relationship to prevalence rates for lifetime PTSD and traumatic load (i.e., the greater number of traumatic events the greater prevalence of lifetime PTSD). Lifetime PTSD was impacted by genotype and traumatic load. The greater the traumatic load the more likely an individual had experienced lifetime PTSD (Kolassa, Kolassa, et al., 2010).

An effect was also found with the Met/Met genotype, traumatic load, and PTSD. There was a greater likelihood of developing PTSD with very few traumatic events for individuals with the Met/Met genotype without an effect on lifetime PTSD prevalence. Traumatic load was also noted as a risk factor for the frequency of lifetime PTSD for genotypes Val/Met and Val/Val. When more than 15 traumatic events occurred,

differences in prevalence rates between genotypes did not occur. Limitations of the study included: the small sample size for Met/Met genotypes and lack of identifying other comorbid Axis I disorders (Kolassa, Kolassa, et al., 2010). Genetic sleep research is beyond the scope of this dissertation however, the COMT (Val 158Met polymorphism) has been studied in a healthy population by Goel et al. (2011) related to chronic partial sleep deprivation (PSD). Goel et al. (2011) suggest the COMT Val158Met region may predict sleep patterns in healthy individuals as well as shed light on sleep related difficulties in other psychiatric conditions.

The nongenetic relationship between sleep and PTSD is well established. Klein et al. (2003) studied 102 participants (26 with PTSD and 76 trauma exposed without PTSD) and 19 patients for one year who were having elective orthopedic surgery. Participants were followed at 1 week, 3 months, and 12 months. PTSD individuals reported more insomnia for week 1 compared to victims of trauma without PTSD or the comparison group. At 1 month, both insomnia and excessive daytime sleepiness (EDS) were significant in the PTSD group compared to the other groups. When the three sleep variables (duration, efficiency, and restfulness) were further examined, PTSD participants had a shorter duration compared to the other groups. This was noted at the three month interval but did not continue to the 12 month time period. At 12 months, the PTSD and non PTSD groups slept longer than the control group. No other correlations were noted. Klein et al. conducted a follow up study one year later with eight PTSD participants and six non-PTSD participants and found no significant differences between participant

groups. The authors suggest sleep misperception may be the primary complaint for PTSD participants.

Lewis et al (2009) studied 541 Australian male Vietnam combat veterans with and without PTSD. Veterans with PTSD had statistically significant mean scores on sleep quality, sleep latency, sleep duration, sleep disturbances, sleep medication, daytime dysfunction, and a global sleep score; only sleep efficiency was not found to have a statistically significant difference. Lewis et al. indicated PTSD veterans had the highest scores compared to other community samples and suggested that sleep problems in veterans may be the result of changes that have to be made in sleep schedules to accommodate the demands of the military or sleep problems, such as nightmares that lead to changes in sleep patterns.

Germain, Hall, Shear, Nofzinger, and Buysse (2006) studied effects sizes in PTSD individuals (n = 10) and a group of healthy controls (n = 5). Medium effect sizes were noted between PTSD cases that had more sleep disturbances and stress symptoms. Variables with the largest effects (either medium to large effect sizes) between the PTSD and the control group were for two specific sleep disturbances: apnea-hypopnea index (AHI) and periodic limb movements with arousal (PLMA); large effect sizes were noted for the percentage of Stage 3 and 4 sleep and for wake time after sleep onset (WASO). On visually scored variables, effects were noted; PTSD cases had longer sleep latency and shorter sleep duration (Germain et al., 2006). REM latency had a large effect size compared to time spent in REM sleep and REM Count. A small effect size was noted for controls in the percentage of time spent in Stage 3, Stage 4, and WASO.

van Liempt et al. (2011) studied obstructive sleep apnea (OSA) in 20 veterans with PTSD, 24 veterans without PTSD (trauma controls), and 17 health controls (HC) matched to PTSD trauma veterans on age, year, and region of deployment. There were 15 apnea hypopnea indices (AHI) in the PTSD group (10%) compared to 13% in trauma controls and 12% in health controls, though no significant results were found. There were greater than 10 AHI per hour noted in 29% of PTSD participants, 21% of trauma controls, and 29% of healthy controls, though no significant results were noted. Participants with AHI scores of greater than10 also had higher PTSD CAP scores compared to those without OSA. CAPS and AHI were significant for PTSD participants; subscales that correlated to CAP scores for PTSD found a relationship with the D subscale related to hyper arousal symptoms (van Liempt et al., 2011).

A meta-analysis of 20 studies representing various types of trauma (i.e., civilian trauma, combat trauma, accident, war related trauma, or weather related trauma) was completed by Kobayashi et al. (2007). They found that PTSD individuals, regardless of gender, had more difficulty with Stage 1 sleep, REM density (REMD), and slow wave sleep (SWS). Male PTSD participants specifically had shorter total sleep time (TST), Stage 2, longer sleep onset latency (SOL), and REMD. Kobayashi et al. indicated in the male group, participants were members of the military. Age, sex, depression, and substance use moderated the effects of sleep on PTSD; Kobayashi et al. suggested that these moderator variables may explain some of the conflicting results obtained from previous studies. Younger individuals with PTSD did not have more sleep difficulties. Kobayashi et al. reported the time since the traumatic experience may have impacted the

findings on age and its effect on sleep. Type of trauma (military vs. nonmilitary) may be associated with greater sleep difficulties (Kobayashi et al., 2007). Substance use moderated PTSD on REM, REM latency with REMD having the most significant impact. Kobayashi et al. suggested that this may be related to the hyper arousal symptoms of PTSD however it is unclear if substance usage occurs before hyper arousal symptoms develop or if substances are used in response to hyper arousal symptoms.

Gellis et al. (2010) examined 201 veterans with PTSD from the Iraq and Afghanistan Operation Iraqi Freedom/Operation Enduring Freedom (OIF/OEF). Sleep difficulties of the veterans included nightmares and difficulties initiating and maintaining sleep (DIMS). Of the 201 participants, 64 participants suffered from severe nightmares, 38 experienced moderate nightmares, and 99 reported little or no nightmares. Severe DIMS was reported by 122 participants: 33 reported moderate DIMS and 46 had no DIMS or little difficulty with DIMS. An association was found between nightmares and DIMS. Additionally, African Americans reported greater DIMS compared to Caucasians. Odds ratios found severe DIMS was associated with nonsleep PTSD severity (*OR* = 1.14) and moderate DIMS (*OR* = 1.06). Nonsleep related PTSD symptoms had higher OR for severe (*OR* = 1.11) and moderate nightmares (*OR* = 1.06). van Liempt et al. (2011) examined nightmares and obstructive sleep apnea (OSA); no significant results were found related to nightmares, obstructive sleep apnea (OSA), and CAP subscales.

Langston et al. (2010) studied 47 children and adolescents that suffered from nightmares. The sample consisted of children and adolescents that experienced

nightmares prior to a traumatic experience (28% reported idiopathic nightmares), nightmares that began following a traumatic experience (23%), or no nightmares (49%). Individuals exposed to a traumatic experience reported weekly nightmares (Langston et al., 2010). Langston et al. found participants with PTSD related nightmares compared to those with no nightmares had more difficulty with time to get to sleep, sad upon waking, global sleep quality, depression, and PTSD. They indicated it is unclear what may be the reason for these differences and suggest the traumatic event may play a role in the differences noted.

Both post trauma nightmares and the idiopathic nightmare group reported a fear of sleep and poor sleep when compared to those without nightmares (Langston et al., 2012). Both groups also reported being "scared of dying, scared of being hurt, felt very scared, and felt unable to stop what was happening" (Langston et al., 2010, p. 351). Compared to idiopathic nightmares, the post trauma nightmare group had more panic symptoms when they woke up (Langston et al., 2010). The authors evaluated if type of trauma impacted nightmares and found no relationship with the exception of sexual abuse. When the traumatic experience was sexual abuse more post trauma nightmares were noted, 55% compared with 9% (Langston et al., 2010).

Limitations of studies related to PTSD and sleep have included small sample sizes, low power, and results that cannot be generalized beyond study participants. Results may be limited by concerns with measurement tools. For example, the DIMS was identified through a single assessment which may not adequately assess insomnia; more sensitive measures may be used to detect OSA; and the self-report nature of the

measurement tools may limit the interpretations as structured interviews did not verify self-reports; child measurement tools were modified from adult tools as child tools were not available; the measurement tool may not accurately collect sleep dimensions; the subjective self-report of sleep was not collected on the days when objective sleep data were collected.

The type of data used (self-report) may present problems. The study design may limit results. In one study only two sample groups were included in the study design, which limited additional evaluation of interaction effects. The time since the trauma was not considered so there may be additional sleep difficulties immediately following trauma that are not considered. Other variables such as depression, traumatic brain injury, and alcohol dependence may impact sleep difficulties independently; comorbid conditions which may be related to sleep problems, or there may be other sleep variables related to sleep and PTSD that were not considered; other potential moderators may be present such as hospitalization, location or type of sleep monitoring, comorbid sleep disorders, trauma related nightmares, and medication use. Diagnosis may also limit results. In one study, the PTSD diagnosis was confirmed at 1 year however, other participants may have met the diagnosis earlier but were not included in the PTSD sample (Gellis et al., 2010; Klein et al., 2003; Kobayashi et al., 2007; Langston et al., 2010; van Liempt et al., 2011).

Walker's (2009) hypothesis suggests sleep disorders associated with emotional disorders may not be a symptom of the disorder but may be the mechanism for the development of the emotional issue. As noted by Langston et al. (2010) children and adolescents that experienced nightmares following a traumatic event experienced more

distress than individuals without nightmares, particularly among individuals that had experienced sexual abuse. Of the different types of traumatic experiences, only sexual abuse was found to be associated with nightmares in Langston et al.'s study. Interestingly, individuals already experiencing nightmares and then having a traumatic experience did not have greater development of PTSD symptoms and did not have greater distress somewhat supporting Walker's hypothesis that sleep problems associated with emotional disorders may be the conduit for the development of the emotional issue (Langston et al., 2010; Walker, 2009).

Walker and van der Helm (2009) suggest that the adrenergic and cholinergic systems as well as the cortex, hippocampus, and amygdala are involved in sleep and memory consolidation. Although sleep neurobiology is beyond the scope of this dissertation, many of these same systems or areas of the brain have also been associated with PTSD through candidate gene or genetic allele studies and may increase an individual's risk for the development of PTSD. Pathways studied related to PTSD include glucocorticoid, dopamine, glutameric, and serotonin systems. Other candidate genes or genetic alleles play a role in the hypothalamic pituitary adrenal (HPA) axis and are involved in the stress response or in fear processing.

Genome-Wide Association Studies (GWAS)

Logue et al. (2012) examined 295 white, non-Hispanic veterans (men and women) with PTSD and 196 of their partners who acted as controls. Replication analysis was done with an African American population from a veteran cohort (PTSD cases = 43 and controls = 41) and the Detroit Neighborhood Health Study (cases = 100 and controls

= 421). Logue et al. found SNP rs8042149, in the retinoid-related orphan receptor alpha gene (RORα) reached genome wide significance and was associated with PTSD. SNP rs8042149 was analyzed to determine if genotype, probability of PTSD, and total number of traumatic events (NTE) were associated. Logue et al. found that "the probability of PTSD diagnosis by genotype indicated subjects with low levels of trauma exposure and a high risk (GG) genotype had a similar likelihood of developing PTSD as those from the high exposure but low risk (AA) genotype" (p. 3). The number of traumatic events was associated with risk for PTSD and gene rs8042149 was also significant. In the African American population, rs8042149 did not reach significance however rs1171588 remained significant in this population even after adjustment for multiple testing. In the DNHS sample, SNP rs16942660 was associated with lifetime PTSD. Logue et al. indicated that different SNPs in RORα may be associated with different ancestries and a function of different linkage disequilibrium patterns. A limitation noted by Logue et al. was the possibility of false associations due to chance and the small sample size.

Xie et al. (2013) conducted a GWAS using a sample that consisted of 1,633 (including 300 PTSD cases) European Americans (EA) and 2,766 (including 444) African Americans (AA); EAs and AAs were recruited from genetic studies on alcohol, cocaine, and opioid dependence recruited from five U.S. sites. The SSADDA was the tool used in this study. One SNP rs406001 on chromosome 7p12 exceeded the threshold for genome wide significance. Cordon-Bleu [COBL] is the gene closest to this region. A sub analysis focusing on a GWAS of PTSD subjects exposed to trauma identified SNP rs406001 on chromosome 7p12 for EAs was the most significant, however none of the

SNPS reached genome wide significance. Xie et al. completed replication analyses and found in the EA population SNP, rs6812849; in the Tolliod-Like 1 gene [TLL1] was successfully replicated. Interestingly, Xie et al. suggested that trauma exposure is less of a contributing factor to the development of PTSD; they indicated the genetic region identified in their study, TLL1, may be a better predictor of PTSD than trauma exposure and indicated this region is a "new susceptibility gene" (p. 656). Xie et al. did not find any genome wide associations among the AA populations studied. Nor did they find any associations that were consistent with other genome wide association studies on PTSD. Limitations noted by Xie et al. included their study population was from a substance dependence study and therefore results may not be generalized to other PTSD populations; additionally, sample sizes were small in this study and specific to European Americans.

Guffanti et al. (2013) conducted a genome wide association study that focused on PTSD, specifically in women. Guffanti et al. suggested that gender played a greater role in the genetics associated with PTSD. They focused their study on African American women from the Detroit Neighborhood Health Study and attempted to replicate their study using a cohort of European American women from the Nurses Health Study II (NHS II). SNP rs10170218 in the ACO68718.1 region reached genome wide significance in the DNHS sample and was tested for evidence of association in the NHS sample. A sub analysis was also done to account for potential differences related to racial differences using a dominant and recessive model (Guffanti et al., 2013). SNP rs10170218 had the strongest signal. The replication analysis found the same SNP with

the minor allele "C" frequency to be 0.27 in the NHS II population compared to 0.22 in the DNHS. Guffanti et al. describe RNA gene ACO68718.1 as lincRNA that has "transposable elements (TE)" which are "discrete pieces of DNA that can move within genomes, inserting themselves into new genomic sites and generating interspersed repeats of the original TEs" (p. 5). SNP rs10170218 maps to Intron 3-4, 236 kb upstream from exon 4 overlapping with class LTR8B; little is known about the functions associated with this genetic region. Limitations noted by Guffanti et al. included: the use of telephonic interviews to determine PTSD that may have biased their results and the study was designed to only find strong genetic effects.

Solovieff et al.'s (2014) study different somewhat from the other three GWAS. They used results from the Psychiatric Genome Wide Association Studies Consortium (PGC) to test 3,742 SNPs across 300 genes in trauma exposed European American women (845 PTSD cases and 1,693 controls). Solovieff et al. tested an association with the SNPs and two PTSD measures: severity score and diagnosis. PTSD diagnosis was found to be associated with SNP rs363276 (OR = 1.4, $p = 2.1 \times 10^{-5}$) in SLC18A2. Nine SNPs in this same region, including the SNP with an association was analyzed in a haplotype analysis. A risk haplotype (CGGCGGAAG, p = 0.0046) was significant and then replicated in an independent cohort of trauma exposed African Americans (p = 0.049). The SLC18A2 has been associated with other psychiatric disorders including major depression but had not been previously identified for PTSD. Solovieff et al. found eight genes (APOE, BDNF, COMT, FKBP5, HTR2A, SLC6A3, SLC6A4, and TPH2), previously identified in the literature associated with either PTSD diagnosis or severity;

none of the results remained significant after adjusting for multiple testing. They also completed separate analyses on each of the regions identified in the other three published GWAS. They replicated only lincRNA ACO68718.1 from Guffanti et al. (2013). Limitations noted included: phenotypic data were identified by telephone allowing for the possibility of misclassification of PTSD and the study had low power to detect weak effects (Solovieff et al., 2014).

The GWA studies published have reported on different areas of the genome. Limitations to the studies may be due to sample size; however, sample sizes have been consistent with other GWAS on PTSD (Guffanti et al., 2013; Logue et al., 2012; Xie et al., 2013). Walker and van der Helm (2009) indicate memory and sleep may be an appropriate theoretical framework to work from when examining PTSD and other psychiatric issues that have sleep problems as part of their presentation. Logue et al. (2012) found that SNP rs8042149, in the retinoid-related orphan receptor alpha gene (RORα) reached genome wide significance and was associated with PTSD. The RORα gene is thought to be a gene involved in circadian rhythm functioning (Akashi & Takumi, 2005). The RORα gene was found to be important in regulating *Bmal1* transcription factor in mice (Akashi & Takumi, 2005). When the RORα did not function properly in promoting *Bmal1*, the circadian rhythm did not function properly; the RORα was considered a "component of the core circadian clock"… and "acts as to promote *Bmal1* transcription, thereby maintaining a robust circadian rhythm" (Akashi & Takumi, 2005, p. 441). The RORα gene may be involved in chronic inflammatory processes such as heart disease or arthritis (Delerive et al., 2001); Boscarino (2008) found individuals with

higher PTSD scores had a 20% increased risk of mortality from heart disease. Logue et al. suggested risk for PTSD from the RORα gene may "confer…a trait negative emotionality…conceptualized as a primary temperamental risk factor" (p. 5). I suggest the RORα gene's impairment leads to the disruption of the normal sleep cycle initiating a cascading effect that impacts memory consolidation and processing of emotional content.

Summary and Conclusions

This literature review provided a comprehensive review of the literature on PTSD including genetic alleles and their relationship to PTSD. The review identified genetic alleles that conferred risk for PTSD, those with mixed results, and genetic alleles that may confer protective factors. Genetic alleles associated with specific PTSD symptoms were reviewed. The literature associated with sleep, a symptom associated with PTSD, and PTSD has been well established and was reviewed to establish the importance of the theoretical hypothesis / framework (Walker & van der Helm, 2009). Recent GWA studies were also reviewed. Chapter 3 reviews the study design, methodology, and statistical analysis for the study.

Chapter 3: Research Method

Introduction

This dissertation study involved the use of a human genome epidemiology approach to focus on discovering the possible genetic risk factors associated with posttraumatic stress disorder using a case control study design and a genome-wide association study method (GWAS) to compare the genome of individuals with PTSD to that of controls. Any genetic allele reaching genome-wide significance was further analyzed in a multi-step process to determine whether there is an association between the allele and the main symptoms of PTSD and then the sub symptom of sleep. Prior research has focused on individual genetic alleles in PTSD rather than the whole genome. The published genome-wide association studies (GWAS) on PTSD have identified new areas of the genome not previously discovered (Guffanti et al., 2013; Logue et al., 2012; Solovieff et al., 2014; Xie et al., 2013). Only one study (Solovieff et al., 2014) replicated the results from other GWAS. However, none of these studies have addressed the symptoms of PTSD, specifically the symptom of sleep, in the context of the genome.

Chapter 3 contains an outline of the design, methodology, and statistical analysis that were used in this study. The chapter begins with a review of the research design and rationale, followed by information on both the independent and dependent variables. The population is reviewed along with the sampling procedure. Procedures for recruitment are reviewed. This study used archival data (both genetic and survey data); information on the use of archival data is provided. The instrumentation and the operationalization of the instrument are reviewed. The chapter continues with a review of threats to validity

and the data analysis plan. The reader is reminded of the research questions under study. Ethical procedures are reviewed. The chapter concludes with implications for the study.

Research Design and Rationale

The gap in the literature at the time of the start of this dissertation suggested that a genome-wide association approach was needed to study the possible complex pathway(s) for PTSD development (Cornelius et al., 2010; Feodorova & Sarafian, 2012; Norrholm & Ressler, 2009). Four GWAS studies have been completed; however, none of these studies have examined PTSD symptoms or specifically the sleep symptom of PTSD and the human genome (Guffanti et al., 2013; Logue et al., 2012; Solovieff et al., 2014; Xie et al., 2013). The genome wide association method was chosen for the study design, as the literature suggested that PTSD had a number of potential genetic risk factors. Whole genome association studies (WGAS) allow the researcher to examine the entire genome, limiting errors related to missed associations that can occur when studying specific candidate genes, and is effective for studying complex diseases with multiple possible contributory genes (National Library of Medicine, 2011).

Independent and Dependent Variables

The independent variable was PTSD status (i.e., individuals [cases] with PTSD vs. controls [individuals without PTSD]). The dependent variables were the main symptoms of PTSD with specific focus and analysis on the variable of sleep, a sub symptom of the hyper arousal symptoms. Following the initial analysis, any genetic allele that reached genome-wide significance moved to a second phase of analysis to discover whether a correlation existed between the genetic allele and the main

symptom(s) of PTSD. The final step in the analysis was to analyze any genetic allele reaching significance with the sleep variable.

Covariates

Covariates identified in the literature have included age, sex, race, socioeconomic status, number of traumas, smoking, lifetime depression, and other Axis I disorders (Change et al., 2012; Shea, Vujanovic, Mansfield, Sevin, & Liu, 2010; Udden et al., 2011). Other covariates include BMI, gender, use of anxiolytics, antidepressants, type of trauma, age at which trauma occurred, intensity of trauma, education, rural/urban, other co-occurring disorders such as major depressive disorder, drug dependence, substance abuse, Axis 2 disorders, and self-esteem (Al-Turkait & Ohaeri**,** 2008; Babson et al., 2011; Frans et al., 2005; Krakow, Germain, et al., 2001; Krakow, Hollifield, et al., 2001; Udden et al., 2011). Nishi et al. (2012) identified that a covariate of watching television coverage of the Great East Japan earthquake of 2001 for more than 4 hours predicted PTSD symptoms among rescue workers.

Mediator Variables

Carlozzi, Reese-Melancon, and Thomas (2011) suggested that depression may be a mediator variable in individuals with PTSD and memory complaints. There were no differences noted between groups for objective memory; however, group differences were noted for subjective memory, with individuals with PTSD reporting more subjective memory complaints. When depression was included as a covariate, subjective memory differences did not occur (Carlozzi et al., 2011).

Moderator Variables

A database from the NCBI that contained genomic data on individuals with PTSD was requested. The database contained data on age, race, ethnicity, gender, sedative dependence, tobacco dependence, stimulant dependence, and substance abuse. These variables were the covariates for the second phase of analysis. The study variables in the database are consistent with what has already been identified in the literature under covariates. Other disorders, particularly substance use disorders, are often co morbid with PTSD. Because the original study, phs000425.v1.p1, "Alcohol Dependence GWAS in European and African Americans," was collected as part of a substance abuse genome set, substance abuse was included as a covariate in this dissertation. In a study by Xie et al. (2010) alcohol dependence had an interactive effect between childhood maltreatment and the FKBP5 polymorphism on increased PTSD risk.

Population

A genome-wide database with de-identified data were requested from the National Library of Medicine's National Center for Biotechnology Information (NCBI). The dbGaP database contained a GWAS dataset that was collected as part of a study on alcohol dependence in European and African Americans (Gelertner & Krazler, 2010); PTSD was included as one of the study variables collected as part of the dataset. The target population was composed of individuals identified with posttraumatic stress disorder (PTSD) whose genomic data were collected as part of a larger genome wide association study on alcohol dependence by dbGAP from the National Center for Biotechnology Information (NCBI). PTSD status was determined from the SSADDA

tool. The study collected genomic data from 2,909 individuals: 1,889 African Americans, 1,020 European Americans, and 491 control subjects. Approximately 500 individuals with PTSD were identified in the original study. The following statement is provided to acknowledge the original study:

> Funding support for the [CIDR-Gelernter Study] was provided through the Center for Inherited Disease Research (CIDR) and the Genetics of Alcohol Dependence in American Populations (CIDR-Gelernter Study)]. [CIDR-Gelernter Study] is a genome-wide association study funded as part of the [Genetics of Alcohol Dependence in American Populations]. Assistance with phenotype harmonization and genotype cleaning, as well as with general study coordination, was provided by the [Genetics of Alcohol Dependence in American Populations]. Assistance with data cleaning was provided by the National Center for Biotechnology Information. Support for collection of datasets and samples were provided by the Genetics of Alcohol Dependence in American Populations (R01 AA011330). Funding support for genotyping, which was performed at the Johns Hopkins University Center for Inherited Disease Research, was provided by the NIH GEI (U01HG004438), the National Institute on Alcohol Abuse and Alcoholism, and the NIH contract 'High throughput genotyping for studying the genetic contributions to human disease' (HHSN268200782096C). The datasets used for the analyses described in this manuscript were obtained from dbGaP at http://www.NCBI.nlm.nih.gov/sites/entrez?Db=gap through dbGaP accession number [phs000425]. (Data Use Certificate Agreement, 2010, pp. 4-5)

Sampling and Sampling Procedures

Using the population of 500 individuals with PTSD with a 95% confidence level and a confidence interval of 4, the sample size required for cases is 273. A 99% confidence interval requires a sample size of 338. Data were collected on 491 controls. Using a 95% confidence level and a confidence interval of 4, the sample size required for the control population is 270. A p value of $\alpha = 95\%$ is consistent with the literature for GWA studies (Qu, Tien, & Polychronakos, 2010). An online sample size calculator was used from (http://www.surveysystem.com/sscalc.htm) to calculate the sample size noted below for both cases and controls.

Table 1

Determining Sample Size—Cases

Confidence Level:	99%
Confidence Interval:	4
Population:	500
Sample size needed:	338

Table 2

Find Confidence Interval—Cases

Confidence Level:	99%
Sample Size:	338
Population:	500
Percentage:	50
Confidence Interval:	4

Table 3

Determining Sample Size—Controls

Confidence Level:	99%
Confidence Interval:	4
Population:	491
Sample size needed:	334

Table 4

Find Confidence Interval—Controls

Confidence Level:	99%
Sample Size:	334
Population:	491
Percentage:	50
Confidence Interval:	4

A request was made for the database of the 500 individuals with PTSD and the 491 controls along with a request for individual, de-identifiable data on both cases and controls. The entire population was used to complete this study. The sample size in other GWA studies have ranged from 94-444 PTSD cases (Guffanti et al., 2013; Logue et al., 2012; Xie et al., 2013). Logue et al. (2012) had 295 cases and 196 controls; Xie et al. (2013) had 300 and 444 PTSD cases respectively; and Guffanti et al. (2013) had 94 PTSD cases. The study design has adequate power using the entire PTSD population and controls. I acknowledge there was no PTSD only population without substance abuse. Substance abuse was evaluated as a covariate and this limitation was noted in the limitations section of the study. Although this is a limitation of the dissertation, this population group, use of PTSD cases collected as part of a substance abuse study was consistent with other published literature (Xie et al., 2013).

Use of Archival Data

A case control genome wide association study was conducted by the National Center for Biotechnology Information (Gelertner & Krazler, 2010). The original study phs000425.v1.p1 titled "Alcohol Dependence GWAS in European and African Americans" collected genomic data on alcohol dependence (NCBI) using the Semi Structured Assessment for Drug Dependence and Alcoholism (SSADDA) questionnaire; which assessed for alcohol and other psychiatric disorders including posttraumatic stress disorder (Gelertner & Krazler, 2010). The study collected genomic data from a total of 2,909 individuals, 1,889 African Americans, 1,020 European Americans, and 491 control subjects, which were collected over three studies that focused on genomic data associated

with alcohol, cocaine, and opioid dependence. Affected sib pairs were collected during the studies when available. Inclusion criteria in the original study were individuals with a DSM-IV diagnosis of alcohol dependence. Excluded were individuals with diagnoses of schizophrenia, bipolar affective disorder, other major psychotic illnesses, or individuals with gross cognitive impairment (Gelertner & Krazler, 2010). A total of 24 variables related to alcohol, phobias, or other psychiatric disorders were collected as part of this study including individuals with PTSD. Additional variables specific to alcohol related issues (i.e., tolerance, withdrawal, and sedative use) were collected. Demographic data collected included gender, age, and race (Gelertner & Krazler, 2010). Whole genome typing was done by Illumina using the HumanOmni1_Quad_v1-0_B platform. Genomic data were collected on approximately 500 individuals with PTSD as part of the original study (Gelertner & Krazler, 2010). Information about the dataset indicated the database should be used in connection with substance abuse research. Only a subset of the database was requested; the data associated with the PTSD variable. A request was made to the National Center for Biotechnology Information for access to only the PTSD section of the database including the individual level dataset that included the responses to the SSADDA questionnaire. To obtain access to the controlled individual-level data, the Principle Investigator (PI) and the Signing Official (SO), which was required to be a representative from the institution, someone with institutional authority, typically this would be a Dean of a program had to cosign the request for data access (eRA Commons, 2013). The information provided on the SO indicated the SO could delegate this function to another person; I requested Dr. Shen, my Chair, be delegated this function (eRA

Commons, 2013). The SO is responsible for ensuring the safety of the genomic data, which is consistent with Dr. Shen's and my role was in the course of this dissertation. The PI directed the research project (eRA Commons, 2013). The request for the database was made to the NIH Data Access Committee at the appropriate NIH Institute or Center, in this case, the Joint Addiction, Aging, and Mental Health DAC. Please note both the PI and the SO were required to have an eRA commons account and co-sign a request for data access. These are the same accounts used to apply for grants. In the eRA commons account when there is a first request for data, a new data request must be filled out by the PI. Form SF 424 (R&R) must be completed. This form was completed and submitted to Dr. Shen and the IRB. IRB approval was required and to be submitted with SF 424. Basic information on the research project was also required that included: the title of the research, the type of research, a statement of no more than 2,000 characters describing the research, a non-technical summary of no more than 1,100 characters, and information on the SO and PI.

I acknowledge that requesting the data constituted an agreement to the terms of use specified in the "Data Use Certification" (DUC) documents provided on the website. The DUC statements outline policies and procedures for using the data, such as limiting use to the project described in the Data Access Request form; not distributing the data beyond those permitted to handle it; not attempting to identify or contact study participants from whom phenotype data and DNA were collected; awareness of the specified principles regarding intellectual property; adhering to policies on the timeframe for publications stemming from the data; and other provisions designed to protect the

confidentiality of study participants and foster scientific advance (Data Use Certificate Agreement, 2010). To ensure applications moved through the submission and review process in a timely way, the SO and PI received various emails providing updates on the status of the request or any required actions. The data access request was then reviewed by the appropriate Data Access Committee(s) at NIH.

Instrumentation and Operationalizing Constructs

Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA)

The original study, phs000425.v1.p1 titled "Alcohol Dependence GWAS in European and African Americans" collected data on PTSD using the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA) (Gelertner & Krazler, 2010). The SSADDA is a tool developed to study genetic association studies involving cocaine and opioid dependence (Pierucci-Lagha et al., 2005). The SSADDA tool was originally developed from the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA); a free copy of the tool is available at http://genetics.bumc.bu.edu/ssadda/ (Boston University, n.d.). "The SSADDA provides extensive coverage of the physical, psychological, social, and psychiatric manifestations of cocaine and opioid abuse and dependence in addition to a number of related Axis I and Axis II disorders" (Samet et al., 2007, p. 28). The PTSD section of the SSADDA collected data on 12 different types of traumatic experiences that included: direct combat in a war; seriously physically attacked or assaulted; physically abused as a child; seriously neglected as a child; raped; sexually molested or assaulted; threatened with a weapon; held captive or kidnapped; witnessed someone being badly injured or killed;

involved in a flood, fire, or other natural disaster; involved in a life-threatening accident; suffered a great shock because one of these events happened to someone close to you; and other (Xie et al., 2009). There are 24 questions with various sub questions that focused on PTSD symptoms. A computerized version of the tool allowed for direct entry of responses by participants as well as a direct upload of data to a database (Boston University, n.d.).

Pierucci–Lagha et al. (2005) examined inter rater reliability and test retest reliability for the SSADDA using 293 participants in two sub studies. For the PTSD section of the tool, they found inter-rater reliability rates to be fair (k = 0.59, 95% CI 0.40-0.77; Y = 0.73) and test retest reliability to be k = 0.76 (95% CI 0.53-0.99), Y = 0.88. PTSD was the only diagnosis whose prevalence rates increased between the test and retest period (p = 0.041; Pierucci-Lagha et al., 2005). The SSADDA tool has been used in studies related to genes and PTSD; Xie et al. (2009) studied interactive effects of PTSD, childhood adversity, adult trauma, gene (5 HTTLPR region), and gene environment interactions. Douglas et al. (2010) used the SSADDA to evaluate adverse childhood experiences (ACE) as risk factors for substance abuse. Ford et al. (2009) used the SSADDA to evaluate co morbid psychiatric and substance disorders treatment utilization in the context of several groups diagnosed with cocaine dependence. Thavichachart et al. (2009) used the SSADDA to identify the presence of chronic PTSD following the December 2004 tsunami off the Andaman Coast in Thailand. The SSADDA has also been used to study attention deficit hyperactivity disorder and substance abuse disorders (Arias et al., 2008); has been used in genome-wide association

studies on substance abuse disorders (Gelernter et al., 2006) and a genome wide association study on PTSD (Xie et al., 2013).

Threats to Validity

External Validity

Testing reactivity was not a factor as the study design was not a pretest posttest study design (Southern Utah University [SUU], n.d.). Interaction effects were not a factor as neither the study population nor the experimental variables were so unique or specialized that results cannot be generalized (SUU, n.d.). Participants in the original study were both Caucasian and African American; Hardy Weinberg equilibrium determined that the groups were comparable. The sample included the entire sample of PTSD cases and controls and had adequate power; I suggest the results are applicable only to the population under study at this time. Reactive effects were minimized as genomic data is not subject to the same biases as survey data. The use of the SSADDA data did not eliminate reactive effects as the original study design was not a naturalistic observation (SUU, n.d.). Multiple treatment interference was not a concern as the study was not examining the effect of any treatment (SUU, n.d.).

Internal Validity

History was not considered a threat to internal validity for genomic data; in terms of the data collection from the questionnaires, history was not be a concern as multiple testing did not occur in the study design (SUU, n.d.). The study was not subject to maturation (testing was not occurring over a long period of time) (SUU, n.d.). Testing (i.e., the Hawthorne effect) was a concern as participants were aware they were

participating in a study (SUU, n.d.). Instrumentation was not a concern for this study as specific instruments were not being used (SUU, n.d.). Statistical regression was not a concern as this was not a repeated measures design (SUU, n.d.). Experimental mortality was not a concern as all the data from the original study have been collected. Selection maturation was not a concern with the population under study (SUU, n.d.).

Threats to Construct or Statistical Conclusion Validity

Sources of bias in genetic epidemiology include selection bias, information bias, and confounding (Garcia-Closas, Wacholder, Caporaso, & Rothman, 2004). The original study included genomic data from participants during three different studies. The potential for information bias related to misclassification or measurement error from the original study phs000425.v1.p1, "Alcohol Dependence GWAS in European and African Americans" (i.e., "biological sample collection, processing and storage, sample labeling, DNA extraction and storage, laboratory assays, data coding, entry and analysis") was addressed by ensuring the two groups were in Hardy Weinberg equilibrium (Garcia-Closas et al., 2004, p. 131; Gelertner & Krazler, 2010). Potential confounders identified in the literature, race, ethnicity, gender, age, sedative dependence, stimulant dependence, tobacco dependence and substance abuse, were evaluated to determine if they should be included as covariates for the second phase of analysis.

Operationalization

The SSADDA tool (Pierucci-Lagha et al., 2005) uses a number of questions that identify symptoms (re-experiencing, avoidance/numbing, hyper arousal, and sleep) associated with PTSD. Participants responded with a "yes" or "no" when answering the

questions. These questions operationalized PTSD symptoms. Examples of some of the questions are noted below:

Re-Experiencing/Intrusion

O2 Did memories, visions, thoughts, or feelings about (EVENT) often keep coming to your mind even though you didn't want them to?

O4 Did you ever suddenly act or feel as if the (EVENT) was happening again? This may include flashbacks, or hallucinations, even if they occur when you are just waking up?

Avoidance/Numbing

O8 Did you ever try to avoid thinking about or having feelings about (EVENT) and find that you couldn't?

O9 Did you ever avoid activities, places, or people that reminded you of (EVENT)?

O12 During that period of time, did you feel more cut off, distant, or separated from people than before the (EVENT) happened?

O13 Were there times when you believed you had lost the ability to experience emotions that you had before (EVENT) happened? For example, did you feel you couldn't have loving feelings or anything like that?

O14 Were there times when you felt that there was no point in planning for the future-that you might not have a rewarding career; a happy family; or a long, good life?

Hyper Arousal

06 Did things that remind you of the (EVENT) make you sweat, tense up, breathe hard, tremble or respond in some other physical way?

O19 Were there times when unexpected noise, movement, or touch startled you more than before (EVENT)?

O20 Were you more watchful or extremely aware of things around you? For example, were you more aware of certain sounds, smells, or sights?

The SSADDA had questions specific to sleep and memory.

Sleep

O3 Did you have unpleasant dreams again and again about (EVENT)?

O16 Did you have more trouble falling asleep or staying asleep than before (EVENT)?

Memory

O10 Did you find that you sometimes could not remember important things about (EVENT)?

The SSADDA collected information related to alcohol and drug usage while experiencing symptoms. These questions were identified as the questions that would be used in the second phase of analysis to control for alcohol and drug abuse in this population. Specifically, on *Question O21*, participants responded Yes-clean only to be included in the response.

Alcohol and Drug-Related Questions

O21 You have told me about things such as reliving the event through dreams, memories, or feelings; avoiding things that reminded you of the event; and problems with sleep, mood, or thinking. Did these experiences last longer than one month? Participants responded Yes-clean only were included in the response.

Additionally, participants responded with a "no" on the two questions related to alcohol and drug use.

Clustering at onset.

A. Around the time you first had these very intense feelings, were you having experiences from 3 or more boxes found on this (ALCOHOL/COCAINE/ OPIATES/ OTHER DRUG) sheet?

   Heavy use when not clustering

B. Around the time you first had these very intense feelings, were you (drinking heavily, using DRUGS) daily or almost daily?

Data Analysis Plan

Whole genome typing was done in the original study by Illumina using the HumanOmni1_Quad_v1-0_B platform (Gelertner & Krazler, 2010). The original database was exported into the most current version of PLINK (Purcell, 2009), which is a free toolset capable of completing analyses on large scale GWAS data. This toolset is appropriate for analyzing phenotype and genotype data. All four GWAS on PTSD have used PLINK software to complete the analysis (Guffanti et al, 2013; Logue et al., 2012;

Solovieff et al., 2014; Xie et al., 2013). Xie et al. (2013) excluded SNPs from study with a call rate of < 98%, with minor allele frequencies of < 1%, and controls whose SNPs were not in Hardy Weinberg equilibrium ($p < 1 \times 10^{-6}$); this was not applied to cases as Xie et al. indicated the presence of disease may impact Hardy Weinberg equilibrium (Anderson et al., 2010, as cited by Xie et al., 2013). Logue et al. (2012) excluded SNPs if > 5% missing genotypes or if SNPs were rare, defined as < 5% minor allele frequency. Guffanti et al. (2013) used SNPs that passed quality control filters that included a call rate > 95%, minor allele frequency > 0.01, Hardy Weinberg equilibrium $p > 1x10^{-6}$). The PLINK program has tools within the program to address missingness of data and quality control procedures; these tools were used and were consistent with other GWAS completed on PTSD.

Analysis was conducted on the data between cases and controls to identify any regions of the genome that reached a genome wide significance threshold. Thresholds that have been established included: $p = 5x10^{-8}$ (Guffanti et al., 2013); $p < 10^{-5}$ for evidence of association and $p < 5x10^{-8}$ for genome wide significance (Logue et al., 2012); and 6.51×10^{-8} for European Americans and 5.75×10^{-8} for African Americans (Xie et al., 2013). A genome wide study by Reiner et al. (2011) on white blood cells used $p < 2.5 \times 10^{-8}$. For the purpose of this study the genome wide association significance was established at $p = 5x10^{-8}$ consistent with both Guffanti et al. (2013) and Logue et al. (2012). The PLINK program can be used with Haploview analysis to provide linkage disequilibrium statistics, including haplotype blocks, population haplotype frequencies, and single marker association statistics, (including r^2as appropriate). PLINK software

has a program to adjust for population stratification. These tools were used as appropriate. An odds ratio for genetic alleles between cases and controls was calculated, as appropriate to account for effect sizes using chi square with an established statistical significance of $p < 0.05$. The SPSS software program was also used to study the effect size correlation between the independent and dependent variables. Gene(s) that reached the threshold were included in the second phase of analysis for additional testing with the main symptoms of PTSD (re-experiencing/intrusion, avoidance/numbing, hyper arousal) and the dependent variable of sleep; the second phase of analysis planned for each dependent variable to be evaluated independently in the most recent version of SPSS using multiple logistical regression to determine if there were any associations. For categorical data from the SSADDA (i.e., yes versus no to the questions) the chi square test statistic had an established statistical significance of $p < 0.05$ (Hebel & McCarter, 2012). Covariates evaluated for the second phase of analysis included: age, gender, race, stimulant dependence, sedative dependence, tobacco dependence, and alcohol abuse. Logistic regression was the statistical tool planned for the second phase of analysis to determine if a correlation existed between an identified gene and each of the dependent variables. Other statistical tools planned for the analysis included: the Odds Ratio for any significant association identified from the *t* test and chi square between PTSD cases and controls.

Research Questions and Hypotheses

The genome wide method is not hypothesis driven but rather discovery driven; research questions to be considered are:

- Research *Question 1*: Are there significant genetic alleles associated with PTSD?
- Research *Question 2*: Is there a significant association between genetic allele(s) and the symptoms of PTSD?
- Research *Question 3*: Is there a significant association between genetic allele(s) and the symptom of sleep? Is either the COMT or RORα, both genes that have been identified in sleep and PTSD, associated with sleep?

Ethical/Privacy Procedures

In the original study, phs000425.v1.p1, "Alcohol Dependence GWAS in European and African Americans" consent was obtained for general research related to alcohol and alcohol related phenotypes/disorders (Gelertner & Krazler, 2010). A data access request (DAR) was made to NCBI; I was the Senior/Key Person under the direction of Dr. Shen, the Chair of this dissertation study. A description of the research, the purpose of the research study, and a request for identifiable participant data from the original GWAS study from the PTSD only variable subset and the control population was requested. I utilized the genomic dataset in accordance with responsible research use and handling of genomic databases and database sharing as set forth in the parameters by the National Institutes of Health [NIH] (n.d.; NIH, 2007).

The Code of Conduct for dbGAP approved users was adhered to in this dissertation as well as the *Policy for Sharing of Data Obtained in NIH Supported or Conducted Genome Wide Association Studies (GWAS)* (Database of Genotypes and Phenotypes, , n.d.). The Code of Conduct required the researcher to:

- Agree to use and distribute the data only as requested in the Data Access Request
- Refrain from contacting participants from the study without IRB approval
- Allow only authorized users access to the data, follow relevant security procedures, and report any breaches of data
- Refrain from publishing information prior to the embargo date
- Acknowledge Intellectual Property Policies of the data
- Refrain from selling and GWAS information
- Provide annual progress reports on the GWAS dataset, as appropriate

(Database of Genotypes and Phenotypes, n.d.; NIH, n.d.)

The risk for identification was minimal as normal data that identifies an individual (i.e., name, date of birth, etc.) was not included in the dataset (NIH, 2007). The dataset contained a random unique code or identifier not associated with an individual's identifiable data to protect an individual's privacy and confidentiality (NIH, 2007). Only the original researchers have access to identifiable data (NIH, 2007); I did not have access to direct identifiers. Concerns still existed that individual data could be compared with an individual's genotype; however no additional datasets were used in this study and all security procedures were followed (NIH, 2007). The study was reviewed and approved by the Walden University IRB (09-10-14-0027013).

Security Procedures

I agreed to follow the data security requirements when using the dataset in accordance with the information contained in the Data Use Certificate Agreement and

completed all required trainings from the NIH Information and Security and Privacy Training Awareness (Database of Genotypes and Phenotypes, 2010). The Data Use Certificate indicated the dataset can be used in conjunction with substance abuse studies. As mentioned only a subsection of the dataset was requested (Database of Genotypes and Phenotypes, 2010).

The Database of Genotypes and Phenotypes (2013) security procedures for accessing de-identified individual (personal) level data were followed to ensure access to the data were limited only to individuals involved in this dissertation. The data were downloaded to a secure computer and saved on an external hard drive that was encrypted and password protected (Database of Genotypes and Phenotypes, 2013). The password was a secure password and was at least 8 characters in length and contained the following: lower case, upper case, numerals and special characters (Database of Genotypes and Phenotypes, 2013). Physical safety was considered with the data that included minimizing access to the data outside of the immediate research area and keeping all data in a locked location (Database of Genotypes and Phenotypes, 2013). The data were accessed in conjunction with the PLINK software that was also downloaded to the hard drive. Analysis of data occurred with programs on the external hard drive. Now that the dissertation is completed all data has been deleted with the exception of the minimum data required to comply with the appropriate data retention policies of the university (Database of Genotypes and Phenotypes, 2013).

Implications

This research provided information about genetic factors associated with PTSD. Positive Social Change implications included bringing attention to the genetic risk factors for PTSD to the research community; Genome wide research on PTSD has not had as much focus as other disorders such as major depression or schizophrenia (Cornelius et al., 2010). With many veterans returning to the United States following the recent war prevention and the importance of effective PTSD diagnosis and treatment is at the forefront of public health. Studying the whole genome identified additional correlations previously undiscovered, which could generate new research areas or topics for future research (Cornelius et al., 2010). Additionally, this research added to the existing GWAS on PTSD and contributed to building the framework for future studies that focus on PTSD.

Summary

A GWA study, using a case control study was used to examine the genomes of cases with PTSD and controls. Any gene that reached genome wide significance went on to phase two of analysis. Chapter 4 provides the results of this study.

Chapter 4: Results

Introduction

This purpose of this dissertation was to examine the genome of individuals with PTSD and the genome of non-PTSD controls to discover whether there were any allele(s) that reach genome-wide significance. Genetic allele(s) reaching genome-wide significance were moved to a second phase of analysis with the symptoms of PTSD, specifically the symptom of sleep. A genome-wide association study method is not hypothesis driven but rather discovery driven; therefore, specific hypotheses were not proposed. The following research questions guided the research:

- Research *Question 1*: Are there significant genetic alleles associated with PTSD?
- Research *Question 2*: Is there a significant association between genetic allele(s) and the symptoms of PTSD?
- Research *Question 3*: Is there a significant association between genetic allele(s) and the symptom of sleep? Is either the COMT or RORα, both genes that have been identified in sleep and PTSD, associated with sleep?

Chapter 4 begins with a review of the process for obtaining the database for the analysis, followed by a review of the study population, including descriptive and univariate statistics. The next section contains a review of covariates to be included in the second analysis should an allele reach genome-wide significance. Results from the genome-wide association are presented along with responses to the research questions. The chapter concludes with a summary of the results and a transition to Chapter 5.

Data Collection

A data access request (DAR) was initially made to and then approved by [Request #32920-2] the National Center for Biotechnology Information Genotypes and Phenotypes (NCBI dbGAP) at the National Institutes of Health for research on alcohol and alcohol-related phenotypes/disorders. The Walden University Office of Research and Sponsored Programs coordinated the application process; I was identified as the senior/key person under the direction of Dr. Shen, Chair. The consent for this research was granted for general research use with respect to alcohol and alcohol-related phenotype disorders. Access of Alcohol Dependence GWAS in European and African-Americans was approved as Project #7620, "A Genome Wide Association Study on the Sleep Symptom of Post Traumatic Stress Disorder." Walden IRB approval was obtained conditionally before requesting the database and received final approval (09-10-14-0027013) following NCBI approval.

The original study, phs000425.v1.p1, "Alcohol Dependence GWAS in European and African Americans," was transferred from dbGAP using the Aspera Connect 3.5.2 secure transfer protocols and downloaded to an external encrypted hard drive (Gelertner & Krazler, 2010). Files were decrypted using the SRA Toolkit (NCBI, 2015) and the repository key provided by NCBI, then extracted twice prior to being ready for use. All analyses were conducted on a Windows 8 operating system.

The original study focused on alcohol dependence, cocaine, and opioid genetics and collected 3,258 genomic samples from individuals over three projects at Yale and Rutgers University and Coriell Institute for Medical Research (Gelernter, 2010; Gelertner

& Krazler, 2010). Quality control measures were implemented that included removing samples with poor performance (30), gender discrepancies (53), unexpected genotyping duplicates (141), and low volume (33). Data cleaning removed another 114 samples, and 43 samples had some other anomaly or discrepancy (Gelernter, 2010). Some samples may have accounted for more than one quality concern (Gelernter, 2010). Gelernter (2010) indicated that "the best indicator for sample data quality for Illumina Infinium chemistry and Genome Studio calling algorithm is the call rate" (p. 1). Following quality control measures, 2,932 samples were identified as high quality (90% call rate).

There were some discrepancies identified from the plan presented in Chapter 3. The original information on the dbGAP website indicated that information was available on 500 PTSD cases and 491controls. When the database was received, the files of 2,909 individuals were reviewed. The affection status of the participants was not coded in the genotyping files. An additional phenotype file included with the study identified the 24 variables noted on the website, including the PTSD phenotype; however, there were no identifiers for control subjects only. Therefore, after consultation with Dr. Shen, the study proceeded with the genomic data from 435 PTSD cases and 2,445 non-PTSD controls. There were 29 participants with unknown phenotype information included in the demographic characteristics section but were removed from the genomic portion of the study. Covariates provided in the phenotype files included age, gender, race, sedative dependence, stimulant dependence, tobacco dependence, and responses to seven questions on alcohol dependence from the SSADDA. Demographic characteristics are provided in Table 5.

Table 5

Descriptive Characteristics of Cases and Controls

	PTSD cases	Non-PTSD controls	Unknown	Group comparison
	N (%) / Mean (*SD*)	*N* (%) / Mean (*SD*)	*N* (%) / Mean (*SD*)	*p* value*
Number of participants	435	2,445	29	
Age	40.6 (9.2)	40.1 (10.2)	42.5 (8.5)	0.27781
Gender				.00 / λ.007
Males	190 (43.7%)	1,421 (58.1%)	13 (44.83%)	
Females	245 (56.3%)	1,024 (41.9%)	16 (55.17)	
Race				0.011 / λ1.0^
Native American		1 (.04%)		
Asian				
Pacific Islander				
African American	258 (59.3 %)	1,651 (67.5%)	18 (62.1%)	
Caucasian	177 (40.7%)	792 (32.4%)	10 (34.5%)	
Other		1 (0.4%)	1 (3.4%)	

*p-value from continuous variables using unpaired t test ($p < .05$); ^Monte Carlo estimate for Pearson chi square used and λ for categorical variables.

The PTSD prevalence rate in the sample population of the study was 15%, which is considerably higher than the prevalence rate of PTSD in the general population, 3.6% (National Comorbidity Survey, 2007). Alonso et al. (2004) found that the prevalence rate for PTSD in Europeans over the past 12 months was 0.9% (95% CI 0.7-1.1). African Americans had lifetime prevalence rates of 8.7% compared to Whites, whose rate was 7.0-7.4% (Roberts et al., 2011). The prevalence rate for this study was more consistent with the long-term PTSD prevalence rate noted by Boscarino et al. (2013), which was 14.3% (95% CI – 11.1-11.8). External validity is limited only to the population under study.

Univariate analyses using SPSS Statistics Student Version 21 examined age, gender, and race between PTSD cases and non-PTSD controls. Age was evaluated using the unpaired *t* test for continuous variables ($p < .05$). Age was not significant ($p = 0.27781$). Monte Carole estimate for Pearson chi square and lambda (λ) was used for nominal categorical variables of gender and race. Gender was significant using chi square, but lambda indicated no relationship ($p = .00$; λ .007). Monte Carlo was significant for race, and lambda indicated a relationship ($p = 0.011$; λ 1.0); race was identified as a covariate for the second analysis.

Confounding variables identified in the literature included BMI, age, gender, race/ethnicity, use of anxiolytics, antidepressants, socioeconomic status, type and intensity of trauma, education, rural/urban, co-occurring disorders such as major depressive disorder, drug dependence, substance abuse, Axis 2 disorders, and age of traumatic exposure (Babson et al., 2011; Frans et al., 2005; Krakow, Germain, et al.,

2001; Krakow, Hollifield, et al., 2001). Initially, it was thought that the database would only include age, gender, race, and substance abuse as potential covariates. However, additional covariates included in the phenotype data included sedative dependence, stimulant dependence, and tobacco dependence. Only tobacco dependence was significant (p = .000 and λ 1.0) among the possible additional covariates and was also identified as a covariate for the second analysis. Table 6 provides details on sedative dependence, stimulant dependence, and tobacco dependence.

Table 6

Covariates: Sedative, Stimulant, and Tobacco Dependence

	PTSD cases	Non-PTSD controls	Unknown	Group comparison
	N / (%)	*N* / (%)	*N* / (%)	*p* value*
Number of participants	435	2,445	29	
Sedative dependence	Abuse – 14 (3%)	Abuse – 60 (2.5%)	Abuse – 6 (21%)	.000 / λ could not be computed
	Unaffected – 398 (92%)	Unaffected – 2,324 (95%)	Unaffected – 23 (79%)	
	Dependence – 23 (5%)	Dependence – 61 (2.5%)	Dependence – 3 (10%)	
Stimulant dependence	Abuse – 6 (1%)	Abuse – 49 (2%)	Abuse – 6 (21%)	.000 / λ could not be computed
	Unaffected – 399 (92%)	Unaffected – 2,326 (95%)	Unaffected – 19 (65%)	
	Dependence – 30 (7%)	Dependence – 70 (3%)	Dependence – 4 (14%)	
Tobacco dependence	Abuse – 0	Abuse – 1	Abuse – 1 (4%)	.000 / λ 1.0
	Unaffected – 92 (21%)	Unaffected – 1,068 (44%)	Unaffected – 0	
	Dependence – 343 (79%)	Dependence – 1,376 (56%)	Dependence – 28 (96%)	

Seven questions on alcohol dependence from the SSADDA were also included in the phenotype files; 2 x 2 tables were created for these questions. Refer to Table 7. Lambda was only calculated on Question 6 – alcohol withdrawal symptoms and showed a weak association. Lambda could not be calculated on any of the other alcohol questions from the SSADDA because the asymptomatic standard error was zero. Alcohol Dependence was not identified as a covariate for the second analysis.

Table 7

Alcohol Dependence

Alcohol dependence item			
Q1 - Excessive time related to substance	PTSD cases (n = 435)	Non-PTSD controls (n = 2,445)	Group comparison - .000 / λ could not be computed
Unaffected	136 (31%)	1,175 (48%)	
Affected	299 (69%)	1,270 (52%)	
Q2 - Unsuccessful attempts to cut down on use			.000 / λ could not be computed
Unaffected	54 (12%)	677 (28%)	
Affected	381 (88%)	1,768 (72%)	
Q3 - Tolerance			.000 / λ could not be computed
Unaffected	93 (21%)	881 (36%)	
Affected	342 (79%)	1,564 (64%)	
Q4 - Impaired social or work activities due to substance			.000 / λ could not be computed
Unaffected	79 (18%)	914 (37%)	
Affected	356 (82%)	1,531 (63%)	
Q5 - Use more than intended			.000 / λ could not be computed
Unaffected	17 (4%)	512 (21%)	
Affected	418 (96%)	1933 (79%)	
Q6 - Alcohol withdrawal symptoms			.000 / .020
Unaffected	194 (45%)	1,563 (64%)	
Affected	241 (55%)	882 (36%)	
Q7 - Use despite physical or psychological consequences			.000 / λ could not be computed
Unaffected	102 (23%)	1,217 (50%)	
Affected	333 (77%)	1,228 (50%)	

Results

Plink Analysis

The NCBI database came with PLINK ready files in the form of bed (bed/bim/fam) files and ped files. Since bed files were already provided by NCBI additional bed files were not required to be made using PLINK. The subject level PLINK ready files were used for quality control measures and the association study. The family file had the affection status for all individuals in the study (n = 2,909) set to missing therefore filtering only controls in the dataset was not possible. A request for an updated file of the non-PTSD individuals identifying "pure controls" (meaning individuals without PTSD or alcohol dependence) for the study was requested but not provided by dbGAP. An alternate phenotype file identifying the affection status of PTSD (n = 435) and non-PTSD controls (n = 2,445) was provided by dbGAP.

The genomic data were analyzed using PLINK software v1.07 (Purcell, 2009; Purcell et al., 2007). The analysis identified 1,140,419 markers to be included from 2,909 individuals. There were 435 cases, 2,445 controls and 29 with missing phenotypes. A total of 2,880 founders were included in the analysis. PLINK set to missing 5,577 heterozygous haplotype genotypes. After Hardy Weinberg equilibrium (HWE p < = 0.001) 216,526 markers were excluded (61,608 from cases and 216,526 from controls). The genotyping rate was 88.52%. There were 129,182 SNPs that failed missingness tests (geno > 0.1) and 292,691 SNPs that failed frequency testing (minor allele frequency, MAF < 0.01). The 29 cases with a missing phenotype status were pruned from the analysis. After frequency and genotyping pruning, 632,686 SNPs were included in the

association analysis with 435 PTSD cases, 2,445 non-PTSD controls that included 1,611 males and 1,269 females. The output file in PLINK generates a row for each SNP association result. The fields include the chromosome (CHR), the SNP identifier, the location of the SNP which is known as the physical distance (bp), allele 1 (or the minor allele), the frequency of the minor allele in cases (F_A) and the frequency of the minor allele in controls (F-U), allele 2 (or the major allele), the chi-square statistic (1 df), the *p* value for this test, and the effect size or odds ratio (OR).

In response to Research Question 1: Are there significant genetic alleles associated with PTSD? The genome wide association threshold that was established for the purpose of this dissertation and to identify these alleles was $p = 5x10^{-8}$ (Guffanti et al., 2013). After quality controls measures mentioned above, there were signals from two SNPs that exceeded the established genome wide threshold: SNP rs13160949 on chromosome 5 ($p = 7.33x10^{-9}$, OR: 1.565) and SNP rs2283877 on chromosome 22 ($p = 2.55x10^{-8}$, OR: 1.748). Results from the case/control association generated a large volume of association results. The top ten SNPs are provided in Table 8.

Table 8

Genome-Wide Association Results

CHR	SNP	BP	A1	F_A	F_U	A2	CHISQ	P*	OR
5	rs13160949	107060238	G	0.3575	0.2623	A	33.45	$7.33x10^{-9}$*	1.565
22	rs2283877	29838195	A	0.1725	0.1065	G	31.02	$2.55x10^{-8}$*	1.748
11	rs12788610	382953	A	0.1767	0.112	G	28.78	$8.12x10^{-8}$	1.701
23	rs6641588	3565302	G	0.4183	0.315	A	27.32	$1.72x10^{-7}$	1.564
2	rs17327578	59851548	A	0.1046	0.05746	G	27.16	$1.87x10^{-7}$	1.916
11	rs2280134	3638095	A	0.3655	0.4609	G	27.16	$1.87x10^{-7}$	0.673
20	rs6075106	16689497	G	0.177	0.1141	A	26.98	$2.06x10^{-7}$	1.67
15	rs776689	40132542	G	0.3828	0.4775	A	26.67	$2.42x10^{-7}$	0.678
11	rs7479832	393939	A	0.149	0.09222	G	26.07	$3.29x10^{-7}$	1.723
11	Rs7941314	$1.24x10^{-8}$	G	0.3425	0.2592	A	25.91	$3.58x10^{-7}$	1.489

*Genome-wide threshold: $p = 5x10^{-8}$

There were concerns the initial significant results for the two SNPS were due to population stratification; therefore the same quality measures were repeated using the tools in PLINK to adjust for population stratification (Purcell et al., 2007). Dadd, Weale, and Lewis (2009) indicate population stratification is a concern for genome wide studies. PLINK uses Genomic Control, Bonferroni, Holm, Sidak, and False Discovery Rate (FDR) to adjust for multiple testing, population stratification, and false positives. According to Clark, Anderson, Pettersson, Cardon, Morris, and Zondervan (2011) both the Bonferroni and Sidak (Single Step – SS; Step Down - SD) can be used for adjustments related to associations identified at the SNP level; the Sidak however, “in

GWA studies comprising dense sets of markers, is unlikely to be true and both corrections are conservative" (p. 123). The Holm can be used in place of Bonferroni but is considered the weaker of the two tests (Clark et al., 2011). The FDR is used to identify false discovery of association results (Clark et al., 2011). Two methods of the FDR are provided by PLINK based on the individuals who developed the tests, BH for Benjamini and Hochberg (1995) and BY for Benjamini and Yekutieli (2001). Genomic Control is considered a reasonable correction method used to control for population stratification (Dadd et al., 2009). For the purpose of this dissertation, Genomic Control was used to correct for population stratification. PLINK identified the genomic inflation factor (based on median chi-squared) as 1.46541; the mean chi-squared statistic was 1.41316. The top ten results from multiple test corrected significance values for 632,686 tests are provided in Table 9.

Table 9

Corrected Significance Values

CHR	SNP	Unadjusted	GC	BONF / HOLM*	SIDAK SS/SD	FRD_BH	FDR_BY
5	rs13160949	$7.33x10^{-9}$*	$1.78x10^{-6}$	0.004638	0.004627	0.004638	0.06463
22	rs2283877	$2.55x10^{-8}$*	$4.20x10^{-6}$	0.01614	0.01601	0.008071	0.1125
11	rs12788610	$8.12x10^{-8}$	$9.36x10^{-6}$	0.05136	0.05006	0.01712	0.2386
23	rs6641588	$1.72x10^{-7}$	$1.58x10^{-5}$	0.109	0.1033	0.01862	0.2595
2	rs1732758	$1.87x10^{-7}$	$1.67x10^{-5}$	0.1185	0.1117	0.01862	0.2595
11	rs2280134	$1.87x10^{-7}$	$1.67x10^{-5}$	0.1185	0.1118	0.01862	0.2595
20	rs6075106	$2.06x10^{-7}$	$1.78x10^{-5}$	0.1304	0.1222	0.01862	0.2595
15	rs776689	$2.42x10^{-7}$	$1.99x10^{-5}$	0.153	0.1419	0.01913	0.2666
11	rs7479832	$3.29x10^{-7}$	$2.46x10^{-5}$	0.2078	0.1877	0.02246	0.3129
11	rs7941314	$3.58x10^{-7}$	$2.62x10^{-5}$	0.2267	0.2029	0.02246	0.3129

*Bonferroni and HOLM rates and SIDAK rates (SS and SD) were identical and combined in Table 9,

Neither SNP rs13160949 nor SNP rs2283877 maintained their genome wide significance following corrected tests for multiple testing, population stratification, and false discovery. The corrected value significance value using genomic control for SNP rs13160949 was $1.78x10^{-6}$ and for SNP rs2283877 was $4.20x10^{-6}$.

Following this initial analysis any allele that exceeded the genome wide association threshold would move on to the second phase of the analysis, which consisted of the remaining two research questions:

- Research *Question 2*: Is there a significant association between genetic allele(s) and the symptoms of PTSD?

- Research *Question 3*: Is there a significant association between genetic allele(s) and the symptom of sleep? Is either the COMT or RORα, both genes that have been identified in sleep and PTSD, associated with sleep?

There were no allele's that maintained genome wide significance following correction for multiple testing therefore there was no second analysis.

Summary

Results from the genome wide association method examining PTSD cases and non-PTSD controls identified two genetic alleles that reached genome wide significance in initial testing but neither survived corrected significance values. In response to Research Q1: there were no significant genetic alleles associated with PTSD. In response to research Q2 and Q3 there were no genetic alleles that moved to the second phase of analysis related to the symptoms associated with PTSD, specifically the symptom of sleep.

Chapter 4 reviewed some of the concerns associated with genome wide association studies, including concerns related to multiple testing, population stratification, and false discovery of positive associations (Type I error). Chapter 5 will review the results and identify additional concerns including Type II error. Limitations to the study, recommendations for future research, and social change implications will conclude Chapter 5.

Chapter 5: Results and Discussion

Introduction

This purpose of this dissertation was to examine the genome of individuals with PTSD and that of non-PTSD controls to discover if there were any allele(s) that reached genome-wide significance. Any genetic allele(s) reaching genome-wide significance would move to a second phase of analysis to determine whether there was an association between the genetic allele(s) and the symptoms of PTSD, specifically the symptom of sleep. The genome-wide association threshold that was established for the purpose of this dissertation was $p = 5x10^{-8}$ (Guffanti et al., 2013). Initial testing done in PLINK identified signals from two SNPs that exceeded the established genome-wide threshold: SNP rs13160949 on chromosome 5 ($p = 7.33x10^{-9}$, OR: 1.565) and SNP rs2283877 on chromosome 22 ($p = 2.55x10^{-8}$, OR: 1.748). There were concerns that the initial significant results might be due to population stratification, multiple testing, or false discovery. Neither SNP maintained its genome-wide significance following tests adjusting for multiple testing, population stratification, and false discovery. The corrected significance value using genomic control for SNP rs13160949 was $1.78x10^{-6}$ and for SNP rs2283877 was $4.20x10^{-6}$.

Following the initial analysis, any allele that exceeded the genome-wide association threshold would move on to a second phase of analysis. There were no alleles that maintained genome-wide significance following correction for multiple testing; therefore, there was no second analysis. In response to Research *Question 1*, there were no significant genetic alleles associated with PTSD. In response to Research

Questions 2 and 3, there were no genetic alleles that moved to the second phase of analysis related to the symptoms associated with PTSD, specifically the symptom of sleep.

Interpretation of the Findings

Clark et al. (2011) indicated, "A significant result in an association test rarely implies that a SNP is directly influencing disease risk" (p. 125). The initial finding of two SNPS reaching genome-wide significance was thought to be spurious and possibly due to population stratification or nonrandom genotyping failure generating false positives (Purcell et al., 2007). Call rates in other GWAS have been 98% (Xie et al., 2013) and 95% (Guffanti et al., 2013). The call rate for this study was 88.5%, which is lower than other GWAS. Additionally, in the methodology section of this dissertation, Hardy Weinberg equilibrium was set at $p > 1x10^{-6}$; however, using this threshold excluded all markers from the study, and therefore the default threshold in PLINK was used in the analysis ($p < =.001$). The Hardy Weinberg equilibrium test assumes that "in a random mating population with no selection, mutation, or migration, allele frequencies and genotype frequencies are constant from generation to generation" (Guo & Thompson, 1992, p. 361). Additional testing was completed to adjust for multiple testing, population stratification, and false discovery. Neither SNP maintained its genome-wide significance threshold following corrected tests. These tests are designed to address Type I error. Although the genome-wide method is not hypothesis driven, these tests were considered useful in interpreting the results.

The literature reviewed in Chapter 2 on PTSD focused on candidate gene and genetic allele studies and GWAS. Results of the studies indicated associations with candidate genes/alleles, mixed results, or no association (Amstadter et al., 2011; Bailey et al., 2010; Change et al., 2012; Dragon & Onisczenko, 2009; Guffanti et al., 2013; Hauer et al., 2011; Lee et al., 2005; Lee et al., 2006; Lee et al., 2011; Logue et al., 2012; Mellman et al., 2009; Morey et al., 2011; Morgan et al., 2003; Pivak et al., 2006; Ressler et al., 2011; Sah et al., 2009; Sarapas et al., 2011; Sayin et al., 2010; ; Segman et al., 2002; Voisey et al., 2009; Voisey et al., 2010; Xie et al., 2013;). The results of this study are consistent with the finding of no association with PTSD. Neither of the two SNPs (rs13160949 nor SNP rs2283877) has been identified from the peer-reviewed literature in Chapter 2. In fact, there was no information available on either SNP in the dbSNP database or via searches in Academic Search Premier, Google Scholar, or PubMed. Opensnp indicated SNP rs13160949 (SNP-ID 701967) on chromosome 5 at position 107696638 has had no mining; no results on PLoS, genome.gov, Personal Genome Project, or Mendeley-results (Opensnp, n.d.). SNP rs2283877 on chromosome 22 was listed in ALFRED (The Allele Frequency Database), allele ID SI441126S, position 31508195 at ribosomal protein S15a pseudogene 37 locus (Kidd, n.d.). No other information was available on either SNP.

The theoretical/conceptual framework for this study was Walker and van der Helm's (2009) "sleep to remember sleep to forget" framework, which focused on sleep as a mechanism for PTSD development. This framework was chosen based on the hypothesis that sleep dysfunction is not just a symptom associated with PTSD but may be

a possible pathway for the development of PTSD (Walker & van der Helm, 2009). There were no genetic alleles reaching genome-wide significance that moved onto the second phase of analysis; therefore, the results do not lend support for or provide evidence to disprove the theoretical/conceptual framework.

This study found no genetic alleles associated with PTSD, which may be true despite limitations noted in the next section. Questions have been raised related to the genome-wide association method, such as the following: Are genotyping technologies sophisticated enough to produce robust datasets? Do different SNP arrays affect genotypes? Do using different algorithms affect data? Do different calling rates impact results (Hong et al., 2010)? Hong et al. (2010) indicated that GWA studies have often not been replicated when using similar populations. The four GWA PTSD studies published each identified novel results with minimal replications of the others' findings (Logue et al., 2012; Guffanti et al., 2013; Solovieff et al., 2014; Xie et al., 2013;). Du, Xie, Chang, Han, and Cao (2012) indicated that GWAS have found associations with genetic alleles; however, heritability studies attribute 37-41% of the variance in PTSD to genetic factors (Bailey et al., 2010; Goenjian et al., 2012). Results from this study, if they are true, suggest that genetic alleles may not be major contributors to PTSD development. Xie et al. (2013) also suggested that common variants may be minimally involved in PTSD risk. Given the literature, genetic factors likely play a role in PTSD risk; however, research efforts may need to be refocused to evaluate genetic factors differently. The most recently published GWAS by Solovieff et al. (2014) did that by specifically examining all the candidate genes identified in the literature and conducting

separate analyses on the genetic alleles identified in the three previously published GWAS (Guffanti et al., 2013; Logue et al., 2012; Xie et al., 2013). They indicated, "PTSD is highly polygenic, influenced by numerous SNPs with weak effects" (Solovieff et al., 2014, p. 1872).

Limitations

Limitations from Type I errors (falsely reporting an association when there is none) in genome-wide association studies include the following: population stratification (occurs when cases and controls are not from the same population), multiple testing, false discovery, biased selection or misclassification of cases and controls, undocumented or cryptic relatedness, and nongenetic confounding variables (Hong et al., 2010; Purcell et al., 2007; Zang & Deng, 2010; Zondervan & Cardon, 2007). Purcell et al. (2007) indicated that multiple testing generates "many highly significant results … by chance alone, making it hard to distinguish signal from noise" (p. 559). Another type of error is due to nonrandom genotyping (Hong et al., 2010). This error can occur when a SNP genotype is called incorrectly or is not called; if the calling is nonrandom, some genotypes (and also phenotypes) may be called more often, leading to a false positive association (Purcell et al., 2007). The initially significant results found in the study may have been from any of these concerns. Initial testing did not adjust for population stratification, false discovery, or multiple testing. The call rate for this study (88%) was lower than call rates noted in other GWAS (98% and 95%; Guffanti et al., 2013; Xie et al., 2013). Covariate analyses indicated that both race and tobacco use should be included in the second analysis. The initial study collected genomic data for substance

dependence. When comparing PTSD cases to non-PTSD controls in the covariate analysis section in Chapter 4, the majority of questions on alcohol dependence were not able to be tested using λ because chi square was .000, suggesting the possibility of confounding. Quality control measures, Hardy Weinberg equilibrium, and corrected significance levels using genomic control adjusted for these concerns. Additional testing to address concerns due to undocumented relatedness was not completed given the results from adjustments.

Type II errors (failure to report an association when there is one) can result from technical errors in genotyping or genotype measurements (Hong et al., 2010). Cooley et al. (2011) identify a number of problems associated with data accuracy including imputation errors when data is entered, differing algorithms being used, genotyping error rates due to duplication, and phenotype misclassification. Errors can also occur at the point of diagnosis (sensitivity and specificity; Cooley et al., 2011). The potential for information bias related to misclassification or measurement error (i.e., "biological sample collection, processing and storage, sample labeling, DNA extraction and storage, laboratory assays, data coding, entry and analysis") was addressed by ensuring the two groups are in Hardy Weinberg equilibrium (HWE) (Garcia-Closas et al., 2004, p. 131). However, as previously mentioned, the default HWE (.001) was used in PLINK rather than the HWE that was established for the study ($p = 1x\ 10^{-6}$) eliminated all SNPs from analysis. Type II errors could also have occurred when the original database did not identify the "true controls", individuals without Alcohol Dependence or PTSD, from the original study. The control population used in the study had 500 individuals without

Alcohol Dependence or PTSD and 1,945 with an Alcohol Dependence. The "true controls" were not identified in the database, which likely introduced bias in the results. These types of errors would have occurred during the collection of and processing of genotype and phenotype information and would not have been addressed by this research study, therefore results should be interpreted with caution.

Another limitation identified with the GWAS method is small to moderate effects or Odd Ratio (OR). Du et al. (2012) indicated that effects from non-genetic risk factors typically generated OR's with two or three fold increases compared to OR's for genome wide studies that only showed modest increases. Stringer, Wray, Kahn, and Derks (2011) stated that GWAS examining single SNPS with dichotomous phenotypes may not identify true effect sizes; they indicated that the variance attributed to genetic factors is low, particularly in psychiatric disorders. "One explanation of the missing heritability is that complex diseases are caused by a large number of causal variants with small effect sizes" (Stringer et al., 2011, p. 1). Galichon, Mesnard, Hertig, Stengel, and Rondeau (2012) indicate that the GWAS method can identify "previously unrecognized sequence homologies caused by SNP microarrays that incorrectly associate a phenotype to a given locus when in fact the linkage is to another distant locus" (p. 4474). Galichon et al. recommend using quality controls measures but also indicate this could eliminate relevant SNPs. They recommend three additional steps that include the following: stratify the data based on sex, check all SNPs identified for "genome wide alignment of the SNP-flanking sequences and of the restriction fragments", and sequence the loci

thought to be associated with the SNP to ensure association with the phenotype (Galichon et al., 2012, p. 4781).

Other sources of bias in genetic epidemiology include selection bias, information bias, and confounding (Garcia-Closas et al., 2004). The original study included genomic data from participants recruited during three different studies. It is unknown if the distribution of genetic factors were similar between cases and controls. The potential for information bias related to misclassification or measurement error (i.e., "biological sample collection, processing and storage, sample labeling, DNA extraction and storage, laboratory assays, data coding, entry and analysis") was addressed by ensuring the two groups are in Hardy Weinberg equilibrium (Garcia-Closas et al., 2004, p. 131). However, as previously mentioned, the default HWE (.001) was used in PLINK rather than the HWE that was established for the study (10^{-6}) which eliminated all SNPs from analysis. Recall bias was minimized as genotypes are stable over time (Botto & Khoury, 2004).

The United States Preventative Services Task Force criteria were identified in Chapter 1 for evaluating internal validity for individual case control studies (National Center for Biotechnology Information, U.S. National Library of Medicine [NCBINLM], n.d.). Internal validity criteria included: valid selection of case, exclusion criteria applied equally to cases and controls, an 80% response rate, the same measurements applied to cases and controls, and addressing confounding variables (NCBINLM, n.d.). These criteria, with the exception of confounding, were not relevant for this study as the requirements were a priori. Confounding was not a concern as the second phase of

analysis in the study did not occur. Other threats to internal validity such as history, maturation, instrumentation, experimental mortality, and selection maturation were not a concern (SUU, n.d.). Testing (i.e. the Hawthorne effect) was identified in Chapter 1 as a concern since participants were aware they were participating in a research study. Recall bias is minimized in GWAS and genotypes are stable over time (Botto & Khoury, 2004).

Results from this study should not be generalized to other populations. The prevalence of PTSD differed in the study population compared to published PTSD prevalence rates and the presence of alcohol dependence in the study population may be different from individuals with PTSD only. The lack of a PTSD only population was previously identified as a limitation. The sample size for PTSD cases was small for a GWAS study (n = 435) suggesting power may be low. Other threats to external validity such as testing reactivity, interaction effects, reactive effects, and multiple treatment interference were not a concern for this study (SUU, n.d.).

Recommendations

The GWAS portion of this study was completed however, there were concerns identified related to the control population and how this population may have affected the results. The control population which, consisted of all non-PTSD individuals, contained a mix of individuals with and without alcohol abuse possibly impacting the identification of alleles that would have been appropriate for phase two of the study. Results of the study neither supported nor provided evidence for the theoretical framework, Walker & van der Helm's (2009) "sleep to remember sleep to forget" framework. Recommendations from the literature do support further investigation of PTSD and sleep

(Krakow, Germain, et al., 2001; Walker & van der Helm, 2009; Yetkin et al., 2010). A GWA study using PTSD cases and non-PTSD controls, both without the presence of alcohol dependence or abuse is recommended. All GWAS published to date have included replication in a different population to substantiate any allele reaching genome-wide significance; this is recommended for any allele(s) reaching genome wide significance (Guffanti et al., 2013; Logue et al., 2012; Solovieff et al., 2014; Xie et al., 2013). Appropriate quality control measures (i.e., an adequate call rate, MAF, and genotyping) should be implemented. HWE and adjustments for population stratification, multiple testing, and false discovery should be used in any future study. Additionally, follow the recommendations from Galichon et al. (2012) to stratify the data by sex, check SNPs flanking sequences and restriction fragments, and sequence the loci thought to be associated with the SNP. Sleep symptoms should be evaluated using a validated measurement tool such as the Pittsburg Sleep Quality Index (PSQI) to collect information on sleep related concerns (Buysse et al., 1989). Additionally, the American Psychiatric Association's Diagnostic and Statistical Manual 5 (2013) has been released and has updated the criteria used for diagnosing PTSD; future studies should use the updated PTSD diagnostic criteria. Confounding should be minimized by controlling for variables identified in the literature: BMI, age, gender, race/ethnicity, use of anxiolytics, antidepressants, socioeconomic status, type and intensity of trauma, education, rural/urban, co-occurring disorders such as major depressive disorder, drug dependence, substance abuse, Axis 2 disorders, and age of traumatic exposure (Babson et al., 2011; Frans et al., 2005; Krakow, Germain, et al., 2001; Krakow, Hollifield, et al., 2001).

Implications

Positive Social Change

PTSD continues to be a public health concern. The effects of PTSD can be experienced years after an event. Effective treatment is needed for individuals who develop this disorder. This dissertation brought attention to the genetic risk factors associated with PTSD to the researcher community; genome wide studies on PTSD have not received as much attention as other more prominent mental health conditions such as depression or schizophrenia (Cornelius et al., 2010). When this dissertation was started there were no published genome wide association studies on PTSD. There are now four published studies (Guffanti et al., 2013; Logue et al., 2012; Solovieff et al., 2014; Xie et al., 2013). This dissertation contributed to bringing awareness of a possible novel pathway for disease development. The idea of sleep as a possible pathway is in its infancy in terms of investigation despite evidence that sleep is intimately involved with PTSD. This dissertation was designed to study PTSD from a broader scope considering multiple factors that may be involved with PTSD development. Initially, by studying the entire genome rather than a single candidate gene, then had phase two been able to be completed an examination of how genetic risk factors may be associated with symptoms and specifically sleep. Finally, this research provided a framework for other GWA studies on PTSD, including limitations with the GWAS method and provided recommendations for future research on the potential relationship between sleep and PTSD development.

Conclusion

This purpose of this dissertation was to complete a two part analysis with the first phase being a genome-wide association study (GWAS) examining the genome of individuals with PTSD compared to non-PTSD controls. Any allele(s) that reached genome wide significance would have moved on to the second phase of analysis, examining the allele with PTSD symptoms, specifically the symptom of sleep. Two SNPs exceeded the genome wide threshold but did not survive adjustments for population stratification, multiple testing, or false discovery therefore there was no second phase of analysis; the results did not lend support for or provide evidence to disprove the theoretical/conceptual framework, Walker & van der Helm's (2009) "sleep to remember sleep to forget". Despite limitations associated with the database, this study demonstrated the principles of public health and epidemiology. Specifically by collaborating with a large community partner (NCBI) to use existing data resources while applying principles of informatics and human genome epidemiology to complete a study typically reserved for a large academic or research setting. This study may open the door for other Walden Students to partner with community stakeholders to complete research studies using existing data sources.

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Appendix

Criterion A: The person has been exposed to a traumatic event in which both of the following have been present:

1) The person has experienced, witnessed, or been confronted with an event or events that involve actual or threatened death or serious injury, or a threat to the physical integrity of oneself or others.

2) The person's response involved intense fear, helplessness, or horror. **Note:** in children, it may be expressed instead by disorganized or agitated behavior.

Criterion B: The traumatic event is persistently re-experienced in at least one (or more) of the following ways:

1) Recurrent and intrusive distressing recollections of the event, including images, thoughts, or perceptions. **Note:** in young children, repetitive play may occur in which themes or aspects of the trauma are expressed.

2) Recurrent distressing dreams of the event. **Note:** in children, there may be frightening dreams without recognizable content

3) Acting or feeling as if the traumatic event were recurring (includes a sense of reliving the experience, illusions, hallucinations, and dissociative flashback episodes, including those that occur upon awakening or when intoxicated). **Note:** in children, trauma-specific reenactment may occur.

4) Intense psychological distress at exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event.

5) Physiologic reactivity upon exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event

Criterion C: Persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness (not present before the trauma), as indicated by at least three (or more) of the following:

1. Efforts to avoid thoughts, feelings, or conversations associated with the trauma
2. Efforts to avoid activities, places, or people that arouse recollections of the trauma
3. Inability to recall an important aspect of the trauma
4. Markedly diminished interest or participation in significant activities
5. Feeling of detachment or estrangement from others
6. Restricted range of affect (e.g., unable to have loving feelings)
7. Sense of foreshortened future (e.g., does not expect to have a career, marriage, children, or a normal life span)

Criterion D: Persistent symptoms of increasing arousal (not present before the trauma), indicated by at least **two** (or more) of the following:

1. Difficulty falling or staying asleep
2. Irritability or outbursts of anger
3. Difficulty concentrating
4. Hyper-vigilance
5. Exaggerated startle response

Criterion E: Duration of the disturbance (symptoms in B, C, and D) is more than one month.

Criterion F: The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Specify if:

Acute: if duration of symptoms is less than three months

Chronic: if duration of symptoms is three months or more

Specify if:

With Delayed Onset: Onset of symptoms at least six months after the stressor

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