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Sleep disturbances and depression: the role of genes and trauma

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

by

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List of Abbreviations

ACE.....adverse childhood experience
AIC.....Akaike Information Criterion
ALDH1A2.....Aldehyde Dehydrogenase 1 Family Member A2
ANOVA .....analysis of variance
BP.....base position
CFA.....confirmatory factor analysis
CIDI .....Composite International Diagnostic Inventory
CONVERGE.....China, Oxford and VCU Experimental Research on Genetic Epidemiology
CSA.....childhood sexual abuse
DSM.....Diagnostic and Statistical Manual
EA .....European American
EEG.....electroencephalography
EFA .....exploratory factor analysis
FDR.....false discovery rate
FWER .....family-wise error rate
GABA .....gamma-aminobutyric acid
GAD.....generalized anxiety disorder
GCTA.....genome-wide complex trait analysis
GRM .....genetic relatedness matrix
GS .....general sleep item
GWAS.....genome-wide association study
GWS.....genome-wide significant
GxE .....gene-environment interaction
HRSD.....Hamilton Rating Scale for Depression
ICD.....International Classification of Disease
KCNK9.....Potassium Two Pore Domain Channel Subfamily K Member 9
LD .....linkage disequilibrium
LDSC .....Linkage Disequilibrium Score regression
MAF.....minor allele frequency
MAO .....monoamine oxidase
MDD .....major depressive disorder
NCS.....National Comorbidity Survey
PC.....principal component
PGC.....Psychiatric Genomics Consortium
PRS .....polygenic risk score
PSQI.....Pittsburgh Sleep Quality Index
PTSD.....posttraumatic stress disorder

QC.....quality control
Q-Q .....quantile-quantile
REML .....restricted maximum likelihood
RMSEA.....root mean square error of approximation
SDS .....sleep within depression score
SLE .....stressful life event
SNP .....single nucleotide polymorphism
UK.....United Kingdom
US .....United States
VATSPSUD.....Virginia Adult Twin Studies of Psychiatric and Substance Use Disorders
WMHSC .....World Mental Health Survey Consortium

Abstract

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Sleep disturbances and insomnia are prevalent, with around 33% of adults indicating that they experience at least one main symptom of insomnia, and bidirectional relationships exist with common psychopathology, particularly major depressive disorder (MDD). However, genetic and environmental (e.g., traumatic event exposure) contributions to the etiology of these phenotypes are not yet well understood. A genetically informative sample of approximately 12,000 Han Chinese women aged 30-60 (50% with recurrent MDD) was used to address several gaps within the sleep literature. Sleep disturbances were assessed in all individuals using a general item addressing sleeplessness (GS). A sleep within depression sum score (SDS) was also created in MDD cases, combining information from the GS and two insomnia items within MDD. A total of 11 traumatic events were assessed and additional information on childhood sexual abuse (CSA) was also obtained. First, factor analyses were conducted to determine trauma

factor structure. The best-fit solution included 3 factors: interpersonal, child interpersonal, and non-assaultive, and composite variables were constructed accordingly. A series of hierarchical regressions were run to examine differential effects of trauma type and timing on sleeplessness. All traumatic events predicted sleeplessness at similar magnitudes, although population models indicated that childhood interpersonal trauma may be particularly potent. An association between CSA and sleeplessness was also replicated. A series of genetic analyses demonstrated that the single nucleotide polymorphism-based heritability of sleep phenotypes did not differ significantly from zero. Further, association analyses did not identify any genome-wide significant loci. However, using a liberal false discovery rate threshold of 0.5, two genes of interest, *KCNK9* and *ALDH1A2*, emerged for the SDS. Polygenic risk score (PRS) analyses demonstrated genetic overlap between the SDS in MDD cases and GS in MDD controls, with PRSs explaining 0.2-0.3% of the variance. A final combined model of both genetic and environmental risk indicated that both PRS and traumatic events were significant predictors of sleeplessness. While genetic results should be interpreted with caution given the lack of heritability, additional research into the genetic and environmental contributions to insomnia, utilizing more standardized phenotypes and properly ascertained samples, is clearly warranted.

Chapter 1: Introduction

Disturbed sleep is a widespread problem in today's society, but the etiologic contributions to this important phenotype are not well understood. Known influences are both environmental (e.g., trauma exposure) and biologic (e.g., genetic) in nature, and studies demonstrate overlap in etiologic sources between sleep difficulties and common internalizing disorders, such as major depressive disorder (MDD). However, questions remain as to how the type (e.g., interpersonal violence versus non-interpersonal violence) and timing (i.e., childhood versus adult onset) of traumatic events influence sleep disturbances. The genetic architecture of insomnia and related phenotypes (e.g., sleep disturbances), including overlap with MDD, is also not well understood. Thus, the purpose of this dissertation is to deepen our understanding of how both traumatic event exposure and molecular genetic contributions influence sleep disturbances. The following section begins with an overview of the prevalence and correlates of insomnia/sleep disturbances and related psychopathology, with a focus on MDD. Following, the epidemiology of traumatic events, an environmental risk factor associated with both poor sleep and MDD, is reviewed with specific attention to trauma type and timing in relation to these phenotypes. Next, a review of studies that examine genetic contributions to insomnia is provided, starting with behavioral genetics (i.e., twin and family studies) and moving on to molecular genetics (candidate gene and genome-wide association studies [GWAS]). Throughout the genetics section, the insomnia literature is compared to that of MDD, with a specific focus on

studies of overlap between these phenotypes. Finally, recent innovations in statistical genetics methods will be discussed, followed by an outline of the aims for this dissertation.

I. Prevalence and correlates of disturbed sleep/insomnia and MDD

Epidemiology of disturbed sleep/insomnia. Insomnia, defined in the Diagnostic and Statistical Manual, 5th edition (DSM-5) as sleep complaints, which include difficulty falling asleep, difficulty maintaining sleep, or early morning awakenings, for at least three nights a week, persisting for a minimum of three months, and causing "significant distress" to the individual[1] and its symptoms are an emerging health concern. Although definitions of insomnia vary within epidemiologic studies (insomnia diagnosis, insomnia symptom(s), subjective sleep quality),[2] disturbed sleep is a widespread problem: General population studies in Western countries indicate that approximately one third of adults endorse a minimum of one of the main nighttime insomnia symptoms (e.g., difficulty falling or staying asleep), with difficulty maintaining sleep typically reported the most often.[2,3] Although sleep disturbances are a widespread problem globally, rates of endorsement do appear to differ by country. An epidemiologic study of the same three insomnia symptoms in China found much lower rates than Western countries, with only 9% of individuals endorsing at least one symptom,[4] and lower rates have also been reported in Nigeria (12%).[5] The first published meta-analysis of insomnia in China identified a point prevalence of 15%, across a wide variety of definitions.[6] Further, the prevalence of lifetime insomnia disorder itself, when measured through DSM or International Classification of Disease (ICD) definitions, is thought to be closer to 6-10%.[2] A recent epidemiologic study comparing insomnia diagnoses between the US and Hong Kong found that estimates were similar across multiple definitions.[7] The epidemiology literature consistently

shows that both age and sex influence insomnia prevalence. Older adults are more likely to report insomnia/sleep disturbances than younger adults (prevalence increasing to 20-48% for older populations)[8-12] and women are more likely to report insomnia than men (risk ratio 1.41).[13]

Sleep and physical health outcomes. Disturbed sleep and insomnia are related to a multitude of negative physical, occupational, and mental health outcomes. In terms of physical health, insomnia has associations with diabetes, hypertension, metabolic syndrome, cardiovascular disease, and neuropsychiatric disease, among other chronic health conditions.[2,14] While many of these studies vary in terms of phenotype used, severity of insomnia, and inclusion of sleep duration, results converge to suggest that disturbed sleep is associated with common health problems. Similar results are also reported in studies from China, which indicate that poor sleep quality or insomnia is also related to higher incidence of diabetes,[15] hypertension,[16] dyslipidemia (in women),[17] heart attack and stroke,[18] metabolic syndrome,[19,20] and even levels of objective measures of metabolic function such as cholesterol and insulin resistance.[21] More globally, there is some evidence that insomnia is related to increased mortality, an association that may be stronger for men.[14,22-25] Finally, there are also important occupational health implications, with studies showing that insomnia results in higher odds of accidents and errors and is costly to the workplace.[26-28] Taken together, these associations between insomnia and health outcomes, although often cross-sectional in nature, highlight the importance of understanding risk factors related to disturbed sleep.

Sleep and psychopathology. In addition to being associated with physical health conditions, disturbed sleep is related to psychiatric phenotypes, particularly internalizing disorders (e.g., MDD, anxiety),[29-32] with approximately 40% of individuals with insomnia endorsing another psychiatric disorder.[3,29,31] MDD is the most common comorbid psychiatric

condition with insomnia.[33] The relationship between disturbed sleep/insomnia and psychiatric disorders is complex. The high rates of comorbidity could be explained by the overlap in symptoms, as insomnia is embedded within the DSM criteria for MDD, generalized anxiety disorder (GAD), and posttraumatic stress disorder (PTSD),[1] manifesting as a key symptom of each disorder.[32,34] Sleep disturbances are prevalent within these disorders, particularly for MDD, where insomnia is thought to be a core symptom[35] and up to 90% of individuals with MDD report sleep problems.[32] Further, experiencing insomnia may be indicative of more severe MDD (e.g., [36,37]). There is also evidence that the relationship between sleep disturbances and psychopathology is bi-directional,[32,34,38] as individuals with insomnia are at a higher risk for developing new-onset psychopathology.[29,30] Two meta-analyses support this conclusion for MDD more specifically,[39,40] as both reported that individuals with insomnia are at least twice as likely to develop MDD as compared to those without the disorder. Longitudinal studies of combat veterans reinforce these relationships, showing that sleep disturbances predict future MDD and PTSD symptoms (e.g., [41,42]). Overall, robust relationships between sleep and psychopathology, particularly MDD, underscore the importance of studying these comorbid conditions together.

Epidemiology of MDD. MDD, the most common internalizing disorder, affects approximately 16% of individuals in the US[43] and 3.3% in China[44] across their lifetimes. This difference in prevalence could be attributed to under-reporting in China, due to cultural differences (e.g., [45,46,47]), although note that many risk factors for MDD, such as stressful life events (SLEs) and childhood sexual abuse (CSA) have been shown to be the same across countries.[48] Like insomnia, MDD is more common in women than men,[49,50] with 8-17% of women and 4-9% of men in Western countries[50] and 4% of women and 3% of men in China[44]

reporting lifetime MDD. The disorder is also extremely costly; MDD is one of the most common causes of disability worldwide[51] and global burden of disease data consistently shows that MDD is one of the leading causes of years lost to disability across many countries.[52,53] There are also associations between MDD and many of the same physical health outcomes as insomnia (e.g., diabetes,[54-58] cardiovascular disease[59-62]), and it is comorbid with many other psychiatric disorders (e.g.,[49,63,64]). Thus, given the high prevalence and overlapping negative consequences of both insomnia and MDD, it is important to examine etiologic factors, both genetic and environmental (e.g., exposure to a traumatic event) in nature, that influence these common and comorbid outcomes. Trauma exposure is an important environmental risk factor to consider, as it is common[65] and has robust associations with both MDD (e.g., [66]) and sleep disturbances (e.g., [67]), which will be outlined below.

II. Environmental influences (i.e., trauma exposure)

a. Trauma epidemiology

Prevalence of traumatic events and sex differences. Exposure to traumatic events is common worldwide. The World Mental Health Survey Consortium (WMHSC) recently published data collected in 24 countries on lifetime exposure to a wide range of traumatic events. The overall prevalence of at least one traumatic event was more than 70%. More specifically, 82.7% of participants assessed in the United States endorsed at least one trauma, which was among the highest prevalence rates. In contrast, most prevalence rates in European countries were below 80%, with the exception being Ukraine (84.6%). Rates were also considerably lower in Asian populations, with 52.5% of individuals in China and 60.7% in Japan indicating that they had experienced at least one of the 29 types of traumatic events. The traumatic event most

commonly endorsed across the full, worldwide sample was unexpected death of a loved one, and the authors note that the pattern of events that were the most common (i.e., top five) was consistent across countries.[65] Overall, traumatic event exposure is more common in men.[68] For example, rates of lifetime trauma exposure were significantly different across the sexes in the National Comorbidity Survey (NCS; 60.7% for males; 51.2 % for females).[69] Men also experienced, on average, more traumatic events than women in a community sample.[70] However, there are important sex differences across specific traumatic events that will be discussed in later sections.

Trauma and physical health outcomes. There are a wide range of post-trauma sequelae that are associated with both mental (e.g., MDD, PTSD; [69,71,72]) and physical health,[73,74] making trauma exposure a significant global public health issue. A number of specific physical health outcomes have been examined in the context of traumatic event exposure. In a large cross-sectional study of 14 countries, lifetime exposure to at least one traumatic event was shown to increase risk for onset of physical health conditions, even after controlling for psychopathology. In general, odds ratios increased as the number of event types increased, indicating a larger effect for multiple traumas. The pattern of results was similar for most individual physical health phenotypes (e.g., arthritis, heart disease, diabetes), and did not typically differ across countries.[75] Additionally, a study of six health outcomes in a community sample of African Americans found that individuals who endorsed eight or more traumatic events reported an age of onset for a physical health condition that was on average 15 years earlier than for those who did not endorse this high level of trauma,[76] emphasizing the potential deleterious relationship between trauma exposure and physical health.

Trauma and psychopathology. The development of psychiatric disorders is also common following traumatic event exposure. Epidemiologic data from adults in the United States participating in the Mental Health Surveillance Study indicates that individuals who had been exposed to at least one lifetime traumatic event were more likely to report any mental illness than those who had not been exposed (prevalence rates were 23.2% vs. 14.3%).[77] Although PTSD, a stress-related disorder requiring traumatic event exposure and encompassing symptoms of re-experiencing the event, avoidance, and hyperarousal,[1] is considered the "flagship" post-trauma psychiatric disorder, a range of psychiatric outcomes that can be both internalizing (e.g., MDD, GAD, PTSD) or externalizing (e.g., alcohol and drug use disorders) in nature[78] are seen following traumatic events.[69,71,72]

MDD in particular is common and highly comorbid with PTSD.[69,71,79,80] Early studies focused on the development of MDD following SLEs, which typically include major life events such as divorce, unemployment, or being fired from one's job, in addition to events that are considered traumas per DSM definition (i.e., "involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others").[81] Broadly, SLE exposure is associated with increased risk of disorder or symptoms, and there is also a dose-response relationship with SLE severity and MDD risk.[82] Similar relationships between SLEs and MDD have been observed in Han Chinese samples.[48,83] MDD is also common following trauma exposure more specifically, with population data demonstrating higher endorsement rates of MDD for individuals reporting at least one traumatic event (10.1% vs. 4.3%).[77] The trauma literature documents MDD risk following many individual traumas, such as the September 11th terrorist attacks,[84,85] earthquakes,[86] and sexual assault.[87,88] Further, women may be more likely than men to develop MDD post-trauma (e.g., [89,90,91]). Note, however, that there is some debate as

to the existence of a "post-traumatic depressive disorder" and whether MDD results from the trauma itself or from the bereavement/loss experienced.[66] Although trauma is broadly associated with these negative health outcomes (i.e., psychopathology including MDD and PTSD, chronic health conditions), differential risk is often seen by type of trauma (e.g., [69]) and the timing of trauma(s) (e.g., [92,93]).

Trauma types. Categorization of traumatic events is important, as the literature indicates differences in outcomes across trauma type. Understanding which forms of trauma may be more deleterious in nature (i.e., more likely to result in negative psychiatric and mental health outcomes) can help to identify individuals who are at the highest risk, inform prevention and intervention efforts, and focus research on specific areas that will have an impact. Traumatic events can be broadly categorized into events that are interpersonal (assaultive) or non-assaultive in nature.[70-72,94] Interpersonal traumas include events such as sexual assault, physical assault, or kidnapping, which have a strong relational component. In contrast, natural disasters and motor vehicle accidents are among events considered to be non-assaultive traumas[70-72,94] and are more random events. There are also important, established sex differences that are seen by trauma type.[65,95] Women are more likely to report exposure to interpersonal traumas, particularly those that are sexual in nature (e.g., rape, sexual molestation, other sexual assault, and childhood physical abuse or neglect).[69,70] In contrast, men are more likely to be exposed to other traumas such as accidents and non-sexual assault.[69,95] This general pattern was also seen within the recent WMHSC data, where women were more likely to endorse intimate partner/sexual violence (OR = 2.3). In contrast, men had higher odds of endorsing various other traumas, including interpersonal violence (which included being beaten up or having witnessed physical fights at home in this study), causing or witnessing bodily harm, and accidents or injuries.[65]

Several published factor analyses support the categorization of traumatic event exposure into two broad categories. Stein and colleagues[94] conducted a principal component analysis of nine traumas in Canadian twins, finding two factors: "assaultive" (robbery, held captive, beat up, sexual assault, other life threat) and "non-assaultive" (sudden family death, motor vehicle accident, fire, natural disaster). In the WMHSC study, the 29 traumatic events examined were also factor analyzed. Five relevant factors were extracted (plus a sixth that encompassed "other" events). One factor represented accidental traumas/injuries (corresponding to non-assaultive), while the remaining factors were all interpersonal in nature (collective violence, caused/witnessed bodily harm [mostly war/combat-related], interpersonal violence, and intimate partner/sexual violence).[65] Given the large number of events examined within this sample, it is not surprising that several more specific interpersonal factors emerged which grouped more similar interpersonal events together. The separation of interpersonal and non-assaultive traumas may be influenced by genetic and personality factors. Unlike other forms of trauma, interpersonal traumas have generally been shown to have genetic influences (e.g., assaultive trauma heritability estimated at 20%;[94] 35-47% for combat;[96] 60% for "high-risk" traumas including rape[97]). Mechanisms may be through personality factors,[98-100] which in turn are heritable.[101] For example, genetic factors contributed to the association between antisocial traits and exposure to assaultive traumas.[100] Thus, one's genetic predisposition, and subsequent effects on characteristics such as personality, may influence selection into environments that increase risk of traumatic event exposure.[102] Within the genetics literature, this concept is known as a gene-environment correlation. In contrast, non-assaultive events tend to be more random occurrences that are out of one's own control (e.g., exposure to a fire, flood, or natural disaster), where gene-environment correlations are likely not at play.[103]

Trauma type and psychopathology. Further supporting the importance of categorizing traumas, there is evidence to suggest that the risk of developing certain psychiatric disorders differs depending on the type of trauma experienced. It is well established within the PTSD literature that the risk of developing PTSD is higher and symptoms are more severe following interpersonal traumas.[69,70,104-108] This is generally supported within the more recent WMHSC data.[109] There is also evidence that interpersonal traumas (vs. non-assaultive/accidental traumas) result in a higher likelihood of MDD diagnosis and higher depressive symptoms.[110-114] A recent study of interpersonal trauma and MDD in Korea found higher rates of interpersonal trauma (sexual or physical in nature) but not non-assaultive trauma, in individuals with MDD. They also found higher depressive and PTSD symptoms within those depressed individuals endorsing interpersonal trauma exposure.[113] These relationships may also be true for externalizing disorders and traits, such as substance use disorders and binge drinking.[111,115] Further, it is not just psychiatric outcomes that are differentially associated with trauma type: a recent review provides evidence that interpersonal traumas may also be linked to worse physical health as measured via self-report (e.g., somatization symptoms, physical well-being).[116]

Trauma timing. Although traumatic experiences can occur across the lifespan, many such events occur during childhood, which is a particularly sensitive developmental window.[92] Around 60% of adults surveyed in the US endorsed at least one adverse childhood experience (ACE).[117-119] Further, a recent review of childhood maltreatment worldwide found specific prevalences ranging from 13% (CSA, combined across sexes) to 36% (emotional abuse).[120] While these may seem like drastically different prevalences, ACEs, like SLEs, typically include a wide range of negative experiences that can occur during childhood which encompass events

that are considered to be traumas (e.g., CSA, physical abuse), as well as those that are not (e.g., divorce/separation of parents, mental illness of someone in the household).

Childhood trauma and psychopathology. The consequences of ACEs have been extensively studied over the past few decades, with a review of the literature pointing to increased risk for psychiatric outcomes, chronic disease, and risky sexual behaviors, and higher mortality.[121] Childhood maltreatment more specifically, including CSA, has associations with many psychiatric disorders (e.g., MDD, GAD, anorexia, panic disorder, alcohol dependence, substance use; e.g., [122,123-125]). A recent meta-analysis summarizes the state of the literature on prospective studies of child maltreatment (sexual abuse, physical abuse, neglect) and depression and anxiety disorders in adults. The authors of this study report that these traumas are responsible for nearly 400 million cases of depression and anxiety disorders worldwide, with odds ratios of 2.03 (for any type of maltreatment) and 2.66 (for CSA) in predicting depression and anxiety disorders.[122] However, note that the majority of these studies were conducted in Western countries. There is a more extensive literature for CSA and MDD outcomes, which demonstrates that CSA severity (i.e., level of physical contact involved) has a dose-response relationship with MDD.[124,126] These findings provide some evidence that specific characteristics of the abuse, such as having multiple perpetrators or endorsing intercourse, may differentially predict MDD risk.[127-129] The relationship between CSA and MDD has also been studied in the Han Chinese population, with similar patterns observed in terms of overall risk, dose-response, and incident characteristics.[130,131] Additionally, there is some evidence to suggest that childhood events are more potent predictors of future depressive and anxiety symptoms in adults than traumas that occur more proximally.[93] Specific time periods may also confer more risk. In a recent study, investigators found that age of first exposure to child maltreatment differentially

predicted MDD and PTSD symptoms.[92] Interestingly, individuals who were first exposed to interpersonal violence between 6-10 years of age had twice the risk for MDD as those who had their first interpersonal violence exposure as adults. In summary, exposure to trauma during childhood is prevalent and often interpersonal in nature, resulting in outcomes that persist many decades following abuse even above and beyond adult trauma load. However, there is a need for more studies within non-Western populations.

b. Trauma and sleep

Overview of the literature. Another common post-trauma outcome that is related to both mental and physical health correlates is disturbed sleep. Traumatic events are thought to disrupt/alter sleep,[67,132] with potential mechanisms related to activation of the stress response and hyperarousal.[132] As far back as the 1970s, a relationship between trauma and sleep has been described in research publications, with the first study using EEG to examine sleep in individuals who participated in the Yom Kippur War.[133] Since then, sleep disturbances and insomnia symptoms have been reported following a wide variety of specific traumatic events, including combat exposure,[134-138] natural disasters such as earthquakes and tornados,[139,140] sexual abuse (including CSA),[141,142] intimate partner violence,[143] motor vehicle accidents,[144,145] and terrorist attacks.[146] Multiple sleep phenotypes have been used as outcomes across these samples, ranging from objective measures like polysomnography (less common; outlined in [132]) to self-report sleep disturbances and insomnia diagnoses (focused on here). Despite the variation in traumas assessed and phenotypes used, the literature supports robust associations between trauma and sleep. Further, while many traumatic events have been analyzed with sleep outcomes, most trauma and sleep studies tend to focus on specific traumas and do not explore potential differential effects of trauma type and/or timing to the extent to which this may have an impact

on sleep. This is an important consideration, as individuals are often exposed to multiple events across the lifespan.

Trauma type and sleep. Investigations of trauma type are beginning to emerge within the trauma and sleep literature. Given that interpersonal traumas are more potent predictors of internalizing psychopathology (MDD, PTSD)[69,70,111,113] and that insomnia is related to these psychiatric disorders,[1] it is possible that similar relationships (i.e., stronger effects for interpersonal traumas) exist for trauma and sleep. A recent investigation of urban young African Americans examined seven specific traumatic events that were both interpersonal and non-assaultive in nature, finding that five of the seven events significantly predicted insomnia (ORs ranging from 1.53-3.27) and that the three interpersonal traumas (sexual trauma, physical assault, sudden violent death) all predicted more severe insomnia (ORs ranging from 2.39-2.86).[147] While this suggests that interpersonal traumas may indeed be predictive of more severe disorder, it is important to note that results changed upon addition of covariates (which included PTSD), with no traumas retaining significance in the more severe insomnia category. Further, note that each traumatic event was examined separately to determine its individual effect on insomnia, not examined in combination. In another recent study, investigators analyzed the individual and combined effects of interpersonal and non-assaultive traumas on self-reported sleep in college students, assessed via a modified version of the Pittsburgh Sleep Quality Index (PSQI).[148] When analyzed hierarchically, only interpersonal trauma had a significant effect on disturbed sleep. Non-assaultive trauma did not contribute uniquely to the variance in sleep symptoms. These are some of the first results comparing multiple traumas, and indicate that interpersonal traumas may have a larger effect on the development of sleep problems.

Trauma timing and sleep. ACEs in particular have been shown to have deleterious effects on sleep even decades after the abuse, most notably for women.[141] In a recent systematic review, the authors examined the effect of ACEs on future sleep problems in adults, summarizing 30 studies.[141] This literature primarily consists of retrospective studies where individuals report on prior ACEs and many of the included studies have female-only samples. There is also a range of sleep phenotypes within the literature, although studies converge on significant associations between child trauma and sleep. For example, a prospective study of adolescents examined ten years after the abuse demonstrated that CSA was related to a higher subjective sleep disturbance score, an association that remained following the addition of both MDD and PTSD symptoms (which themselves can influence sleep).[149] In another study by Greenfield and colleagues,[150] individuals endorsing the most severe class of abuse, which included sexual abuse, reported increased odds of poor subjective sleep measures from the PSQI. More specifically, individuals were more likely to experience more sleep disturbances, worse sleep quality, and more daytime dysfunction, in addition to being more likely to utilize sleep medication (all odds ratios > 2). A recent paper[151] added to the literature by examining sleep and CSA in a large twin sample, finding that for both males and females, CSA increased the odds of experiencing sleep symptoms by 1.7. Additionally, incident characteristics, including severity, in females endorsing CSA were examined, but none of these items were differential predictors of worse sleep. Further, there may be sex differences, with a newer study demonstrating that women are more likely to experience poor sleep in adulthood following childhood adversity.[152] Thus, it is possible that both type and timing interact. In a recent paper, scientists showed that insomnia symptoms in adolescents were highest with a history of interpersonal violence (vs. accidents/injuries, social/network events, other).[153] While most studies report associations with sleep, one notable limitation of the child

trauma and sleep literature is that the majority of these studies have been conducted in North American or European populations, with no studies in African or East Asian countries including China.

Trauma, sleep, and psychopathology. An important consideration in interpreting the trauma and sleep literature is that many studies examine sleep problems along with (or as symptoms of) comorbid psychopathology. Thus, while it is well established that trauma is related to sleep disturbances,[67] results are often confounded by psychopathology. There is a substantial literature of longitudinal investigations that establish temporal relationships between sleep and psychopathology, demonstrating that sleep disturbances both pre- and post-trauma can have an impact on psychiatric outcomes.[41,42,67,140,154-158] For example, Bryant and colleagues[156] examined the relationship between sleep disturbances pre-injury and later psychiatric disorders in a sample of patients admitted to the hospital. Most disorders (e.g., PTSD, MDD, obsessive compulsive disorder, substance use) were significantly associated with pre-trauma sleep disturbance, even after excluding prior psychopathology. Extending results to an Army sample, Gehrman and colleagues[41] looked at associations between pre-deployment insomnia and sleep variables and the development of psychopathology after deployment. Results indicated that insomnia symptoms were associated with a higher risk of new-onset MDD, PTSD, and anxiety. Notably, the effect size of insomnia was similar to that of the trauma (i.e., combat exposure) in all models. There are also studies of post-trauma symptoms. Wright and colleagues[157] looked at insomnia, MDD, and PTSD at two time points post-deployment, finding that insomnia symptoms at Time 1 predicted PTSD and MDD symptoms at Time 2. More recently, a study of Chinese adolescents demonstrated that sleep disturbances predicted MDD and PTSD at multiple time points

following a major earthquake.[159] Thus, both pre- and post-trauma sleep symptoms can influence psychopathology.

Summary and future directions for environmental influences on sleep. There are four main points to be drawn from this review of the complex trauma, sleep, and psychopathology literature: 1) trauma exposure is associated with disturbed sleep/insomnia, although many studies include comorbid psychopathology as an outcome or covariate; 2) disturbed/sleep insomnia is a predictor of later psychiatric symptoms in individuals exposed to traumatic events; 3) an emerging literature is investigating contributions of trauma type to insomnia, with early results suggesting that interpersonal trauma is a more potent predictor; and 4) trauma timing is also important, with evidence that early trauma influences sleep in adults. However, the extant literature has a number of limitations that represent important future directions. First, few studies examine multiple different trauma types together, focusing instead on specific events. Examining multiple events will allow for a comparison of traumas, improve our understanding of how events cluster together, and establish important differences in trauma type. Second, there is a need for more studies that focus specifically on how trauma impacts sleep, apart from psychopathology. Third, with few studies parsing out effects of both childhood and adult traumas together on sleep, trauma timing should continue to be examined. Finally, while the childhood trauma and sleep literature has increased substantially, there is clearly a lack of studies using non-European populations, such as the Han Chinese, which should be addressed.[141] While trauma exposure is an environmental risk factor that impacts disturbed sleep and insomnia, there are other etiologic sources, particularly those that are biologic in nature, to consider in understanding these phenotypes. One such factor is genetics, and there is a long history of

examining the genetic contributions to insomnia, which will be discussed in the following section.

III. Genetic components influence both sleep and MDD

a. Behavioral genetics

Family studies. Twin and family studies demonstrate that genetic influences are an important etiological risk factor for insomnia. Five early family studies of insomnia exist, with the first dating back to the 1960s.[160-164] Higher rates of insomnia in family members of individuals with the disorder were found across all studies, supporting familial aggregation of insomnia. Studies did differ in terms of timing of insomnia assessed (childhood vs. adulthood; two studies looked at childhood sleep patterns) and outcome phenotype examined (insomnia vs. sleep patterns), but overall results suggest a familial component. A more recent family study[165] utilized more sophisticated measures to analyze familial aggregation and heritability, finding that risk ratios for current and lifetime insomnia were > 2 in first-degree relatives and that heritability estimates were relatively high, even when controlling for psychopathology (0.48 for current; 0.58 for lifetime). Additionally, a new family study reported a risk ratio of 1.80, consistent with prior studies.[166]

Twin studies. The current twin literature for insomnia is much larger than that of family studies. The twin approach has several notable advantages, including the calculation of specific estimates of genetic contributions (i.e., heritability), and the ability to parse out shared environmental components that make individuals similar on a given phenotype. To date, there are more than 20 twin studies of insomnia and related symptoms (excluding sleep duration), 14 of which were conducted in young adult/adult samples, with the remaining studies coming from

pediatric samples, and the majority of participants being European or of European-American descent. Overall, most adult studies report heritability estimates between 20% and 60%.[167] The vast majority of adult twin studies have utilized subjective sleep phenotypes, with the exception being an early study that used EEG data on sleep latency and wake time.[168] It is important to note that these estimates are consistent despite the wide variety of phenotypic definitions used across the literature (see Table 2 of Lind and Gehrman (2016)[167] for specific estimates). Most twin studies have used definitions based on individual symptoms or combinations of symptoms, and these definitions have been heterogeneous with little standardization across studies.[167,169]

The wide range of heritability estimates found in the literature may be due to methodological differences across twin studies. For example, many of the higher estimates that have been found for insomnia sum/composite scores are in younger samples (e.g., Watson and colleagues[170] reported an insomnia heritability of 57% (95% CI: 47% to 63%) with an average age of 32; Drake and colleagues[171] found that the heritability of insomnia was 55% in women and 43% in men in with an average age of 23). In contrast, studies of older twins have yielded lower estimates. For example, Hur and colleagues,[172] reported an insomnia symptom heritability of 28% (95% CI: 25% to 31%) with an average age of 50, and the Vietnam Era Twin Study of Aging found the heritability of a global sleep composite score to be 34% (95% CI: 25% to 42%) in males with a mean age of 55.[173] Given that sleep problems become more prevalent as individuals age,[2] it is possible that there is less variation attributable to genetic effects. This could be a result of the high prevalence of comorbid health conditions associated with age that also affect sleep (e.g., arthritis, back pain). Higher heritability has also been reported using longitudinal data, a design that helps mitigate the effects of measurement error; although note that higher estimates are expected based on study design. Specifically, while Lind et al.[174] found

that time-specific estimates of insomnia heritability were only around 20%, estimates increased to 59% (females; 95% CI: 44% to 69%) and 38% (males; 95% CI: 27% to 48%) when both time points were modeled simultaneously. The degree of insomnia heritability may differ across the sexes, referred to as a quantitative sex effect, which is consistent with studies of MDD,[175] and highlights the importance of conducting analyses by gender since genes may be more relevant for insomnia in women.

The twin literature also includes studies that have investigated individual insomnia symptoms, such as difficulty falling asleep or difficulty staying asleep, instead of or in combination with insomnia composite scores. In general, these estimates range from 25-45% and tend to be lower than estimates for insomnia overall.[167,171,176] For example, a study of Vietnam veterans[176] found heritabilities of 28% (trouble falling asleep), 42% (trouble staying asleep), and 26% (waking up several times) among individual symptoms. A Finnish twin study of sleep and mortality reported estimates that were similar but a bit higher, with heritability at 41% (difficulty initiating sleep; 95% CI: 36% to 46%), and 45% (nocturnal awakening; 95% CI: 41% to 49%).[22] Interestingly, Drake and colleagues[171] reported similar estimates for DSM insomnia symptoms of difficulty staying asleep (25% in males and 35% in females) and non-refreshing sleep (34% in males and 35% in females), but no genetic influences on difficulty falling asleep for both genders. Heritability estimates from the PSQI, the gold standard self-report measure for sleep quality, have also converged with the other estimates reported. Two studies in adults have analyzed the PSQI and its subscales,[173,177] finding estimates of 23% (95% CI: 9% to 36%) and 39% (95% CI: 2% to 53%) for the sleep disturbances subscale, which has individuals describe how often certain events (e.g., trouble falling asleep, waking up, having bad dreams) contribute to trouble sleeping.[178] Overall, the twin literature supports that insomnia, whether measured by a

composite/total score or by individual symptoms, is moderately influenced by genetic factors. The highest heritability estimates are seen for insomnia composite scores in younger samples, which may be related to the fact that sleep disturbances become more common with age.[2]

The sleep literature is consistent with that of the substantial genetic epidemiologic literature on MDD in that MDD is also moderately heritable (37-38%).[179-181] There is also evidence that MDD heritability differs across the sexes, with higher estimates in females (40-42%) vs. males (29-39%).[182,183] Several groups have examined the heritability of individual MDD symptoms as well, indicating that the sleep symptom more specifically is moderately heritable (35% and 19%).[184,185] Given bidirectional relationships between MDD and insomnia,[32] there is a growing twin literature that examines the overlap in genetic and environmental influences on insomnia and internalizing disorders, particularly MDD (e.g., [186,187]). Initial examinations of etiologic overlap were conducted primarily in samples of children,[188-194] with several studies reporting high estimates of overlap between insomnia phenotypes and MDD (e.g., Gehrman and colleagues[189] reported complete genetic overlap; Gregory and colleagues[193] reported a genetic correlation of 0.64). Emerging longitudinal data in adults supports these relationships. In a longitudinal examination of overlap between insomnia and common psychiatric disorders, substantial overlap was found, with 56% (females) and 72% (males) of insomnia's latent heritability shared with that of MDD.[187] Additionally, a recent young adult study modeled the genetic and environmental overlap between insomnia and MDD longitudinally using a correlated factors model, once again finding high genetic correlations between the two traits (0.73-0.89).[186] In sum, family and twin studies have laid the groundwork for molecular genetic studies of both insomnia and MDD, which aim to identify specific genes

contributing to these disorders. Further, evidence for shared genetic influences on insomnia and MDD indicates that this overlap should be considered at the molecular level as well.

b. Molecular genetics

Candidate gene studies. In the section that follows, a brief overview of select candidate gene studies is presented, followed by a more detailed discussion of the more contemporary genome-wide designs. The main focus will be on studies conducted on insomnia phenotypes, but the molecular genetics literature of MDD will be referenced for comparison. In the candidate gene approach, gene(s) of interest are selected *a priori* based on biological mechanisms/prior research and variants within the gene(s) are examined in association with the phenotype of interest (e.g., insomnia vs. control, quantitative measure of sleep disturbances). Next, statistical analyses are conducted to determine whether or not the variant of interest occurs more frequently in individuals with the phenotype (vs. without). Initial candidate gene studies for insomnia and related phenotypes focused mainly on genes that were plausible based on mechanism. Variants within circadian rhythm genes, such as *CLOCK*, *PER*, and *TIMELESS*, were examined in early studies, given that circadian processes are important for sleep. Serotonin, a neurotransmitter that plays a role in sleep regulation[195] and is also widely studied in MDD,[196] has also been examined in sleep. These investigations have included assessments of polymorphisms in the serotonin transporter (e.g., *5-HTTLPR*; e.g., [197,198]) as well as in enzymes that degrade serotonin (monoamine oxidase; MAO) (e.g., [199,200]). More recently, new systems that have been studied in relation to insomnia include dopamine, apolipoprotein, PGC-1α, and the aryl hydrocarbon receptor.[201-204] Overall, the candidate gene literature for insomnia is small compared to psychiatric phenotypes such as MDD (where around 200 different genes have been analyzed and multiple meta-analyses conducted[205]). While the results of some of these insomnia studies show

significant or suggestive results, results are mixed, particularly for serotonin. Investigations of serotonin and *CLOCK* polymorphisms often examine insomnia symptoms in individuals with MDD or receiving treatment, which makes it challenging to parse out the effects of the variants on insomnia more specifically.

Limitations of the candidate gene approach. While candidate gene studies utilize a hypothesis-driven approach that incorporates variants in genes thought to be biologically relevant, there are several major limitations to consider,[206,207] especially given the lack of replication seen across studies.[208] First, the candidate gene approach relies on the appropriate choice of gene and variants, which is critical yet can be difficult given our minimal understanding of the biologic etiology of many phenotypes.[207] Further, in order to achieve the best interpretability, variants should be chosen to maximize function (i.e., within coding regions; related to gene expression, or at least tagging other functional variant(s),[206,207,209] but are often chosen based on ease of genotyping and are limited by our knowledge of the genome.[207] Second, although discrepant results could be due to real differences in study population or phenotype,[207] the use of small sample sizes and low power suggest that many results may be invalid (i.e., false positives, false negatives in replication attempts).[210] Within the MDD literature, meta-analyses of the most commonly studied genes have not converged on strong predictions (even with refined phenotypes),[211] and, despite having enough power to detect these effect sizes in more agnostic approaches (i.e., GWAS), hypothesized genes are not significant.[205] In contrast, it is difficult to even meta-analyze genes within the insomnia literature, as few studies examine the same variants and phenotypes are heterogeneous.[167] Finally, another important issue is population stratification, as spurious results could be due to differences in allele frequency across ancestries.[206] Quantitative approaches to classifying ancestry can improve this, but were not used in many

early studies.[212] Thus, while the candidate gene literature provides some evidence of a role for specific genes in these phenotypes, results do not converge and additional approaches are needed.

Introduction to GWAS of insomnia and MDD. GWAS approaches, which allow for the simultaneous examination of millions of variants (measured or imputed) across the genome to identify potential loci contributing to a phenotype, have been used to gain new insight into the genetic contributions to insomnia. To date, there have only been five published GWAS of insomnia or related sleep phenotypes[213-217] and one replication study.[218] Studies that analyze only sleep duration are excluded from this total. This total is in contrast to other psychiatric phenotypes like MDD, where a mega-analysis of eight GWAS was published in 2013[219] and multiple additional large GWAS have been published since then (e.g., [220-223]). Although the MDD mega-analysis combined data on over 9,000 cases and 9,000 controls of European ancestry, no genome-wide significant (GWS) loci were found.[219] However, more recent GWAS of MDD have focused on different phenotypes (recurrent MDD, "broad" depression) and/or large sample sizes, with more success (e.g., [220-223]). Within a sample of Han Chinese women (N ~ 11, 000), two novel GWS loci were found for recurrent MDD: one near *SIRT1* and one in *LHPP*.[220] More recently, a meta-analytic GWAS (N ~ 70,000) conducted on a "broad" depression phenotype that encompassed both diagnosis and symptoms, was able to identify and replicate a single nucleotide polymorphism (SNP) with the *FHIT* gene.[222] Finally, in what is the largest GWAS of MDD to date (over 70,000 cases/200,000 controls), investigators from 23andMe identified novel loci for MDD.[223] When this data was meta-analyzed with Psychiatric Genomics Consortium (PGC) MDD data and combined with an additional 23andMe replication sample, a total of 17 SNPs in 15 loci reached genome-wide significance for MDD.

Specific GWAS of insomnia. In comparison with MDD, there are fewer loci that have been identified for insomnia and most studies have used much smaller sample sizes; results are outlined in the following sections. Note that measurement issues remain within this literature, as only three of the five GWAS include specific "insomnia" phenotypes and the majority of papers utilize self-report data (with the exception of Spada and colleagues,[217] who used objective phenotypes from actigraphy). The first GWAS of insomnia, published by Ban and colleagues,[214] utilized genetic and phenotypic data on 8,719 individuals from a Korean epidemiologic sample who reported on their insomnia status via self-report. Although no loci reached genome-wide significance, several top SNPs were determined to be of interest (out of 3354 SNPs with $p < 0.005$), and had prior associations with psychiatric disorders. *ROR1* included the most significant SNP, and there were multiple other SNPs of marginal significance within this gene, which has been linked to bipolar disorder.[224] *PLCB1* was the next-most significant (a total of 17 SNPs in this gene reached the marginal significance cutoff), and the gene has associations with schizophrenia.[225,226] Next, Byrne and colleagues[215] utilized an Australian twin sample (N = 2323) to conduct a GWAS of an insomnia factor score and other sleep phenotypes (sleep latency, sleep time, sleep quality, sleep depth, sleep duration). No SNPs passed genome-wide significance for any phenotypes, including the insomnia factor score. Of interest in this study was a set of SNPs in linkage disequilibrium (LD) (i.e., recombination occurs between these loci more frequently than would be predicted by chance[227]) in the *CACNA1C* gene, which were nominally significant in predicting sleep latency. However, these associations did not reach significance in replication samples either. Of note, another SNP in *CACNA1C* was of interest for sleep quality. Similar to the genes suggested by Ban and colleagues,[214] above, this gene is associated with both schizophrenia and bipolar disorder, in addition to MDD.[228,229] Parsons and colleagues[218] sought

to replicate the *CACNA1C* association in a British sample (N = 952) that had PSQI data on sleep quality, duration and latency. This was successful: the SNP was associated with sleep latency in the new sample, which provides additional evidence for its relevance in sleep phenotypes. Despite being a different variant than the one identified in the initial study, this *CACNA1C* SNP was also associated with sleep quality in the replication sample, further supporting the role of this gene in sleep.

Although these initial GWAS studies were not successful in identifying GWS variants for insomnia, they were able to suggest novel variants that were not previously investigated in candidate gene studies, with some evidence of replication. Additionally, all SNPs of interest from these studies seem to be relevant for other psychiatric disorders as well. Newer studies using different phenotypic approaches and larger sample sizes are adding more genes of interest to the literature and are finding GWS results. Spada and colleagues[217] were the only group to analyze objective phenotypes, using sleep parameters derived from actigraphy data in a sample of 956 adults in Germany. Some of these, like sleep quality and sleep latency, correspond to subjective phenotypes measured in other GWAS. There were significant SNPs in multiple parameters, including sleep efficiency on weekdays (*UFL1*, a circadian gene), sleep latency (*DMRT1*), and sleep offset (*SMYD1*). However, these results should be interpreted in light of several key limitations: 1) there was no correction for the examination of multiple phenotypes; and 2) a diagnosis of insomnia is made using self-report information on symptoms; to date there are no clear patterns within objective data from polysomnography or actigraphy.[230-232] Another new study[213] identified the *RBFOX3* gene as important for self-reported sleep latency in a large combined sample (N = 4242) of Europeans. The heritability of sleep latency in this sample was estimated at approximately 20% using SNP-based approaches, reviewed in more depth below.

Three correlated SNPs within this gene were found to be significant and could be replicated in additional samples. Gene network analysis was also conducted, and results indicated associations between *RBFOX3* expression and expression of genes involved in calcium channels and gamma-amino-butyric acid (GABA) signaling (gene expression and methylation data was also collected on a subset of individuals). GABA is an inhibitory neurotransmitter that is important for sleep regulation,[233] supporting the plausibility of *RBFOX3*'s role in sleep latency. *RBFOX3* was also related to other neurotransmitter release (glutamate, serotonin, dopamine), through these processes. The results of these first four GWAS of insomnia phenotypes, while identifying different genes and SNPs of interest, do converge in terms of gene function: processes involved in excitability or sleep reactivity (i.e., quality of sleep in response to a stressful event[171]) are seen across these genes.[167]

Most recently, the largest GWAS of sleep phenotypes to date was published using the United Kingdom (UK) Biobank data set, which is comprised of health data from over 500,000 volunteers from the UK.[216] Data on sleep duration, insomnia symptoms (measured via a single ordinal item that asked about frequency of difficulty falling and staying asleep, with only the extremes of "never/rarely" and "usually" used for analysis), and daytime sleepiness were collected. Insomnia analyses were conducted on 31,767 cases and 29,935 controls. Variants in five novel genes (including two that may be sex-specific) were identified in this study for insomnia, and results held when including covariates such as depression. These included *MEIS1* (which was also found to be significant when a multi-trait sleep GWAS was conducted using data on sleep duration, insomnia, and daytime sleepiness), *TMEM132E*, *CYCL1*, *TGFBI* (females), and *WDR27* (males). Interestingly, *MEIS1* has been implicated in restless legs syndrome, another sleep disorder,[234] and the same *CYCL1* SNP reached nominal significance in a

GWAS of comorbid alcohol dependence and depression.[235] *TMEM132E* is relevant given its relationship to psychopathology, as variants near *TMEM132E* have previously been associated with bipolar disorder[236] and variants in another gene in the same family, *TMEM132D*, have been replicated across several GWAS of panic disorder.[237,238] Finally, the two sex-specific genes of interest, *TGFB1* and *WDR27*, have been linked to Type 1 diabetes,[239-241] suggesting that immune processes may be involved in insomnia. Taken together, the results of this newest GWAS indicate that genes involved in insomnia may be shared not only with other psychiatric disorders but also with complex traits. However, an important caveat is that the insomnia phenotype was a single ordinal item and not an established sleep measure or diagnosis.

Summary of molecular literature. Overall, molecular genetic studies of insomnia have demonstrated mixed results in candidate gene studies, and notably, there have not been any times in which a GWAS of insomnia has identified a gene that has been studied in a candidate-gene framework. The early GWAS studies failed to find GWS loci, but are beginning to identify genes of interest as data are combined, resulting in increases in sample sizes and associated statistical power. Genes of interest tend to show prior associations with psychopathology.[167] This pattern of gene-finding efforts parallels that of other psychiatric disorders, although the insomnia literature lags behind (i.e., there are far fewer identified GWS loci for insomnia compared to schizophrenia and MDD; e.g., [220-223,242]). Given this and the small number of published studies to date, it remains important to continue gene identification as well as replication efforts for insomnia in different samples. Other than the Korean GWAS,[214] all studies were conducted in samples of European origin, which limits generalizability of findings. Further, while twin studies do converge on heritability estimates despite diverse phenotypes, consistent phenotyping remains a problem in gene identification studies, as phenotypic heterogeneity can contribute to lack of

replication and makes it difficult to synthesize results. To date, no GWAS of insomnia utilize DSM diagnoses. It will be important for the field to decide on suitable phenotypes and attempt to conduct genetic analyses appropriately.

Introduction to aggregate molecular approaches. Advances in statistical genetics have resulted in the development of methods that estimate heritability from available genomic data on unrelated individuals. One such approach is genome-wide complex trait analysis (GCTA), which utilizes the estimated genetic relationships between unrelated individuals to estimate the variance in a trait that is due to the additive effect of SNPs that are available.[243] This is done through the creation of a genetic relatedness matrix (GRM), which includes correlations for all individuals across all SNPs, which is then regressed on phenotype using a restricted maximum likelihood (REML) method. This widely popular method has now been used across a variety of complex traits (e.g., [244-248]), although heritability estimates tend to be lower than twin estimates, and GCTA represents a lower bound.[245,249] The method is not without its limitations, particularly since large sample sizes are needed to have adequate power to detect heritability.[250] Analyses of case-control traits in particular can result in biased estimates since heritability must be transformed onto a liability scale[251] and one recent critique suggests that GCTA is not accurate even when all assumptions are met, due to problems with the GRM (e.g., noise, over fitting).[252] As described within the definition, GCTA can only take into account SNPs that are measured (or imputed) and other variants that are in LD with them. As this generally encompasses common SNPs, rarer variants are not represented in GCTA, although they could be contributing to the disorder. GCTA also assumes additive effects, and thus does not take into account other genetic effects such as dominance effects, epistasis, or gene-environment interactions (GxE).[245,249] Further, there are instances where the genetic architecture of a trait is such that GCTA would not

detect heritability, despite the trait having large genetic effects. This could occur if there are one or two significant loci of large effect and then all other variants have small effects (e.g., closer to Mendelian inheritance). Despite these limitations, GCTA does have several advantages, in that it is not biased by sample size or to specific effects and conclusions can be made about the trait as a whole.[245] Genetic correlations between traits can also be obtained through GCTA (bivariate), although there are other methods, such as LD Score regression (LDSC),[253] that can estimate correlations (and heritability) through summary statistics.

There are also additional approaches that leverage molecular data to examine aggregate genetic risk and overlap across phenotypes, often without needing raw genetic data for all phenotypes involved. Polygenic risk scores (PRSs), which utilize summary statistics from a discovery dataset to calculate weighted risk scores that are then applied to individuals in a target sample, represent one approach. A key difference between GCTA and PRSs, which are both aggregate approaches, is that PRSs take into account specific SNP effects, weighting contributions based on effect size from GWAS, while GCTA assumes that effects are random. PRSs are based on the idea that many traits are polygenic in nature (i.e., many genes of small effect that do not pass GWS thresholds via conventional GWAS methods contribute to the trait).[245,246,254,255] The assumption is that within the variants that make up a risk score, there are some with true effects. This approach was initially used to show that genetic risk scores for schizophrenia predicted bipolar disorder (and vice-versa),[254] and since the publication of this first paper, the method has been applied to many phenotypes (e.g., [247,248]). Commonly referred to as the Purcell method, an important step in this method of creating genetic risk scores is pruning the list of SNPs based on LD before score creation, as including highly correlated SNPs could bias the risk scores (although pruning can be done through several approaches[256]). The Purcell

method also incorporates *p*-value thresholds, such that multiple scores are created using SNPs within different ranges of *p*-values, ranging from very small to encompassing all SNPs.

New methods for computing PRSs (e.g., LDpred,[257] PRSice[258]) are emerging to address limitations of the initial method. One main criticism of the Purcell method, described above, is that important information is lost due to LD pruning. To address this limitation, the program LDpred incorporates LD information from a reference panel and takes into account LD, and is thus able to utilize all available SNPs in the score.[257] The program also estimates scores differently, in that it utilizes a Bayesian prior for the genetic architecture of the trait based on an estimate of the trait's heritability and proportion of variants (i.e., fraction) that are causally contributing to the trait to generate scores across different fractions. The method does have its own limitations, although most of these are related to PRSs in general (e.g., controlling for ancestry, appropriate LD reference). Like GCTA, PRSs are limited by the SNPs that are available (i.e., in common across discovery and target samples) and do not generally take into account non-additive effects, GxE, epistasis, or rare variation.[245,249] Sample size is also important, particularly for the discovery sample, as larger discovery samples will give better estimates.[245,259] Another problem within the PRS literature is the heterogeneity in score creation,[256] making it difficult to compare scores across studies.

Applications and future directions. While these methods have increased our understanding of genetic contributions to many complex traits, sleep phenotypes are under-studied. To date, there are only three studies that have examined SNP-based heritability of insomnia-related outcomes. Estimates range from 30% for the insomnia symptom of MDD[260] to 20% for sleep latency,[213] and 21% for insomnia within the UK Biobank GWAS.[216] Note that these estimates were obtained using methods similar to GCTA (i.e., heritability was based on

SNPs) but not the GCTA software, described above. The estimate obtained for insomnia within MDD[260] is consistent with the twin literature. In contrast, estimates of the two more general insomnia-related phenotypes are on the lower end when compared to twin estimates of insomnia, which is consistent with what is seen across the literature for other phenotypes.[245,261] Further, despite evidence for biometric overlap between insomnia and MDD (e.g., [186,187,189]), and the presence of some candidate gene studies that examine genetic contributions to MDD with insomnia/sleep symptoms (e.g., [199,262-266]), few studies examine genetic overlap and/or incorporate a PRS approach. In the 23andMe MDD GWAS,[223] a genetic risk score was created for MDD (from the 17 identified SNPs) and used to predict a range of other related phenotypes, including insomnia (assessed via a yes/no question about difficulty "getting to sleep"), where it was a significant predictor. Within the UK Biobank sample, LDSC was used to examine genetic correlations between sleep variables and 20 other phenotypes, finding a significant correlation between insomnia and depression (as well as several metabolic traits).[216] Overall, studies that utilize aggregate molecular approaches for insomnia-related phenotypes are only just beginning to emerge, and more investigations are warranted, particularly using more refined insomnia phenotypes. The use of statistical methods described here, in addition to other post-GWAS approaches, such as gene and pathway analysis, will be important in developing our understanding of the genetic architecture of insomnia and how it relates to MDD, as this may have implications for gene-finding efforts.

IV. Aims

Sleep disturbances and insomnia affect the well-being of many individuals worldwide, making an in-depth understanding of their etiologic influences (both genetic and environmental

in nature), above and beyond what has been examined in the extant literature, essential for improving population health. This dissertation aims to address several important and understudied areas within the sleep literature using the China, Oxford, and VCU Collaborative Research on Genetic Epidemiology (CONVERGE) dataset, which contains detailed phenotypic and genetic data on approximately 12,000 Han Chinese women, half with recurrent MDD. Since few investigators have examined specific effects of trauma type and timing on sleep outcomes across multiple trauma types while accounting for psychopathology in non-Western populations, Aim 1 is to determine the relationship between type of trauma exposure (interpersonal vs. non-assaultive) and timing of trauma exposure (childhood vs. adult onset), and sleep disturbances (Chapter 3). It is hypothesized that interpersonal traumas will be stronger predictors of sleep than non-assaultive traumas, and that childhood events will be more potent than those that have initial onset during adulthood. Next, as genetic influences are also important for insomnia, but molecular studies are still in their infancy, with few examining SNP-based heritability or looking at sleep in the context of or overlapping with MDD, Aim 2a is to conduct analyses of SNP-based heritability of disturbed sleep (Chapter 4), and to identify potential genetic variants associated with sleep phenotypes in CONVERGE (both within and independent of MDD) (Aim 2b, Chapter 5). It is hypothesized that sleep phenotypes will be heritable and that genetic loci of interest will be identified for these phenotypes. The goal of Aim 2c is to examine molecular overlap between the different sleep phenotypes as well as between sleep and MDD within this sample by utilizing two different methods of PRSs (LDpred and the Purcell method) (Chapter 6). It is expected that PRSs for sleep within MDD will significantly predict sleep in MDD controls (and vice-versa) and that sleep and MDD risk scores will significantly predict each other. Finally, in an effort to combine both genetic and environmental influences in order to obtain a more complete etiologic

model of sleep, the Exploratory Aim (Chapter 7) is to conduct cumulative models of combined genetic and environmental risk for sleep disturbances using results generated in Aims 1 and 2 (e.g., PRS and trauma variables as predictors of sleep). For this aim, it is expected that both genetic and environmental risk factors will have significant contributions to sleep disturbances.

Chapter 2: Methods

I. Sample

The CONVERGE dataset was used for analysis.[48] CONVERGE is a large genetically informative sample comprised of approximately 12,000 Han Chinese women aged 30-60, 50% with recurrent MDD (cases) and 50% matched screened controls, which was initially ascertained to conduct detailed genetic and phenotypic analyses of MDD. Genetic data passing all quality control (QC) standards (described in more detail below) is available for ~88% of the sample (N=10,502). Data was collected from 59 hospitals from 45 cities in 23 provinces and municipalities in China via clinical interviews. Exclusion criteria for cases were i) history of bipolar illness, ii) psychosis outside depressive episodes, iii) drug or alcohol dependence with onset prior to MDD, iv) developmental disability, and v) blood relative of another case. CONVERGE included only individuals as cases who presented to psychiatric services for depression and received treatment. The entry criteria for controls were i) female, ii) current age 40-60 (to reduce the likelihood that these individuals would later develop MDD), iii) no lifetime history of MDD, and iv) all four grandparents Han Chinese. Controls were excluded if they had a developmental disability, had a known history of bipolar illness or psychosis, or were a blood relative of a case.

II. Measures

Identification of traumatic events. Individuals were asked about 16 SLEs (via yes/no responses), with each item including a follow up question about the age at the first time this event occurred. These items were modified from those used within the Virginia Adult Twin Studies of Psychiatric and Substance Use Disorders (VATSPSUD).[175] Ten of these events were considered to be potentially traumatic events, based on the DSM-5 definition that a trauma must involve a potential life threat or a threat to the integrity of self/others.[1] An additional childhood trauma variable, CSA, was assessed separately with six items that queried its occurrence and severity in response to the following question: "Before the age of 16, did any adult, or another person older than yourself, involve you in any unwanted incidents like…" The six items included the following question stems: a) Inviting or requesting you to do something sexual; b) Kissing or hugging you in a sexual way; c) Touching or fondling your private parts; d) Showing their sex organs to you; e) Making you touch them in a sexual way; f) Attempting or having sexual intercourse. Response options to these questions included 0 (never), 1 (once), and 2 (more than once). A broad yes/no CSA variable was created by coding individuals who endorsed at least one of the six items at least once as 1 and those who did not endorse any of the items as 0 (this is consistent with prior analyses of CSA[124,129,151]).

The 11 traumatic events included in subsequent analyses were: 1) death of a spouse, child, or sibling; 2) serious illness (self); 3) life-threatening accident (self); 4) fire, flood, or natural disaster (self); 5) witnessed someone being badly injured or killed; 6) rape; 7) physical attack or assault (self; other than events already indicated); 8) physical abuse as a child (other than events already indicated), 9) serious neglect as a child; 10) CSA (binary variable); and 11) threatened with a weapon, held captive, or kidnapped (self; other than events already indicated).

Assessment of CSA characteristics. Additional CSA items, modified from those used in the VATSPSUD,[175] were also assessed. An ordinal categorical CSA severity variable was created using this data: (1) no genital contact (sexual invitation, sexual kissing, exposing), (2) genital contact but no intercourse (fondling and sexual touching), and (3) intercourse. Further, there were a number of items assessing incident characteristics that were also used for analysis.[129] These included: intercourse vs. other types of abuse (coded as 1 and 0, respectively, using the CSA severity variable created above), age at time of first abuse (continuous variable), age of perpetrator (5 categories: under 15 years old, 15-18, 19-24, 25-49, $\geq$ 50; each analyzed separately with 1 coding for that age group being endorsed and 0 for non-endorsement), gender of perpetrator (male [0] vs. female or both sexes [1]), relationship to the perpetrator (relative [1] vs. non-relative [0]), feeling forced or threatened by the person(s) involved (yes [2] and somewhat [1] vs. no [0], treated in the regression as a factor with three levels), and how much the incident affected the victim at the time (on a 7-point scale, with 1 not endorsing being affected and 7 endorsing that the experience affected you a great deal, recoded to 0-6); if more than one incident occurred participants were asked to rate the worst one).

MDD assessment. Participants were assessed for recurrent MDD (lifetime) using the Composite International Diagnostic Interview (CIDI; World Health Organization lifetime version 2.1; Chinese version) section for the disorder expanded to include a "deep" assessment of the DSM-IV A criteria for MDD (32 questions for the 9 criteria including culturally sensitive probes for sad mood and loss of interest). Symptoms were reported for the worst lifetime episode of MDD, as indicated by the individual.

Sleep assessment.

General sleep item (GS). All individuals (both MDD cases and MDD controls) were asked a lifetime question about general sleep patterns, "Do you suffer from sleeplessness?" within the Neuroticism subscale of the Eysenck Personality Questionnaire,[267] which was translated into Mandarin. This is a binary/yes no item, and is used as the main sleep outcome in many analyses, as it is the only sleep-related item asked of both MDD cases and MDD controls.

Sleep items within MDD. Two binary sleep items were also asked in the context of worst MDD episode: "During that worst time (when you were feeling depressed/had lost interest/lacked energy), did you have trouble sleeping almost every night for two weeks or more -- either trouble falling asleep, waking in the middle of the night, or waking up too early?" [Item E8]; "Did you often wake up in the early morning before you wanted to get up and be unable to get back to sleep?" [Item E8.A].

Composite sleep variables. i. Sleep within depression score (SDS): The two MDD sleep items, listed above, along with the general sleep item, were summed within MDD cases to obtain a sleep within depression score, an ordinal variable with levels ranging from 0-3. This variable was treated as a quasi-continuous variable in genetic analyses (note that it was highly negatively skewed; see Figure 1 for a histogram showing the distribution of this variable). However, log transformation did not improve skewness, so the variable was used without transformation. ii. Adjusted GS: This is a binary sleep variable created for use in genetic analyses that incorporates all available sleep information for both MDD cases and MDD controls. For MDD controls, it is the same as the GS, above. For MDD cases, individuals were coded as a '1' if any of the three sleep items was positively endorsed (i.e., a 1, 2, or 3 on SDS) and a 0 if none of these items were endorsed (i.e., a 0 on SDS).

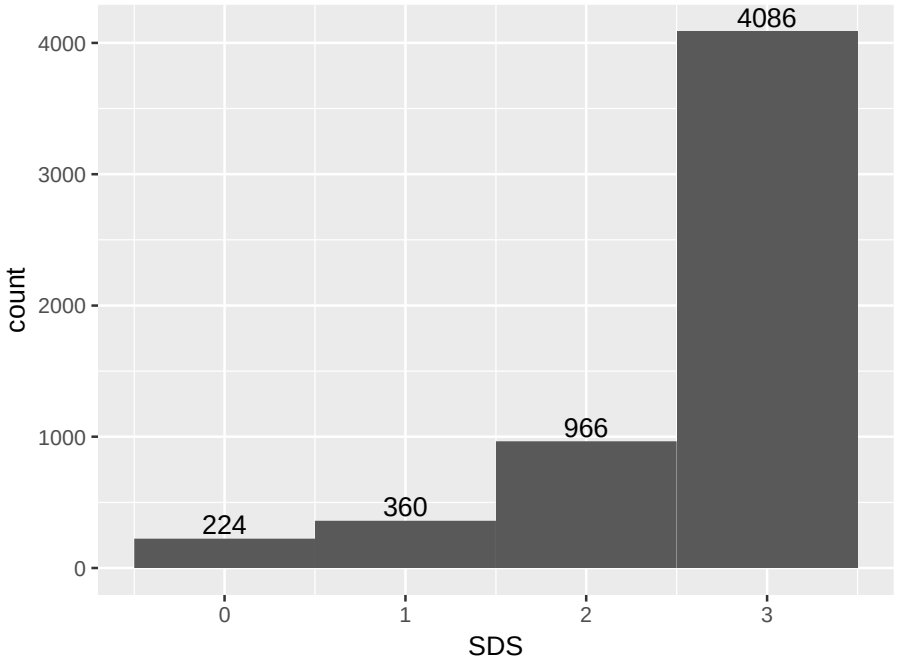


Figure 1: Distribution of sleep within depression (SDS) variable. This histogram displays the endorsement of the SDS variable, with the count for each category included on top of each bar.

Demographic variables. Standard demographic variables were obtained (e.g., age, level of education, current job status, social class, and marital status). Within phenotypic analyses, only age was used as a covariate, as level of education (and other demographic variables relevant to socioeconomic status) had high missingness.

III. Genetic data

DNA sequencing and imputation. Detailed information on genetic methods can be found in the original MDD GWAS.[220] DNA was collected using saliva samples and extracted using the Oragene protocol. Samples were sequenced on Illumina Hiseq machines, which were aligned to Genome Reference Consortium Human Build 37 patch release 5 (GRCh37.p5) with Stampy (v1.0.17)[268] with default parameters. Any reads with base quality ≤ 5 or containing adaptor

sequencing were removed. Missing genotype data was imputed in two rounds using BEAGLE version 3.3.2[269] using Asian samples from the 1000 Genomes Project Phase1 East Asian reference panel.[270] This resulted in a total of approximately 20.5 million imputed SNPs. The imputation method was confirmed to be accurate in calling genotypes: 12 samples were re-sequenced at greater depth (10x), 72 samples were called on a commercial genotyping array, and all samples were genotyped at 21 random sites using a Sequenom mass spectrometry method (overall concordance ~98%).[220]

Population structure. Principal component analysis was conducted to reduce population stratification within the sample using EIGENSOFT 3.0[271] and SMARTPCA.[272] Before inclusion in analysis, SNPs were pruned at $r^2 > 0.7$ to correct for LD.[272] Ten intracontinental principal components (PCs) were constructed using information from 144,929 Autosomal SNPs with Pr(G) > 0.9 and < 1% missing rate. Only PCs 1 and 2, which represent North-South regional differences and technical artifacts, were used as covariates in genetic analyses, as these PCs were used in prior MDD analyses.[220]

Sample selection. Individuals with an excess number of private variants or an excess number of heteroplasmic sites in their mitochondrial genome were removed to eliminate contamination. Individuals were also removed if they had low imputation quality in > 10% of sites, were first-degree relative of another individual (assessed through identity by state), and/or had incomplete phenotype information. This resulted in a maximum of 10,502 independent individuals that could be used for GWAS of sleep phenotypes. Note that this number differs from the original GWAS of MDD within CONVERGE (N = 10,640),[220] as additional related individuals were removed. Specific Ns for each GCTA and GWAS run are presented Tables 13 and 14, column 2, as this differed for each analysis based on missingness across sleep items.

Chapter 3: Analyses of trauma type and timing (Aim 1)

I. Data analytic plan

All analyses were conducted using R version 3.3.0.[273] All figures (except for Figures 2 and 3) were created using custom code in ggplot2, version 2.2.1.[274] Descriptive statistics were run, and the Psych package version 1.6.9[275] was used to obtain tetrachoric correlations across trauma types.

Factor analyses of trauma type. Several standard techniques for evaluating dimensionality (i.e., Kaiser rule,[276] Scree test,[277] parallel analysis;[278] all implemented in the Psych package[275]) were used to determine how many latent factors might be extracted when performing exploratory factor analyses (EFAs). EFAs were then run using the Psych package[275] to estimate different factor structures for the binary trauma type variables. Following this, the factor loadings, cross-loadings, and inter-factor correlations for each EFA were evaluated to provide guidance about specifying and confirmatory factor analysis (CFA) models (i.e., if there were high cross loadings and/or poorly identified factors, the model was not retained). Next, the lavaan package version 0.5-22[279] was used to carry out CFA to more rigorously test restrictive factor models within a structural equation model framework. The best-fit CFA model was chosen based on root mean square error of approximation (RMSEA) and X^2.[280] Finally, the best-fit factor solution identified above was used to inform the creation of new trauma type and timing variables for use in the next step, explained in greater detail within the Results section, below.

Effects of trauma type and timing on sleeplessness. A series of hierarchical logistic regression models were run to examine the effects of trauma type and timing on sleeplessness. For each phenotype, Step 1 examined the effect of demographic covariates (i.e., age, MDD when necessary) on sleeplessness. In Step 2, child interpersonal and adult interpersonal trauma variables were included as predictors. In Step 3, child non-assaultive and adult non-assaultive trauma variables were added to determine whether or not they had unique effects on sleeplessness above/beyond interpersonal traumas. Model fit statistics (i.e., Akaike Information Criteria [AIC], which balances parsimony and model misfit[281]) and Nagelkerke's pseudo R^2 (where possible; calculated by the *pscl* package version 1.4.9[282]) were used to compare models. Following the first set of hierarchical regressions, a second set of stepwise regressions was run to examine potential interactions between trauma type and timing on sleeplessness. The initial model in these analyses was the final model from Step 3 (all types of trauma included), and then interaction terms for child x adult interpersonal trauma and child x adult non-assaultive trauma were added in sequentially (Steps 2 and 3, respectively).

In order to appropriately address ascertainment bias within the CONVERGE sample, these regression models were run in three different ways to account for MDD status. First, all regressions were run in the full sample with MDD as a covariate. Second, a survey-based approach was utilized using the R package *survey*,[283,284] which assigned different weights to individuals with and without MDD to model a population prevalence of 8% (i.e., individuals without MDD were given more weight than individuals with MDD). Third, regressions were run separately in MDD cases and MDD controls for comparison.

Effects of CSA specifically on sleeplessness. Logistic regressions were run within the full sample (with MDD and age as covariates) to examine the effect of one particularly potent form

of child trauma, CSA, on sleep, replicating analyses conducted within the VATSPSUD sample.[151] A binary broad CSA variable was used here to predict sleeplessness in all individuals. The same analysis was also conducted using the survey method to verify results, but MDD cases and controls were not examined separately given that individuals with MDD did not differ across CSA and sleeplessness (i.e., rates of endorsing sleeplessness were similar in those with and without CSA within this subset of the sample). Next, an examination of CSA severity and incident characteristics was conducted in the subset of individuals endorsing CSA. Here, CSA severity, as well as all incident characteristics described earlier in the methods, were used as predictors of sleeplessness. Survey methods were not used, as a reliable expected population prevalence of MDD within individuals endorsing CSA would be needed. Given that only 2.7% of MDD controls endorsed CSA, analyses of incident characteristics were not conducted given scarcity of cell size for certain variables (e.g., five or fewer individuals endorsing certain characteristics).

II. Results

Descriptive statistics. The sample consisted of 11,673 Han Chinese women, including 5,864 (~50%) cases with recurrent MDD, and 5,783 (~50%) controls. The mean (SD) age of the sample was 46.1 (7.6). Approximately 58% of the full sample (N=6,439) endorsed suffering from sleeplessness. Not surprisingly, endorsement of sleeplessness was significantly lower in MDD controls (29%) vs. MDD cases (86%) ($X^2 = 4{,}000$, df = 1, $p < 0.0001$). Prevalence for individual trauma types are shown in Table 1 for the full sample, MDD cases, and MDD controls. Endorsement of most traumatic events was higher in MDD cases than MDD controls, with the two exceptions being fire, flood, or natural disaster and witnessed someone being badly

injured or killed, whereas MDD cases did not significantly differ from MDD controls on endorsement of these events. Table 2 shows correlations across individual trauma types (in the full sample). The pattern of correlations among trauma types was as expected, with high correlations between trauma types that were interpersonal in nature (*r*s ranging from 0.45-0.53, all *p* values < 0.01 for rape, physical attack or assault, and threatened, held captive, or kidnapped), particularly the child trauma specific items (*r*s ranging from 0.48-0.73, all *p* values < 0.01 for CSA, childhood physical abuse, and serious neglect, and *r*s ranging from 0.36-0.50, all *p* values < 0.01 across child and other interpersonal traumas). Non-interpersonal events (e.g., fire, flood, or natural disaster) were less correlated with interpersonal traumas (*r*s ranging from -0.01-0.33, not all correlations significant), as well as with each other (*r*s ranging from 0.10-0.24, all *p* values < 0.01). Given that endorsement of interpersonal traumas was low in MDD controls (e.g., only 3 individuals (0.1%) endorsed rape and 22 (0.4%) endorsed being threatened, held captive, kidnapped; see Table 1 below) and correlation patterns were broadly similar when examining cases and controls separately, factor analyses were run within the full sample.

Table 1. Prevalences of specific traumatic events by MDD case status

	Total (N, %)	**MDD Ca (N, %)**	**MDD Co (N, %)**	**X^2**	***p*-value**
1. Physical attack/assault	541 (5.0)	393 (7.3)	148 (2.7)	120.20	< 0.0001
2. Threatened/held captive/kidnapped	109 (1.0)	87 (1.6)	22 (0.4)	39.06	< 0.0001
3. Rape[1]	75 (0.7)	72 (1.3)	3 (0.1)	63.24	< 0.0001
4. Physical abuse (childhood)	300 (2.8)	248 (4.6)	52 (1.0)	133.76	< 0.0001
5. Serious neglect (childhood)	683 (6.3)	572 (10.7)	111 (2.0)	338.99	< 0.0001
6. Childhood sexual abuse	733 (6.8)	588 (11.0)	145 (2.7)	292.47	< 0.0001
7. Life-threatening accident	828 (7.6)	463 (8.6)	365 (6.7)	14.11	0.0002
8. Witness injury/death	868 (8.0)	451 (8.4)	417 (7.6)	2.03	0.1539
9. Fire, flood, or natural disaster	1196 (11.0)	590 (11.0)	606 (11.1)	0.02	0.8793
10. Serious illness	1160 (10.1)	694 (12.1)	466 (8.1)	52.08	< 0.0001
11. Death of a loved one	2112 (18.4)	1149 (20.1)	963 (16.7)	22.38	< 0.0001

Abbreviations: Ca = case; Co = control; MDD = major depressive disorder.

[1]This variable was constructed so that it does not overlap with CSA (i.e., if rape was endorsed before the age of 16, this was counted as CSA).

Note that all chi-squared tests were run on the 2x2 tables using a Yates continuity correction.

Table 2. Cross-trauma type correlations (full sample)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1. Rape	1										
2. Physical attack/assault	0.45**	1									
3. Threatened, held captive, kidnapped	0.50**	0.53**	1								
4. Physical abuse (child)	0.41**	0.50**	0.45**	1							
5. Serious neglect (child)	0.41**	0.45**	0.36**	0.73**	1						
6. Childhood sexual abuse	0.36**	0.43**	0.36**	0.49**	0.48**	1					
7. Death of a loved one	0.02	0.06*	0.04	0.01	0.05	-0.01	1				
8. Serious illness	0.09	0.16**	0.05	0.12*	0.17**	0.16**	0.13**	1			
9. Life-threatening accident	0.30**	0.33**	0.29**	0.24**	0.25**	0.25**	0.10**	0.22**	1		
10. Fire, flood, or natural disaster	0.19*	0.17**	0.21**	0.17**	0.12**	0.05	0.14**	0.15**	0.24**	1	
11. Witnessed injury/death	0.11	0.24**	0.23**	0.27**	0.23**	0.17**	0.12**	0.14**	0.21**	0.23**	1

*$p < 0.05$; **$p < 0.01$.

Note that correlations are tetrachoric, as all items are binary (yes/no).

Factor analysis of trauma type. To determine the factor structure of the different trauma type variables within this sample and provide support for the creation of composite trauma variables for use in later regression analyses, exploratory and confirmatory factor analyses were conducted within the full CONVERGE sample (both MDD cases and MDD controls). Parallel analysis was run to ascertain how many dimensions may be present for the associations among the different trauma types. A Scree plot showing these parallel analysis results, as well as the Kaiser rule (line marking eigenvalues equal to 1) is shown in Figure 2. Parallel analysis suggested the presence of 4 factors, as seen here.

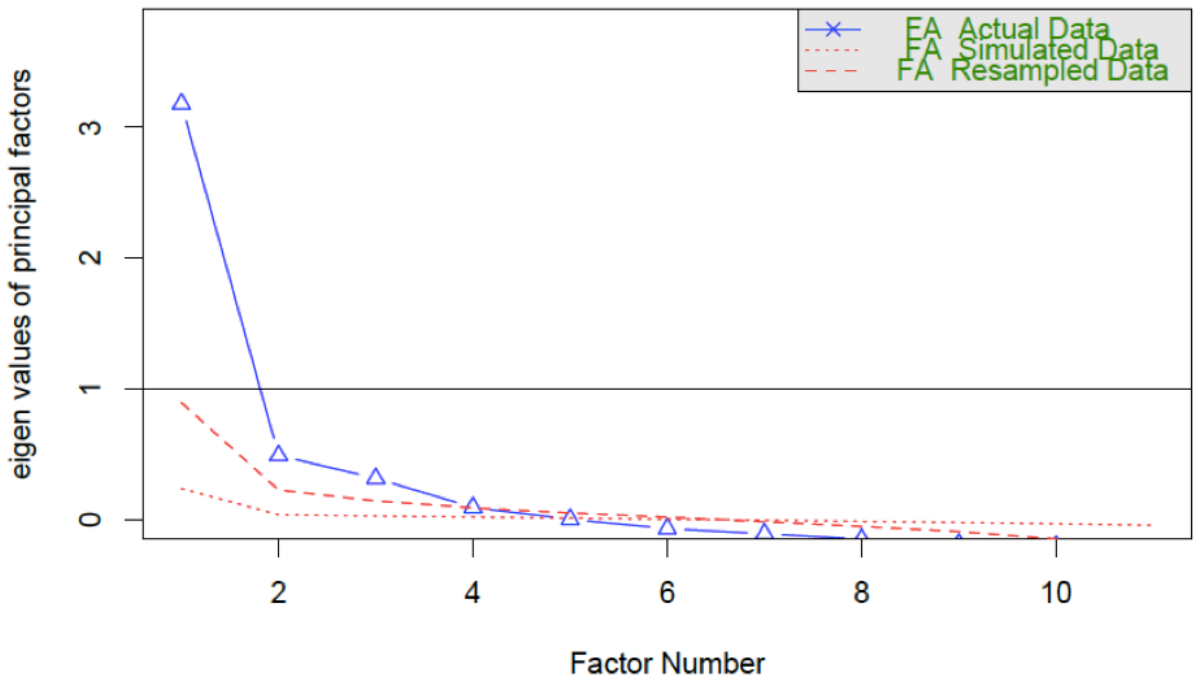


Figure 2: Scree plot and parallel analysis for EFA of trauma type. This figure shows a Scree plot with the number of factors on the y-axis and the eigen values of the principal factors on the y-axis. The solid black line represents an eigen value cutoff of 1. As indicated in the legend, the blue triangles mark the eigen values from the actual data, while the dashed red lines show simulated and resampled data, representing results of parallel analysis.

Based on these results, 1-, 2-, 3-, 4-, and 5-factor EFAs were run using the Psych package[275] in R. The EFAs were run using a minimum residual (ordinary least squares) method, missing data was treated "pairwise", and tetrachoric correlations were estimated since all trauma variables were coded as binary. Additionally, a GeominQ (oblique) rotation was implemented because it was expected that the factors would be correlated (see Table 3 for factor loadings and Table 4 for factor correlations for all EFAs). Next, CFAs were fit for 1-, 2-, and 3-common factor model specifications (the 4- and 5-factor solutions were not examined in the CFAs, since these solutions resulted in some poorly identified factors), using the R package lavaan, which is a structural equation modeling package.[279] Models were developed based on the data driven EFA results, treating the observed trauma variables as ordered categorical indicators and utilizing a WLSMV estimator with a theta parameterization. CFA model fitting results indicated that a 3-factor solution, with interpersonal (3 items), child interpersonal (3 items), and non-assaultive (5 items) factors, had the best fit to the data, since it had the lowest RMSEA and X^2. Model fit comparisons for the 1-, 2-, and 3- factor CFAs are shown in Table 5. The trauma items that loaded predominantly on each of the factors, as well as the standardized factor loadings for the 3-factor solution and factor correlations are shown in Figure 3.

Table 3. Factor loadings from 1-, 2-, 3-, 4-, and 5-factor EFAs

	Factor	Physical attack/ assault	Rape	Threatened/ held captive/ kidnapped	Serious neglect (child)	Physical abuse (child)	Childhood sexual abuse	Fire/flood/ natural disaster	Serious illness	Life-threatening accident	Witnessed someone injured/ killed	Death of a loved one
1-factor	1	**0.67**	**0.59**	**0.62**	**0.76**	**0.79**	**0.61**	**0.25**	**0.21**	**0.41**	**0.34**	**0.07**
2-factor	1	**0.54**	**0.52**	**0.62**	0.06	-0.02	0.18	**0.48**	**0.22**	**0.55**	**0.31**	**0.22**
	2	0.21	0.15	0.08	**0.83**	**0.79**	**0.47**	-0.17	0.02	-0.07	0.08	-0.13
3-factor	1	**0.80**	**0.57**	**0.55**	-0.03	0.16	0.26	0.15	-0.11	0.25	0.07	-0.07
	2	0.18	0.12	-0.02	**0.89**	**0.74**	**0.42**	-0.08	0.12	0.01	0.12	-0.04
	3	0.09	0.00	-0.02	0.02	-0.01	-0.01	**0.42**	**0.41**	**0.40**	**0.34**	**0.34**
4-factor	1	**0.58**	**0.61**	**0.80**	0.06	-0.02	**0.37**	0.03	-0.02	0.31	0.02	-0.05
	2	0.13	0.05	-0.01	**0.73**	**0.96**	0.28	0.04	0.01	-0.05	0.19	-0.04
	3	0.09	0.02	-0.01	0.04	-0.01	-0.02	**0.49**	**0.42**	**0.39**	**0.34**	**0.32**
	4	0.02	-0.02	-0.27	0.16	-0.05	0.27	-0.25	0.25	0.07	-0.10	-0.02
5-factor	1	**0.66**	0.03	**0.74**	-0.02	0.03	**0.37**	-0.01	0.01	**0.30**	0.19	-0.01
	2	-0.01	**0.97**	0.04	0.07	-0.01	0.01	0.06	0.00	0.05	-0.15	-0.02
	3	0.12	0.03	-0.01	**0.79**	**0.90**	0.32	0.02	0.03	-0.03	0.16	-0.04
	4	0.01	0.01	0.02	0.00	0.03	-0.14	**0.58**	**0.29**	**0.29**	**0.32**	**0.29**
	5	0.00	0.00	-0.25	0.07	-0.14	0.17	-0.07	**0.42**	0.19	0.00	0.12

Abbreviations: EFA = exploratory factor analysis.
Loadings for each specific factor are in **bold**.

Table 4. Factor correlations from 1-, 2-, 3-, 4-, and 5-factor EFAs

	1*2	1*3	2*3	1*4	2*4	3*4	1*5	2*5	3*5	4*5
1-Factor	--	--	--	--	--	--	--	--	--	--
2-Factor	0.67	--	--	--	--	--	--	--	--	--
3-Factor	0.57	0.37	0.32	--	--	--	--	--	--	--
4-Factor	0.65	0.36	0.30	0.08	0.11	0.03	--	--	--	--
5-Factor	0.60	0.64	0.41	0.35	0.20	0.22	0.15	0.02	0.19	-0.02

Abbreviations: EFA = exploratory factor analysis.

Table 5. Model comparisons for CFA

	X^2	df	*p*-value	RMSEA (90% CI)
1-Factor Model	276.60	44	< 0.0001	0.022 (0.020-0.025)
2-Factor Model	175.65	43	< 0.0001	0.017 (0.014-0.020)
3-Factor Model	**91.19**	**41**	**< 0.0001**	**0.011 (0.008-0.014)**

Abbreviations: CFA = confirmatory factor analysis; df = degrees of freedom; RMSEA = root mean square error of approximation.
Note that the best-fit solution is bolded.

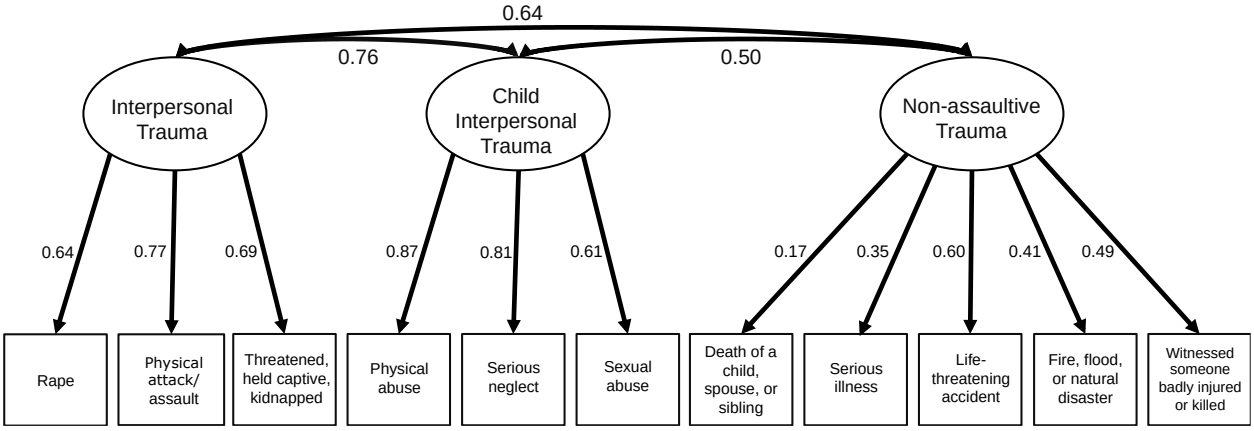


Figure 3: Results of the best-fit 3-factor CFA solution for trauma type. The identified factors were interpersonal, child interpersonal, and non-assaultive traumas. The items that comprise each factor are displayed, with individual loadings shown on the paths. Factor correlations are presented on double-headed arrows at the top.

Creation of trauma type and timing variables. Using results of the CFA, trauma sum score variables that incorporated the trauma type structure determined above, in addition to the age at first onset information available for each trauma, were created. Childhood events were defined as occurring before the age of 16. Thus, the following four trauma variables were constructed: 1) child interpersonal; 2) child non-assaultive; 3) adult interpersonal; and 4) adult non-assaultive. Interpersonal traumas represented across both age groups included rape, physical attack or assault, and threatened, held captive, or kidnapped. The three child interpersonal items (from the CFA) were included in the child interpersonal variable only (i.e., CSA, physical abuse,

serious neglect). The remaining five traumas (death of a spouse, child, or sibling; serious illness; life-threatening accident; fire, flood, or natural disaster; and witnessed someone badly injured or killed) were considered to be non-assaultive traumatic events. Given sum score distributions (most individuals endorsed either 0 or 1 for each event category), each trauma variable was collapsed into a binary yes/no item, with "yes" indicating that the individual endorsed at least one event in that category, due to scarcity of cell sizes.

Descriptive statistics for the computed trauma category variables, within the full sample and separated by MDD case status, are shown in Table 6. Adult non-assaultive traumas were the most commonly endorsed category. The prevalences of all four variables were significantly higher in MDD cases (vs. MDD controls). Figure 4 displays the endorsement of sleeplessness across each trauma category for the full sample, MDD cases only, and MDD controls only. Significantly more individuals with at least one child interpersonal trauma in the full sample and MDD control group endorsed sleeplessness, but this did not differ for MDD cases. For all other trauma categories (child non-assaultive, adult interpersonal, adult non-assaultive), individuals endorsing at least one event from that trauma category endorsed sleeplessness at a higher rate, and this was true for all subsets of the sample examined (full, MDD cases, MDD controls), with the exception of adult interpersonal trauma in MDD controls, which was nominally significant ($p < 0.10$). Finally, correlations between trauma and sleeplessness across the sample are presented in Table 7. There were modest but significant correlations (all p values < 0.01) between all trauma variables and sleeplessness within the full sample, and these were higher for interpersonal traumas (0.31 for child, 0.25 for adult) than non-assaultive (0.12 for child, 0.14 for adult) traumas. For MDD cases, all traumas except for child interpersonal were significantly correlated with sleeplessness (range 0.07-0.13, p values <0.05, for those that were significant). In contrast,

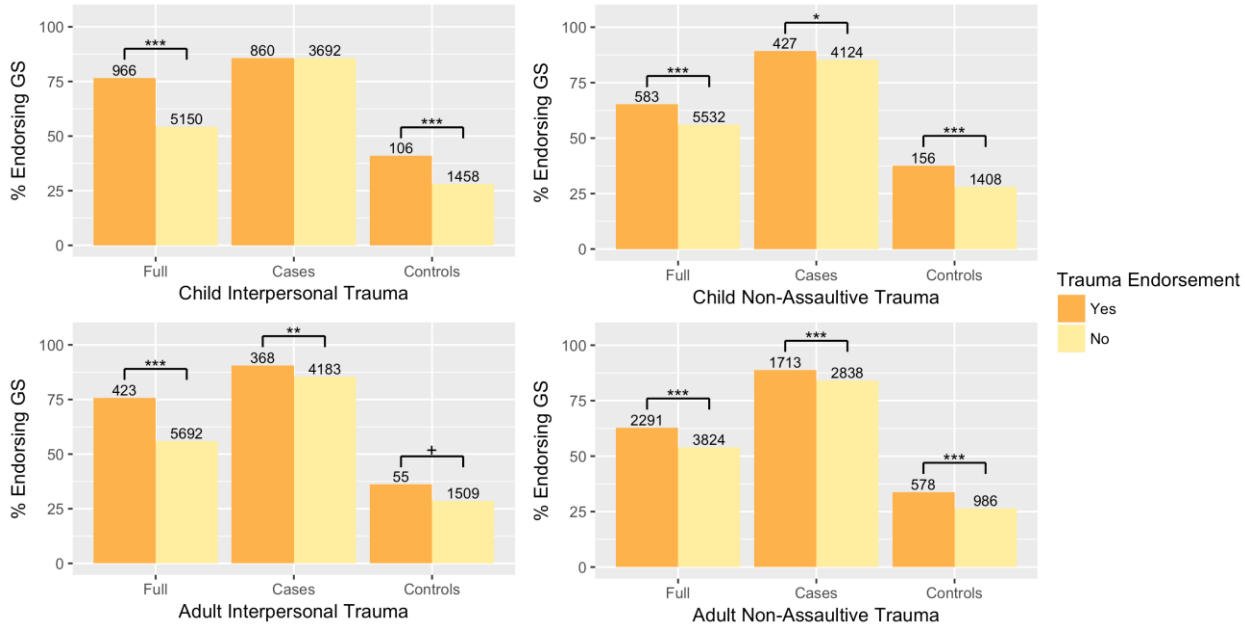
all traumas except for adult interpersonal (range 0.13-0.15, all *p* values < 0.01) were significantly correlated with sleeplessness in MDD controls.

Table 6. Prevalence of composite trauma variables by MDD case status

	Total (N, %)	**MDD Ca (N, %)**	**MDD Co (N, %)**	**X^2**	***p*-value**
Child IP	1268 (11.7)	1008 (18.8)	260 (4.8)	512.78	< 0.0001
Child N-A	900 (8.3)	483 (9.0)	417 (7.6)	127.43	< 0.0001
Adult IP	563 (5.2)	410 (7.6)	153 (2.8)	6.35	0.0117
Adult N-A	3669 (33.9)	1954 (36.4)	1715 (31.4)	29.65	< 0.0001

Abbreviations: Ca = case; Co = control; IP = interpersonal trauma; MDD = major depressive disorder; N-A = non-assaultive trauma.

Note that all chi-squared tests were run using a Yates continuity correction. Child IP includes CSA, childhood physical abuse, severe neglect, physical assault, and threatened held/captive/kidnapped with age of first occurrence before age 16. Similarly, adult IP includes rape, physical assault, and threatened/held captive/kidnapped with onset at age 16 or older. Child N-A includes fire, flood, or natural disaster, serious illness, life-threatening accident, witnessed someone badly injured or killed, and death of a child, spouse, or sibling, endorsed before age 16. Adult N-A includes the same traumas as child N-A, but only those that had age of onset at 16 or older.



+ $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 4: Sleep endorsement across trauma type in different subsets of the sample. This figure shows endorsement of sleeplessness by trauma type (child interpersonal, child non-assaultive, adult interpersonal, adult non-assaultive) for each subset of the sample (full sample, MDD cases, MDD controls), with percentage shown on the y-axis and N on each bar. Asterisks indicate significance of chi-squared tests across groups.

Table 7. Tetrachoric correlations between trauma variables and sleeplessness

	GS, Full sample	GS, MDD Ca	GS, MDD Co
Child IP	0.31 (0.27-0.34)**	0.00 (-0.07-0.06)	0.16 (0.09-0.23)**
Child N-A	0.12 (0.07-0.17)**	0.09 (0.01-0.18)*	0.13 (0.06-0.19)**
Adult IP	0.25 (0.19-0.30)**	0.13 (0.05-0.21)**	0.09 (-0.01-0.19)
Adult N-A	0.14 (0.11-0.17)**	0.07 (0.13-0.18)**	0.13 (0.08-0.18)**

*$p < 0.05$; **$p < 0.01$.
Abbreviations: Ca = case; Co = control; GS = general sleep item; IP = interpersonal trauma; MDD = major depressive disorder; N-A = non-assaultive trauma.

Hierarchical logistic regression models. Hierarchical logistic regressions were conducted to examine the effects of child and adult trauma types, and their potential interactions, on sleeplessness. Analyses were conducted three separate ways in order to best examine effects of and account for ascertainment bias. First, in the primary model, the full sample was used, with

MDD status as a covariate. This served as the basis for later genetic analyses within the full sample to maximize power. Second, a survey approach was used to simulate an MDD prevalence of 8% and served as a validity check for the first approach, determining whether results were stable. Finally, analyses were run separately in MDD cases and MDD controls given differences in ascertainment. This was also done to facilitate the creation of a combined genetic and environmental model, as PRS analyses presented in Chapter 6 necessitated splitting the sample in half based on case status. These results will be reviewed in turn.

Full sample, MDD covariate. Results for sleeplessness regressions run in the full sample are presented in Table 8. Demographic covariates (i.e., age and case status), entered in Step 1, were both significant predictors of higher odds of reporting suffering from sleeplessness (MDD OR = 16.39, 95% CI = 14.84-18.14, $p < 0.0001$; age OR = 1.02, 95% CI = 1.02-1.03, $p < 0.0001$) and Nagelkerke's pseudo R^2 was 0.41. In Step 2, both child (OR = 1.30, 95% CI = 1.11-1.54, $p = 0.0017$) and adult (OR = 1.53, 95% CI = 1.21-1.95, $p = 0.0005$) interpersonal trauma variables were significantly associated with higher likelihood of endorsement of sleeplessness. Both age and MDD remained significant at similar magnitudes to Step 1. The addition of the interpersonal trauma variables increased the amount of variance in sleeplessness explained in comparison to Step 1 (new pseudo $R^2 = 0.44$). In Step 3, non-assaultive traumas, both child and adult, were added into the final model. Both were significant predictors of sleeplessness (OR = 1.44, 95% CI = 1.21-1.72, $p < 0.0001$ for child; OR = 1.33, 95% CI = 1.20-1.47, $p < 0.0001$ for adult). In this model, both child and adult interpersonal traumas remained significant, with similar ORs, as did MDD and age (pseudo $R^2 = 0.45$). Model AIC decreased as trauma predictors were added in Steps 2-3, suggesting a decrease in model misfit and providing support for the inclusion of all traumas within the final model. The second set of hierarchical regressions aimed at examining

the potential interactive effect of type and timing began with the final model from above, which included all trauma categories. Interaction terms for child x adult interpersonal traumas and child x adult non-assaultive traumas were then added to this model in a step-wise fashion. Neither of these interaction terms was significant ($p = 0.0755$ [interpersonal] and $p = 0.3347$ [non-assaultive] in the final model).

Table 8. Results of hierarchical logistic regressions examining the effects of trauma type and timing on sleeplessness in the full sample

		OR	95% CI	*p*-value	Pseudo R^2	AIC	ΔAIC
Step 1: Demographics	MDD	16.39	14.84-18.14	< 0.0001	0.41	11109.65	--
	Age	1.02	1.02-1.03	< 0.0001			
Step 2: Interpersonal	MDD	15.85	14.29-17.60	< 0.0001	0.44	10769.77	-339.88
	Age	1.03	1.02-1.03	< 0.0001			
	Child IP	1.30	1.11-1.54	0.0017			
	Adult IP	1.53	1.21-1.95	0.0005			
Step 3: Non-assaultive	MDD	15.81	14.25-17.57	< 0.0001	0.45	10722.71	-47.06
	Age	1.02	1.02-1.03	< 0.0001			
	Child IP	1.23	1.04-1.45	0.0140			
	Adult IP	1.43	1.13-1.82	0.0034			
	Child N-A	1.44	1.21-1.72	< 0.0001			
	Adult N-A	1.33	1.20-1.47	< 0.0001			

Abbreviations: AIC = Akaike Information Criterion; IP = interpersonal trauma; MDD = major depressive disorder; N-A = non-assaultive trauma.
Note: Pseudo R^2 is Nagelkerke's.

Full sample, survey approach. Results for regressions using the survey method (R package: *survey*[283,284]), conducted to examine effects of trauma on sleeplessness in a more representative sample, are presented in Table 9. Survey weights were computed such that MDD cases counted for 8% of the sample and MDD controls the remaining 92%. This allowed for a more population-based estimate without the loss of data that would occur using random sampling (i.e., take all MDD controls and a random subset of MDD cases). MDD was not used as a covariate since the population prevalence of MDD was already incorporated into the models.

Since this is a variation of a generalized linear model, pseudo R^2 values cannot be computed. However, AIC can be calculated using specific algorithms (see *survey* package documentation) and is used here to compare models, as was done across all three approaches presented. In Step 1, age was not a significant predictor of sleeplessness. Both child (OR = 2.26, 95% CI = 1.85-2.75, $p < 0.0001$) and adult (OR = 1.60, 95% CI = 1.22-2.10, $p = 0.0007$) interpersonal traumas were significant predictors of sleeplessness when added in Step 2. Finally, both child (OR = 1.39, 95% CI = 1.15-1.67, $p = 0.0006$) and adult (OR = 1.35, 95% CI = 1.21-1.51, $p < 0.0001$) non-assaultive traumas significantly predicted sleeplessness in Step 3, and child and adult interpersonal traumas remained significant at similar magnitudes. The AIC decreased in Steps 2 and 3, suggesting that the final model, which contains all trauma categories, results in a decrease in overall misfit. When interaction terms were added hierarchically, as done above, there were no significant interactions between child and adult interpersonal or child and adult non-assaultive traumas (*p* values of 0.9687 and 0.5880, respectively).

Table 9. Results of hierarchical logistic regressions examining the effects of trauma type and timing on sleeplessness utilizing a survey-based approach

		OR	**95% CI**	***p*-value**	**AIC**	**ΔAIC**
Step 1: Demographics	Age	1.00	1.00-1.01	0.5138	13963.84	--
Step 2: Interpersonal	Age	1.01	1.00-1.02	0.0956	13724.33	-239.51
	Child IP	2.26	1.85-2.75	< 0.0001		
	Adult IP	1.60	1.22-2.10	0.0007		
Step 3: Non-assaultive	Age	1.00	1.00-1.01	0.3292	13657.79	-66.54
	Child IP	2.12	1.73-2.58	< 0.0001		
	Adult IP	1.50	1.14-1.97	0.0037		
	Child N-A	1.39	1.15-1.67	0.0006		
	Adult N-A	1.35	1.21-1.51	< 0.0001		

Abbreviations: AIC = Akaike Information Criterion; IP = interpersonal trauma; N-A = non-assaultive trauma.
Note: Pseudo R^2 not available due to survey approach.

Separate case/control analyses. *a) Controls.* Results for MDD controls only are presented in Table 10. Age was a significant predictor of sleeplessness in Step 1 (OR = 1.02, 95% CI = 1.01-1.03, $p < 0.0001$), with a model pseudo R^2 of 0.01. In Step 2, child interpersonal trauma (OR = 1.77, 95% CI = 1.37-2.29, $p < 0.0001$) significantly predicted sleeplessness, but adult interpersonal trauma was only nominally significant (OR = 1.35, 95% CI = 0.95-1.89, $p = 0.0882$). Age remained significant in this model and the pseudo R^2 increased to 0.02. In Step 3, where all traumas were included, both child (OR = 1.45, 95% CI = 1.17-1.79, $p = 0.0005$) and adult (OR = 1.32, 95% CI = 1.16-4.33, $p < 0.0001$) non-assaultive traumas were significant, age and child interpersonal trauma remained significant, and adult interpersonal trauma was no longer nominally significant. The pseudo R^2 was 0.02 in the final model. Once again, there were substantial decreases in AIC across models, indicating that the model containing all traumas is appropriate. No interaction terms were significant when added to this final model (*p* values = 0.8936 [interpersonal] and 0.8858 [non-assaultive]) and thus are not reported in the tables.

Table 10. Results of hierarchical logistic regressions examining the effects of trauma type and timing on sleeplessness in MDD controls only

		OR	**95% CI**	***p*-value**	**Pseudo R^2**	**AIC**	**ΔAIC**
Step 1: Demographics	Age	1.02	1.01-1.03	< 0.0001	0.01	6496.11	--
Step 2: Interpersonal	Age	1.03	1.01-1.04	< 0.0001	0.02	6475.68	-20.43
	Child IP	1.77	1.37-2.29	< 0.0001			
	Adult IP	1.35	0.95-1.89	0.0882			
Step 3: Non-assaultive	Age	1.02	1.01-1.03	< 0.0001	0.02	6445.92	-29.76
	Child IP	1.65	1.27-2.13	0.0002			
	Adult IP	1.27	0.89-1.78	0.1801			
	Child N-A	1.45	1.17-1.79	0.0005			
	Adult N-A	1.32	1.16-4.33	< 0.0001			

Abbreviations: AIC = Akaike Information Criterion; IP = interpersonal trauma; N-A = non-assaultive trauma.
Note: Pseudo R^2 is Nagelkerke's.

b) Cases. Results for MDD cases only are presented in Table 11. Age was a significant predictor of sleeplessness in Step 1 (OR = 1.02, 95% CI = 1.02-1.03, $p < 0.0001$), with a pseudo R^2 of 0.02. In Step 2, adult interpersonal (OR = 1.75, 95% CI = 1.25-2.51, $p = 0.0016$) but not child interpersonal trauma was significant, and age continued to predict sleeplessness with a similar magnitude. Nagelkerke's pseudo R^2 increased to 0.11. When the non-assaultive trauma terms were added in Step 3, both were significant (OR = 1.41, 95% CI = 1.05-1.94, $p = 0.0263$ for child; OR = 1.33, 95% CI = 1.12-1.58, $p = 0.0013$ for adult). The other variables remained similar to Step 2 and the pseudo R^2 increased to 0.12. AIC decreased across models, similar to prior regressions, indicating that the final model is more parsimonious and has less misfit than initial models. Interestingly, when a multiplicative interaction term was added for child x adult interpersonal trauma, this was significant. This remained significant in the final model where the child x adult non-assaultive trauma multiplicative interaction (itself non-significant) was added, with a final effect of OR = 0.43 (95% CI = 0.21-0.87, $p = 0.0190$, AIC = 4273.61, change in AIC from model with no interaction = -4.57). This suggests that the combined effect of child interpersonal and adult interpersonal traumas is less than multiplicative on the odds-ratio scale, but will not be discussed further since it is scale-dependent and the decrease in model misfit (i.e., decrease in AIC) may not be biologically meaningful.

Table 11. Results of hierarchical logistic regressions examining the effects of trauma type and timing on sleeplessness in MDD cases only

		OR	95% CI	*p*-value	Pseudo R^2	AIC	ΔAIC
Step 1: Demographics	Age	1.02	1.02-1.03	< 0.0001	0.02	4615.54	--
Step 2: Interpersonal	Age	1.03	1.02-1.04	< 0.0001	0.11	4290.44	-325.10
	Child IP	1.07	0.88-1.32	0.4905			
	Adult IP	1.75	1.25-2.51	0.0016			
Step 3: Non-assaultive	Age	1.02	1.01-1.03	< 0.0001	0.12	4278.18	-12.26
	Child IP	1.03	0.84-1.27	0.7963			
	Adult IP	1.63	1.17-2.35	0.0059			
	Child N-A	1.41	1.05-1.94	0.0263			
	Adult N-A	1.33	1.12-1.58	0.0013			

Abbreviations: AIC = Akaike Information Criterion; IP = interpersonal trauma; N-A = non-assaultive trauma.
Note: Pseudo R^2 is Nagelkerke's.

CSA analyses. To examine the effects of one particularly potent form of childhood trauma, CSA, and incident characteristics associated with it, on sleeplessness, a series of univariate logistic regressions were conducted. Results of CSA analyses within the full sample, using the primary method (co-varying for MDD status), are presented in Table 12. MDD and age were included as covariates in these models. Broad CSA, examined across all individuals, was a significant predictor of higher odds of endorsing sleeplessness (OR = 1.28, 95% CI = 1.04-1.58, $p = 0.0202$) within the full sample. Similar results, although with a larger effect for CSA, were seen using the survey method (OR = 2.30, 95% CI = 1.77-2.98, $p < 0.0001$). The subsequent analyses examining incident characteristics were restricted to only individuals endorsing CSA (N = 730), as this was a requirement for answering the follow-up questions, and are also shown in Table 12. CSA severity did not differentially predict sleeplessness, and none of the other incident characteristics were significant predictors of sleeplessness. Given this, no items were included in a combined regression. A survey approach was not used here due to the restriction of the sample to CSA cases only. As endorsement of CSA was much lower in MDD controls than MDD cases

and there was scarcity of cells across many incident characteristics for MDD controls only, analyses were not conducted separately.

Table 12. Regression models examining the effects of CSA and CSA characteristics on sleeplessness in the full sample with MDD covariate

Item	Prevalence	Univariate regression (OR)
Childhood sexual abuse (CSA)	**733 (6.8)**	**1.28 (1.04-1.58)***
CSA severity		
Genital	287 (39.3)	0.84 (0.54-1.28)
Intercourse	176 (24.1)	1.32 (0.78-2.30)
Intercourse vs. other forms of CSA	176 (24.1)	1.45 (0.90-2.41)
Age at time of abuse	12.22 ± 5.19	1.01 (0.98-1.05)
Age of perpetrator		
Under 15 years	91 (12.5)	0.79 (0.46-1.40)
15-18 years	140 (19.2)	1.43 (0.87-2.41)
19-24 years	158 (21.6)	1.07 (0.68-1.71)
25-50 years	316 (43.3)	1.00 (0.68-1.47)
≥ 50 years	57 (7.8)	0.58 (0.31-1.12)
Gender of perpetrator		
Male vs. female or both	670 (91.8)	1.09 (0.54-2.10)
Abuse by a relative		
Relative vs. non-relative or stranger	227 (31.1)	0.9 (0.63-1.45)
Forced or threatened		
Maybe	121 (16.6)	0.92 (0.55-1.56)
Definitely	102 (14.0)	1.35 (0.74-2.57)
How affected at the time	2.01 ± 2.17	1.05 (0.95-1.15)

*$p < 0.05$.

Note that the all analyses were run with both age and MDD as covariates.

III. Discussion

Here, a novel examination of trauma type and timing within a large Han Chinese sample is presented, and this information is used to examine the relationship between trauma and sleep in more detail. This is one of the first studies to date to explicitly examine whether sleeplessness differs across trauma types and to examine the factor structure of trauma type within a Chinese sample. There are three main findings to be discussed in this section: 1) a three-factor solution was the best-fit for trauma type, with the three factors representing interpersonal trauma, child interpersonal trauma, and non-assaultive trauma; 2) hierarchical regressions indicated that all trauma categories (child interpersonal, adult interpersonal, child non-assaultive, adult non-assaultive) were significant, unique predictors of sleep within this sample, and that effect sizes were similar across trauma categories, although there were some differences when cases and controls were modeled separately and when using a population-based approach; and 3) broad CSA, but not individual incident characteristics, was a significant predictor of sleeplessness within this sample.

Prevalence of traumatic events. Overall, endorsement of traumatic events within CONVERGE is lower than what is seen in population samples such as the WMHSC[65] and the NCS.[69] However, within the WMHSC data (where men/women were combined), the prevalence of any traumatic event in China (52.5%) was lower than the overall prevalence worldwide (70%), and individuals from China were less likely to report traumas across most events/categories, including those that are interpersonal in nature (i.e., interpersonal violence, intimate partner/sexual violence). Exceptions included collective violence and man-made disaster, where ORs did not differ for individuals from China. This should be considered when making comparisons. Note that death of a loved one was the trauma with the highest

endorsement in both CONVERGE (18.4% in the full sample) and the WMHSC (31%; higher OR for women). Particularly striking is the low prevalence of interpersonal traumas within the CONVERGE sample, even when examined in MDD cases only. For example, being mugged/threatened with a weapon was endorsed by 14.5% of individuals in WMHSC (not separated by sex; although being female was protective for endorsement of this event) and 6.8% of women in the NCS, but only 1.0% of the total sample (and 1.6% of MDD cases) here endorsed being threatened, held captive, or kidnapped. Further, only 0.7% of CONVERGE (1.3% of MDD cases) endorsed rape, while reported estimates for rape were 3.2% in the WMHSC and 9.2% for women in the NCS. In sum, endorsement of traumatic events is low in CONVERGE. One might expect that rates of interpersonal traumas would be higher given that half the sample has MDD and SLEs are strongly linked to the disorder (e.g., [82]) and the sample is all female,[69,70] but it is possible that under-reporting and cultural differences are at play, similar to what is hypothesized for lower MDD prevalence (e.g., [45,46,47]).

Factor analysis of trauma type. Traumatic events within CONVERGE were factor analyzed in order to better understand how these events cluster within the population, as this has not been done yet for a Chinese sample. A 3-factor solution for trauma type, with interpersonal, child interpersonal, and non-assaultive factors was the best fit, with the interpersonal and child interpersonal factors highly correlated, as expected (0.76). These results are similar to other factor analyses of trauma type in different samples that show a separation of interpersonal and non-assaultive traumas. For example, Stein and colleagues[94] found a 2-factor solution from a principal component analysis of nine traumas in a veteran twin sample (comprised of both males and females), with Factor 1 representing "assaultive" events (e.g., robbery; sexual assault) and Factor 2 representing "non-assaultive events (e.g., motor vehicle accident; tornado, flood,

earthquake). Note that none of these traumas were specific to childhood. Benjet and colleagues[65] had access to a much wider range of traumatic events within the WMHSC data (29 in total) than available in CONVERGE (and Stein et al.[94]) and EFAs were conducted using data on both sexes. Five distinct factors (with a sixth that encompassed other events) were found: exposure to collective violence (e.g., civilian in war zone, refugee); causing/witnessing bodily harm to others (e.g., combat); interpersonal violence (e.g., beaten up; this factor includes events in childhood); intimate partner or sexual violence (e.g., rape); accidents and injuries (e.g., natural disasters). The factor solution presented here is similar to the interpersonal violence, intimate partner or sexual violence, and accidents and injuries factors, with some exceptions as to what items loaded where (e.g., traumatic event to loved one loaded on the intimate partner or sexual violence factor in Benjet et al.,[65] while similar items from CONVERGE, such as death of a loved one, loaded onto the non-assaultive factor).

These results are unique in that a separate factor for child interpersonal traumas was identified in the context of other traumatic events that occur mostly during adulthood, indicating that these childhood events in particular cluster together. Prior studies of childhood adversities demonstrate that events are related and often co-occur, with individuals exposed to one event more likely to be exposed to others.[285-288] In several studies of population data, sexual abuse, physical abuse, and neglect all loaded strongly onto one factor representing "maladaptive family functioning" along with several other child adversity variables,[285,287,288] supporting shared etiology and aligning with the results demonstrated here. Another recent study of 18 childhood events (defined as occurring before age 18) considered to be "traumatic or extremely stressful" also found that CSA, physical abuse, and neglect/poverty loaded onto one factor, along with emotional abuse, bullying, and domestic violence.[93] Thus, childhood traumas (and their resultant

sequelae) are likely a product of the overall environment that occurs in childhood (factors influencing this can be related to the specific child, parent, family as a whole, community, and even society[289]) and thus often do not occur in isolation.

Further, this child trauma factor was highly correlated with the interpersonal trauma factor. This is consistent with prior studies of revictimization, which suggest that individuals exposed to sexual abuse in childhood are more likely to experience sexual assault as adolescents or adults.[290-294] A wide range of approaches have been used to investigate correlates of revictimization, examining predictors such as prior psychopathology (e.g., PTSD, alcohol use) and risky sexual behavior, among other intrapersonal factors (e.g., [290]). Personality may also contribute. Several studies show that childhood abuse is related to higher neuroticism and openness,[295,296] and openness could result in higher risk-taking behavior, thus selecting individuals into situations that could increase the likelihood of sexual assault. This could be a result of gene-environment correlation, as personality dimensions have been shown to be heritable.[101] Revictimization may also have a genetic influence in that both childhood abuse and rape/sexual assault could share similar genetic underpinnings, which is in line with the literature suggesting that interpersonal traumas are heritable.[94,96,97] Mechanisms could also be acting through the environment. There is a wealth of literature suggesting that early exposure to traumatic events (i.e., during sensitive periods) has lasting effects on the stress response system, resulting in sensitization and changes in how the individual responds to future stressors (e.g., [297-299]). A limitation of these results is that age for traumas other than CSA, childhood physical abuse, and serious neglect was not used within the factor analysis. Overall, factor analyses demonstrate that the structure of traumatic events is similar in the Chinese population, despite low endorsement of many events, and highlight the significance of both type and timing (i.e.,

separate child interpersonal factor). Based on factor analytic results, both trauma type and timing were incorporated into final trauma type categories such that effects of timing on each type of event were considered.

Hierarchical regressions, trauma and sleeplessness. Results of hierarchical logistic regressions indicated that all trauma types (child interpersonal, adult interpersonal, child non-assaultive, adult non-assaultive) had unique effects on the endorsement of sleeplessness, and that the relative potency of each event category (i.e., through comparison of ORs and 95% CIs) was similar, with exposure to at least one traumatic event in that category resulting in higher risk for sleeplessness. Results remained similar when a survey approach was used to simulate a population prevalence of 8% for MDD (i.e., control individuals were weighted so that they encompassed 92% of the sample and cases were weighted to encompass 8% in order to more closely model a population sample, as done in CONVERGE genetic analyses,[300] instead of using MDD as a covariate), although there was potency found for child interpersonal trauma as related to sleeplessness using this method. Further, there were some differences observed when analyses were run separately in cases and controls, discussed below. In general, these results were contrary to initial hypotheses, where it was expected that interpersonal traumas would be stronger predictors than non-assaultive traumas and that child traumas would be more potent than adult traumas. This may differ depending on subset of the sample used, which highlights the importance of appropriate sample selection. The different approaches will be discussed in turn.

Within analyses of the full sample (including MDD covariate), all traumatic event categories (child interpersonal, child non-assaultive, adult interpersonal, adult non-assaultive) were significant predictors of sleeplessness at similar magnitudes. This is in contrast to the small body of trauma and sleep literature where interpersonal traumas have been shown to be more

potent predictors of sleep disturbances than accidental traumas.[147,148] In a recent paper examining trauma and sleep in college students, it was shown that although accidental traumas were significant predictors of sleep individually, they were no longer significant when included in a model with interpersonal traumas.[148] Interpersonal events also predicted more severe insomnia within an urban sample[147] and a study of childhood adversity and insomnia in adolescence found that exposure to interpersonal violence (e.g., rape) resulted in the highest risk for insomnia.[153] The lack of differential predictions seen here could be due several different factors. First, ascertainment may be contributing, as this sample is not representative of the more general Chinese population and the MDD covariate does not necessarily account for differences. Additionally, while one might expect that the age range of the study (30-60) would result in more variation in sleep responses since the endorsement of sleep problems increases with age,[166] high rates of endorsement for sleep within MDD could be masking effects. Second, the sleeplessness item may not be an appropriate proxy for insomnia. It could be that these relationships are only seen at a more clinically significant level of sleep disturbances. Third, as discussed earlier, population prevalences of traumatic events were lower across the sample, as were correlations between trauma and sleep, which could be contributing to smaller effects seen here, especially if acting in combination with the non-specific sleep item used here. Despite the lack of specificity for trauma type, note that effect sizes for each trauma category were relatively modest (ORs ranged from 1.23 – 1.44 in the final model) in comparison to the large effect of MDD specifically (OR = 15.81 in the final model) on sleeplessness, yet trauma exposure was still significantly associated with higher odds of experiencing sleeplessness. MDD within this sample is likely more severe than that of other samples examining trauma and sleep and taking into account psychopathology (e.g., [153]).

Additionally, child interpersonal traumas more specifically did not have larger effects on sleep than other traumas within these analyses, which is in contrast to the extant literature for sleep and MDD (e.g., [92,93,153]). Two papers using detailed analyses of type and timing showed that childhood traumas/stressors resulted in higher risk for depression and anxiety when compared to adult traumas, highlighting the importance of both type and timing.[92,93] Further, a recent study by Wang and colleagues[153] examined trauma types and insomnia risk across development (early childhood, middle childhood, adolescence), finding higher risk for insomnia in individuals exposed to interpersonal violence during early childhood or adolescence (but not middle childhood), although these results are difficult to interpret and cannot be extended to adults. Findings could be due to ascertainment, particularly since there were no differences across child interpersonal trauma and sleep for MDD cases, or an effect could be masked by more proximal traumatic events accounting for the effects of earlier traumas. There were also no significant interactions between child and adult interpersonal and child and adult non-assaultive traumas. While initial hypotheses were based on the idea that individuals with child interpersonal trauma might be more likely to endorse adult interpersonal traumas (i.e., revictimization) and that this would then have more of an effect on sleep than endorsing one of these categories alone, this was not the case here. In the study by Chu et al.,[93] described above, interactions between early life stressors and adult stressors were also tested, and were not significant predictors of depression or anxiety. Thus, while many traumatic events do co-occur, their effects on sleep may be distinct.

In contrast to analyses within the full sample, presented above, results from the survey method demonstrated that child interpersonal trauma did have a larger effect than the other trauma types (OR = 2.12, 95% CI= 1.73-2.58) on sleeplessness, although adult interpersonal

trauma had the same magnitude as the others (all traumas were significant). This approach minimized the contributions of MDD cases and modeled a population sample where MDD cases and MDD controls were appropriately weighted. These results provide some support for larger effects of child interpersonal trauma on sleep and align more closely with the literature presented above (e.g., [147,148]). The lack of difference in magnitude for adult interpersonal trauma suggests that larger effects of interpersonal trauma on sleep seen in the literature may be driven by exposure to child interpersonal traumas only. Indeed, prior studies showing larger effects for interpersonal traumas on sleep did not focus on timing, and thus variables for events such as sexual assault could encompass events that occurred in childhood.[147,148] Given that revictimization is common for sexual assault, it could be that this initial event has a stronger, more persistent effect on disturbed sleep than subsequent traumas. Moreover, these results highlight the importance of sample ascertainment in drawing conclusions and provide some insight into the contradictory findings presented earlier. Based on this, it is likely that results seen in the full sample are due to ascertainment biases that cannot be corrected for by including MDD status.

There were also differences for interpersonal traumas when cases and controls were analyzed separately. Adult interpersonal trauma was not a significant predictor of sleeplessness in MDD controls. While this may seem contrary to prior results, the prevalence of the adult interpersonal trauma variable was even lower within this subset of the sample (See Table 4) and endorsement of the sleep item did not significantly differ by trauma endorsement. Thus, it is possible that there is not enough power to detect effects of interpersonal traumas within this subset of the sample, given low endorsement. Further, although the prevalence of sleep disturbances within MDD controls broadly aligns with population estimates (although it is higher

than some of what has been reported in China,[2,4,6]) it is possible that due to selecting specifically for control individuals without recurrent MDD (and also without substance use and psychosis), the sample does not represent insomnia risk in the general Han Chinese population. This could result in underestimates of trauma prevalence, which could in turn be why effects on sleep are not seen. On the other hand, it could also be that adult interpersonal traumas are not significantly related to sleep when including other types of traumas, particularly when child interpersonal traumas are included as well (see discussion in the prior paragraph regarding lack of differential effects). Finally, as discussed earlier, the lack of specificity of the sleep item is also important to consider.

For individuals with MDD, child interpersonal trauma was not a significant predictor of sleeplessness. The endorsement of child interpersonal trauma did not differ based on sleeplessness (refer to Figure 4), which is reflected in the regression results. This subset of the sample is highly selected, such that all individuals have recurrent MDD and the majority endorses sleeplessness, and thus there is not much variation here. Further, prevalence rates for child interpersonal traumas are much higher in MDD cases than MDD controls. Thus, it is probable that the lack of a significant finding is due to ascertainment rather than lack of effect, as many studies across the literature report significant effects of child interpersonal traumas (e.g., CSA) on sleep,[141,142] and some indicate that childhood interpersonal trauma is a more potent predictor than all other traumas in predicting depression and anxiety (e.g., [93]). However, in a recent study, investigators found that childhood trauma was significantly associated with cognitive dimensions of depression only, not other dimensions, which included insomnia.[301] Within MDD cases, however, all other forms of trauma examined (adult interpersonal, child non-assaultive, and adult non-assaultive) were significant predictors of sleeplessness, aligning with

earlier results and prior literature (i.e., that individuals endorsing any form of trauma vs. none endorse more sleep disturbances),[153], although there were no differences in terms of magnitude of effect across these events. Thus, trauma endorsement, more broadly, is associated with more sleeplessness in MDD cases, but it is difficult to understand the contributions of childhood interpersonal trauma due to ascertainment.

In sum, examinations of trauma and sleeplessness showed associations between the phenotypes within this large Han Chinese sample. Most trauma types were important at similar magnitudes across all analyses run, with several exceptions: 1) child interpersonal trauma did not predict sleeplessness in MDD cases; 2) adult interpersonal trauma did not predict sleeplessness in MDD controls; and 3) child interpersonal trauma may have a larger effect on sleeplessness than other traumas for individuals in a more general population (modeled via the survey approach). Evidence for the potency of child interpersonal trauma in predicting sleeplessness supports a more detailed examination of CSA.

CSA analyses. While it is well established that CSA has an effect on sleep in adults decades after the abuse,[141,142] this is the first study to examine the effects of this particularly potent traumatic event on sleep in a Han Chinese population, as earlier studies have primarily used European and European American samples. Similar to prior analyses of child interpersonal trauma, broad CSA was a significant predictor of sleeplessness. The odds ratio of 1.28 (95% CI = 1.04-1.58; co-varying for MDD status) for CSA predicting sleep here is similar to, although a bit less than, what was found for CSA predicting insomnia symptoms in female twins within the VATSPSUD sample (OR = 1.67, 95% CI = 1.35-2.06),[151] despite sample differences. Note that both samples were assessed for broad CSA using identical questions, but that CONVERGE has a much lower CSA prevalence (6.8%) than the female twin sample used from VATSPSUD

(30.1%),[151] even when restricting to MDD cases only (11.0%). The samples also differed in terms of sleep assessment (binary item assessing sleeplessness vs. quasi-continuous insomnia symptom variable). The OR for CSA predicting sleeplessness in CONVERGE did increase to 2.30 (95% CI = 1.77-2.98) when the survey approach was used to model population prevalence, suggesting that the effect may in fact be larger in a more population-based sample that is not oversampled for MDD. This is closer to ORs for CSA predicting MDD in CONVERGE,[130,131] and provides some evidence that a particularly potent child interpersonal trauma like CSA may have a larger effect on sleep then other, more recent traumas, which is in line with the literature outlined above.[93] Overall, these results support that findings across CSA and sleep are robust and do extend to the Han Chinese population, despite lower endorsement.

Upon examination of incident characteristics within individuals endorsing CSA in CONVERGE, none of these items (or CSA severity) were significant predictors of sleeplessness. Within the MDD literature, a dose-response relationship with CSA severity is documented (e.g., [124,126]) and shown to replicate in CONVERGE.[130,131] However, so far this has not been shown for insomnia; it was not identified within VATSPSUD.[151] Some argue that CSA severity is related to both emotional and physical factors, and that these physical factors alone are not enough to explain severity.[302] Further, studies of incident characteristics in relation to sleep outcomes are mixed,[149,151,303,304] with some showing differential predictions. There is also variation within the MDD literature, where this has been studied in more detail, with some evidence that certain characteristics may be associated with greater risk for MDD following CSA (e.g., [128,303]), while others do not demonstrate differential impacts (e.g., [125]). It could be that differential effects of CSA severity and/or incident characteristics on sleep do exist, but cannot be detected here because they are mediated through MDD/MDD symptoms. The severity of the abuse could be

reflected in the development of early MDD, which could in turn result in more sleep disturbances (see [305] for analyses of psychopathology mediating the relationship between broad CSA and sleep). Additional analyses would be needed to further explore this and would require a sample that is not oversampled for recurrent MDD.

Limitations. All results should be interpreted in light of five main limitations. First, there is ascertainment bias, as the CONVERGE sample has been selected such that half of the women have recurrent MDD, while the other half do not. As a result, the endorsement of sleeplessness was much higher in MDD cases (86%) than in MDD controls (28%). This was addressed by running trauma and sleep analyses using three different approaches, although there were also differences that occurred in the endorsement of traumatic events (almost all higher in MDD cases, but lower than population samples[65,69]) and across both trauma and sleep (MDD cases with and without child interpersonal traumas endorsed sleeplessness at similar rates; MDD controls with and without adult interpersonal traumas endorsed sleeplessness at similar rates), which make it difficult to draw conclusions. Further, the MDD controls did not have a history of psychosis, bipolar disorder, or substance use, which may not reflect the general population and recurrent MDD may represent a more severe subset of individuals with the disorder. Second, although there is detailed information on trauma type and timing and trauma assessment was conducted through clinical interview, this information was collected retrospectively and the exact temporal order of all lifetime traumas was unknown (e.g., if an event has occurred more than once, only the age at first onset is known). There could also be reporting bias, given the retrospective nature of assessment (i.e., reporting age of onset), and there may be under-reporting, particularly for sensitive childhood events (e.g., CSA).[306] The trauma list is not exhaustive, and all events may not truly be DSM-IV Criterion A traumas, as this was not

assessed. Third, and perhaps most importantly, the assessment of sleep within this sample was not ideal, with only one subjective binary sleep item available on all individuals, assessed at the lifetime level, which was not part of a verified sleep scale. However, assessments of disturbed sleep tend to have high face validity.[169] It is also recognized that endorsement of this general sleep item may reflect other underlying sleep disorders, such as circadian rhythm disorders. Fourth, analyses presented here are correlational and causal conclusions cannot be drawn. Finally, generalizability is limited, as the sample is comprised solely of Han Chinese women.

There are several strengths worth noting. First, few studies specifically examining trauma type and timing and sleep exist, making this investigation novel. Second, the detailed assessment of traumatic events permitted the examination of factor structure and thus the specific influences of type and timing within this sample. Third, the Han Chinese are an understudied population with regard to analysis of trauma types and the relationship between CSA and sleep. Fourth, the use of an all female sample is appropriate given that the prevalence of interpersonal traumas,[69,70] sleep disturbances,[2,13] and MDD[44,49,50] are all higher in women. Fifth, since controls in CONVERGE were ascertained to minimize the likelihood of developing recurrent MDD in the future, the general sleep item endorsed in MDD controls is unlikely to be confounded by experiencing sleep disturbances within MDD (or psychopathology such as substance use, psychosis, or bipolar disorder).

Conclusions and Future Directions. Traumatic events separate into interpersonal and non-assaultive types in a large, Han Chinese sample, and child interpersonal items (physical abuse, neglect, CSA) in particular may represent a unique factor. This is largely consistent with the prior literature, despite lower prevalences.[65,69] However, regression analyses of trauma type and timing predicting sleeplessness did not find differential effects for separate types of traumas,

which is in contrast to the prior literature for sleep and psychopathology (e.g., [69,105,106,113,147,148]). However, results may differ in a more population-based sample, as there was some evidence through survey-based methods (and examining MDD controls only) that child interpersonal events in particular may exert stronger effects on sleep. Finally, a relationship between CSA and sleep was replicated within this sample, but there was no evidence for specific effects of severity or incident characteristics. Taken together, these results indicate that although certain trauma types (i.e., interpersonal) are more potent predictors of internalizing disorders, effects on sleep may vary depending on sample ascertainment (i.e., oversampling for MDD results in higher endorsement of both trauma and sleep problems). Nevertheless, findings across CSA and sleep do appear to extend to diverse populations and highlight the importance of assessing sleep disturbances in individuals with CSA histories. More work is needed to examine relationships between trauma type and timing and sleep in population samples with less ascertainment bias, better sleep phenotypes, and trauma assessments with additional temporal information, and a focus on child interpersonal trauma in particular may be warranted. This line of research has the potential to identify individuals who may be at higher risk for experiencing poor sleep following trauma and target interventions accordingly, and may also help prevent the development of psychopathology.

Chapter 4: Estimating heritability of sleep phenotypes through GCTA

I. Data analytic plan

The software program GCTA, which estimates the heritability of a trait based on the additive effect of SNPs present within available genome-wide data, was used to obtain heritability estimates. This method creates a GRM based on SNPs for all pairs of individuals in the sample. The GRM is then used to predict phenotypic relatedness using a REML approach, resulting in an estimate of the variance in the phenotype of interest that is due to SNPs.[243] For binary traits, the variance can be transformed to a liability scale by indicating the expected population prevalence.[307] Here, GCTA version 1.24.7 was used to create the GRM and obtain heritability estimates. The GRM was constructed from 4.7 million hard-called SNPs that had a genotype probability $\geq$ 0.9, missing rate < 1%, MAF > 1%, and Hardy-Weinberg p value > 10^{-6} (see [300] for more details). Two north-south ancestry PCs (PC1 and PC2) out of 10 total were used as covariates in all analyses (as done in prior genetic analyses of this sample[220,300]), and an MDD covariate was used as appropriate (i.e., for analyses including both MDD cases and MDD controls). The SDS variable was analyzed as a quantitative trait (quasi-continuous variable), while all other sleep phenotypes were analyzed as binary variables, with the estimated population prevalence of the trait included in all analyses to transform the estimates onto the liability scale (see Table 13 for prevalence estimates used across variables).

Case-control power analyses were run in the GCTA-GREML power calculator, http://cnsgenomics.com/shiny/gctaPower/). There was over 80% power to detect heritability for

the SDS,[250] and 100% power to detect heritability for combined MDD cases and MDD controls. However, for separate analyses in MDD cases and MDD controls, power calculations indicated only 52% power for the GS in MDD cases and 74% for the GS in MDD controls. Power estimates were even lower (<30%) for the Adjusted GS and Item E8, given high endorsement within the sample.

II. Results

To determine the SNP-based heritability of sleep phenotypes and to prioritize the variables in subsequent GWAS analyses (Aim 2b), GCTA of all sleep phenotypes were conducted. Results are shown in Table 13. The first four rows show estimates for sleep variables that exist only in MDD cases, starting with the quasi-continuous trait (SDS), followed by binary sleep variables. None of these GCTA analyses yielded heritability estimates of sleep variables that were significant, with large standard errors and all *p*-values > 0.05. Similar results were seen in the fifth row for the GS in MDD controls. The final four rows show results within the combined sample of both MDD cases and MDD controls, utilizing the GS and the Adjusted GS (which contained additional sleep information for MDD cases). When there was no adjustment for MDD case status, both versions of the GS were heritable (8% for GS and 14% for Adjusted GS, *p*-values < 0.05), as can be seen in lines 7 and 9 of Table 13, respectively. Including the MDD covariate resulted in decreases in both estimates (~0 for GS and 1% for Adjusted GS), with neither estimate remaining significant. Although there was not support for SNP-based heritability for any of the sleep variables examined in CONVERGE, the SDS in MDD cases (quantitative trait and thus higher power than case/control analyses with a large number of cases) and the Adjusted GS (with MDD covariate) in the full sample (which incorporates the most

information across all individuals) are discussed in detail in subsequent GWAS analyses.

Table 13. Estimates of SNP-based heritability for sleep phenotypes generated from GCTA*

	N (Ca/Co)	Covariates[1]	Specified disease prevalence	Sample prevalence	H^2 (scaled to prevalence)	SE	*p*-value
MDD Cases only							
SDS[2]	5069 2.58 (0.79)	PCs	--	--	0.00	0.06	0.50
GS	5116 (4375/741)	PCs	0.85	0.86	0.00	0.13	0.50
Adjusted GS	5244 (5039/205)	PCs	0.85	0.96	0.00	0.41	0.50
Item E8	5221 (4813/408)	PCs	0.85	0.92	0.02	0.21	0.46
MDD Controls only							
GS	4885 (1404/3481)	PCs	0.30	0.29	0.00	0.10	0.50
Full sample							
GS	10001 (5779/4222)	PCs	0.35	0.58	0.08	0.04	0.02
GS with MDD	10001 (5779/4222)	PCs, MDD	0.35	0.58	0.00	0.04	0.50
Adjusted GS	10129 (6443/3686)	PCs	0.35	0.64	0.14	0.05	<0.001
Adjusted GS, with MDD	10129 (6443/3686)	PCs, MDD	0.35	0.64	0.01	0.04	0.41

Abbreviations: Ca = sleep case; Co = sleep control; GCTA = genome-wide complex trait analysis; GS = general sleep item; H^2 = heritability; MDD = major depressive disorder; PC = principal component; SDS = sleep within depression; SE = standard error; SNP = single nucleotide polymorphism.

*Note that GCTA was also run on the original MDD variable to ensure that code worked properly.

[1] Although age was a significant predictor of sleep variables in phenotypic analyses, it accounted for a small proportion of variance, particularly within controls, so it was not included within genetic analyses presented here. When age was included as a covariate in the analyses in rows 1-5 presented above, estimates remained similar. For combined case/control samples, age and case status are confounded due to ascertainment within CONVERGE, so it was not included for the full sample either.

[2] This variable was treated as a quantitative trait so no prevalence was specified.

III. Discussion

To determine the extent to which sleep phenotypes utilized with CONVERGE are under genetic influence, and subsequently determine the SNP-based heritability of these traits, GCTA was conducted on sleep variables, both in the full sample and separately in MDD cases and MDD controls. Overall, heritability estimates were not significant, with estimated variance due to additive genetic effects close to zero for the SDS (quantitative trait) in MDD cases, GS in MDD cases, and GS in MDD controls (see Table 13) and only 2% for the main insomnia item from MDD assessment (Item E8) in MDD cases. None of these estimates were significantly different from 0. In contrast, a significant heritability estimate (8%) was obtained when MDD cases and controls were combined together for the GS, but this effect was no longer significant when accounting for MDD status, suggesting that the variance detected was due to MDD. Similar results were obtained with the Adjusted GS variable, which incorporated all available sleep information for MDD cases, although the initial estimate was higher (14%). This estimate also became non-significant when MDD status was included as a covariate.

Taken together, these results suggest that sleep phenotypes within the CONVERGE sample are not heritable, which contradicts the extant literature for insomnia. The twin literature indicates that insomnia phenotypes are moderately heritable, with the lower bound of published estimates at around 20% and the highest estimates close to 60%.[167] Several published GWAS of insomnia have included SNP-based heritability estimates within their results, and these are broadly consistent with, but on the lower end of, twin estimates: The UK Biobank GWAS of sleep phenotypes[216] reported that their binary insomnia phenotype had a heritability of 21% (using BOLT-REML variance components analysis[308]), and the sleep latency GWAS by Amin and colleagues[213] reported an estimate of 20% for sleep latency, estimated through GenAbel.[309]

The heritability estimate obtained within CONVERGE when combining MDD cases and MDD controls for the adjusted sleep variable (~14%) is closest to these estimates, although this is heavily influenced by ascertainment and MDD status, and thus all genetic variance is accounted for by MDD. Estimates within MDD cases and MDD controls separately were robust to the effects of age and were reported without age covariates. Differences in the phenotype from CONVERGE, as well as ascertainment within the sample, could be contributing to the inconsistency with the current literature, and will be discussed in greater detail below.

Another paper specifically investigated the SNP-based heritability of MDD symptoms, which included insomnia-related items.[260] Principal component analysis was conducted on the 17 items that made up the Hamilton Rating Scale for Depression (HRSD) to determine how the specific items sorted into clusters. Interestingly, insomnia was among the most heritable symptom clusters, with an estimate of 30%, surpassing heritability for anxiety (5%) and core MDD symptoms (14%), but equal to that of appetite. This heritability is very similar to the twin estimates for sleep within depression (19-35%; [184,185]). Given the detailed phenotyping for MDD that occurred within CONVERGE, replication should have been possible within this sample for sleep variables within depression, especially given the larger sample size (over 5,000 when restricted to MDD cases, compared to under 2,000 in Pearson et al.[260]), but this was not the case. The HRSD asked three insomnia items specific to MDD, while CONVERGE only included two MDD-specific insomnia items (the third item used in the SDS was a general item). There are other differences between the sample presented here and that used within the Pearson study that may be contributing to these divergent findings. Pearson and colleagues[260] utilized a sample of treatment-seeking individuals of European ancestry who were diagnosed with MDD per DSM-IV criteria. The study reported a much wider age range than CONVERGE (18-75 years old vs.

30-60 years old) and included both genders (~60% female, vs. 100%). Given that both insomnia[174] and MDD[175] have been shown to have higher heritability in women, this should increase the likelihood of detecting heritability in CONVERGE. However, note that there are no twin estimates of MDD symptom heritability in Asian samples, so it is assumed that estimates parallel those in the existing literature, which could be incorrect. Further, since individuals within CONVERGE were selected for recurrent MDD, it could also be that endorsement of the sleep items in this sample (~90%) were too high and there was not enough variation to obtain estimates.

Power. In addition to placing these findings in the context of the extant literature, there are several major points to consider in interpreting these GCTA results, including 1) power; 2) phenotype; and 3) method. First, the ability to detect significant heritability estimates for these phenotypes within the CONVERGE sample is dependent on power, which for GCTA is, in turn, dependent on sample size and the prevalence of the trait. Power may be influencing some of the results seen here, especially given the high endorsement of sleep variables within the sample. For the quasi-quantitative SDS trait, there was over 80% power,[250] which should have been adequate to detect the expected heritability of approximately 30%. However, note that the variable is highly negatively skewed (and thus not normally distributed even after log transformation; refer to Figure 1 for distribution), and this could result in a biased estimate.[251] When both cases and controls were combined for the GS item, there was also adequate power (~100%) given the large sample size (over 10,000). These estimates were significantly different from 0 without an MDD covariate, but decreased when MDD was included. However, power is definitely a concern when looking at sleep variables separately in MDD cases and MDD controls, where the sample size is half as large as for the combined analyses. For all of these binary sleep items, power was below

80%, and in some cases even below 30%. This suggests that heritability may not be identified for these traits due to low power, which is related to the population prevalence and high endorsement within this sample.

Phenotype. Second, there are problems with the sleep phenotypes used that could be contributing to the lack of genetic influences. The only item available for both MDD cases and MDD controls is the GS, which consists of one binary item that asks, "Do you suffer from sleeplessness?" This particular question is not part of DSM criteria for insomnia[1] nor does it come from an established insomnia (e.g., Insomnia Severity Index[310]) or sleep quality (PSQI[178]) scale. However, note that there is variability in terms of insomnia phenotypes used across the two samples[213,216] that have estimated SNP heritability so far. The UK Biobank utilized one ordinal (3-level) insomnia item that was collapsed into a binary yes/no variable (i.e., endorsement of "never/rarely" indicated a control; "usually" were cases; "sometimes" were excluded).[216] This question encompassed both trouble falling asleep and waking up at night, which are two of the main DSM insomnia symptoms.[1] In contrast, Amin and colleagues[213] utilized a quantitative sleep latency phenotype, which measured time to fall asleep, in minutes, which may reflect the difficulty falling asleep component of insomnia (i.e., individuals with difficulty falling asleep would report a longer sleep latency). Note that neither of these studies used an insomnia diagnosis. The general sleep phenotype used here, although binary and not from a standardized measure, is consistent with prior analyses given that it measures an insomnia symptom (trouble sleeping), not disorder. Further, within the subset of MDD controls, it is certain that these individuals do not have recurrent MDD and will likely not develop it in the future (i.e., genetic risk is minimized). Given bidirectional relationships between sleep and psychopathology,[32,34,38] it is useful to know that for these individuals, the endorsement of this

sleep item is not confounded by MDD. This means that any genetic contributions detected are sleep-specific. However, this could also be a disadvantage in that sleep in these individuals is not representative of the general population (i.e., they could be "super controls"), and thus could partially explain the lack of insomnia heritability for MDD controls seen here.

In comparison, in MDD cases, sleep is appropriately assessed within the context of MDD, as CONVERGE was ascertained for studying the genetic contributions to MDD and items come from diagnostic criteria.[48] Note that individuals were given the option to endorse hypersomnia as well as insomnia, although insomnia is the focus here (in general, estimates did not differ with hypersomnia included as a covariate). The endorsement of these sleep items is high within CONVERGE, with 92% of individuals with MDD who were included in GCTA endorsing the main MDD sleep item (Item E8; difficulty falling or staying asleep). Further, 87% of these individuals endorsed early morning awakenings. These estimates are definitely high but not surprising, given associations that have been shown between insomnia and depression severity (e.g., [36,37]). Since this is a sample with recurrent MDD, it is reasonable that insomnia symptoms are very prevalent. The literature points to sleep as a core symptom of MDD[35] and up to 90% of individuals with MDD report experiencing a sleep symptom.[32] Further, in a recent South Korean sample, endorsement of insomnia items within MDD was 93%.[37] A quasi-quantitative trait for sleep within depression (SDS) in MDD cases was also analyzed, created by summing up responses on the main MDD sleep item (Item E8), early morning awakenings (Item E8.A), and the general sleep item, which also had high endorsement in MDD cases (86%). This was based on results from a prior paper that showed that three insomnia symptoms (difficulty falling asleep, difficulty staying asleep, and early morning awakenings) loaded highly onto a single factor in a phenotypic factor analysis, although the three items used in this paper were

asked on a 1-5 Likert scale.[174] Thus, it is possible that the SDS sum score variable used here is not adequately measuring sleep within the sample, and as noted earlier, the variable is skewed. The skewness of the variable and high endorsement (and thus low variation) could lead to biased estimates of heritability.

Method. Overall, estimates of SNP-based heritability have been lower than twin estimates across a range of phenotypes, both psychiatric and non-psychiatric in nature.[245,311-314] While promising, this method has been unable to resolve all of the so-called "missing heritability" (a concept also discussed in the context of the total variance explained by known GWS loci).[251,315,316] GCTA assumes an additive effect for all variants (and does not incorporate effect sizes) when estimating heritability.[243,245] While additivity is an assumption also made in the twin literature (unless dominance is modeled), it does not encompass all possible types of genetic contributions to the trait, such as dominance effects and epistasis (gene-gene interactions).[317] Some researchers have proposed that heritability is not "missing," but is instead "phantom" heritability that results from the overestimation of the total variance, since GxE and gene-gene interactions, among other genetic effects, are not measured.[316] Generally, GCTA estimates narrow-sense heritability and estimates are considered to be a lower bound for the true heritability, given that all genetic variation is not encompassed.[245,249] GCTA is also limited by the available SNPs in the study sample, which includes those that are measured or imputed, as the estimate can only be influenced by these SNPs and variants that are in LD with them.[245,249] As a result, GCTA captures effects of mostly common variants, since rarer variants are not in LD with what is measured or imputed.[318] Within the analyses presented here, no variants with minor allele frequency (MAF) < 1% were included in GCTA, although note that the majority of common variation has been measured, given that genetic data collected here is sequence-based.

Based on this, variants that are not included in GCTA (i.e., rarer variants) could be contributing to missing heritability[251], however this is not likely to account for the majority of the missing heritability.

Further, there are some assumptions of GCTA related to case-control phenotypes that warrant discussion. GCTA was initially designed for quantitative traits[319] and then adapted for use in case-control methods.[307] For a binary trait, heritability estimates are dependent on sample size, prevalence of the trait in the population, proportion of cases, true heritability, and number of SNPs.[251] Thus any of these variables could affect the estimate. The approaches are similar, but in the case-control method, the initial scale is 0,1 (not liability, as seen for quantitative traits), ascertainment results in many more cases than would be seen in a population sample, and estimates are more sensitive to artifacts.[307] To address this first point, it is assumed that there is an underlying latent liability for case-control traits, as indicated by the liability threshold model (i.e., the latent trait is continuous/normally distributed and once individuals reach a certain threshold they can be considered cases). To calculate the SNP-based heritability for a binary trait, GCTA takes the phenotype, coded as 0/1, uses REML to calculate the observed heritability, and then converts it to the liability scale based on population prevalence *k*.[243] While this is technically unbiased, there are several assumptions of REML that are violated in the case of a binary trait. First, the underlying distributions of variables are not normal (genes, environment, liability). For the case of liability in particular, case-control ascertainment generally collects more cases than one would expect in the general population, resulting in a non-normal distribution. GCTA does correct for this, transforming using information on population prevalence,[243] but Golan and colleagues[251] argue that this may not be sufficient. Second, REML assumes that genetic and environment influences are uncorrelated, yet the case-control design

itself can introduce an "induced" GxE, which is problematic.[251] Additionally, Golan et al.[251] also argue that including covariates into a case-control design (i.e., by adding fixed-effects) can further bias the estimates. These assumptions and their violations, which have the greatest effects when the N is large and population prevalence is low and are believed to result in biased (i.e., underestimated) estimates, should be taken into consideration. The most relevant of these limitations to the CONVERGE sample include 1) bias if the ascertainment correction is not appropriate; and 2) bias introduced by the case-control design, which could be introducing GxE. Additionally, there is a recent paper that argues that GCTA results in biased estimates for quantitative traits, even without violations of assumptions, due to noise and over-fitting of the GRM.[252]

Summary. In sum, SNP-based heritability for sleep items within CONVERGE was not detected using GCTA, which in contrast to the prior literature.[213,216] Future directions (aside from utilizing a sample with better sleep phenotypes and less ascertainment bias) include utilizing other programs to estimate heritability and see if predictions improve. For example, LDSC incorporates LD information to provide heritability estimates that are not biased by the LD between markers and causal variants,[253] which is one criticism of GCTA that its creators acknowledge.[319] The lack of heritability indicates that results of subsequent genetic analyses should be interpreted with caution. If significant estimates were obtained, sleep phenotypes would be able to be compared better to those within the literature. Further, there would be more justification in proceeding with gene-finding efforts and PRSs within this sample and the subsequent interpretation of these results could be done with more certainty.

Chapter 5: Identification of variants contributing to sleep phenotypes through GWAS (Aim 2b)

I. Data analytic plan

GWAS were conducted in Plink, version 1.07,[320] using dosage data and run by chromosome. All sleep phenotypes were treated as binary (case/control), except for the SDS variable, which was analyzed as a quantitative trait. Covariates included two ancestry PCs (used within the original MDD GWAS and GCTA papers[220,300]) and MDD case status, where appropriate (i.e., full sample). Included SNPs were filtered such that all had a Hardy-Weinberg *p*-value > $1x10^{-7}$, INFO > 0.9, and MAF > 0.01 and < 0.99. This resulted in a total of approximately 6.1 million SNPs retained for subsequent analysis (see Table 15 for exact numbers). Manhattan and Q-Q plots were constructed using custom code in ggplot2[274] in R, adapted from scripts used within the Molecular and Statistical Genetics course at VCU, HGEN 603. The genomic inflation factor, lambda, was also calculated using custom code in R. The p.adjust function in the R package *stats* (specifying "fdr" and default values) was used to calculate false discovery rate [FDR]-based q-values for all SNPs that passed QC, and only SNPs with q-values < 0.5 were further examined via annotation. Notably, an FDR of 0.5 is very liberal, as this means that approximately half of the values below this cut-off are false positives. A more stringent FDR (e.g., 0.05, 5% false positives) would be scientifically and statistically ideal in order for results to be believable (i.e., to have confidence that the variant(s) of interest may actually be influencing the phenotype and is/are not just false positives), and to justify more

detailed examination of specific genes. However, given the goal of balancing rigorous science with choosing an FDR that would realistically provide the ability for training and experience in further probing of top variants, this level was put forward.

Annotation of these top SNPs was conducted using the UCSC genome browser[321] hg19 geneKey, 20151017, adapted from code used in the Spit for Science genetic analysis pipeline. For initial examination, SNPs were grouped into clusters such that a cluster contained SNPs that were on the same chromosome and located within 10 kb of each other. Following this, clusters were then created such that SNPs < 75 kb from each other were collapsed. The gene key, above, was used to determine if each cluster included specific annotated gene(s). The clusters were also examined for other nearby genes (i.e., genes located 50 kb upstream or downstream from the cluster start/end). LocusZoom,[322] a regional association plotting program available online, was used to visualize genes of interest (i.e., that contained variants with a minimum *p*-value < 10^{-6}). The hg19 genome build with 1000 genomes Mar 2012 ASN LD reference was used to create all LocusZoom plots. $-\log_{10}$P values for all SNPs +/- 200 kb from the specified gene of interest were plotted, along with their LD correlations in relation to the index SNP (defined here as the SNP with the lowest *p*-value). Finally, replication of GWS SNPs (or SNPs indicated to be of interest due to nominally significant *p*-values in the original studies) from the five prior GWAS of insomnia-related phenotypes was conducted, examining the effects of 7 (out of 15 total) previously identified SNPs within the summary statistics of the Adjusted GS with MDD covariate in CONVERGE. This phenotype was chosen for replication, as it is more similar to general insomnia than the SDS variable. Effect allele, effect size, and *p*-values were compared across the original insomnia GWAS and CONVERGE.

Power calculations for the two main GWAS phenotypes (SDS in MDD cases and Adjusted GS with MDD covariate in the full sample) are shown in Table 14. For the SDS (quasi-quantitative trait), power was calculated across different estimates of variance explained using custom code in R. There was adequate power (i.e., > 80%; see first half of Table 14) to detect individual variant(s) that explain ≥ 0.8% of the variance in the trait, but not for variants with smaller effects. For the Adjusted GS with MDD covariate (binary), power was calculated across several MAFs and genotype relative risks using the Genetic Association Study Power Calculator, http://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/index.html; see second half of Table 14. Here, power was adequate to detect variants with genotype relative risk ≥ 1.15 and MAF ≥ 0.25 (prevalence was set at 35%).

Table 14. Power to detect variants in GWAS across main sleep phenotypes

	SDS, MDD cases only (Quantitative trait, N = 5073) Variance explained				Adjusted GS, MDD covariate (Binary trait, N = 6,450 Ca, 3,704 Co) Genotype relative risk			
MAF	0.001	0.005	0.008	0.01	1.05	1.10	1.15	1.20
0.05	0.7%	33.9%	82.1%	95.3%	0%	0.1%	2.0%	17.2%
0.25	0.7%	33.9%	82.1%	95.3%	0.1%	13.6%	82.3%	99.8%
0.5	0.7%	33.9%	82.1%	95.3%	0.2%	25.7%	92.5%	100%

Abbreviations: Ca = case; Co = control; GS = general sleep item; MAF = minor allele frequency; MDD = major depressive disorder; SDS = sleep within depression variable.

II. Results

GWAS of main phenotypes. In order to determine if specific genetic variants contributed to risk for sleep traits within CONVERGE, GWAS were conducted with PC1 and PC2, as well as MDD where appropriate, as covariates. Association analyses focus on the SDS in MDD cases and Adjusted GS with MDD covariate within the full sample, given discussion following the pattern of GCTA findings. An overview of results is presented in Table 15, including covariates

used, sample size, number of SNPs passing quality control (QC) filters, genomic inflation factor, minimum *p*-value obtained, and number of SNPs with FDR < 0.5. Association results for the main phenotypes are presented in the top 2 rows. Manhattan plots and Q-Q plots are presented for these variables (SDS, Figures 5 and 6; Adjusted GS with MDD covariate, Figures 7 and 8) and will be discussed. No *p*-values reached genome-wide significance ($5x10^{-8}$) for either of the two phenotypes, although there was a *p*–value range (between $-\log_{10}P$ value of 4 and 6) in the Q-Q plot of the SDS that contained more values than expected by chance. Several *p*–values passed the threshold of nominal significance ($-\log_{10}P > 6$) for this trait. Both lambda values were just below 1 (0.998 for both), indicating that overall, the distributions of *p*–values are slightly under inflated (i.e., more larger *p*-values than would be expected by chance). FDR q-values were used to identify SNPs and regions of interest for the SDS that passed the *a priori* threshold (< 0.5). There were 312 SNPs with q-values < 0.5 (i.e., less than 50% chance that the SNP is a false positive) for the SDS, thus warranting further examination. No SNPs passed q < 0.5 for the Adjusted GS with MDD covariate.

Table 15. Sample sizes and summary of GWAS results for all phenotypes run

	Covariates?	GWAS N	SNPs passing QC filter	Genomic inflation (λ)	Minimum *p*-value	SNPs with FDR < 0.5
Main phenotypes						
SDS[1]	PCs	5073	6,105,870	0.998	3.72E-07	312
Adjusted GS, All	PCs, MDD	10154 (6450 Ca, 3704 Co)	6,111,327	0.998	2.28E-06	0
Other phenotypes						
GS, MDD controls	PCs	4906 (1409 Ca, 3497 Co)	6,112,139	1.000	5.75E-07	0
GS, MDD cases	PCs	5120 (4376, Ca, 744 Co)	6,106,412	1.005	1.97E-06	0
Adjusted GS, MDD cases	PCs	5248 (5041 Ca, 207 Co)	6,105,541	0.993	3.27E-07	0
Item E8, MDD cases	PCs	5225 (4815 Ca, 410 Co)	6,104,655	1.010	1.30E-06	0
GS, All	PCs	10026 (5785 Ca, 4241 Co)	6,111,579	1.015	2.67E-06	0
Adjusted GS, All	PCs	10154 (6450 Ca, 3704 Co)	6,111,327	1.025	7.08E-07	489

Abbreviations: Ca = sleep case; Co = sleep control; FDR = false discovery rate; GS = general sleep item; GWAS = genome-wide association study; MDD = major depressive disorder; PC = principal component; QC = quality control; SDS = sleep within depression; SNP = single nucleotide polymorphism.

[1] This variable was treated as a quantitative trait.

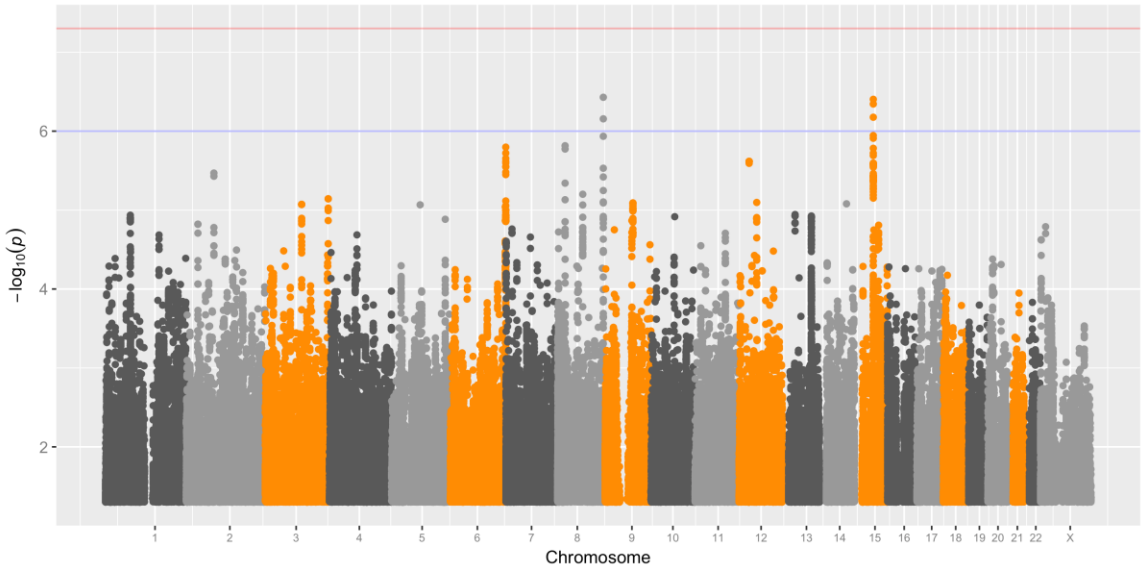


Figure 5: Manhattan plot for SDS in MDD cases. This figure plots the –log10(*p*) values of associations for the SDS by chromosome. The red line represents genome-wide significance (*p* = 5x10E-08), while the blue line indicates nominal significance (*p* = 10^{-6}).

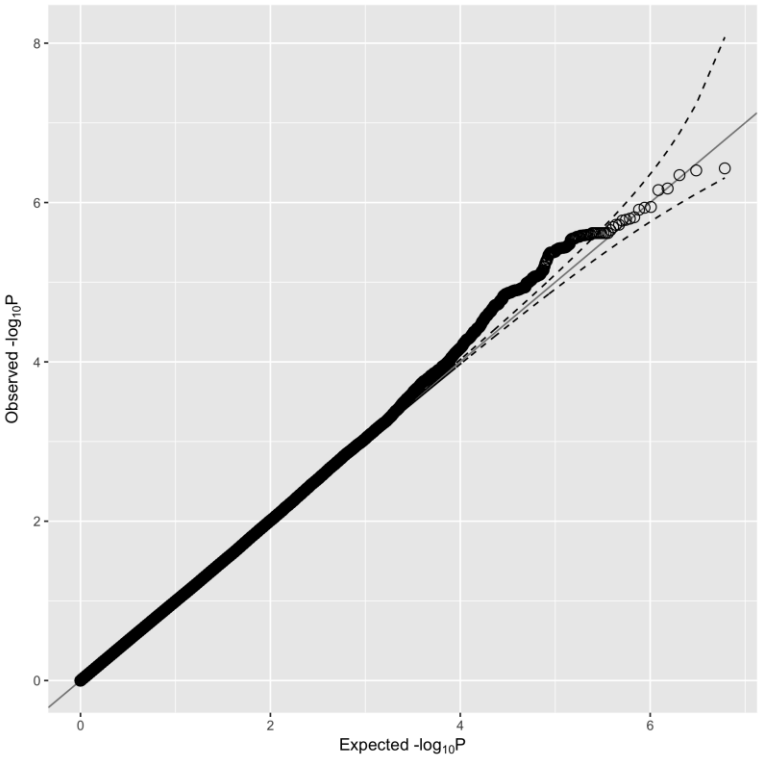


Figure 6: Q-Q plot for SDS in MDD cases. The expected distribution of *p*-values is shown on the x-axis, while the observed distribution of *p*-values from GWAS of the SDS is shown on the y-axis. All *p*-values are represented as –log10(P). The dashed lines represent 95% confidence intervals.

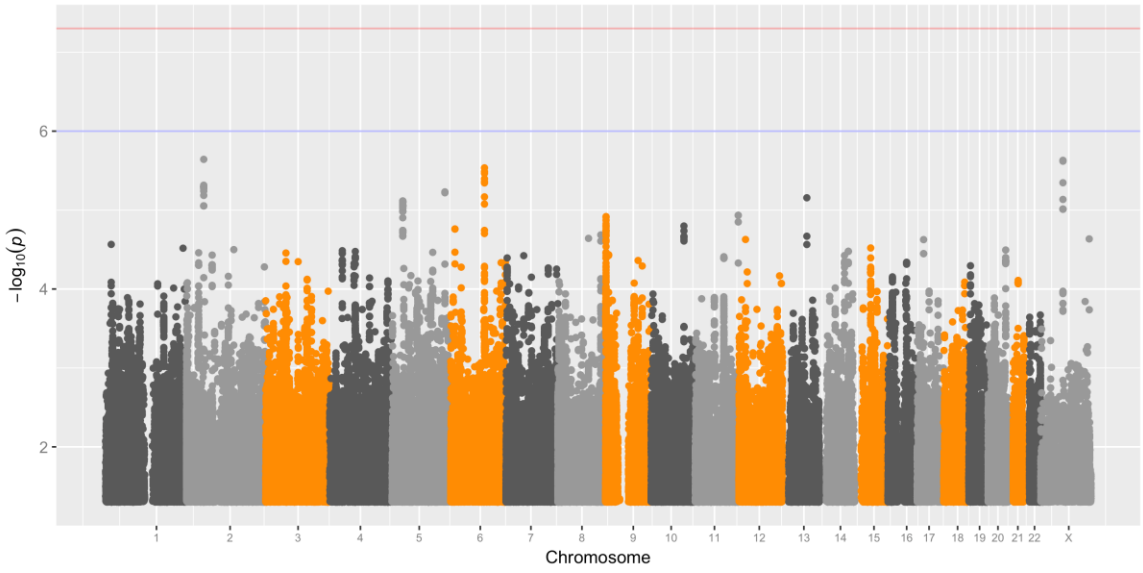


Figure 7: Manhattan plot for Adjusted GS in full sample, MDD covariate. This figure plots the –log10(*p*) values of associations for the Adjusted GS with MDD covariate by chromosome. The red line represents genome-wide significance (*p* = 5x10E-08), while the blue line indicates nominal significance ($p = 10^{-6}$).

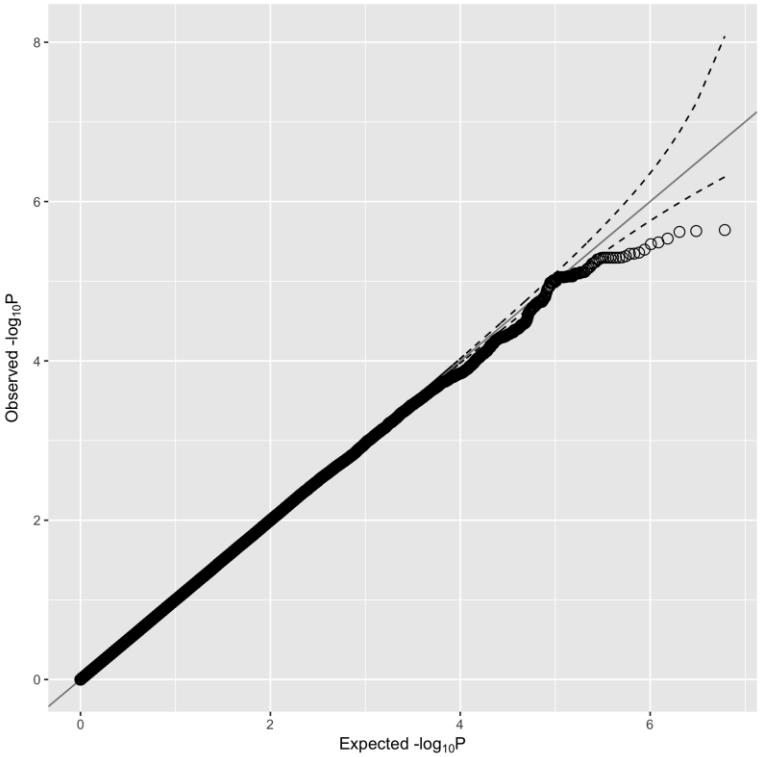


Figure 8: Q-Q plot for Adjusted GS in full sample, MDD covariate. The expected distribution of *p*-values is shown on the x-axis, while the observed distribution of *p*-values from GWAS of the Adjusted GS with MDD covariate is shown on the y-axis. All *p*-values are represented as –log10(P). The dashed lines represent 95% confidence intervals.

Top SNPs for SDS. Table 16 displays 33 clusters of SNPs with q < 0.5 (SNPs that are part of a cluster are located on the same chromosome and within 75 kb of each other) for the SDS, annotated with UCSC hg19 data. Chromosome, position start and end, number of SNPs included in the cluster, minimum *p*-value and minimum q-value from that cluster, associated genes, and local genes are all located in this table. Genes with a minimum *p*-value less than 10^{-6} include Potassium Two Pore Domain Channel Subfamily K Member 9 (*KCNK9*) on Chromosome 8 (16 SNPs) and Aldehyde Dehydrogenase 1 Family Member A2 (*ALDH1A2*) on Chromosome 15 (54 SNPs). LocusZoom plots[322] that show *KCNK9* and *ALDH1A2*, with 200 kb flanking either side of the gene, are presented in Figures 9 and 10, respectively. The SNP with the smallest *p*-value (as indicated in Table 15) is used as the index SNP in both plots. These plots show that for each gene of interest, there are a number of SNPs that are in high LD with the top SNP.

Table 16. Annotated clusters of top SNPs for SDS phenotype, ordered by chromosome and position

Chr	Start BP	End BP	# of SNPs	Min P	Min Q	Genes	Local genes
1	76922551	76943605	11	1.16E-05	0.42	*ST6GALNAC3*	None
1	165166002	165170408	2	2.07E-05	0.46	None	*LMX1A*
2	36195077	36199327	3	1.51E-05	0.42	None	None
2	85289866	85315647	4	3.40E-06	0.38	None	*KCMF1*, *TCF7L1*
3	112018435	112047314	13	8.48E-06	0.42	*BC041484*	*BC041484*, *CD200*, *SLC9C1*
3	193670290	193684988	7	7.20E-06	0.42	*LOC647323*	*DPPA2P3*, *LOC647323*
4	84599815	84599815	1	2.06E-05	0.46	None	None
5	87981557	87981557	1	8.58E-06	0.42	None	*LINC00461*, *MEF2C*, *MIR9-2*
5	165108213	165108213	1	1.31E-05	0.42	None	None
6	168889305	168918704	5	9.12E-06	0.42	*SMOC2*	None
6	170339330	170349370	43	1.60E-06	0.38	None	None
7	968785	968785	1	2.49E-05	0.49	*ADAP1*	*ADAP1*, *COX19*, *GET4*
7	18777804	18782969	4	1.74E-05	0.45	*HDAC9*	None
7	75426938	75426938	1	2.20E-05	0.47	None	*CCL24*, *CCL26*
8	2994124	2994124	1	1.89E-05	0.45	*CSMD1*	None
8	23200605	23224711	10	1.53E-06	0.38	*LOC100507156*, *LOXL2*	*BC128546*, *ENTPD4*, *LOC100507156*, *LOXL2*, *R3HCC1*
8	77437702	77483797	24	6.30E-06	0.42	None	*ZFHX4-AS1*
8	**140590172**	**140615287**	**16**	**3.72E-07**	**0.38**	***KCNK9***	**None**
9	28263705	28263705	1	1.78E-05	0.45	*LINGO2*	None
9	82121130	82170990	4	1.36E-05	0.42	None	*TLE4*
9	85332673	85359511	27	8.11E-06	0.42	None	None
10	72887202	72887202	1	1.21E-05	0.42	None	None
11	93104594	93138334	3	1.97E-05	0.45	*CCDC67*	None
12	31285810	31298362	9	2.42E-06	0.38	*OVOS2*	*DDX11*, *DKFZp434C0631*, *OVOS2*
12	54578882	54584028	12	8.01E-06	0.42	*SMUG1*	*AX747003*, *CBX5*, *MIR3198-2*, *SMUG1*
13	38967904	38997952	12	1.14E-05	0.42	None	*UFM1*
13	89020561	89057520	29	1.19E-05	0.42	None	None
14	82510563	82510563	1	8.33E-06	0.42	None	None
15	**58209736**	**58353510**	**54**	**3.95E-07**	**0.38**	***ALDH1A2***	**None**

Chr	Start BP	End BP	# of SNPs	Min P	Min Q	Genes	Local genes
15	60017791	60017791	1	1.79E-05	0.45	None	*BNIP2*
15	74613097	74671931	5	1.56E-05	0.44	*BC013681*, *CCDC33*, *CYP11A1*, *LOC729739*	*BC013681*, *CCDC33*, *CYP11A1*, *LOC729739*, *SEMA7A*
X	2273319	2273482	3	2.38E-05	0.49	None	None
X	15830587	15891711	2	1.63E-05	0.45	None	None

Abbreviations: BP = base position; Chr = chromosome number; Min = minimum.
Rows that contain nominally significant *p*-values ($p < 10^{-6}$) are in **bold**.

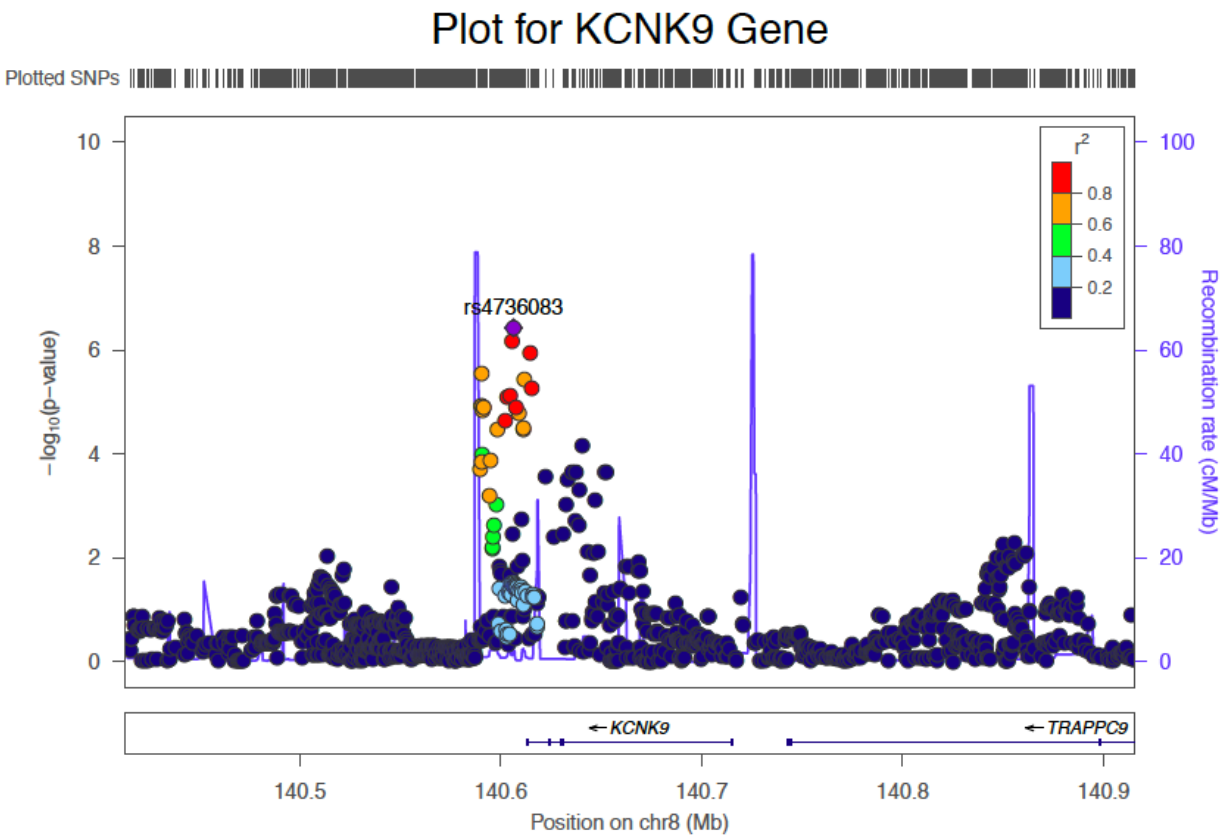


Figure 9: LocusZoom plot for SDS gene of interest *KCNK9*. Associations for SNPs within/surrounding the gene of interest *KCNK9* (+/- 200 kb) from the SDS GWAS are shown here. rs4736083, the SNP with the smallest *p*-value, was used as the index SNP. The x-axis shows the position of each SNP, while the y-axis reflects the *p*-value, transformed to –log10(*p*). Magnitude of LD for each SNP with the index SNP (r^2) is represented by different colors, with red being highest and blue being lowest.

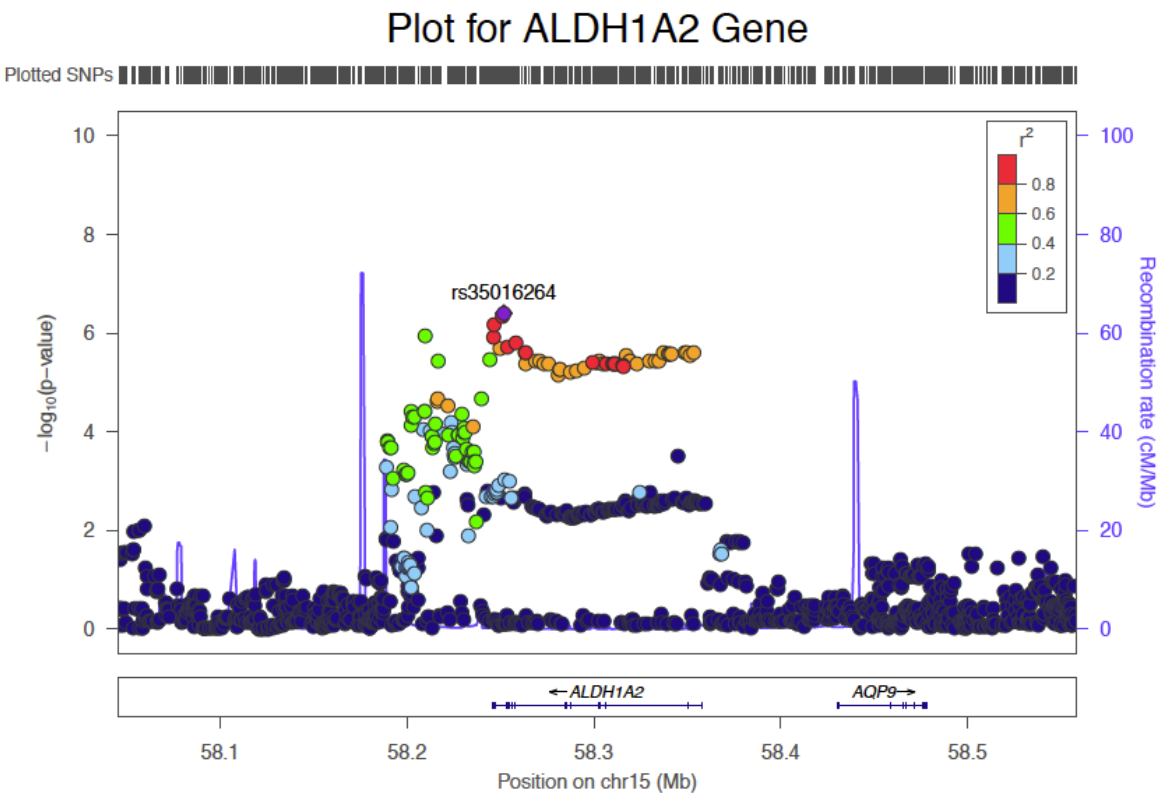


Figure 10: LocusZoom plot for SDS gene of interest *ALDH1A2*. Associations for SNPs within/surrounding the gene of interest *ALDH1A2* (+/- 200 kb) from the SDS GWAS are shown here. rs35016264, the SNP with the smallest *p*-value, was used as the index SNP. The x-axis shows the position of each SNP, while the y-axis reflects the *p*-value, transformed to –log10(*p*). Magnitude of LD for each SNP with the index SNP (r^2) is represented by different colors, with red being highest and blue being lowest.

Attempted replication of previously identified GWAS loci for insomnia within results for Adjusted GS. Table 17 lists SNPs that were found to be GWS (or of interest) in prior GWAS of insomnia phenotypes and are also available within the filtered CONVERGE summary statistics for the Adjusted GS with MDD covariate in the full sample. Information on the SNP (including the initial phenotype where association was found, rs number, chromosome, BP, and alleles) is shown in the first five columns. Next, summary statistics for the SNP from the original sample are shown (N, effect allele frequency, INFO, effect [OR/beta], 95% CI/SE, and *p*-value) in columns 6-11. Finally, the same information from the Adjusted GS with MDD covariate summary statistics is shown in the final six columns. Most SNPs had different MAFs and directions of effect than in the original samples and were not significant. However, rs2302729 (see row 4), was nominally significant in CONVERGE (OR = 1.12, $p = 0.01$).

Table 17. Summary of replication attempts for existing insomnia-related GWAS results within the Adjusted GS, MDD covariate

					Original sample						**CONVERGE Adjusted GS, MDD covariate**					
Phenotype	SNP	CHR	BP	Alleles	N	EAF	INFO	OR/ Beta	95% CI/SE	P	N	EAF	INFO	OR	SE	P
Insomnia[216]	rs5922858	X	82971008	G/T	58676	0.85	0.99	1.12	1.07-1.16	1.3E-8	10154	0.70	1.00	1.01	0.04	0.90
Insomnia (females)[216]	rs3792900	5	135393754	C/T*	58702	0.47	0.99	1.10	1.07-1.14	2.2E-8	10154	0.44	0.97	1.00	0.04	0.91
Sleep latency[215]	rs7304986	12	2438105	C/T*	2323	0.01	NR	0.49	0.11	1.4E-6	10154	0.98	0.98	1.02	0.16	0.89
Sleep quality[215]	rs2302729	12	2783972	T/C	2323	0.17	NR	0.17	0.04	4.4E-6	10154	0.35	0.98	1.12	0.04	0.01
Insomnia[214]	rs11208305	1	64088067	C/G*	8719	0.34	NR	1.60	NR	5.6E-6	10154	0.97	1.01	1.05	0.11	0.68
Insomnia[214]	rs718712	20	8714008	A/G*	8719	0.32	NR	0.82	NR	8.5E-6	10154	0.67	0.99	1.00	0.04	0.92
Sleep offset[217]	rs2919869	2	88404547	G/A*	956	0.26	NR	0.27	0.05	3.5E-8	10154	0.21	1.00	0.95	0.05	0.29

Abbreviations: BP = base position; EAF = effect allele frequency; GS = general sleep item; MDD = major depressive disorder; SNP = single nucleotide polymorphism.

*Effect allele is not the same in CONVERGE.

GWAS of additional phenotypes. Results of GWAS for six additional sleep phenotypes are also presented in Table 15 (see "Other phenotypes" heading). Manhattan and Q-Q plots for these variables are shown in Figures 11-22, below, for reference. Approximately 6.1 million SNPs were included in each analysis, and there was no genomic inflation (λ ranged from 1.00-1.025). No SNPs reached genome-wide significance in any of these six phenotypes, and only one analysis resulted in any SNPs that had an FDR < 0.5 (Adjusted GS without MDD covariate, a region of interest in the Q-Q can be seen in Figure 23; however, these results are confounded with MDD).

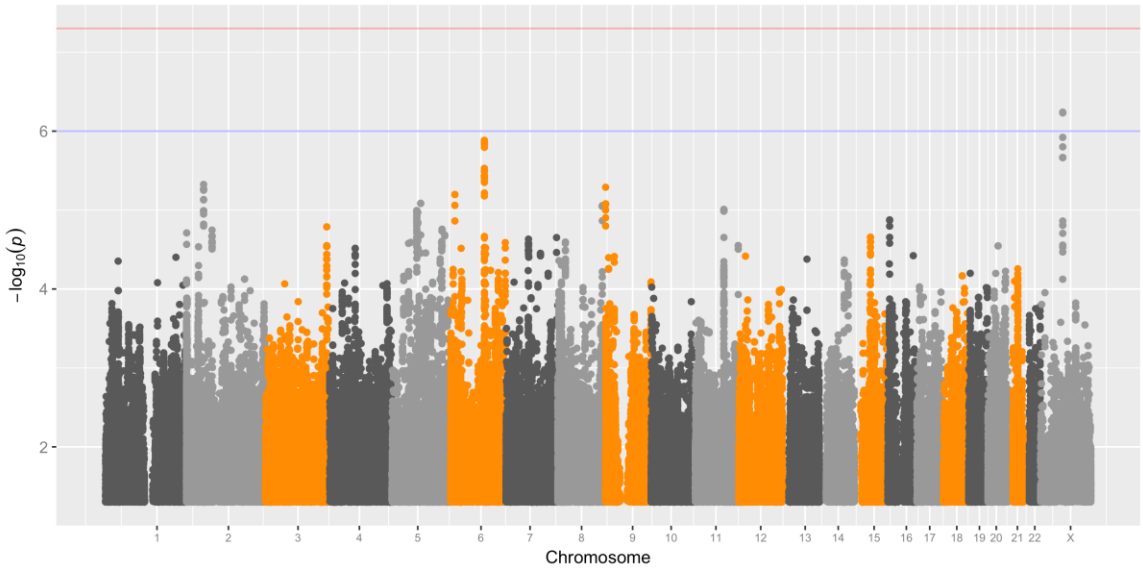


Figure 11: Manhattan plot for GS in MDD controls. This figure plots the –log10(*p*) values of associations for the GS in MDD controls by chromosome. The red line represents genome-wide significance (*p* = 5x10E-08), while the blue line indicates nominal significance (*p* = 10^{-6}).

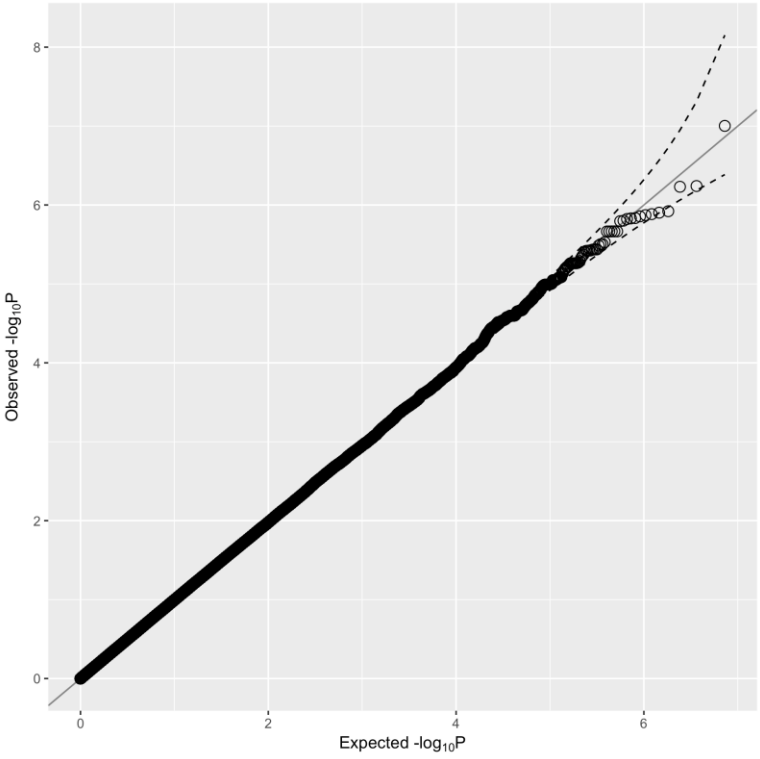


Figure 12: Q-Q plot for GS in MDD controls. The expected distribution of *p*-values is shown on the x-axis, while the observed distribution of *p*-values from GWAS of the GS in MDD controls is shown on the y-axis. All *p*-values are represented as –log10(*p*). The dashed lines represent 95% confidence intervals.

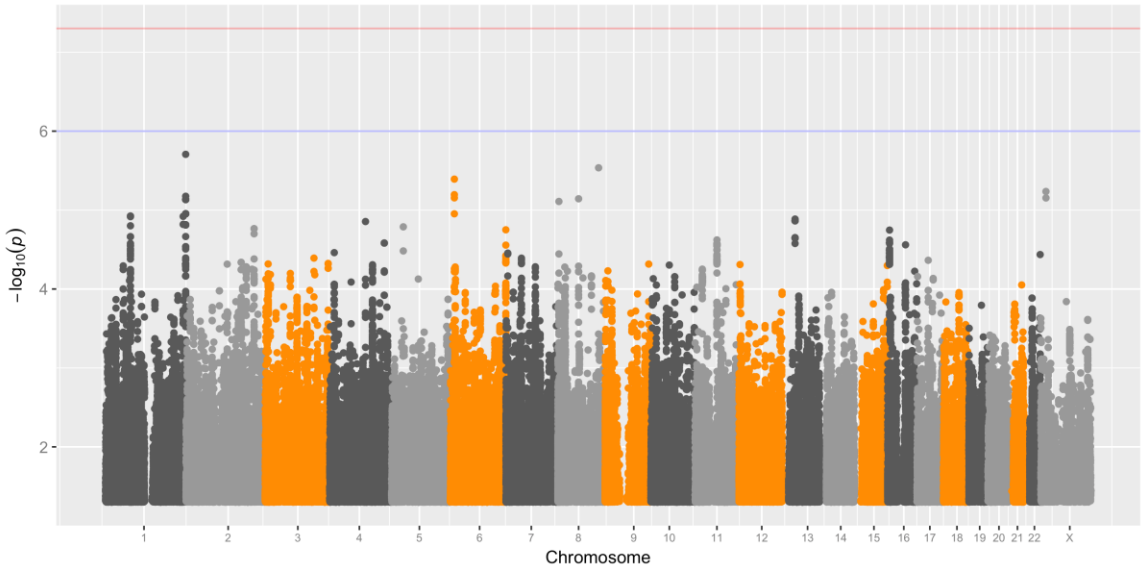


Figure 13: Manhattan plot for GS in MDD cases. This figure plots the –log10(*p*) values of associations for the GS in MDD cases by chromosome. The red line represents genome-wide significance (*p* = 5x10E-08), while the blue line indicates nominal significance ($p = 10^{-6}$).

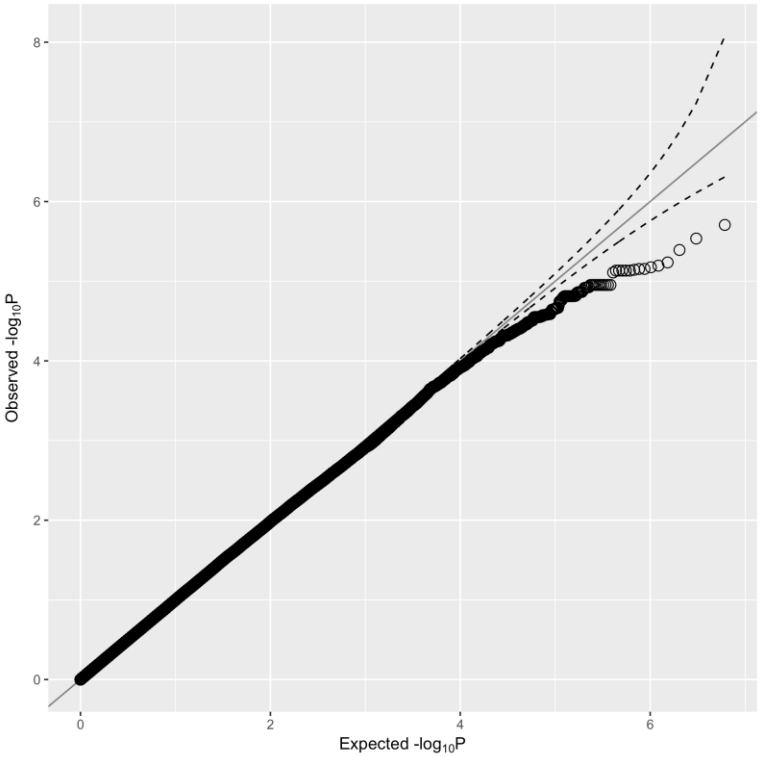


Figure 14: Q-Q plot for GS in MDD cases. The expected distribution of *p*-values is shown on the x-axis, while the observed distribution of *p*-values from GWAS of the GS in MDD cases is shown on the y-axis. All *p*-values are represented as –log10(*p*). The dashed lines represent 95% confidence intervals.

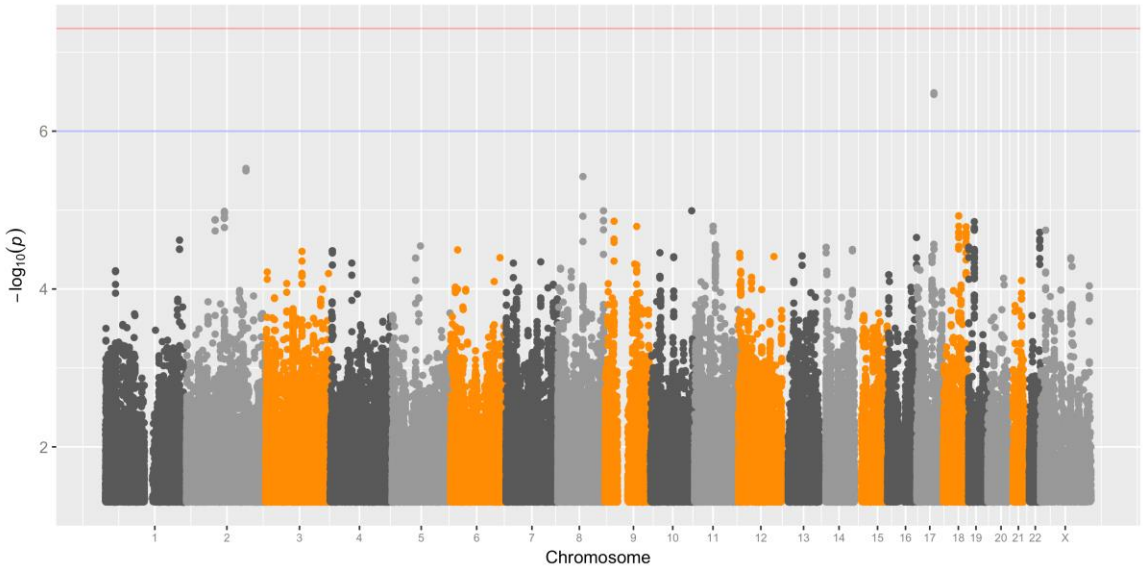


Figure 15: Manhattan plot for Adjusted GS in MDD cases. This figure plots the –log10(*p*) values of associations for the Adjusted GS in MDD cases by chromosome. The red line represents genome-wide significance (*p* = 5x10E-08), while the blue line indicates nominal significance ($p = 10^{-6}$).

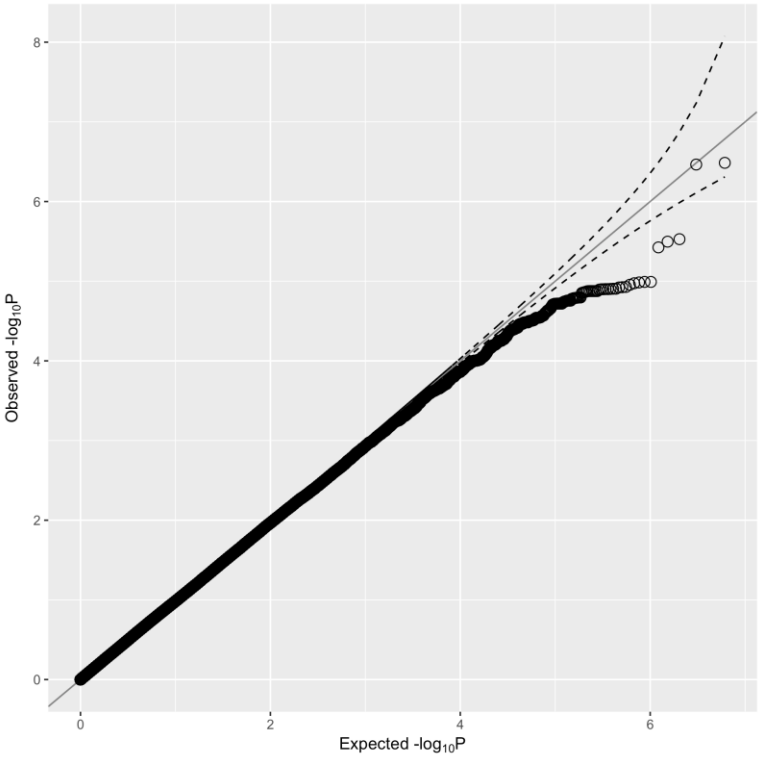


Figure 16: Q-Q plot for Adjusted GS in MDD cases. The expected distribution of *p*-values is shown on the x-axis, while the observed distribution of *p*-values from GWAS of the Adjusted GS in MDD cases is shown on the y-axis. All *p*-values are represented as –log10(*p*). The dashed lines represent 95% confidence intervals.

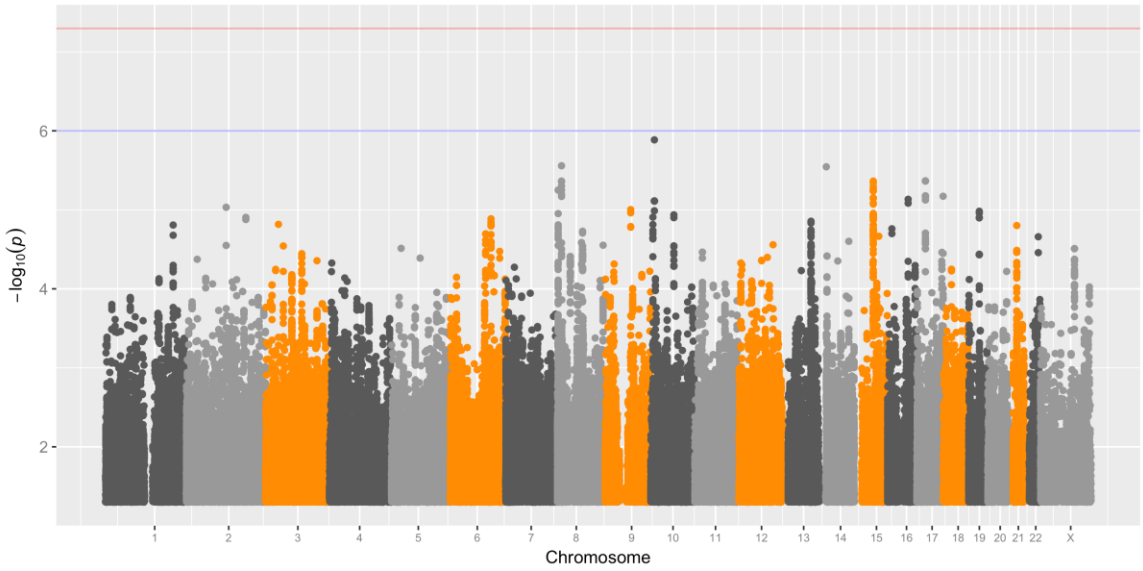


Figure 17: Manhattan plot for Item E8 in MDD cases. This figure plots the –log10(*p*) values of associations for the Item E8 in MDD cases by chromosome. The red line represents genome-wide significance (*p* = 5x10E-08), while the blue line indicates nominal significance ($p = 10^{-6}$).

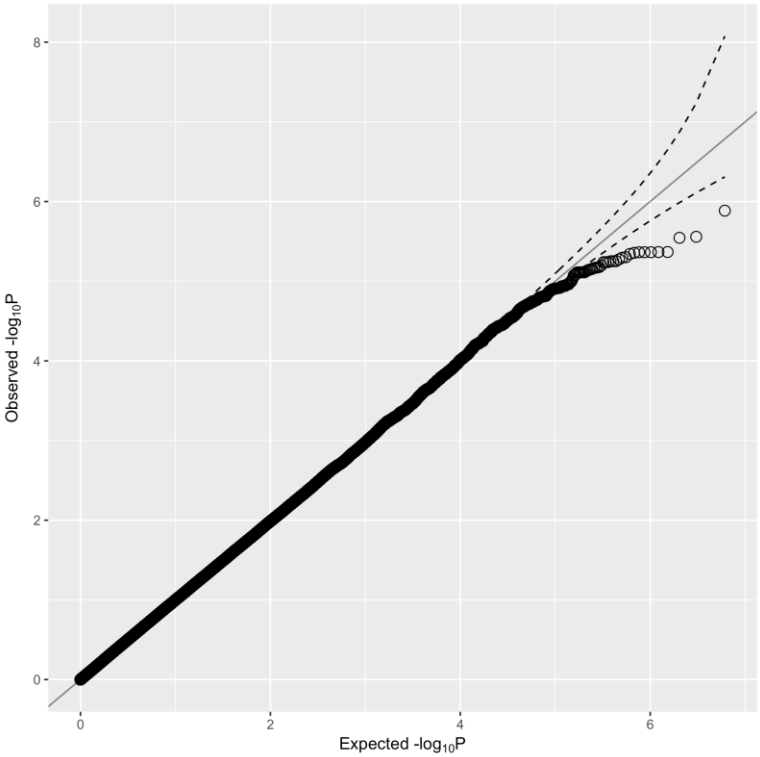


Figure 18: Q-Q plot for Item E8 in MDD cases. The expected distribution of *p*-values is shown on the x-axis, while the observed distribution of *p*-values from GWAS of Item E8 in MDD cases is shown on the y-axis. All *p*-values are represented as –log10(*p*). The dashed lines represent 95% confidence intervals.

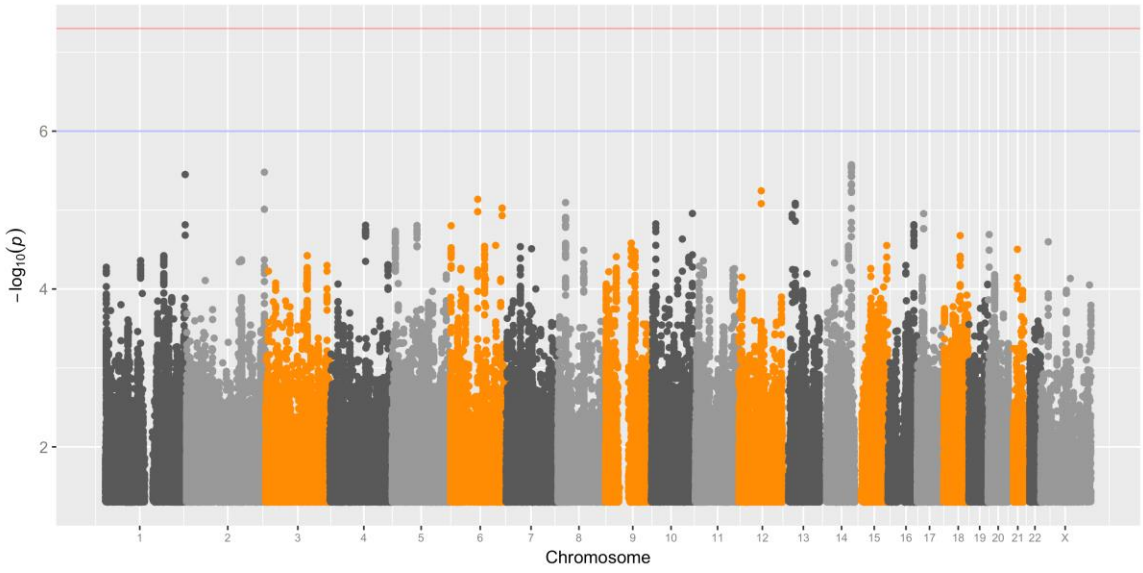


Figure 19: Manhattan plot for GS in full sample, no MDD covariate. This figure plots the –log10(*p*) values of associations for the GS in the full sample with no MDD covariate by chromosome. The red line represents genome-wide significance (*p* = 5x10E-08), while the blue line indicates nominal significance ($p = 10^{-6}$).

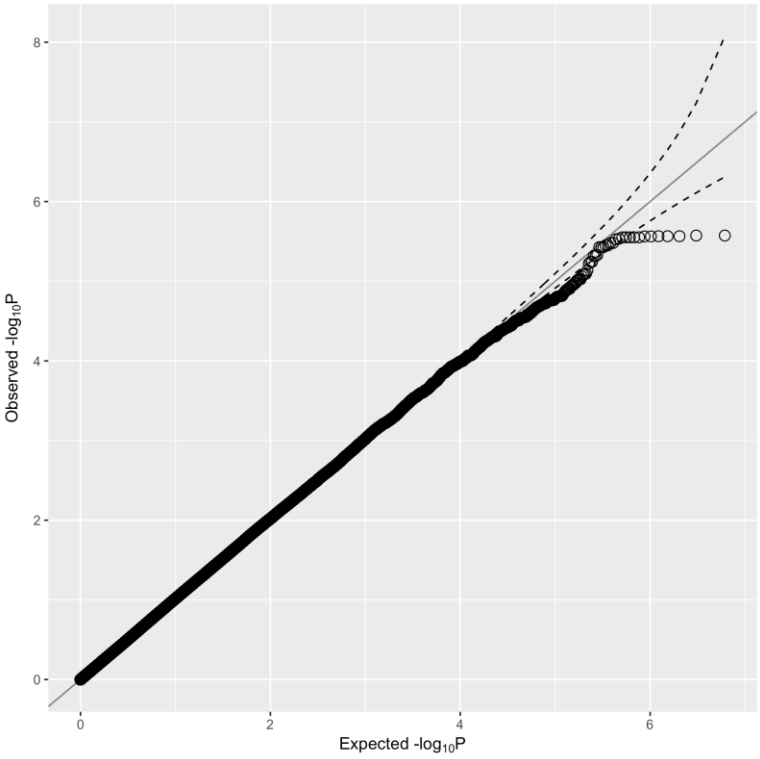


Figure 20: Q-Q plot for GS in full sample, no MDD covariate. The expected distribution of *p*-values is shown on the x-axis, while the observed distribution of *p*-values from GWAS of the GS in the full sample with no MDD covariate is shown on the y-axis. All *p*-values are represented as –log10(*p*). The dashed lines represent 95% confidence intervals.

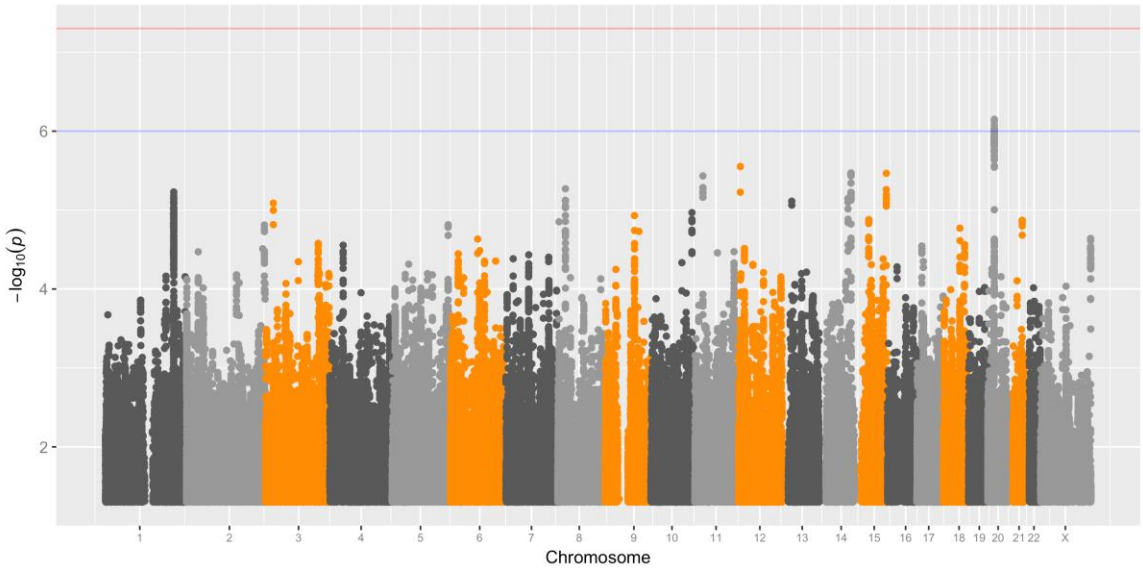


Figure 21: Manhattan plot for Adjusted GS in full sample, no MDD covariate. This figure plots the –log10(*p*) values of associations for the Adjusted GS in the full sample with no MDD covariate by chromosome. The red line represents genome-wide significance (*p* = 5x10E-08), while the blue line indicates nominal significance ($p = 10^{-6}$).

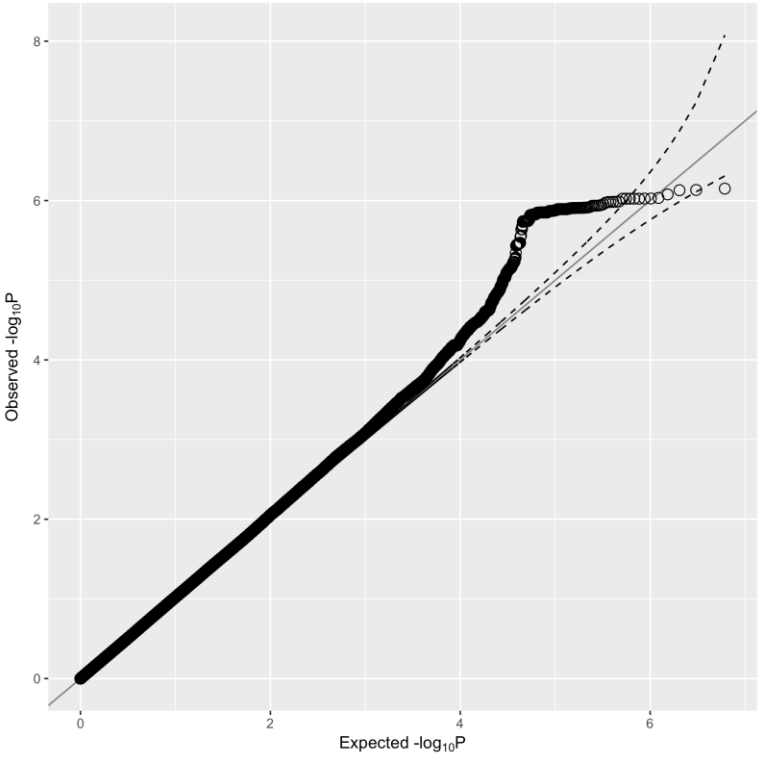


Figure 22: Q-Q plot for Adjusted GS in full sample, no MDD covariate. The expected distribution of *p*-values is shown on the x-axis, while the observed distribution of *p*-values from GWAS of the Adjusted GS in the full sample with no MDD covariate is shown on the y-axis. All *p*-values are represented as –log10(*p*). The dashed lines represent 95% confidence intervals.

III. Discussion

GWAS were conducted to determine if any specific genetic variants contribute to sleep disturbances within depression in MDD cases, as well as sleeplessness in general. Findings should be interpreted with the caveat that SNP-based heritability estimates of these phenotypes were not significantly different from zero, as discussed in the prior chapter. This discussion will focus on results for the SDS phenotype (quantitative trait), as well as the Adjusted GS with MDD covariate, with other phenotypes discussed briefly since there were no GWS results or SNPs with q-values < 0.5 in these additional variables. Overall, there were also no variants that reached genome-wide significance in the SDS or Adjusted GS with MDD as a covariate and Q-Q plots showed under inflation generally (lambda < 1.0). However, as indicated in the results above, there was an area of interest within the SDS phenotype where there were more *p*-values than expected by chance (between $-\log_{10}P$ of 4 and 6). Although the most significant *p*-values were not within this region, SNPs in this region could be in LD with top SNPs, which suggests that some of these variants could be of interest for sleep within depression. The genome-wide significance cut-off of $5x10^{-8}$ represents a conservative approach to limiting the number of false positives, and is based on the concept of family-wise error rate (FWER). A FWER reflects the probability of having any false positive across the millions of associations tested. In contrast, other methods for controlling for false positives, such as the FDR, permit the individual to set the percentage of false positive results that are acceptable. Although a higher rate of Type 1 errors is permissible through the FDR method, power is maximized.[323] As discussed above, a very liberal FDR was adopted (< 0.5) to provide the opportunity for training on further examination of GWAS findings.

Results of interest for SDS. The 312 SNPs that had q-values < 0.5 for the SDS were further annotated and examined. As discussed in the results, there were two genes, *KCNK9* and *ALDH1A2,* with minimum *p*-values < 10^{-6}. However, these should be interpreted with caution given that FDR < 0.5 is liberal. Additional support (e.g., significance in gene-based tests, identification in a larger sample of sleep within depression with a better phenotype) would be needed in order to confirm the role of these genes in sleep within MDD. As mentioned in the Results, the Locus Zoom plots showed multiple SNPs in high LD with the most significant SNP in each of these genes, thus representing potential genes that may contribute to sleep disturbances in individuals with MDD. The minimum q-value within these results was 0.38. *KCNK9,* which contains two SNPs and begins a maximum of 22.9 kb downstream from the remaining thirteen SNPs within that cluster (this includes rs4736083, which has the smallest *p*-value [*p* = 3.72E-07]), encodes a pH-dependent potassium channel.[324,325] The gene is imprinted such that the maternal allele is usually expressed, and a mutation within this gene results in Birk-Barel mental retardation dysmorphism syndrome, which is characterized by mental retardation, hypotonia, hyperactivity, and facial dysmorphism.[326] *KCNK9* has also been implicated in cancer (through overexpression/amplification[327-329]), as well as in metabolic traits like hypertension and body mass index (e.g., [330,331]). Interestingly, knockout mouse models show that the gene may be involved in sleep, demonstrating slower transitions from wake to sleep and more fragmented REM sleep,[332] in addition to increased activity during the dark period[333] in knock out animals. *ALDH1A2* contained the most SNPs with q < 0.5, including rs35016264, which had the next smallest *p*-value. This gene codes for the enzyme that catalyzes the synthesis of retinoic acid, a signaling molecule that is important for embryonic development.[334] Like *KCNK9*, there are also associations between *ALDH1A2* and cancer (e.g., [335,336]). Further, there is some evidence that this

gene may be involved in psychiatric disorders. A haplotype containing two *ALDH1A2* SNPs (neither of which were included in the top hits in this sample) was found to be protective for schizophrenia in a Han Chinese sample[337] and methylation near *ALDH1A2* was associated (negatively) with intoxication/loss of control, suggesting a role in alcohol problems.[338]

Sleep within depression literature. There are no GWAS to date that have examined sleep within MDD, making this investigation novel. However, there is a small candidate gene literature looking at sleep in the context of MDD. Gass and colleagues[262] examined adenosine-related genes in the context of depression with and without sleep symptoms, finding that *SLC29A3* was associated with depression with early morning awakenings although this finding did not remain significant following Bonferroni correction. There were also some associations with depression including these sleep variables in men (with *SLC28A1*), but these were not significant after applying a correction for multiple testing. Another analysis by the same group examined 18 circadian genes with these same phenotypes, finding that *TIMELESS* was associated with depression with fatigue and depression with early morning awakenings in both men and women (although there was some gender specificity), and replicated this association with seasonal symptoms.[266] Another study found that MDD with late insomnia was associated with variants in pre-miR-182, which targets *CLOCK*.[264] Finally, there are some investigations of polymorphisms in several genes (*CLOCK*, *MAO-A*) and sleep in individuals with depression, although results are mixed, with only some studies identifying significant genetic effects.[199,265,339] Note that none of these candidate genes correspond to the genes of interest from the SDS GWAS, although this is not surprising given issues with power, phenotype, and ascertainment within the CONVERGE dataset and the fact that insomnia candidate genes do not replicate in insomnia GWAS either.[167] It is interesting to note, however, that the top gene in the SDS results, *KCNK9*, codes for an ion

channel, and only around 1% of protein coding genes are ion channels.[340] *CACNA1C*, identified by Byrne and colleagues[215] for sleep latency, as well as *ABCC9*, implicated in sleep duration[218,341] are also involved in ion channels, providing further evidence for an excitatory mechanism for insomnia/poor sleep.[167]

Adjusted GS and additional phenotypes. While there were no loci potentially associated with the GS in the full sample (or any of the additional GWAS of the GS in MDD controls or full sample), other GWAS of insomnia have identified genes of interest. Note that analyses presented here represent the first GWAS of an insomnia phenotype in a Han Chinese sample, although the first insomnia GWAS was in a Korean sample.[214] Only one of the prior studies utilized a larger sample size than was available in CONVERGE.[216] A more detailed overview of the insomnia GWAS findings to date can be found in Chapter 1, Section IIIa, Molecular Genetics. A brief overview of results will be presented here. The first two GWAS of insomnia phenotypes did not identify GWS hits but were able to identify several genes of interest,[214,215] one of which could be replicated.[218] The three remaining studies identified a total of nine loci that reached genome-wide significance, all using different phenotypes (described below in the Phenotype limitation).[213,216,217] When summarizing prior GWAS of insomnia, it is important to consider that none of these studies identified GWS variants in the same genes and minimal replication has occurred. Identified SNPs from these earlier insomnia GWAS were examined in CONVERGE results for the Adjusted GS with MDD covariate, where available (refer to Table 17 for results). Most SNPs did not replicate, but rs2302729, which was nominally associated with sleep latency (Beta = 0.20, p = 4.63E-06) in women in the GWAS by Byrne and colleagues[215] (2013), was also nominally related within the CONVERGE sample (OR = 1.12, p = 0.01).

Power. There are several major limitations to consider that could explain the inability to

identify GWS hits for sleeplessness and sleep within MDD. These include power, phenotype, and method, paralleling what was discussed in the prior chapter for GCTA. For GWAS, important determinants of power include sample size, effect size, and MAF. As sample size increases, so does the ability to detect less common variants and/or those with smaller effects. Phenotype is also important for power, as rarer traits that are stable and have high heritability will require smaller Ns to detect common effects.[342] As described in the data analytic plan, there was adequate power (> 80%) to detect variants with ORs $\geq$ 1.15 and MAFs $\geq$ 0.25, but not for smaller genotype relative risks and MAFs, within analyses of the Adjusted GS with MDD covariate. The inclusion of covariates (here, two ancestry PCs and MDD) may decrease the power in case-control analyses, but should not be an issue here given the large sample size.[343] However, note that most of the variance within this trait is likely accounted for by the MDD covariate, as seen in GCTA, potentially explaining the lack of GWS results. For the SDS, analyses were well-powered to detect individual variants that explain $\geq$ 0.8% of the variance in sleep within MDD, but not for variants with smaller effects. Further, as discussed previously, the variable was highly skewed. The majority of individuals endorsed higher values (i.e., 2-3). In order to maximize the power to identify genetic variants contributing to sleep within MDD, a new sample with balanced ascertainment with regard to sleep variables would be needed. Specifically selecting for individuals with MDD who do not endorse sleep problems, as well as those who endorse multiple sleep problems would be a way to increase variation and maximize the power, as selecting from extremes improves power for a quantitative trait.[342]

Phenotype. Phenotypic issues for GWAS are similar to those described in detail for GCTA (see Chapter 4, Section III, Phenotype), and will be expanded upon here to compare with the extant GWAS literature. To recap, the fact that the GS consists of a single sleep item that

does not come from DSM criteria or a standard sleep scale is problematic. However, phenotypic heterogeneity is a concern across the five insomnia GWAS conducted to date, which used different phenotypes. Ban and colleagues[214] utilized a binary self-report insomnia phenotype, while Byrne and colleagues,[215] who did create an insomnia factor, found main results of interest for self-reported sleep latency and quality. The three GWAS identifying GWS loci used phenotypes ranging from one subjective insomnia item,[216] to a quantitative phenotype of sleep latency[213] and objective actigraphy parameters.[217] Thus, while the insomnia phenotype used here is not ideal, neither are those utilized within the literature, as none reflect full diagnostic criteria or a standardized sleep measure (although sleep latency and quality are components of scales such as the PSQI). For the SDS variable, the GS and sleep items in MDD were summed to create a quasi-quantitative trait. As no GWAS of sleep in MDD have been done, phenotypes cannot be compared as done for GWAS. Interestingly, several of the candidate gene studies of sleep within MDD compared depressed individuals with sleep problems to controls,[262,266] while others examined sleep only within those who were depressed.[263,265,339]

Method. While the GWAS approach allows for the examination of many variants without specific pre-determined hypotheses, there are five important limitations to consider. First, GWAS analyzes common variants. Rarer variants (MAF < 0.01) were filtered out in the results presented here. This is common across GWAS, as rare variants are imputed with less certainty.[342,344] With improvements in sequencing approaches and imputation, analyzing variants of lower frequency is becoming possible. Since CONVERGE is comprised of whole-genome sequence data, it does contain rarer (and rare) variants. However, the power to detect effects of these lower frequency variants is likely less than that for common variants, particularly with smaller sample sizes (i.e., less than 6,000) across most phenotypes here. Specific types of

analyses exist for examination of rare variants in aggregate,[345] which could be a future direction. Second, identified GWS variants have not been shown to account for a large proportion of the hypothesized heritability across many psychiatric traits (e.g., [220,251,315,316]). Overall, effect sizes are small and missing heritability remains a problem, as discussed within the GCTA section (Chapter 4, Section I, Method). Third, like GCTA, GWAS does not account for non-additive genetic effects or interactions, such as epistasis and GxE. Fourth, results can be influenced by population stratification (i.e., results are spurious/false positives) if ancestry components are not properly controlled for. This is likely not a problem here, since CONVERGE is Han Chinese and ancestry PCs were used as covariates across all genetic analyses. Finally, GWAS examines variants individually, and thus does not account for the potential polygenic nature of the trait of interest. As there is evidence that many psychiatric disorders, such as MDD, are polygenic in nature,[219,245,246,254,255] it may be more useful to examine the effects of variants in aggregate using a PRS approach, which is different than GCTA in that it takes into account effect sizes of variants in creating risk scores,[245] as will be discussed in the next chapter.

Summary. Overall, no GWS variants contributing to these sleep phenotypes independent of MDD were identified, although there were two potential genes of interest for the SDS (*KCNK9, ALDH1A2*). Significant results for sleep in MDD would be particularly novel, given that no GWAS have examined this phenotype, and could provide insight into the heterogeneity of symptoms experienced within this disorder. Identified variants could be considered "modifier variants", influencing the symptom presentation of the disorder.[346] Had significant variants been identified, the next step would be replication in independent sample(s) using similar sleep phenotypes. Following replication, functional analyses on the specific gene/SNP of interest would be useful to determine how the findings fit into known biology. For example, in the sleep

latency GWAS that identified *RBFOX3*, functional analyses of co-expression showed a role for the gene in neurotransmitter release (including GABA, an important inhibitory neurotransmitter for sleep/wake regulation), highlighting a potential mechanism by which *RBFOX3* variants could affect variation in sleep latency.[213] Further, given hypothetically significant results, running additional bioinformatics analyses (e.g., gene-based enrichment, pathway analysis) on the results would then be warranted to increase understanding of related processes and functions. For example, pathway analysis (e.g., using Ingenuity Pathway Analysis software, http://www.ingenuity.com) involves utilizing GWAS summary statistics to determine if specific pathways are over-represented within results from the phenotype of interest.

Chapter 6: Examining genetic overlap between sleep in MDD and sleep in general, as well as sleep and MDD, using PRSs (Aim 2c)

I. Data analytic plan

Two methods for generating PRSs were used and compared. First, LDpred,[257] a software program written in Python that generates risk scores from GWAS summary statistics, was used. LDpred utilizes Bayesian priors on the genetic architecture of the trait of interest (calculated from heritability [as estimated by the program] and fraction of causal variants) and LD information from a reference sample, to estimate PRSs at different fractions (i.e., models of inheritance). In the seminal paper, the authors demonstrated that this method results in PRSs that account for greater proportions of variance in the phenotype of interest, when compared to prior PRS methods.[257] The LDpred software was downloaded from http://bitbucket.org/bjarni_vilhjalmsson/ldpred and Python version 2.6.6[347] was used for all steps. A subset of hard-called genotypes from CONVERGE (converted from dosage files using PLINK) were used as the target sample in LDpred. In order to be included in the hard-called subset, INFO had to be at least 0.99. Every genotype call for each SNP and person had to include one genotype probability of at least 0.9, or that SNP was set to missing for that person. LDpred filters on MAF < 0.01 and removes ambiguous SNPs (i.e., SNPs where the two potential alleles are complementary to each other, such as A/T; when this occurs it is difficult to know if the strand needs to be flipped, and thus the individual's genotype at that location may be unclear). The CONVERGE sample was used as its own LD reference, and the LD radius (i.e., "the number

of SNPs…[to]…adjust for on each side of a given SNP"; see page 579 of Vilhjalmsson et al.[257]) used across all analyses was 1157 (calculated as instructed in LDpred; total number of SNPs from initial step/3000). Default fractions (i.e., percentage of variants contributing causally to the trait of interest) were used to model genetic architecture (0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, infinitesimal).

Scores were created using summary statistics from GWAS above of the SDS (MDD cases), Item E8 (MDD cases), GS (MDD cases), and GS (MDD controls). The use of MDD cases and MDD controls separately is referred to here as the "natural split," which permitted the examination of overlap between sleep in MDD cases and sleep in MDD controls. Additionally, the sample was randomly split in half in order to look at genetic overlap between sleeplessness and MDD (referred to here as the "random split" approach). Half of individuals were assigned to an MDD GWAS, while the other half were assigned to sleep GWAS (GS and Adjusted GS, with MDD covariate), and scores were created using these summary statistics. Following their creation, scores were used to predict sleep phenotypes in the opposite half of the sample (i.e., scores created from the GS in MDD controls were used to predict sleep variables in MDD cases [SDS, GS, Item E8] and vice-versa in the natural split; scores from MDD were used to predict sleep [GS, Adjusted GS] and vice-versa in the random split). This was done using logistic regressions (glm for binary, polr for ordinal) conducted in R, with Nagelkerke's pseudo R^2 (as determined by the *pscl* package[282]) and *p*-values used to evaluate the model prediction. Covariates included two PCs (PC1 and PC2) in all analyses, and MDD in the random split approach when sleep was the outcome.

The second method used was the Purcell method.[254] This method uses Plink to create weighted risk scores from summary statistics following pruning of variants in LD. In order to

facilitate comparisons between Plink and LDpred scores, hard called genotypes were also used for Plink, ambiguous SNPs were removed from the summary statistics, and SNPs were required to have MAF < 0.01. For the Purcell method, risk scores were generated in Plink version 1.07,[320] using a tutorial from the Psychiatric Genetics department at QIMR Berghofer as a guide for data management.[348] Once a complete list of available SNPs was compiled, pruning was performed in Plink to remove SNPs that were in high LD with each other (pairwise $r^2 > 0.3$, 50 BP window). Following, risk scores were created from the pruned SNPs across a range of *p*-value thresholds (0.001, 0.01, 0.1, 0.5, 1.0; modeled off of a risk score paper examining seasonality).[246] The number of SNPs that went into each Plink score is shown in Tables 16 and 18. The same logistic regression analyses as above were conducted using the Plink scores (sleep in MDD cases to predict sleep in MDD controls and vice-versa; MDD to sleep and vice-versa). Once all analyses were conducted across both methods, a Benjamini-Hockberg FDR was applied separately for *p*-values generated from the natural split (MDD case/control) and random split (MDD/sleep) analyses to determine what method/phenotype/threshold passed multiple testing correction, and thus could be carried forward into a combined analysis with trauma variables, examined earlier.

II. Results

Natural split. To determine whether there was genetic overlap between sleep in MDD cases and sleep in MDD controls, PRSs were constructed both using LDpred and the Purcell method across three sleep variables in MDD cases (GS [sleeplessness], Item E8 [difficulty falling or staying asleep within MDD], and SDS [sleep within depression; quasi-quantitative trait]) and one in MDD controls (GS [sleeplessness]) using GWAS results from Chapter 5. The number of SNPs that went into Plink scores at each threshold is shown in Table 18. Results are

displayed in Table 19 (across all phenotypes and methods), and Figures 23 (LDpred) and 24 (Plink). Information provided in Table 19 includes method (LDpred or Plink), discovery phenotype, target phenotype, threshold or fraction used, effect of risk score, *p*-value for the effect, total variance explained by the model (Nagelkerke's pseudo R^2), change in variance from a base model containing only PCs (ΔR^2, %), and q-value (from Benjamini-Hockberg FDR, used to correct for multiple testing). The figures provide a visual representation of how the variance explained differs across thresholds and phenotypes for each method, and *p*-values for the PRS effect are presented above each bar. Overall, none of the predictions were significant using LDpred scores, with analyses using the GS in MDD cases/GS in MDD controls demonstrating the smallest change in variance (nearly 0). However, for the SDS in MDD cases (see Figure 23, top right graph), genetic risk scores were nominally significant predictors of the GS in MDD controls (*p* values ranging from 0.04 [Inf] to 0.081 [0.001]), resulting in a ~0.1% increase in R^2. For predictions done in Plink (see Figure 24), there were nominally significant results across higher thresholds ($p < 0.1$, $p < 0.5$, $p = 1$) for the GS in MDD cases predicting GS in MDD controls (and vice versa), resulting in a change in variance ranging from 0.10-0.15%. Predictions for Item E8 in MDD cases predicting GS in MDD controls were also nominally significant across most thresholds (ΔR^2 = ~0.1%). Finally, genetic risk scores were significant (and passed multiple testing correction) when the SDS in MDD cases was used to predict GS in MDD controls and vice-versa at higher thresholds ($p < 0.1$, $p < 0.5$, $p = 1$; ΔR^2 = 0.2-0.3%)

Table 18. Number of SNPs that are part of each Plink PRS score; natural split

Threshold	SDS Cases	E8 Cases	GS Cases	GS Controls
p <0.001	156	159	130	187
p <0.01	1696	1623	1631	1686
p <0.1	16964	16841	16958	17017
p <0.5	85031	85476	85388	85594
p <=1	170579	170517	170568	170804

Abbreviations: E8 = depression item E8; GS = general sleep item; SDS = sleep within depression

Table 19. Results of PRSs for sleep in MDD cases predicting sleep in MDD controls (and vice-versa), conducted in both LDpred and Plink

Method	Discov.	Target	Thresh. or Fraction	OR[a] (95% CI)	R^2 (%)[b]	ΔR^2 (%)	*p*-value	q-value[c]
				LDpred				
LDpred	SDS Ca	GS Co	Inf	1.07 (1.00-1.14)	0.842	0.1222	0.040	0.281
LDpred	SDS Ca	GS Co	1	1.06 (0.99-1.13)	0.812	0.0924	0.075	0.281
LDpred	SDS Ca	GS Co	0.3	1.06 (0.99-1.13)	0.812	0.0924	0.074	0.281
LDpred	SDS Ca	GS Co	0.1	1.06 (0.99-1.13)	0.811	0.0920	0.075	0.281
LDpred	SDS Ca	GS Co	0.03	1.06 (0.99-1.12)	0.808	0.0884	0.081	0.281
LDpred	SDS Ca	GS Co	0.01	1.06 (0.99-1.13)	0.812	0.0924	0.074	0.281
LDpred	SDS Ca	GS Co	0.003	1.06 (0.99-1.13)	0.811	0.0921	0.075	0.281
LDpred	SDS Ca	GS Co	0.001	1.06 (0.99-1.13)	0.811	0.0915	0.076	0.281
LDpred	E8 Ca	GS Co	Inf	1.06 (1.00-1.13)	0.815	0.0953	0.070	0.281
LDpred	E8 Ca	GS Co	1	1.04 (0.98-1.11)	0.766	0.0471	0.203	0.304
LDpred	E8 Ca	GS Co	0.3	1.04 (0.98-1.11)	0.767	0.0471	0.203	0.304
LDpred	E8 Ca	GS Co	0.1	1.04 (0.98-1.11)	0.767	0.0474	0.201	0.304
LDpred	E8 Ca	GS Co	0.03	1.04 (0.98-1.11)	0.770	0.0505	0.187	0.304
LDpred	E8 Ca	GS Co	0.01	1.04 (0.98-1.11)	0.767	0.0471	0.203	0.304
LDpred	E8 Ca	GS Co	0.003	1.04 (0.98-1.11)	0.767	0.0472	0.202	0.304
LDpred	E8 Ca	GS Co	0.001	1.04 (0.98-1.11)	0.768	0.0483	0.197	0.304
LDpred	GS Ca	GS Co	Inf	1.01 (0.95-1.07)	0.721	0.0014	0.829	0.972
LDpred	GS Ca	GS Co	1	1.00 (0.94-1.07)	0.720	0.0002	0.940	0.972
LDpred	GS Ca	GS Co	0.3	1.00 (0.94-1.07)	0.720	0.0002	0.940	0.972
LDpred	GS Ca	GS Co	0.1	1.00 (0.94-1.07)	0.720	0.0001	0.948	0.972
LDpred	GS Ca	GS Co	0.03	1.00 (0.94-1.06)	0.719	0.0000	0.984	0.984
LDpred	GS Ca	GS Co	0.01	1.00 (0.94-1.07)	0.720	0.0002	0.940	0.972
LDpred	GS Ca	GS Co	0.003	1.00 (0.94-1.07)	0.720	0.0002	0.943	0.972
LDpred	GS Ca	GS Co	0.001	1.00 (0.94-1.07)	0.719	0.0001	0.964	0.976
LDpred	GS Co	SDS Ca[d]	Inf	1.06 (0.99-1.12)	0.138	0.0749	0.078	0.281
LDpred	GS Co	SDS Ca[d]	1	1.05 (0.99-1.11)	0.119	0.0560	0.128	0.285
LDpred	GS Co	SDS Ca[d]	0.3	1.05 (0.99-1.11)	0.119	0.0560	0.128	0.285
LDpred	GS Co	SDS Ca[d]	0.1	1.05 (0.99-1.11)	0.120	0.0567	0.126	0.285
LDpred	GS Co	SDS Ca[d]	0.03	1.05 (0.99-1.12)	0.124	0.0606	0.113	0.285
LDpred	GS Co	SDS Ca[d]	0.01	1.05 (0.99-1.11)	0.119	0.0560	0.128	0.285
LDpred	GS Co	SDS Ca[d]	0.003	1.05 (0.99-1.11)	0.119	0.0562	0.127	0.285
LDpred	GS Co	SDS Ca[d]	0.001	1.05 (0.99-1.12)	0.121	0.0578	0.122	0.285
LDpred	GS Co	E8 Ca	Inf	1.10 (1.00-1.22)	0.193	0.1598	0.060	0.281
LDpred	GS Co	E8 Ca	1	1.07 (0.97-1.18)	0.109	0.0761	0.195	0.304
LDpred	GS Co	E8 Ca	0.3	1.07 (0.97-1.18)	0.109	0.0761	0.195	0.304
LDpred	GS Co	E8 Ca	0.1	1.07 (0.97-1.18)	0.110	0.0766	0.193	0.304
LDpred	GS Co	E8 Ca	0.03	1.07 (0.97-1.18)	0.110	0.0775	0.191	0.304
LDpred	GS Co	E8 Ca	0.01	1.07 (0.97-1.18)	0.109	0.0761	0.195	0.304
LDpred	GS Co	E8 Ca	0.003	1.07 (0.97-1.18)	0.109	0.0765	0.194	0.304
LDpred	GS Co	E8 Ca	0.001	1.07 (0.97-1.18)	0.109	0.0765	0.194	0.304
LDpred	GS Co	GS Ca	Inf	1.01 (0.93-1.09)	0.220	0.0023	0.797	0.972
LDpred	GS Co	GS Ca	1	1.00 (0.93-1.09)	0.219	0.0005	0.904	0.972

Method	Discov.	Target	Thresh. or Fraction	OR[a] (95% CI)	R^2 (%)[b]	ΔR^2 (%)	*p*-value	q-value[c]
				LDpred				
LDpred	GS Co	GS Ca	0.3	1.00 (0.93-1.09)	0.219	0.0005	0.905	0.972
LDpred	GS Co	GS Ca	0.1	1.01 (0.93-1.09)	0.219	0.0005	0.900	0.972
LDpred	GS Co	GS Ca	0.03	1.01 (0.93-1.09)	0.219	0.0007	0.886	0.972
LDpred	GS Co	GS Ca	0.01	1.00 (0.93-1.09)	0.219	0.0005	0.904	0.972
LDpred	GS Co	GS Ca	0.003	1.00 (0.93-1.09)	0.219	0.0005	0.904	0.972
LDpred	GS Co	GS Ca	0.001	1.01 (0.93-1.09)	0.219	0.0006	0.893	0.972
				Plink				
Plink	SDS Ca	GS Co	p < 0.001	0.99 (0.93-1.05)	0.725	0.0060	0.648	0.843
Plink	SDS Ca	GS Co	p < 0.01	1.02 (0.96-1.09)	0.733	0.0133	0.498	0.658
Plink	**SDS Ca**	**GS Co**	**p < 0.1**	**1.09 (1.03-1.16)**	**0.948**	**0.2289**	**0.005**	**0.065**
Plink	**SDS Ca**	**GS Co**	**p < 0.5**	**1.11 (1.04-1.18)**	**1.028**	**0.3081**	**0.001**	**0.035**
Plink	**SDS Ca**	**GS Co**	**p <= 1**	**1.11 (1.04-1.18)**	**1.010**	**0.2910**	**0.002**	**0.035**
Plink	E8 Ca	GS Co	p < 0.001	1.06 (1.00-1.13)	0.830	0.1109	0.051	0.281
Plink	E8 Ca	GS Co	p < 0.01	1.04 (0.98-1.11)	0.767	0.0475	0.201	0.304
Plink	E8 Ca	GS Co	p < 0.1	1.06 (0.99-1.12)	0.806	0.0868	0.084	0.281
Plink	E8 Ca	GS Co	p < 0.5	1.05 (0.99-1.12)	0.793	0.0735	0.112	0.285
Plink	E8 Ca	GS Co	p <= 1	1.05 (0.99-1.12)	0.797	0.0774	0.103	0.285
Plink	GS Ca	GS Co	p < 0.001	0.98 (0.92-1.04)	0.733	0.0139	0.488	0.657
Plink	GS Ca	GS Co	p < 0.01	1.00 (0.94-1.07)	0.720	0.0004	0.902	0.972
Plink	GS Ca	GS Co	p < 0.1	1.05 (0.98-1.11)	0.781	0.0617	0.145	0.304
Plink	GS Ca	GS Co	p < 0.5	1.06 (0.99-1.12)	0.806	0.0867	0.084	0.281
Plink	GS Ca	GS Co	p <= 1	1.07 (1.00-1.13)	0.835	0.1155	0.046	0.281
Plink	GS Co	SDS Ca[d]	p < 0.001	0.96 (0.90-1.02)	0.116	0.0525	0.141	0.304
Plink	GS Co	SDS Ca[d]	p < 0.01	1.03 (0.97-1.10)	0.089	0.0257	0.302	0.444
Plink	**GS Co**	**SDS Ca[d]**	**p < 0.1**	**1.10 (1.04-1.17)**	**0.305**	**0.2421**	**0.002**	**0.035**
Plink	**GS Co**	**SDS Ca[d]**	**p < 0.5**	**1.10 (1.04-1.17)**	**0.299**	**0.2362**	**0.002**	**0.035**
Plink	**GS Co**	**SDS Ca[d]**	**p <= 1**	**1.10 (1.03-1.17)**	**0.277**	**0.2135**	**0.003**	**0.046**
Plink	GS Co	E8 Ca	p < 0.001	0.95 (0.86-1.05)	0.077	0.0436	0.327	0.472
Plink	GS Co	E8 Ca	p < 0.01	1.04 (0.94-1.15)	0.057	0.0240	0.467	0.638
Plink	GS Co	E8 Ca	p < 0.1	1.05 (0.95-1.16)	0.074	0.0415	0.338	0.479
Plink	GS Co	E8 Ca	p < 0.5	1.09 (0.98-1.20)	0.154	0.1206	0.102	0.285
Plink	GS Co	E8 Ca	p <= 1	1.08 (0.98-1.20)	0.142	0.1088	0.121	0.285
Plink	GS Co	GS Ca	p < 0.001	0.97 (0.90-1.05)	0.240	0.0223	0.423	0.589
Plink	GS Co	GS Ca	p < 0.01	1.02 (0.94-1.10)	0.224	0.0062	0.673	0.861
Plink	GS Co	GS Ca	p < 0.1	1.08 (1.00-1.17)	0.360	0.1422	0.043	0.281
Plink	GS Co	GS Ca	p < 0.5	1.08 (1.00-1.16)	0.335	0.1165	0.067	0.281
Plink	GS Co	GS Ca	p <= 1	1.07 (0.99-1.16)	0.320	0.1017	0.086	0.281

Abbreviations: Ca = cases; Co = controls; Discov. = discovery sample; E8 = depression item E8; GS = general sleep item; Inf = infinitesimal model; SDS = sleep within depression; Thresh. = *p*–value threshold.

[a]All scores have been standardized such that the odds ratio can be interpreted as the increase in the likelihood of endorsing sleeplessness for a 1 SD increase in PRS; [b]R^2 = Nagelkerke's pseudo R^2; [c]Benjamini-Hockberg FDR was used; [d]When analyzing the SDS as an ordinal outcome, PC2 violated the proportional odds test. Given this, a linear model that treats the phenotype as quasi-continuous was also run. Results were similar in both models, so the ordinal regression is shown here for consistency.

Bold text is used to indicate results passing multiple testing correction (q < 0.10).

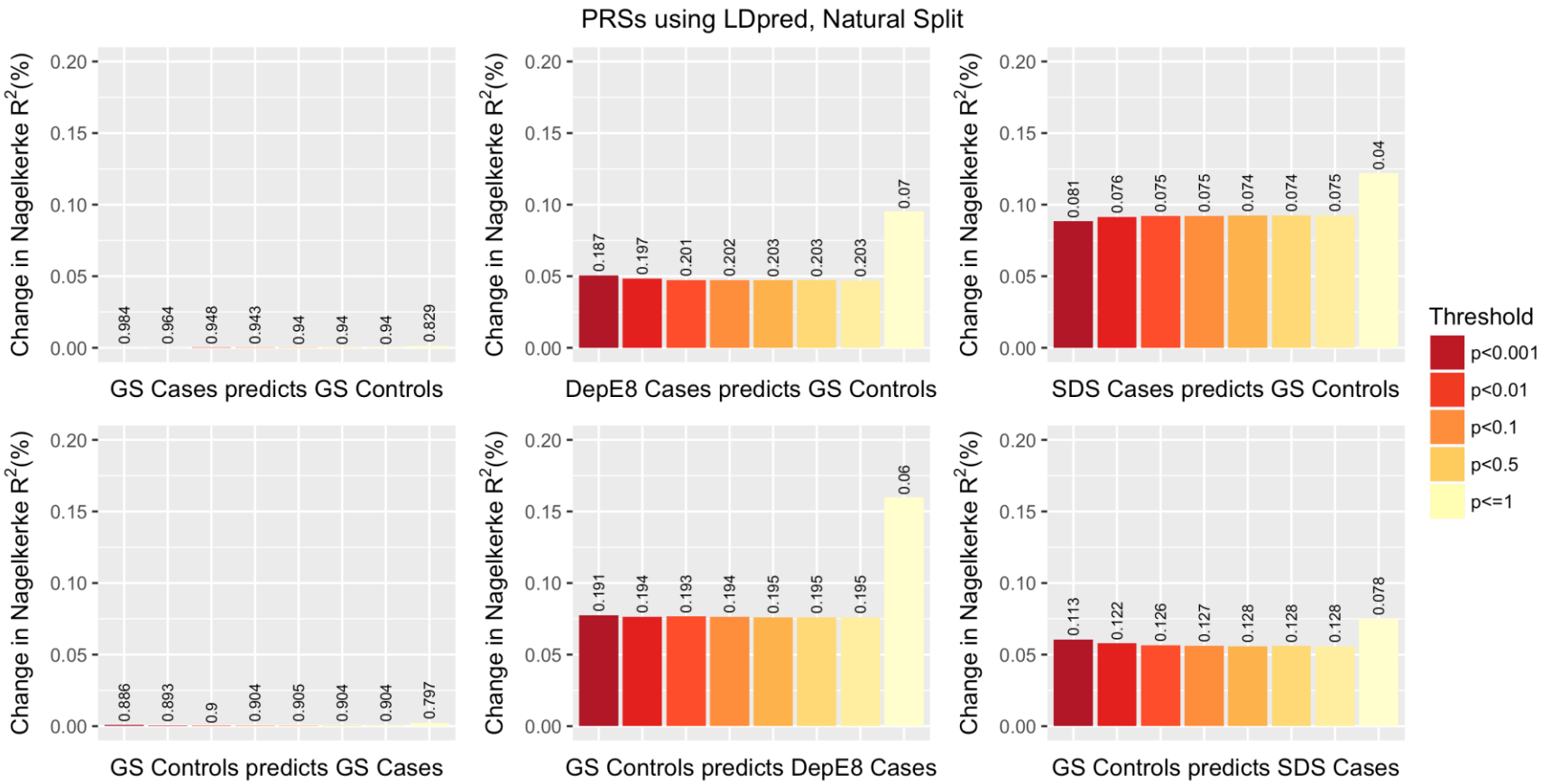


Figure 23: Results of PRS analyses for sleep in MDD cases predicting sleep in MDD controls (and vice-versa), using LDpred software. Graphs for risk scores created from sleep variables in MDD cases predicting sleeplessness in MDD controls are shown in the top row, while graphs for the reverse, risk scores created from sleeplessness in MDD controls predicting sleep variables in MDD cases, are shown in the bottom row. Within each individual graph, the fraction of variants modeled as contributing to the trait of interest is shown on the y-axis (see legend) and the change in Nagelkerke's pseudo R^2 (in percent) from a base model containing only principal component covariates is shown on the x-axis. The *p*-value of each PRS effect is shown on top of each individual bar.

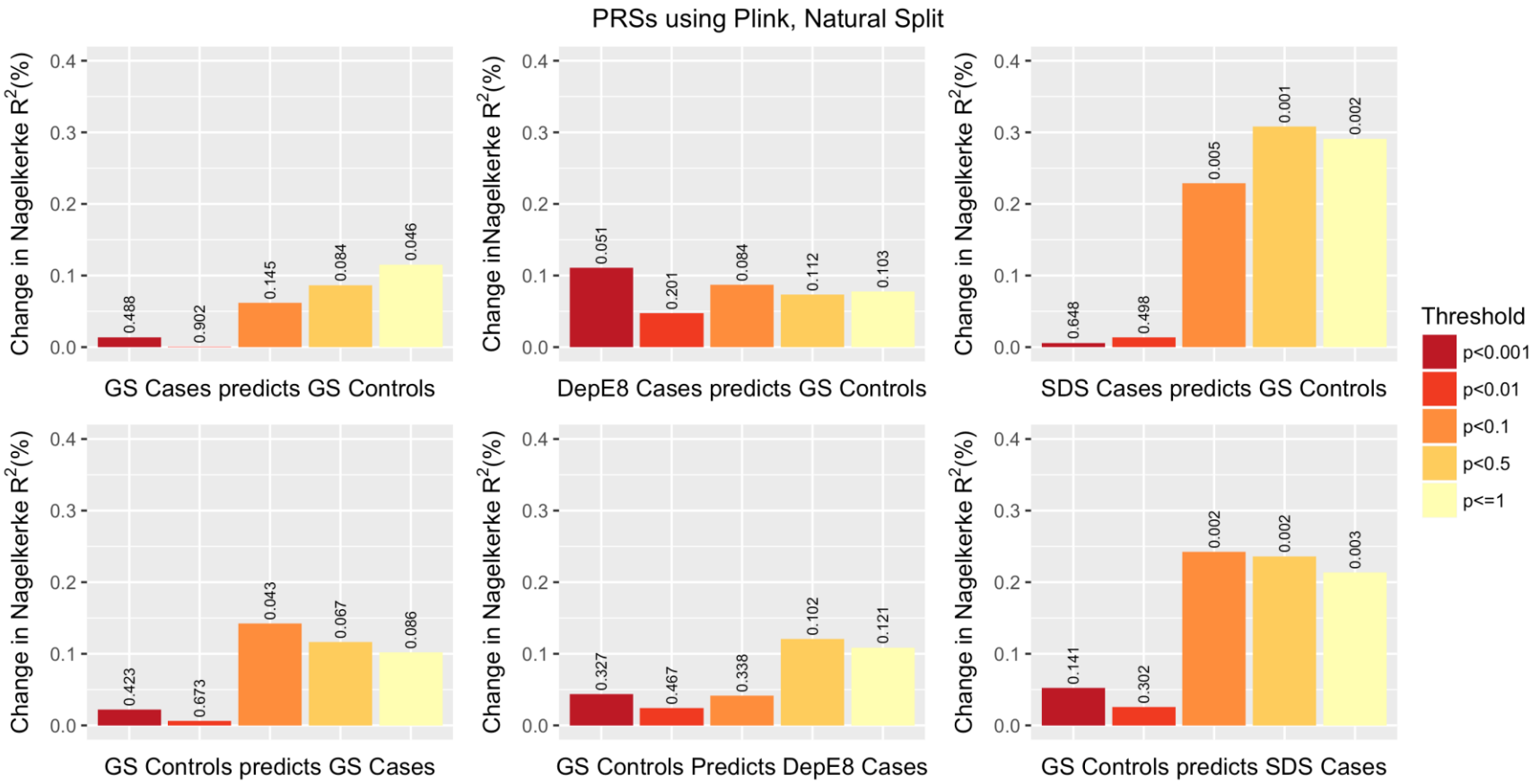


Figure 24: Results of PRS analyses for sleep in MDD cases predicting sleep in MDD controls (and vice-versa), using the Purcell method in Plink. Graphs for risk scores created from sleep variables in MDD cases predicting sleeplessness in MDD controls are shown in the top row, while graphs for the reverse, risk scores created from sleeplessness in MDD controls predicting sleep variables in MDD cases, are shown in the bottom row. Within each individual graph, the *p*-value threshold used is shown on the y-axis (see legend) and the change in Nagelkerke's pseudo R^2 (in percent) from a base model containing only principal component covariates is shown on the x-axis. The *p*-value of each PRS effect is shown on top of each individual bar.

Random split. To examine overlap between sleeplessness and MDD, PRSs were created for MDD, GS, and Adjusted GS (incorporating additional information on sleep for MDD cases) within random halves of the sample (i.e., randomly assigned to GWAS so that individuals included in the MDD and GS/Adjusted GS samples did not overlap), also utilizing both methods. Similar to the natural split, described above, Table 20 shows the number of SNPs that went into Plink scores at each threshold. Table 21 provides information on method, phenotypes, effect/*p*-value, and variance at each threshold. Note that FDR q-values are not shown since none of the predictions were significant or nominally significant. Results converged across the two methods, with no risk scores emerging as significant predictors (for MDD predicting either GS and vice-versa), and very small ΔR^2 values were obtained (see Table 21 and Figures 25 and 26; all $\Delta R^2 <$ 0.04%). Note that for all analyses with MDD predicting GS, an MDD covariate was used (in addition to PCs), since many of the individuals utilized within the sleep GWAS were MDD cases. Finally, Figure 27 provides a comparison of the percent variance explained across samples and methods, displaying the maximum variance explained by risk scores for the natural split and random split analyses using LDpred and Plink. As can be seen in the figure, the maximum variance explained by Plink scores was higher than that of LDpred scores for the natural split, but both methods were similar for the random split. More variance was explained in natural split than random split analyses.

Table 20. Number of SNPs that are part of each Plink PRS score; random split.

Threshold	MDD	GS	Adj. GS
p <0.001	181	186	156
p <0.01	1920	1672	1732
p <0.1	17701	17197	17016
p <0.5	86523	85384	85750
p <=1	170659	170634	170578

Abbreviations: GS = general sleep item; Adj. GS = Adjusted GS item, such that all sleep items were incorporated for cases; MDD = major depressive disorder.

Table 21. Results of PRSs for MDD predicting sleep (and vice-versa; random split halves), conducted in both LDpred and Plink

Method	Discov.	Target	Threshold or Fraction	OR[a] (95% CI)	R^2 (%)[b]	ΔR^2 (%)	*p*-value
LDpred							
LDpred	MDD	GS	Inf	1.04 (1.97-1.12)	41.59	0.0272	0.226
LDpred	MDD	GS	1	1.00 (0.93-1.07)	41.56	0.0000	0.980
LDpred	MDD	GS	0.3	1.00 (0.93-1.07)	41.56	0.0000	0.981
LDpred	MDD	GS	0.1	1.00 (0.93-1.07)	41.56	0.0000	0.992
LDpred	MDD	GS	0.03	0.99 (0.92-1.06)	41.57	0.0021	0.736
LDpred	MDD	GS	0.01	1.00 (0.93-1.07)	41.56	0.0000	0.981
LDpred	MDD	GS	0.003	1.00 (0.93-1.07)	41.56	0.0000	0.988
LDpred	MDD	GS	0.001	1.00 (0.93-1.07)	41.56	0.0001	0.945
LDpred	MDD	Adj. GS	Inf	1.01 (0.93-1.10)	60.56	0.0017	0.738
LDpred	MDD	Adj. GS	1	0.96 (0.88-1.04)	60.57	0.0132	0.349
LDpred	MDD	Adj. GS	0.3	0.96 (0.88-1.04)	60.57	0.0132	0.349
LDpred	MDD	Adj. GS	0.1	0.96 (0.88-1.04)	60.57	0.0144	0.328
LDpred	MDD	Adj. GS	0.03	0.95 (0.87-1.03)	60.58	0.0249	0.198
LDpred	MDD	Adj. GS	0.01	0.96 (0.88-1.04)	60.57	0.0132	0.348
LDpred	MDD	Adj. GS	0.003	0.96 (0.88-1.04)	60.57	0.0135	0.344
LDpred	MDD	Adj. GS	0.001	0.96 (0.88-1.04)	60.57	0.0164	0.297
LDpred	GS	MDD	Inf	1.04 (0.98-1.09)	0.734	0.0400	0.208
LDpred	GS	MDD	1	1.00 (0.95-1.06)	0.694	0.0001	0.948
LDpred	GS	MDD	0.3	1.00 (0.95-1.06)	0.694	0.0001	0.948
LDpred	GS	MDD	0.1	1.00 (0.95-1.06)	0.694	0.0001	0.948
LDpred	GS	MDD	0.03	1.00 (0.95-1.06)	0.694	0.0001	0.939
LDpred	GS	MDD	0.01	1.00 (0.95-1.06)	0.694	0.0001	0.948
LDpred	GS	MDD	0.003	1.00 (0.95-1.06)	0.694	0.0001	0.948
LDpred	GS	MDD	0.001	1.00 (0.95-1.06)	0.694	0.0001	0.945
LDpred	Adj. GS	MDD	Inf	1.01 (0.96-1.07)	0.697	0.0032	0.722
LDpred	Adj. GS	MDD	1	0.97 (0.92-1.03)	0.720	0.0262	0.309
LDpred	Adj. GS	MDD	0.3	0.97 (0.92-1.03)	0.720	0.0262	0.309
LDpred	Adj. GS	MDD	0.1	0.97 (0.92-1.03)	0.720	0.0262	0.309
LDpred	Adj. GS	MDD	0.03	0.97 (0.92-1.03)	0.720	0.0262	0.309
LDpred	Adj. GS	MDD	0.01	0.97 (0.92-1.03)	0.720	0.0262	0.309
LDpred	Adj. GS	MDD	0.003	0.97 (0.92-1.03)	0.720	0.0262	0.309
LDpred	Adj. GS	MDD	0.001	0.97 (0.92-1.03)	0.720	0.0262	0.309
Plink							
Plink	MDD	GS	p < 0.001	0.95 (0.89-1.02)	41.60	0.0350	0.170
Plink	MDD	GS	p < 0.01	0.99 (0.92-1.06)	41.57	0.0011	0.804
Plink	MDD	GS	p < 0.1	1.03 (0.96-1.11)	41.58	0.0152	0.366
Plink	MDD	GS	p < 0.5	1.01 (0.95-1.09)	41.57	0.0031	0.682
Plink	MDD	GS	p <= 1	1.02 (0.95-1.10)	41.57	0.0066	0.551
Plink	MDD	Adj. GS	p < 0.001	0.95 (0.87-1.03)	60.58	0.0262	0.187
Plink	MDD	Adj. GS	p < 0.01	0.97 (0.89-1.05)	60.57	0.0095	0.426

Method	Discov.	Target	Threshold or Fraction	OR[a] (95% CI)	R^2 (%)[b]	ΔR^2 (%)	*p*-value
				Plink			
Plink	MDD	Adj. GS	p < 0.1	0.99 (0.91-1.08)	60.56	0.0005	0.849
Plink	MDD	Adj. GS	p < 0.5	1.01 (0.93-1.10)	60.56	0.0006	0.844
Plink	MDD	Adj. GS	p <= 1	1.02 (0.94-1.10)	60.56	0.0021	0.707
Plink	GS	MDD	p < 0.001	0.98 (0.93-1.03)	0.709	0.0151	0.439
Plink	GS	MDD	p < 0.01	1.01 (0.95-1.06	0.696	0.0016	0.798
Plink	GS	MDD	p < 0.1	1.03 (0.97-1.08)	0.715	0.0209	0.363
Plink	GS	MDD	p < 0.5	1.00 (0.95-1.06)	0.694	0.0000	0.967
Plink	GS	MDD	p <= 1	1.01 (0.95-1.06)	0.696	0.0021	0.772
Plink	Adj. GS	MDD	p < 0.001	1.03 (0.98-1.09)	0.725	0.0316	0.264
Plink	Adj. GS	MDD	p < 0.01	0.99 (0.94-1.05)	0.696	0.0016	0.800
Plink	Adj. GS	MDD	p < 0.1	1.02 (0.96-1.07)	0.704	0.0100	0.530
Plink	Adj. GS	MDD	p < 0.5	1.00 (0.95-1.06)	0.694	0.0004	0.906
Plink	Adj. GS	MDD	p <= 1	1.00 (0.95-1.06)	0.695	0.0006	0.876

Abbreviations: Ca = cases; Co = controls; GS = general sleep item; Adj. GS = Adjusted GS item, such that all sleep items were incorporated for cases; Inf = infinitesimal model; MDD = major depressive disorder.
[a]All scores have been standardized such that the odds ratio can be interpreted as the increase in the likelihood of endorsing sleeplessness for a 1 SD increase in PRS; [b]R^2 = Nagelkerke's pseudo R^2.

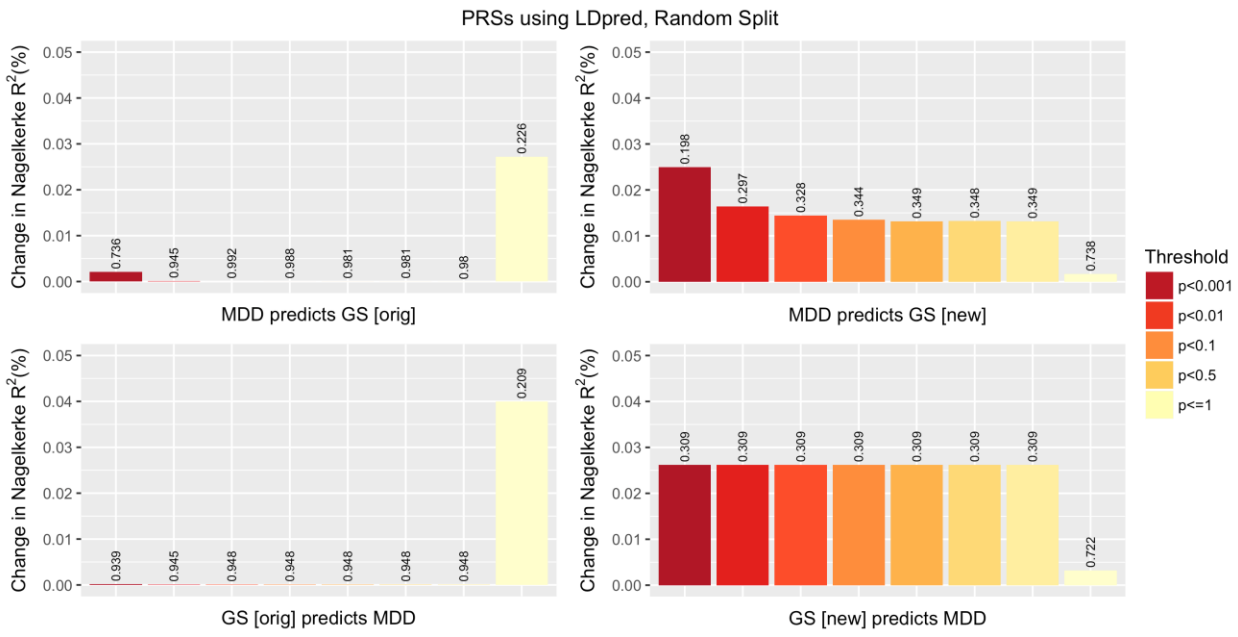


Figure 25: Results of PRS analyses for MDD predicting sleeplessness (and vice-versa) through random sampling, using LDpred. Graphs for risk scores created from MDD predicting sleeplessness are shown in the top row, while graphs for the reverse, risk scores created from sleeplessness predicting MDD, are shown in the bottom row. Within each individual graph, the fraction of variants modeled as contributing to the trait of interest is shown on the y-axis (see legend) and the change in Nagelkerke's pseudo R^2 (in percent) from a base model containing only principal component covariates is shown on the x-axis. The *p*-value of each PRS effect is shown on top of each individual bar.

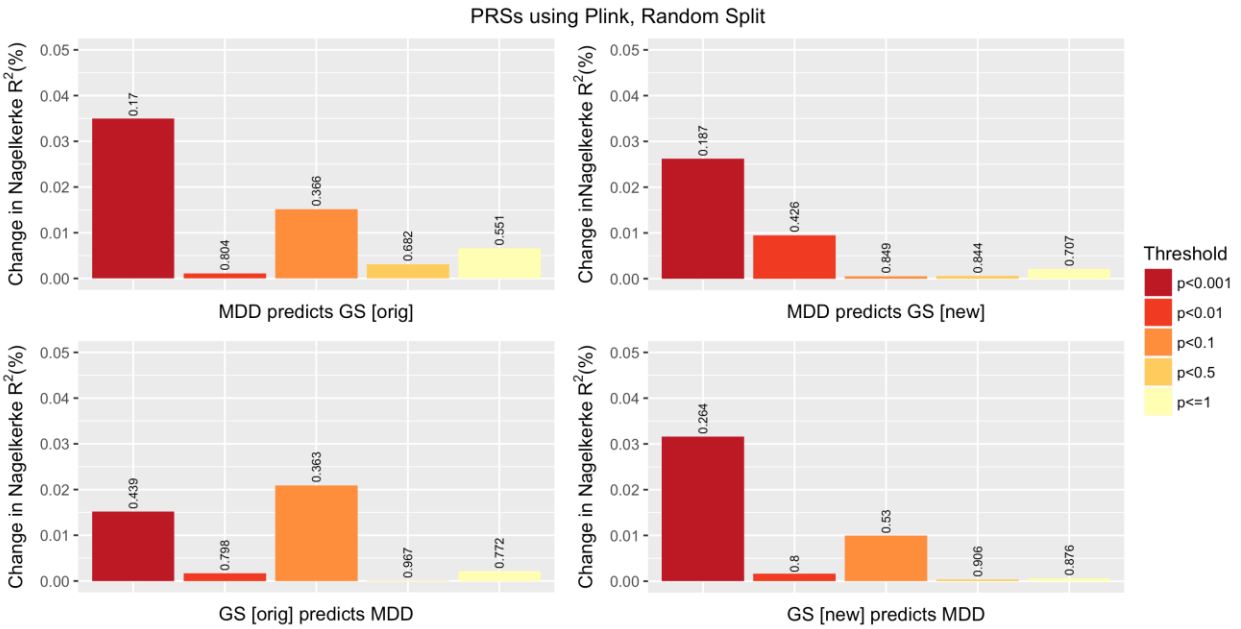


Figure 26: Results PRS analyses for MDD predicting sleeplessness (and vice-versa) through random sampling, using the Purcell method in Plink. Graphs for risk scores created from MDD predicting sleeplessness are shown in the top row, while graphs for the reverse, risk scores created from sleeplessness predicting MDD, are shown in the bottom row. Within each individual graph, the *p*-value threshold used is shown on the y-axis (see legend) and the change in Nagelkerke's pseudo R^2 (in percent) from a base model containing only principal component covariates is shown on the x-axis. The *p*-value of each PRS effect is shown on top of each individual bar.

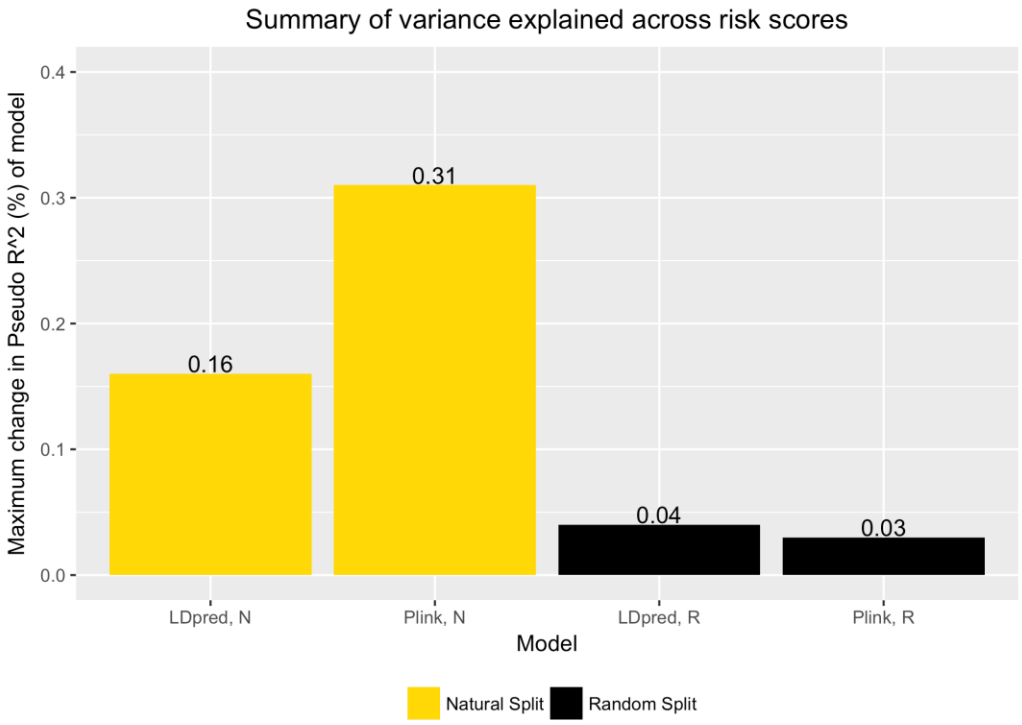


Figure 27: Summary of variance explained by PRSs in CONVERGE. The maximum change in variance explained by each PRS method across both splits of the sample is shown here. The x-axis shows the method (Plink or LDpred) and sample split (natural or random), while the y-axis show the maximum change in Nagelkerke's pseudo R^2 (in percent). The percent change is also written on the individual bars.

Discussion

Despite the lack of demonstrated SNP-based heritability and no significant GWAS hits within this sample, it is likely that the genetic architecture of insomnia is polygenic in nature, such that many variants of small effect (and thus not detectable/reaching genome-wide significance in GWAS at current sample sizes)[245,254] contribute to the variance in sleep traits when examined in aggregate. Taking into account polygenicity, PRS methods utilize summary statistics from GWAS of a trait of interest (discovery sample) and use this information to create weighted risk scores in a target sample (where genetic information is available), which are then used to predict another (or the same) phenotype. PRSs assume that in summing up effects of

many variants, some true effects are included. As the number of SNPs included grows, the increase in noise produced by adding more SNPs is balanced by the likelihood that some of these SNPs will have real effects.[245,254] The analyses presented here are novel, as few PRS studies of sleep phenotypes exist. Significant predictions were found within the natural split analyses (sleep in MDD cases predicting sleeplessness in MDD controls), which also demonstrated a larger maximum change in variance (by an order of magnitude) than in random split analyses (examining overlap between sleeplessness and MDD). Further, Plink scores explained nearly twice the variance as LDpred scores within the natural split analyses, although estimates were similar across methods within the random split. Results from each sample split, as well as a comparison of methods, will be discussed in turn.

Sleep in MDD cases and MDD controls, natural split. Results provide some evidence that the genetic influences on sleep within MDD (particularly via the SDS variable) significantly predict sleeplessness in MDD controls, resulting in an increase in variance of ~0.3% from the baseline model, using risk scores created in Plink. This prediction also occurred in the reverse, with the GS in MDD controls predicting the SDS in MDD cases as well (~0.2% increase in variance). For both of the Plink analyses, the significant *p*-value thresholds were those that encompassed more variants ($p < 0.1$, $p < 0.5$, all SNPs), the effects indicated an increase in risk for sleeplessness (OR > 1), and the *p*-values from these thresholds remained significant following an FDR correction that accounted for 78 tests run (three phenotype combinations, two directions, thirteen thresholds across two methods). Additionally, LDpred scores for the SDS in MDD cases predicting GS in MDD controls were nominally significant (before correction), supporting Plink findings for the SDS. There was also suggestive evidence in Plink for other sleep within MDD variables (i.e., some *p*-values that did not pass multiple testing correction but

were at least nominally significant; this occurred for the GS in both directions and Item E8 predicting GS in MDD controls). The amount of variance explained in the Plink models with the SDS (0.2-0.3%) is lower than what has been reported in the literature using bipolar disorder and schizophrenia (~1-3%).[254] However, it is similar to what Byrne and colleagues[246] found when using bipolar disorder scores to predict seasonality (0.4%; although note that they found higher predictions for schizophrenia, up to 3%, and no prediction for MDD) and roughly twice what Maciejewksi et al.[248] recently identified for MDD predicting suicidal ideation (0.1-0.16%). The fact that significant predictions were present for thresholds incorporating more *p* values highlights the polygenic nature of these phenotypes, and is consistent with prior literature indicating that this seems to be the case for psychiatric phenotypes (when compared to other complex traits like cardiovascular disease).[245,256]

This is the first examination of molecular genetic overlap between sleep in depressed and non-depressed individuals, making these findings novel. As discussed in detail within the introduction, twin studies have looked at heritability of insomnia symptoms within the context of MDD (e.g., [184,185]), as well as genetic overlap between insomnia and MDD (e.g., [186,187]), finding similar estimates to insomnia in general and a high degree of overlap. Further, at the molecular level, candidate gene studies have attempted to identify genes related to sleep symptoms in MDD (e.g., [262,266]). However, here, it was uniquely possible to answer the question of whether or not the same genes contribute to an insomnia phenotype in non-depressed vs. depressed individuals. Sleep within depression and sleeplessness in women without MDD have a shared genetic etiology, according to analyses presented here. While the amount of variance explained by the genetic risk scores may seem small (0.2-0.3%), it should be noted that for MDD, risk scores have only been able to explain a maximum of 1% of the variance (e.g., [219,220]). As phenotype remains

an issue throughout all genetic analyses presented here, it is possible that more variance could be explained with an improved phenotype.

Sleeplessness and MDD; random split. PRS analyses were also conducted to examine overlap between sleeplessness and MDD. Within these analyses, there was no support for genetic overlap between sleeplessness and MDD in either direction, with MDD case status appropriately controlled for. Results were consistent across Plink and LDpred. These results differ from the extant literature, given that biometric studies demonstrate large (i.e., over 50%[186-188]) genetic overlap between insomnia and MDD in adults, which suggests that there may be few insomnia-specific genes that are not also involved in MDD[187] and thus significant predictions would be expected. Results are in contrast to two existing studies that have utilized molecular data to examine genetic overlap between insomnia and MDD, although only one incorporated a PRS method. First, in a recent GWAS of MDD[223] in the large 23andMe dataset, the authors created a risk score from 17 MDD SNPs identified as GWS within the sample and used this to predict insomnia, finding a significant effect. Note that the sample size here was large, with predictions made in nearly 250,000 individuals that were either insomnia cases or controls. In addition to the sizeable N, this PRS approach was different in that the risk score was constructed using GWS SNPs only. It is possible that using GWS SNPs reduced the amount of noise within the score, resulting in improved prediction over the use of SNPs across a range of *p*-value thresholds, as done here. However, a recent overview of heterogeneity in the PRS literature found that using GWS SNPs (vs. no correction/pruning/clumping) resulted in the smallest amount of variance explained across four traits of interest, which included MDD.[256] Second, within their GWAS of insomnia in the UK Biobank, Lane and colleagues[216] examined the relationship between insomnia and MDD using LDSC, identifying a significant genetic correlation of 0.3. LDSC

utilizes all available SNPs and accounts for the LD between them, so the aggregate effects of many variants were used in determining the correlation here (i.e., many more variants used than in the Hyde et al. PRSs). Note that this is lower than most genetic correlations between insomnia and MDD reported in the twin literature for adults (most are over 50%).[186-188] Thus, while there is some molecular evidence for overlap between insomnia and MDD, this is an underdeveloped literature where more analyses are clearly needed.

There are several factors that could be contributing to the lack of predictions seen here. First, while MDD was well-phenotyped within the sample, sleep was not. As touched on in the prior sections of the discussion, the binary item measuring sleeplessness is not the ideal measure of insomnia. Second, in order to examine this overlap within CONVERGE, the sample had to be randomly split in half. This resulted in sample sizes of ~5,000 for both phenotypes. While 5,000 individuals should be more than enough for the target sample, a larger discovery sample is desirable for best predictions.[245,259] Ideally, access to an outside genetically informed sample for insomnia, preferably within an Asian population, would provide the best way to assess genetic overlap between insomnia and MDD using CONVERGE. This would address the phenotypic issues within insomnia and maximize the use of MDD data. With appropriate insomnia data, significant cross-predictions would be expected for insomnia and MDD, in addition to a robust genetic correlation.

Comparison of methods. As discussed earlier, significant predictions were only found within Plink analyses of phenotypes in the natural split (sleep in MDD cases/sleeplessness in MDD controls), and here, Plink scores explained approximately twice the variance as LDpred scores. The maximum change in variance explained by risk scores was much larger within the natural split analyses than in random split analyses, where there were no significant results. The

larger amount of variance explained by Plink (and lack of significant results in LDpred) was surprising, given that the initial paper describing LDpred demonstrated that scores generated by this program performed better than other scores (e.g., Plink) in risk prediction.[257] LDpred was designed to address the loss of information that occurs due to LD pruning during the Purcell method, with the idea that including more SNPs (while still properly accounting for LD) will improve prediction. Thus, among the differences between these two methods, a key point to consider is the number of SNPs included in each set of scores. For the scores constructed using LDpred, over 3 million SNPs were included, since the software uses all available SNPs (that are in common between target and discovery samples) and accounts for the LD between them using information from a reference panel (in this case, the same sample).[257] In contrast, fewer SNPs were included in Plink scores, as pruning was performed prior to score creation and different sets of *p*-value thresholds were used. The number of SNPs included ranged from several hundred (smallest threshold, $p < 0.001$) to around 160,000 (all SNPs) (see Tables 18 and 20 for additional details on number of SNPs per specific Plink score). Note that in general, prediction improved at higher thresholds in Plink; this suggests that a larger number of variants are contributing to risk, as discussed before.[254] A similar pattern was seen across the LDpred results for phenotypes where scores accounted for some variance (i.e., Item E8 and SDS); the largest amounts of change in variance explained (and lowest *p*-values; see Figure 25) were within the infinitesimal models (i.e., where an infinite number of causal variants are contributing to risk in the phenotype; note, however that *p*-value thresholds cannot be directly compared to fractions of causal variants, although as the fractions increase, more variants are thought to contribute to the trait, just as higher *p*-value thresholds contain more SNPs). Another major methodological difference between Plink and LDpred is that LDpred utilizes the heritability of the trait based on SNPs

(from the input summary statistics) in its calculation of risk scores, which could be an issue since initial GCTAs showed low to no heritability. In several instances, the sample size had to be increased in order to obtain an LDpred estimate, which is likely contributing to the smaller amount of variance explained by LDpred scores. Finally, LDpred assumes that a point normal prior is appropriate for modeling the genetic architecture of the trait of interest, which could be incorrect depending on the true genetic architecture of the trait.[257]

Limitations of PRSs. There are several limitations to discuss in the context of PRS methods. The first is that larger discovery samples give better predictions and thus have more power.[245,246,254,259] Many of the initial (and current) analyses utilize large consortium data as the discovery sample.[246,247,254] In comparison, the discovery sample sizes utilized here (~5,000 since the sample was split in half) are of modest size. Second, PRS methods are not standardized and individual studies do not often report the exact methods used. Across the literature, studies utilizing the Purcell method have addressed LD in different ways. Most do either pruning or clumping (using a variety of parameters) within Plink, although some choose not to adjust at all. Additionally, *p*-value thresholds used to bin SNPs also vary based on study. A recent publication outlined the issue of heterogeneity in PRS methods, running different variations of Purcell PRSs for several traits (height, weight, educational attainment, and depression; over 400 analyses in total) within a large publicly available dataset.[256] Among the researchers' conclusions was that while scores created using GWS SNPs were worse, no specific method of pruning/clumping resulted in the highest variance. In fact, many of these scores at the same thresholds had low correlations with each other. Based on this, the authors actually recommend the use of no LD pruning or clumping so that results are replicable.[256] Third, PRS methods cannot capture all genetic influences on a trait, as rare variation, GxE, or gene-gene interactions are not included.

Fourth, methods assume that larger effects carry more weight, which may not necessarily be the case (see cholesterol as an example, where a variant explains a small portion of variance in the trait of interest but has a large effect on enzyme metabolism; e.g., [349]). Lastly, the PRS methods used here are not all-encompassing, as new software continues to develop (e.g., PRSice) and approaches are fine-tuned.

Summary. Overall, results provide some evidence that sleep within MDD can predict sleep within MDD controls, which had not been examined prior to this study. However, there is no support for overlap between sleeplessness and MDD within the sample. Results should be interpreted with the caveat that GCTAs of sleep items did not demonstrate heritability and no significant hits were identified through GWAS. Given robust genetic correlations seen in prior twin studies, additional examination of molecular genetic overlap between sleep and MDD is needed, using samples with better phenotypes.

Chapter 7: Combined phenotypic and genetic analyses (Exploratory Aim)

I. Data analytic plan

To examine the contributions of both genetic and environmental risk factors to sleeplessness, a combined model was conducted. Based on the overall results from the phenotypic and PRS analyses, the final trauma regression for MDD controls from Chapter 3 (Table 10, Step 3; contains age and four trauma variables) was the basis for this combined model that added the significant PRS (created from the SDS) from Chapter 6 (Table 19). Three scores for the SDS in MDD cases predicting sleeplessness in MDD controls passed multiple testing correction (Plink method with thresholds $p < 0.1$, $p < 0.5$, all SNPs), but the score from the threshold of $p < 0.5$ was chosen for the final model since it explained the largest proportion of variance. The final model (age, all trauma variables, PCs, PRS) was compared to the initial phenotypic model (age, all trauma variables) through the change in Nagelkerke's pseudo R^2, change in AIC, and analysis of variance (ANOVA). The effect of PRSs alone was determined by comparing a model with age, traumas, and PCs to the final model listed above. All regressions were conducted in R, using the same methods as described in Chapters 3 and 6.

II. Results

As shown in Table 22, effect sizes remained similar to the initial model, with age and all traumas, except for adult interpersonal, significant. PC2, but not PC1, was a significant predictor of sleeplessness, as seen in prior PRS models. The PRS was a significant predictor of

sleeplessness in the final combined model as well, such that a one standard deviation increase in PRS results in a 10% increase in risk (OR of 1.10, 95% CI = 1.03-1.17, $p = 0.0037$). The pseudo R^2 increased to 0.0332 (from 0.0244[1]; change of 0.009) and model AIC decreased by 24.62 (5811.19[1] to 5786.57). Comparison of models through ANOVA indicated that adding the PRS and PCs resulted in a significant change ($X^2 = 30.62$, $p < 0.0001$) suggesting that adding the PRS does improve model fit/decrease model misfit. The change in variance explained by PRSs alone (above and beyond an intermediate model including age, traumas, and PCs, AIC = 5793.02; model not shown) was 0.002 (from 0.0308 to 0.0332), with a significant change also identified through ANOVA ($X^2 = 30.62$, $p = 0.0037$).

Table 22. Results of final combined model examining the effects of both genetic and environmental influences on sleeplessness in MDD controls (using the top PRS created from the SDS in MDD cases)

	OR (95% CI)	*p*-value	Model R^2	Model AIC
Age	1.03 (1.01-1.04)	< 0.0001	0.0332	5786.57
PC1	0.96 (0.90-1.02)	0.2053		
PC2	1.16 (1.09-1.23)	< 0.0001		
Child IP	1.63 (1.24-2.14)	0.0004		
Adult IP	1.25 (0.87-1.80)	0.2221		
Child NA	1.41 (1.12-1.75)	0.0026		
Adult NA	1.30 (1.14-1.48)	0.0001		
PRS	1.10 (1.03-1.17)	0.0037		

Abbreviations: AIC = Akaike Information Criterion; IP = interpersonal trauma; MDD = major depressive disorder; NA = non-assaultive trauma; PC = principal component; PRS = polygenic risk score; SDS = sleep within depression.
Notes: Nagelkerke's pseudo R^2 is reported. PC1, PC2, and PRS have all been standardized to facilitate interpretation.

[1] Note that this is slightly different from Table 10, as the model was restricted to individuals with genetic data in order to allow for model comparison.

III. Discussion

Overall, results for this combined model demonstrate that both genetic (via PRS) and environmental (i.e., traumatic event exposure) influences are significant predictors of sleeplessness in MDD controls within the CONVERGE sample and account for unique variance. These analyses are novel in that they are the first to integrate molecular genetic risk scores (i.e., PRS) and environmental risk factors in relation to sleep phenotypes. Here, a genetic risk score created in Plink from GWAS results of the sleep within depression (SDS) variable and containing SNPs with $p < 0.5$ after pruning predicts sleeplessness in MDD controls, above and beyond the effects of traumatic events (child interpersonal, adult interpersonal, child non-assaultive, adult non-assaultive). Within this final model, effects of all trauma variables (and the PRS) are consistent with prior models. Note that while the PRS was significant and the model fit improved with the inclusion of the genetic data, with an increase in variance of 0.9%, the risk scores themselves only explained an additional 0.2% of the variance in sleeplessness, similar to what was observed in PRS analyses described in Chapter 6.

Despite these novel results, there are several caveats to note upon interpretation. First, the amount of variance in sleeplessness that is explained by the genetic risk score is small. Thus, even though the PRS reflects the effects of thousands of SNPs across the genome, explanatory power is limited. This suggests that there are other important factors contributing to sleeplessness that are not captured here. Second, while it is straightforward to interpret the meaning of an odds ratio for trauma type (e.g., experiencing at least one interpersonal traumatic event results in a 30% increase in risk of endorsing sleeplessness), interpretation of genetic risk scores becomes more complicated, as the PRS is a summation of the aggregate effects of all included SNPs. The PRS used here has been standardized, such that a one standard deviation increase in the PRS

predicts a 10% increase in risk of endorsing sleeplessness within this sample, but exactly what this means on a more molecular level is unclear, as this does not implicate specific variants or combinations of variants. Third, the model presented here was limited to MDD controls only. Risk scores within this dissertation were created in split halves of the sample (MDD cases only or MDD controls only) to avoid overlap between individuals, and thus could only be combined with trauma variables for those same individuals. For a more complete model of combined genetic and environmental risk for sleeplessness in this data, a risk score for insomnia created from an external phenotype would be needed so that the full sample could be used. Finally, the same sample limitations discussed in prior phenotypic and genetic sections (i.e., phenotype and ascertainment) should also be considered in relation to the final model presented here, particularly since a combined model is only shown for MDD controls.

Chapter 8: Overall discussion and future directions

This dissertation explored both genetic and environmental contributions to sleep phenotypes. To recap, phenotypic analyses showed that the factor structure of trauma type was consistent with prior literature, different trauma types are related to sleeplessness at similar magnitudes (although childhood interpersonal traumas may be more potent), and the relationship between CSA and sleep was replicated in Han Chinese women. Next, genetic analyses of sleep phenotypes (both general and within the context of MDD) did not indicate that there were robust genetic contributions to sleeplessness or sleep within MDD, which was in contrast to the extant literature (e.g., [213,216]). There were several suggestive GWAS findings for sleep within MDD (in *KCNK9* and *ALDH1A2*) and some evidence for genetic overlap between sleep in MDD and sleeplessness in general from PRS analyses. However, these results should be interpreted with caution given that the GCTA estimates of heritability did not differ significantly from zero. Finally, genetic risk remained a significant predictor of sleeplessness in MDD controls when combined into a model with trauma types, indicating that both genetic and environmental risk factors contribute to sleeplessness. As outlined in earlier chapters, there are several key limitations, which remain an issue across all sets of analyses and represent areas of future direction for subsequent investigations of insomnia.

First, appropriate phenotypes are critical. Sleep phenotypes used within all analyses presented here were limited. The only sleep item available across both MDD cases and MDD controls was the binary GS item, reflecting "sleeplessness." To reiterate prior discussions, this

item is not from a formal insomnia definition (i.e., DSM or ICD) nor is it from a standardized scale for measuring sleep quality or insomnia. Further, endorsement was high within MDD cases (86%), resulting in less variation than would be expected in a general sample. This high endorsement was problematic across all analyses. Sleep items within MDD, although from diagnostic criteria, were also endorsed at high rates given MDD severity (i.e., recurrent MDD) within the sample. The sum score created for sleep in MDD (SDS) was highly negatively skewed, since it summed up responses across three widely endorsed items, and it is possible that this score does not reflect a unique phenotype. Taken together, these phenotypic issues are likely contributing to results presented here. However, the state of the insomnia literature as a whole is also limited. Epidemiologic studies of insomnia use definitions that vary in timing and severity (e.g., [2,3]) and extant insomnia GWAS use mostly self-report phenotypes (see discussion in Chapter 5 for more details) as opposed to clinical DSM based diagnoses. Thus, the measurement of insomnia has not been ideal and this represents an important area to address, particularly for genetic studies. While large consortia that combine data are becoming the standard for genetic studies, there is not currently a consortium for insomnia. As stated earlier, fewer GWAS of insomnia phenotypes exist (compared to other psychiatric disorders like MDD and schizophrenia), and phenotypic heterogeneity will need to be resolved to some extent in order to make such a large-scale collaboration a viable option.

Second, ascertainment was a major problem within CONVERGE, as the sample was designed for genetic analyses of MDD. Reduced phenotypic heterogeneity across MDD (recurrent, homogeneous sample) resulted in the identification of MDD-relevant loci,[220] but was clearly a disadvantage in the analyses of insomnia presented here. The full sample did not represent a population sample for insomnia, and combining individuals from both cases and

controls resulted in little to no heritability once the effect of MDD was regressed out. Further, the ascertainment of cases resulted in individuals with recurrent MDD, which relates to the high prevalence of sleep variables seen here, given that insomnia is a key symptom of the disorder and may reflect severity (e.g., [36,37]). Controls, although they did not have recurrent MDD nor were they likely to develop it, may be "super controls" and thus not reflective of the population either. Ascertainment issues were highlighted within phenotypic analyses as well, as some traumas were non-significant predictors of sleep when restricted to specific subsets of the sample (i.e., adult interpersonal trauma was not significant in MDD controls, child interpersonal trauma was not significant in MDD cases). Modeling a population prevalence of MDD of 8% indicated that child interpersonal traumas might be more potent predictors of sleep, a finding that was not seen using other methods. Study designs with appropriately matched cases and controls for insomnia (or depressed individuals with and without sleep problems for studying insomnia within MDD), as opposed to samples of convenience or samples used to study other phenotypes, will be essential for studying genetic influences on insomnia and the contributions of trauma to sleep disturbances. Moreover, the ideal sample for genetic analysis may not be ideal for phenotypic analyses, particularly if trying to understand a specific population.

Related to ascertainment is phenotypic heterogeneity, which may be a key contributor to missing heritability.[245] For example, heritability estimates may be higher in clinical samples vs. population based[245] (this is seen in the twin literature for psychiatric disorders like schizophrenia; e.g., [350-353]). Heterogeneity as it relates to clinical subtypes may also be important.[245] Within insomnia, this could reflect differences in the genetic contributions to sleep-onset vs. maintenance subtypes of the disorder. This has not been explored at the molecular level, as genetic studies of insomnia generally use composite phenotypes or questions that incorporate

both onset and maintenance. Within the twin literature, some studies do examine different symptoms separately,[167] but detailed comparisons have not been made and some twin studies have even found no heritability for some symptoms (e.g., [171]). Examining subtypes may make it easier to detect important variants, as effect sizes are likely larger in these more specific samples.[245] It also is possible that the incorporation of other intermediate phenotypes may aid in understanding the genetic architecture of insomnia. For example, the concept of sleep reactivity (change in sleep in response to a stressor), which has been identified as a robust risk factor for insomnia,[354,355] may also have shared genetic influences with the disorder[171]. Difficulties in using other phenotypes like this is that they may also influence other disorders like MDD,[245] as is the case for sleep reactivity,[356] which could make it difficult to isolate insomnia-specific effects (although note that twin studies suggest there may not be many insomnia-specific genes [e.g., [186,187]], making the examination of other phenotypes a good strategy).

Third, large sample size and the number of cases and controls is also important for genetic analyses (sample sizes were adequate for phenotypic analyses, with the exception of some CSA-specific analyses). Although the overall sample size in CONVERGE was large, the study was not designed as a case-control study for insomnia, resulting in an imbalance in cases and controls for sleeplessness when the full sample was used (i.e., 86 % of MDD cases endorsed sleeplessness, see Section III, Discussion in Chapters 4 and 5). Further, samples sizes were modest for GWAS (~5,000) when split into MDD cases and MDD controls, and endorsement for sleep within depression remained problematic. In general, as sample size increases, so does the power to detect significant variants, particularly those of smaller effect sizes. Large GWAS of other phenotypes, such as schizophrenia, have identified more loci as the numbers of cases and controls have increased (e.g., for schizophrenia, there were seven GWS loci in a sample of

~9,000 cases and 19,000 controls, the first PGC,[357] increasing to 108 loci identified using ~37,000 cases and 108,000 controls in the most recent PGC analysis).[242] The most recent GWAS of sleep phenotypes, which included insomnia assessment in ~60,000 individuals, was able to identify five top loci for the trait.[216] This provides support for the notion that adding more individuals will help gene identification efforts for insomnia as well, given that its genetic architecture is likely polygenic.

Once large, well-phenotyped samples for insomnia exist, this will also permit further examination of overlap with other psychiatric and non-psychiatric traits and increase our understanding of shared molecular underpinnings, which should in turn add to knowledge of the disorder. One strategy is that used within the UK Biobank insomnia GWAS,[216] where LDSC was used to look at genetic correlations between sleep and other phenotypes. However, replications are needed using different approaches, particularly PRSs, since no studies to date, excluding this dissertation, have created insomnia-related PRSs. A focus on overlap with other psychiatric disorders in particular is warranted, given biometric studies of shared genetic contributions (e.g., [186,187]). Although overlap was examined here (with some evidence for overlap between sleep in MDD and sleep in general but none for sleeplessness and MDD), sample limitations discussed earlier (with regard to phenotype and ascertainment) make it difficult to interpret results. Risk scores created from other established phenotypes (i.e., from PGC consortia) should also be used to examine overlap with insomnia, as a good discovery sample results in greater power to detect shared genetic effects, and target samples do not need to be as large.[245,259] The CONVERGE MDD results could be used in this way, but an appropriate insomnia GWAS (e.g., from combined data, if possible) would also be needed. Examination of GWS hits (or scores created

from GWS hits, similar to what was done in the 23andMe MDD GWAS[223]) for related phenotypes may also prove useful.

Further, as our understanding of genetic contributions to insomnia increases, the inclusion of both genetic and environmental influences in an etiologic model (e.g., as done in the exploratory aim here) will be important, as the ultimate goal is to utilize this information to inform prevention and intervention efforts for insomnia. A recent overview of the literature suggests that incorporating well-characterized environmental factors and considering phenomena such as GxE is important from a public health perspective, as it may improve understanding of genetic effects and their underlying mechanisms.[358] Although this sounds simple in theory, there are multiple reviews that outline the difficulty in incorporating large-scale genetic information for psychiatric traits into useful interventions.[358-360] Unlike the fields of cancer and pharmacogenomics, the genetic architecture of the traits being studied (e.g., insomnia, most psychiatric traits) is not well understood.[358] For phenotypes with identified variants of larger effect, examining risk conferred by these variants has clinical relevance, but this is not the case for psychiatric traits like insomnia and MDD. Additionally, determining exactly how to utilize genetic data is difficult. Currently, PRSs have the most potential in terms of prediction (i.e., it may be that individuals with certain combinations of risk alleles are more likely to develop the disorder or respond to a certain treatment or medication), given what is known about the polygenic nature of psychiatric traits.[245,358] However, the aggregate nature of the method, its assumption of additive effects, and its restriction to common variants represent important limitations to consider in utilizing PRSs. As statistical genetic approaches continue to develop, they will inform the field's understanding of how to best integrate genetic and phenotypic results, and it may be that PRSs are not ideal. Knowledge of genomics is constantly changing,

and statistical innovations are coming online that can be used in studying insomnia. For example, the field of epigenetics has gained popularity in recent years, given that these DNA modifications are (at least partially) a result of environment, which may be particularly relevant for insomnia.[361] Other non-additive contributions, such as gene-gene interactions, may be relevant once more research has been done. One area that has been promising for insomnia is gene expression, where studies have found differences in genes expressed within insomnia following specific insomnia treatments (e.g., [362,363])

Final conclusions. This dissertation aimed to understand the genetic and environmental influences on sleep disturbances, both within and outside of MDD, in an understudied population. There were several novel findings in both phenotypic (i.e., verification of trauma structure in Han Chinese, replication of the effect of CSA on sleep in a Chinese population) and genetic (i.e., identification of several suggestive genes based off of the liberal q-value threshold that could be relevant for sleep within MDD, demonstration of shared genetic influences between sleep within MDD and sleeplessness in general) analyses and a final model incorporated both trauma and aggregate genetic risk. However, limitations of the sleep phenotypes and sample ascertainment greatly limited the ability to answer the proposed questions, bringing up concerns that are relevant to the insomnia literature as a whole. Many questions remain unanswered, particularly in understanding insomnia in the context of MDD and the extent to which molecular genetic influences overlap between insomnia and MDD. This underscores the need for additional studies of well-defined insomnia phenotypes that examine the differential effects of traumatic event exposure, additional environmental variables (e.g., overall physical health/exercise), and comorbid psychiatric conditions, in addition to incorporating genomic data, in order to advance our understanding of this prevalent health concern.

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Vita

Mackenzie Jean Lind was born on May 4, 1988 in Lancaster, Pennsylvania. She graduated *summa cum laude* with Honors from the University of Scranton in May 2010 with a B. S. in Neuroscience and French and Francophone Studies, and a minor in Biochemistry. Her honors thesis, "The effects of acute ethanol exposure just prior to dark cycle onset on sleep continuity in the fruit fly *Drosophila melanogaster*," was conducted under the supervision of Dr. J. Timothy Cannon, PhD. While completing her French coursework, Ms. Lind studied abroad at the Sorbonne in Paris, France during the Spring 2008 semester, earning a Certificat de Langue et Civilization Françaises, level B2. She received the Joseph K. Brunner Excellence in Foreign Languages Award as well as the Award for Excellence in Neuroscience upon graduation. She also applied for and received a Fulbright-University of Helsinki Graduate Student Award to conduct research at the University of Helsinki Institute of Biomedicine, Department of Physiology, in Helsinki, Finland for the 2010-2011 academic year. Ms. Lind's project proposal, "The neuroscience of sleep and circadian shifts," allowed her to conduct research on mouse sleep and metabolism under the supervision of Dr. Tarja Porkka-Heiskanen, MD, PhD. After returning from Finland, Ms. Lind moved to Richmond to begin VCU's MD/PhD program. During the first two years of medical school, she received the M1 Foundations of Clinical Medicine Course Award and served as co-president of both the Student Psychiatry Society and the Student Interest Group in Neurology. Ms. Lind began her graduate work in Clinical and Translational Sciences, with a concentration in Psychiatric, Behavioral, and Statistical Genetics, in Summer 2013, under

the supervision of Dr. Ananda B. Amstadter, PhD, with additional mentorship by Dr. Kenneth S. Kendler, MD. Her research broadly explores the etiology (both genetic and environmental) of and relationships between trauma exposure, disturbed sleep/insomnia, and psychopathology (particularly internalizing disorders). During her graduate work so far, Ms. Lind has published 8 peer-reviewed papers (7 first author) and 3 book chapters (2 first author) and has given talks or poster presentations at multiple local and national conferences. In recognition of her work, she has received the First Place Poster Award at the Virginia Academy of Sleep Medicine conference (November 2015) and a VCU Graduate Student Travel grant (Fall 2015). She has been supported by the "NIMH Training Program in Psychiatric and Statistical Genetics at the Virginia Institute for Psychiatric and Behavioral Genetics" NRSA T32 Grant during the 2016-2017 academic year. As a graduate student, she has also served the university in multiple roles: Student co-president for VCU's MD/PhD program (2014-2015) and treasurer of the American Physician Scientists Association chapter at VCU (2015-2016). She is also involved in the admissions committee for the MD/PhD program. Following completion of medical school, Ms. Lind hopes to pursue further training in Psychiatry and Sleep Medicine and continue her line of research into the genetic and environmental contributions to insomnia and related psychiatric disorders.