Whole Blood Transcriptome Sequencing in Individuals with Insomnia

BY

Sara Mithani
B.S.N. Case Western Reserve University, 2016

THESIS

Submitted as partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Nursing Sciences in the Graduate
College of the University of Illinois at Chicago, 2020

Chicago, Illinois

Defense Committee:

Anne Fink, Chair and Advisor
Jessica Gill, National Institutes of Health- National Institutes of Nursing Research
Holli DeVon, Professor Emerita, Department of Biobehavioral Health Science
Michael Calik
Eileen Collins
Mary Kapella

This thesis is dedicated to my parents, Mansoorali and Rozina Mithani: for teaching me to trust in God, for providing me with the best available opportunities beyond their means, for supporting me through every step of my journey and for inspiring me always to aim higher.

ACKNOWLEDGEMENTS

"No honour is like knowledge. No belief is like modesty and patience, no attainment is like humility, no power is like forbearance, and no support is more reliable than consultation."—Hazrat Ali ibn Abi Talib

First and foremost, I would like to acknowledge the National Institutes of Health (NIH)-National Institutes of Nursing Research Graduate Partnership Program and the University of Illinois at Chicago College of Nursing for establishing a partnership to support my dissertation research.

I want to acknowledge my dissertation chair, mentor, and advisor, Dr. Anne Fink. Thank you for your constant support and dedication since the first day of my Ph.D. program. She prioritized investing in me as an individual which has enabled me to succeed in diverse environments.

Dr. Jessica Gill, thank you for taking me into your lab during my first summer research experience at the NIH. Little did we know at the time that I would come back to the NIH to complete my dissertation under your mentorship. I am beyond grateful for your guidance and always supporting my research dreams. I am constantly in awe of your dedication and work ethic and I aspire to be a researcher and leader like you. It has been my greatest honor to work closely with you and I am excited to see where our research takes us in the future. I would also like to acknowledge our collaborator, Dr. Philip Gehrman, from the University of Pennsylvania Department of Psychiatry, for his gracious support during my dissertation.

To my dissertation committee, Drs. Holli Devon, Michael Calik, Eileen Collins and Mary Kapella, thank you for your patience and encouragement as I completed my dissertation at the NIH. I sincerely appreciate the time devoted to helping me through my project and the reinforcement all of you have provided over the years.

I would also like to thank other faculty members who I have been fortunate enough to have as mentors. To my constant cheerleader at UIC, Dr. Carrol Ferrans, your support has uplifted me when I

have needed it the most. I want to thank Drs. Collen Corte, Catherine Vincent and Lauretta Quinn for providing moral support and always listening and providing solutions to problems I faced along the way.

Additionally, I would like to thank my UIC colleagues, who have become some of my close friends. In particular, Susan Hovers, Katherine Breen, Katherine Erbe, Judie Hollman and Katherine Maki, my journey through this program would not be the same without all of you loving souls.

My dissertation would also not have been possible without the researchers, staff, and colleagues at NIH. Drs. Hyung-Suk Kim, Lai Chen and Ms. Chelsea Wagner, you make working in the lab easier and enjoyable. Thank you for always being a reliable resource and answering my questions about lab processing. Dr. SijungYun, thank you for being so patient and working with me on sequencing data. I appreciate the countless hours you have spent assisting me with analyzing data. Dr. Pamela Tamez, thank you for your support in all matters related to my training at the NIH. To my fellow officemate, Jackie Leete, thank you for all of your help with graphics and always being a breath of fresh air in times of stress. A special thank you to my dear friend, Dr. Meghan Murry, for supporting me through the ups and downs of life over the past year.

To my favorite Australian researcher and friend, Dr. Cassandra Pattinson, your work ethic and enthusiasm have always inspired me and I am grateful for our fun times together. Thank you for being my mentor and fellow sleep researcher and providing guidance during my dissertation. I am excited to see your career grow and I can’t wait to see you again in the near future.

I would also like to thank my colleague, mentor and science enthusiast, Dr. Vivian Guedes. My experience at the NIH would not be the same without you. I am always grateful to brainstorm new research ideas with you, learn new techniques that are always way more involved than we think and grab a latte with you. You bring so much positive energy into my life and continuously motivate me to not shy away from things that may be difficult.

Lastly, and perhaps most importantly, I am wholeheartedly grateful for my family and friends who provided the much-needed emotional support that makes this process remotely possible. To my brother, Asif, you have been my rock since day one, support my every dream and make sure I am also enjoying life along the way. To my sister-in-law, Azmina, thank you for being a blessing in our house and supporting my needs during my education. To my childhood best friend and sister, Sarah Adtani, thank you for being a constant source of happiness over the many years. To my friends from Case Western Reserve University, Holly Johnson, Emily Kugel, Belle Perez, Shreya Sekaran, Liam McCormick, Zach March, Anthony Nguyen and Melissa Kelley, thank you for always encouraging me from day one and never letting distance affect our friendship.

To my fiancé and forever best friend, Fahad, I don't think I can ever convey how grateful I am to have your support through my education and training. Thank you for motivating me daily, encouraging me to pursue my dreams and being an anchor in my life.

Above all, my parents, Mansoorali and Rozina Mithani, there are no words to thank you for every sacrifice you have made to make sure I received the best education and opportunities possible. Thank you for always encouraging me to pursue my interests, wiping my tears at my lows and celebrating my success, no matter how big or small. Everything I am and have achieved is a direct reflection of you.

Thank you, God, for all your blessing to my family and me; for the strength you give me each day; for the opportunity to get an education and for all the people around me who make my life more meaningful.

Funding

This author was supported by funding from the Graduate Partnership Program at the National Institutes of Health and Jonas Philanthropies.

SMM

Table of Contents

CHAPTER PAGE

1. Introduction 1
   1.1. Background 1
   1.2. Pathophysiology of Sleep and Insomnia 2
   1.3. Chronic Illnesses related to Insomnia 3
   1.4. Treatment of Insomnia 4
   1.5. Transcriptome Profiling in Human Disease 5
   1.6. Study Objectives and Hypothesis 7
   CITED LITERATURE 8

2. Whole Blood Transcriptome Analysis in Individuals with Insomnia: A RNA-Sequencing Study 10
   2.1. Introduction 10
   2.2. Methods 11
      2.2.1. Sample Processing 12
      2.2.2. Statistical Analysis 12
   2.3. Results 13
      2.3.1. Demographics and Clinical Characteristic 13
      2.3.2. RNA-seq and Network analysis 13
         1. Network 1
         2. Network 2
         3. Network 3
   2.4. Discussion 15
   2.5. Conclusion 18
   CITED LITERATURE 19

3. A Review of the Glymphatic System and its Implications for Alzheimer's Disease and Brain Injury Research 41
   3.1. Introduction 41
   3.2. What is the glymphatic system? 41
   3.3. Neurodegeneration: Alzheimer's Disease 42
      3.3.1. Sleep-Wake Disruption in AD 43
      3.3.2. Role of glymphatic system in AD 43
      3.3.3. Biomarkers in AD 44
   3.4. Neurovascular: Traumatic Brain Injury 45
      3.4.1. Sleep-wake Disruption in TBI 45
      3.4.2. Role of glymphatic system in TBI 45
      3.4.3. Biomarkers in TBI 46
   3.5. Conclusion and Future Direction 47
   CITED LITERATURE 51

   APPENDIX 56
      APPENDIX A. Human Research Protections Program: Intramural Research Program 56
   VITA 71

LIST OF TABLES

TABLE PAGE

TABLE I 22

TABLE II 23

TABLE III 24

TABLE IV 30

LIST OF FIGURES

FIGURE PAGE

Figure 1. RNA-sequencing Pipeline. 9

Figure 2. Pie chart of protein coding and non-protein coding gene. 36

Figure 3. Volcano plot of dysregualted genes identifyed by RNA-sequencing. 37

Figure 4. IPA Network 1. 38

Figure 5. IPA Network 2. 39

Figure 6. IPA Network 3. 40

Figure 7. Glymphatic flow of healthy and injured brain. 55

Figure 8. Metabolic clearance during wakefulness and sleep. 56

LIST OF ABBREVIATIONS

Aβ	Amyloid-beta
AD	Alzheimer's disease
AQP4	Aquaporin-4
BBB	Blood brain barrier
BMI	Body mass index
CBT	Cognitive Behavioral Therapy
cDNA	Complimentary deoxyribonucleic acid
CNS	Central nervous system
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
ELISA	Enzyme-linked immunosorbent assay
FDR	False discovery rate
ICF	Intracellular fluid
IPA	Ingenuity Pathway Analysis
ISF	Interstitial fluid
ISI	Insomnia severity index
LFC	Log fold change
LPS	Lipopolysaccharides
NADH	Nicotinamide adenine dinucleotide hydrogenase
NF-kB	Nuclear factor kappa light chain enhancer of activated B cells
NfL	Neurofilament light chain
NIH	National Institutes of Health
NREM	Non-rapid eye movement
p-Tau	Phosphorylated tau
REM	Rapid Eye Movement
RNA	Ribonucleic acid
RNA-seq	Ribonucleic acid sequencing
SD	Standard deviation
SF-8	General Health Survey (short form)
Simoa	Single molecule array

SNP	Single nucleotide polymorphism
t-tau	Total tau
TBI	Traumatic brain injury

SUMMARY

The purpose of this dissertation was to identify a transcriptomic profile of individuals with insomnia and explore the role of the glymphatic system and sleep disturbances in neurodegenerative disease and traumatic brain injury. The first paper reports whole blood transcriptomic analysis in individuals with insomnia using RNA sequencing technology. The study incorporated innovative high throughput sequencing platform with extended pathway analysis using QIAGEN's Ingenuity® Pathway Analysis software to identify differentially expressed genes and pathways in individuals with insomnia. The second paper provides a critical review of the sleep-wake process and the glymphatic system and how these processes contribute to neurodegenerative disorders and traumatic brain injury. The research addresses an important gap in knowledge about underlying mechanism of insomnia and how sleep disturbance, the glymphatic pathway and neurologic conditions may be interrelated.

1. Introduction

1.1 Background

In the United States, it is estimated that 50 to 70 million individuals of all ages and socioeconomic classes are affected by sleep-related problems. Insomnia occurs in approximately one-third of the population with 6-10% meeting the diagnosis for insomnia disorder.[1,2] The Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5)[3] defines insomnia as a predominant complaint of dissatisfaction with sleep quantity or quality associated with one of more of the following symptoms: 1) difficulty initiating sleep 2) difficulty maintaining sleep—characterized by frequent awakenings or problems returning to sleep after awakening and/or 3) early-morning awakening with the inability to return to sleep. The diagnosis is met if the sleep difficulty occurs at least 3 nights per week despite adequate opportunity to sleep and is not attributed to other physiological effects of substances such as drug abuse, alcohol or medications.[4] The National Institutes of Health (NIH) has recognized that there is controversy around including nonrestorative sleep or sleep quality as part of the definition of insomnia as it is difficult to objectively characterize these terms.[5] Although the definition of insomnia is still heavily debated, insomnia remains a highly prevalent disorder with substantial negative impacts on an individual's quality of life. In a study of 19,711 adults where health-related quality of life was measured with the short-form 8 (SF-8) questionnaire, individuals with insomnia had significantly lower physical and mental scores and a greater impairment score compared to individuals without insomnia.[6] Moreover, the economic burden of insomnia is sizable, costing between 30 to 108 billion dollars per year with direct and indirect costs through prescription or over-the-counter medications,[7] reduced work capacity and/or productivity,[8-11] low levels of work performance, increased absences from work, and injuries and illnesses.[12,13] Although insomnia has debilitating consequences, little is known about the pathophysiological mechanisms of the disorder, under what conditions it progresses, or potential targets for prevention and treatment.

1.2 Pathophysiology of Sleep and Insomnia

A widely used operational definition of sleep is that it is a natural, reversible and self-regulated state characterized by a reduction in voluntary motor activity and decreased response to stimulation.[14] The sleep-wake cycle is divided into three predominant states: wakefulness, non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep. NREM sleep is comprised of stages 1-3, with each stage involving unique characteristics. Following stage 3 NREM sleep, there is a transition to REM sleep. REM sleep includes rapid eye movements; increase variability in heart rate and blood pressure; and breathing is rapid, irregular, and shallow.[15] The two-process model of sleep regulation proposes that homeostatic and circadian processes drive sleep.[16] The homeostatic process (Process S), which builds during wakefulness and declines during sleep, interacts with a circadian process (Process C), which is independent of sleep and wake. During wakefulness, Process S increases exponentially, eventually reaching an upper threshold—the maximum propensity for sleep.[17] Process S decreases during periods of sleep, reaching the lower threshold after approximately 8 hours of sleeping.[18]

The neural circuitry underlying the regulation of sleep and wakefulness is discrete but is uniquely interdependent. Wakefulness is maintained by an ascending reticular activating system which originates in the upper brainstem adjacent to the pons, midbrain and diencephalon where it then separates into two branches. The first branch innervates the thalamus and plays a gating role that can block the generation of thalamocortial rhythms and promote wakefulness.[19] The second branch projects into the lateral hypothalamus, basal forebrain and cerebral cortex and plays a role in the discharging of neurons during wakefulness, NREM and REM sleep. Specifically, orexin/hypocretin neurons play a strong active role during REM sleep, and cholinergic neurons of the basil forebrain discharge at their maximal rates during REM sleep and active wake.[19] Sleep-promoting neurons (e.g., histamine, dopamine, norepinephrine, serotonin, glutamate, orexin and acetylcholine) in the preoptic area, posterior lateral hypothalamus and in the lower brainstem inhibit the firing of neurons in the arousal area during sleep. Designed like a seesaw, the “flip flop” switch/circuit is created by mutual inhibition between the sleep promoting and the wake

promoting neurons, which allows for a rapid and complete transition between sleep and wakefulness. Understanding the pathways of the sleep-wake cycle allows us to better understand how this cycle is altered in sleep disorders, chronic illnesses and drug interactions. Often considered to be a condition of hyperarousal, insomnia is associated with increased somatic, cognitive and cortical activation.[20] Physiological arousal may arise in both the central (cortical) and peripheral (autonomic) nervous system. In addition, the neurobiological and physiological factors contributing to insomnia are likely multifaceted and may include additional factors such as environment, genetics, behavior, cognition and emotions. Despite the high prevalence of insomnia, little is understood about the underlying mechanisms or how primary insomnia can be isolated compared to insomnia as a secondary symptom.

Recent studies show that sleep state is unique in the sense that it plays a key role in the clearance of metabolic waste in the brain that is collected during wakefulness.[21] This system, known as the glymphatic system, utilizes a unique system of perivascular tunnels formed by astroglia cells to promote efficient elimination of aggrege proteins that can toxic if accumulated in the brain. Xie et al. in 2013 demonstrated in both sleep and anesthetized mice that interstitial space volume was expanded to 22-24% compared to 13-15% during wakefulness.[22] This observation indicates that the glymphatic function is particularly active during sleep and that the brain clears its waste products most affectively at this time. The developments of the glymphatic functions have been of particular importance to researchers as it may provide insight into how protein aggregation occurs in the brain thus leading to neurodegenerative conditions. Additionally, the combination of monitoring sleep and glymphatic alterations can serve as a diagnostic marker for identifying individuals who may be at risk of developing further injury related complications.[21,23] Further studies in human subjects need to focus on the neuroprotective properties of sleep as it relates to the glymphatic function in healthy controls and different disease processes.

1.3 Chronic Illnesses related to Insomnia

Numerous research studies have demonstrated increased prevalence of insomnia in individuals with somatic diseases[12,21-23] and psychiatric disorders with anxiety and depression being the most common in individuals with insomnia[24-27] For example, in an epidemiologic study with 7954 respondents, 40% of individuals with insomnia had a comorbid psychiatric disorder compared with 16.4% of those with no sleep complains.[28] Additionally, complaints of insomnia are highly prevalent in individuals with cancer, chronic pain, and cardiovascular disease. A review article of 15 studies reported that 30% to 50% of individuals with cancer experience symptoms of insomnia.[29] A meta-analysis of 13 studies showed that individuals suffering from insomnia had a significantly increased risk (45%) of developing or dying from cardiovascular disease.[30] In recent years, sleep disturbances have been associated with neurological and neurodegenerative disorders, including Parkinson's disease and Alzheimer's disease. The cause of insomnia in neurodegenerative disease is multifactorial and includes the use of medications related sleep alterations (e.g., dopaminergic and anticholinergic treatment), the physiological effects from degenerative processes, and the natural aging process.[31] Often, there is a bidirectional relationship between insomnia and these disorders, making it difficult to distinguish sleep disturbance as a primary disorder or secondary to another cause. Further research that explores these physiological alterations and mechanisms is needed to elucidate the role of sleep alterations in chronic illnesses.

1.4 Treatments of Insomnia

Treatment availability for insomnia include psychological, pharmacological, and alternative therapies. In 2005, a National Institutes of Health State-of-the-Science panel recognized cognitive-behavioral therapy (CBT) as a first-line treatment for insomnia. This therapy is aimed at modifying behavioral and thinking patterns that are presumed to prolong or exacerbate insomnia. Participants receive guidance to change sleep habits, sleep schedules and thinking pattern by trained mental-health clinicians.[32] Although there is extensive data supporting the benefits of CBT-Insomnia (CBT-I) for sleep-onset and maintenance insomnia, contraindications of the therapy include daytime sleepiness, early

morning awakenings and adverse effects to sleep restriction such as manic phase or seizures. There are many different medications available to manage insomnia, but discovering the optimal pharmacological treatment that minimizes the treatment risk-benefit ratio varies for each patient based on the specific nature of their insomnia. Alternative therapies such as acupuncture are not generally recommended for the management of insomnia due to the lack of evidence about their potential benefits and risks.

Despite advances in pharmacological and behavioral treatments, many individuals with insomnia have recurrent symptoms, and some chronic and severe forms of insomnia are impervious to treatment. An increased understanding of the molecular mechanisms underlying insomnia is necessary to design novel and effective treatments for this disorder.

1.5 Transcriptome Profiling in Human Disease

The transcriptome contains the full information of all ribonucleic acid (RNA) transcribed in a specific tissue or cell type and their quantity for a specific developmental stage or physiological condition. The transcriptome allows for a comprehensive overview of gene structure, function, expression and plasticity.[33] Additionally, the transcriptome may reveal alterations in the biological processes triggering human disease thus offering insight into underlying mechanisms and also potential targets for diagnosis and clinical therapy. The recent development of high-throughput sequencing has provided new methods for mapping and quantifying the transcriptome. RNA-sequencing (RNA-seq) is the first sequencing method that allows for the entire transcriptome to be surveyed in a highly specific and quantitative manner. The complete RNA-seq procedure consists of three main components: experiment, bioinformatics, and additional analysis (Figure 1).[34] During the experiment preparation, RNA is isolated from tissue or blood samples and is converted to complimentary deoxyribonucleic acid (cDNA) using reverse transcriptase. A library is constructed to identify and quantify all types of RNAs, and the cDNA is sequenced in a high-throughput manner to obtain short sequences at one end or both ends of the fragment. After the sequencing, the bioinformatics arm of the procedure is introduced. The sequence reads are put through a series of quality controls. The reads are then aligned to a genome based on the origin of the

sample with an RNA-seq aligner. During this process, the transcriptome is constructed. The constructed transcriptome is normalized and then statistical tests to identify differential genes are completed. Further analysis (e.g. cluster, visualization, pathway and network analysis and mathematical modeling) can be completed on the constructed transcriptome to gain further insight into the data.[34]

1.6 Study Objectives and Hypothesis

The objective of this study was to identify differentially expressed genes and pathways associated with insomnia using RNA-seq technology. The high throughput sequencing method enables hundreds of millions of DNA molecules to be sequenced at one time. The advantage of this technology is that it can generate more comprehensive insight into the cellular genomic and transcriptomic signature of the disease and its developmental stages. Additionally, a nonsystematic review was conducted to review and synthesize new information of the glymphatic system and sleep. We also show how these mechanisms may be involved in two neurologic conditions, Alzheimer's disease and sleep, and its clinical relevance through discussing potential biomarkers for the conditions. The novelty of the review paper is that it is the first of its kind to tie together the overlap of sleep, glymphatic in respects to Alzheimer's disease and brain injury as it relates to clinically relevant biomarkers.

CITED LITERATURE

1. Morin CM, Bootzin RR, Buysse DJ, Edinger JD, Espie CA, Lichstein KL. Psychological and behavioral treatment of insomnia:update of the recent evidence (1998-2004). *Sleep.* 2006;29(11):1398-1414.
2. Ohayon MM, Roth T. Place of chronic insomnia in the course of depressive and anxiety disorders. *Journal of psychiatric research.* 2003;37(1):9-15.
3. Association AP. *Diagnostic and Statistical Manual of Mental Disorders.* 5th ed. Washington, DC2013.
4. (US) SAaMHSA. Impact of the DSM-IV to DSM-5 Changes on the National Survey on Drug Use and Health [Internet]. In: *Substance Abuse and Mental Health Services Administration.* Rockville, MD2016.
5. Health NIo. National Institutes of Health State of the Science Conference statement on Manifestations and Management of Chronic Insomnia in Adults, June 13-15, 2005. *Sleep.* 2005;28(9):1049-1057.
6. Bolge SC, Doan JF, Kannan H, Baran RW. Association of insomnia with quality of life, work productivity, and activity impairment. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation.* 2009;18(4):415-422.
7. Hatoum HT, Kong SX, Kania CM, Wong JM, Mendelson WB. Insomnia, health-related quality of life and healthcare resource consumption. A study of managed-care organisation enrollees. *PharmacoEconomics.* 1998;14(6):629-637.
8. Godet-Cayre V, Pelletier-Fleury N, Le Vaillant M, Dinet J, Massuel MA, Leger D. Insomnia and absenteeism at work. Who pays the cost? *Sleep.* 2006;29(2):179-184.
9. Leger D, Massuel MA, Metlaine A. Professional correlates of insomnia. *Sleep.* 2006;29(2):171-178.
10. Linton SJ, Bryngelsson L. Insomnia and its relationship to work and health in a working-age population. *Journal of Occupational Rehabilitation.* 2000;10(2):169-183.
11. Ozminkowski RJ, Wang S, Walsh JK. The direct and indirect costs of untreated insomnia in adults in the United States. *Sleep.* 2007;30(3):263-273.
12. Katz DA, McHorney CA. Clinical correlates of insomnia in patients with chronic illness. *Archives of internal medicine.* 1998;158(10):1099-1107.
13. Taylor DJ, Lichstein KL, Durrence HH, Reidel BW, Bush AJ. Epidemiology of insomnia, depression, and anxiety. *Sleep.* 2005;28(11):1457-1464.
14. Fuller PM, Gooley JJ, Saper CB. Neurobiology of the sleep-wake cycle: sleep architecture, circadian regulation, and regulatory feedback. *J Biol Rhythms.* 2006;21(6):482-493.
15. Saper CB. The neurobiology of sleep. *Continuum (Minneapolis, Minn).* 2013;19(1 Sleep Disorders):19-31.
16. Borbely AA. A two process model of sleep regulation. *Human neurobiology.* 1982;1(3):195-204.
17. Borbely AAA, P. . Sleep Homeostasis and Models of Sleep Regulation. 1999.

18. Mithani S, Fink AM. Mathematical Models of Sleep and Circadian Rhythms: A Case for Using the 2-Process Model in Neuroscience Nursing. *Journal of Neuroscience Nursing.* 2019;51(1):48-53.

19. Schwartz JR, Roth T. Neurophysiology of sleep and wakefulness: basic science and clinical implications. *Current neuropharmacology.* 2008;6(4):367-378.

20. Levenson JC, Kay DB, Buysse DJ. The pathophysiology of insomnia. *Chest.* 2015;147(4):1179-1192.

21. Jessen NA, Munk AS, Lundgaard I, Nedergaard M. The Glymphatic System: A Beginner's Guide. *Neurochemical research.* 2015;40(12):2583-2599.

22. Xie L, Kang H, Xu Q, et al. Sleep drives metabolite clearance from the adult brain. *Science.* 2013;342(6156):373-377.

23. Iliff JJ, Thrane AS, Nedergaard M. Chapter 3 - The Glymphatic System and Brain Interstitial Fluid Homeostasis. In: Caplan LR, Biller J, Leary MC, et al., eds. *Primer on Cerebrovascular Diseases (Second Edition).* San Diego: Academic Press; 2017:17-25.

24. Kuppermann M, Lubeck DP, Mazonson PD, et al. Sleep problems and their correlates in a working population. *Journal of general internal medicine.* 1995;10(1):25-32.

25. Martikainen K, Partinen M, Hasan J, Laippala P, Urponen H, Vuori I. The impact of somatic health problems on insomnia in middle age. *Sleep Med.* 2003;4(3):201-206.

26. Gislason T, Almqvist M. Somatic diseases and sleep complaints. An epidemiological study of 3,201 Swedish men. *Acta medica Scandinavica.* 1987;221(5):475-481.

27. Ohayon MM, Caulet M, Philip P, Guilleminault C, Priest RG. How sleep and mental disorders are related to complaints of daytime sleepiness. *Arch Intern Med.* 1997;157(22):2645-2652.

28. Ohayon MM, Roth T. Place of chronic insomnia in the course of depressive and anxiety disorders. *J Psychiatr Res.* 2003;37(1):9-15.

29. Taylor BC, Hagel EM, Carlson KF, et al. Prevalence and Costs of Co-occurring Traumatic Brain Injury With and Without Psychiatric Disturbance and Pain Among Afghanistan and Iraq War Veteran VA Users. *Medical Care.* 2012;50(4):342-346.

30. Phillips B, Mannino DM. Does insomnia kill? *Sleep.* 2005;28(8):965-971.

31. Ford DE, Kamerow DB. Epidemiologic study of sleep disturbances and psychiatric disorders. An opportunity for prevention? *Jama.* 1989;262(11):1479-1484.

32. Savard J, Simard S, Blanchet J, Ivers H, Morin CM. Prevalence, clinical characteristics, and risk factors for insomnia in the context of breast cancer. *Sleep.* 2001;24(5):583-590.

33. Sofi F, Cesari F, Casini A, Macchi C, Abbate R, Gensini GF. Insomnia and risk of cardiovascular disease: a meta-analysis. *Eur J Prev Cardiol.* 2014;21(1):57-64.

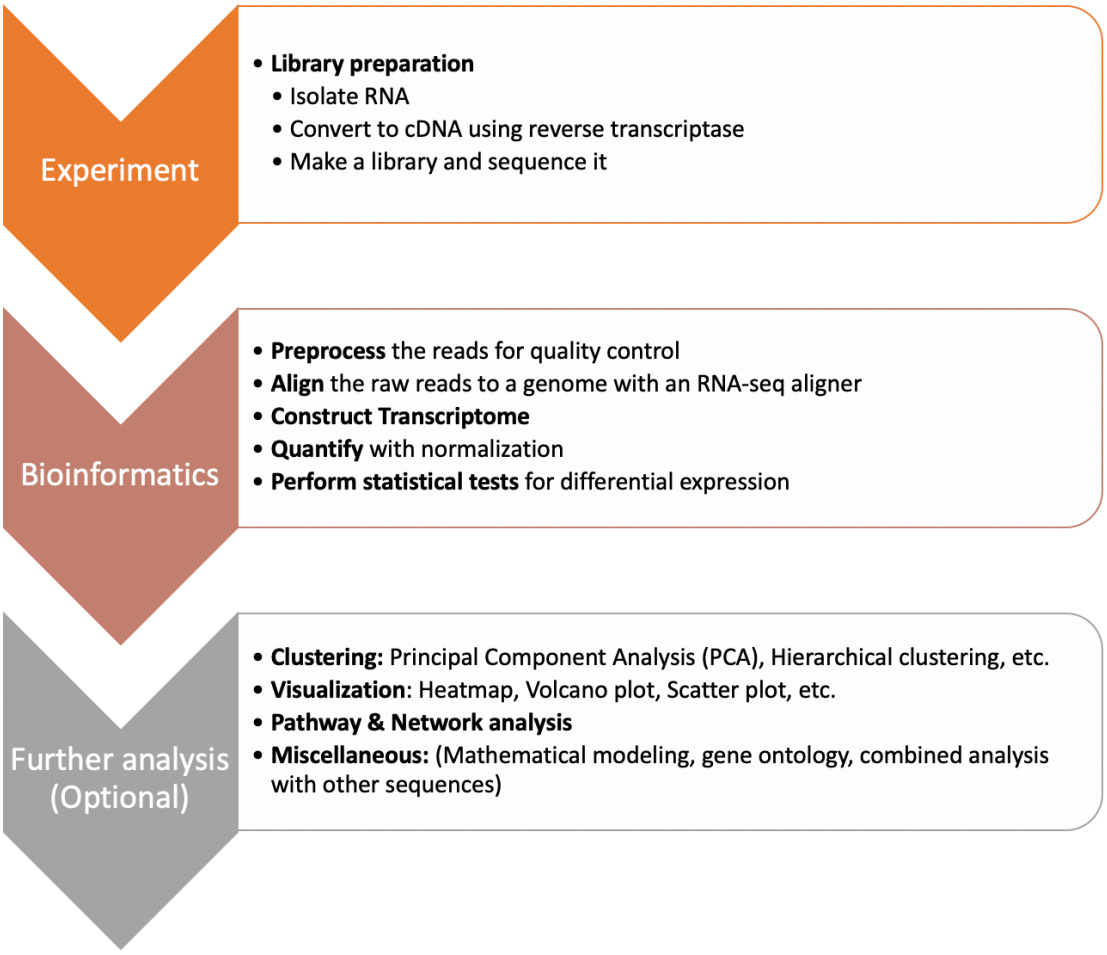
34. Dauvilliers Y. Insomnia in patients with neurodegenerative conditions. *Sleep Med.* 2007;8 Suppl 4:S27-34.

35. Taylor DJ, Pruiksma KE. Cognitive and behavioural therapy for insomnia (CBT-I) in psychiatric populations: a systematic review. *International review of psychiatry.* 2014;26(2):205-213.

36. Casamassimi A, Federico A, Rienzo M, Esposito S, Ciccodicola A. Transcriptome Profiling in Human Diseases: New Advances and Perspectives. *Int J Mol Sci.* 2017;18(8).

37. Hrdlickova R, Toloue M, Tian B. RNA-Seq methods for transcriptome analysis. *Wiley Interdiscip Rev RNA.* 2017;8(1).

Figure 1. RNA-sequencing Pipeline.



2. Whole Blood Transcriptome Analysis in Individuals with Insomnia: An RNA-Sequencing Study

2.1 Introduction

Insomnia—difficulty falling and staying sleep—is a prevalent sleep disorder in the United States affecting between 10% and 20% of the population.[1,2] The chronic sleep loss caused by insomnia can have many deleterious effects on health including effects on mood and anxiety disorders [3,4]; respiratory [5] and neurologic conditions [6,7]; chronic pain [1,8]; metabolic and cardiovascular disease [9,10]; and diabetes.[11,12] Insomnia has a significant economic burden, costing approximately $30 to 108 billion dollars per year, a figure based on health consequences, work absences, and reduced productivity.[13] Furthermore, it is well-documented that insomnia has a positive association with a broad range of negative health outcomes and decreased quality of life.[14] Treatment options remains elusive, and the failure to treat insomnia is related to the lack of information about the biological mechanisms underlying this sleep disorder. An increased understanding of insomnia pathophysiology is needed before effective therapies can be developed.

Given the well-established associations of specific circadian (Clock, PER1, PER2, PER3, and Bmal1), inflammatory (cytokines/immune NF-kB, TNF, IL-1B, IL-6, IL10) and growth (growth hormone releasing hormone, Ghrelin Rho and Leptin epidermal growth factor) genes with sleep regulation identified in genome-wide association studies (GWAS), question arise of how changes in the expression of these genes may be associated with insomnia. GWAS identified five new genes (MEIS1, HHEX, RHCG, IPO7 and TSNARE1) and one locus (encompassing MEIS1) associated with insomnia.[15,16] Specifically, the MEIS1 association signal identified was confirmed in all of UK Biobank samples (n=75,508 cases of frequent insomnia symptoms and 64,403 control).[17] Saus et al., reported a common variant, rs76481776, located in the precursor form of miR-182 (found on CLOCK gene), was significantly associated with insomnia in patients with major depression.[18] While these studies suggest a significant role for genes in insomnia, few studies have examined the expression of genes, specifically how gene-activity pathways relate to insomnia. Understanding patterns of gene expression, through transcriptomics, may provide insight into the multifaceted variations in traits that relate to insomnia, yet

these possible links remain largely underdetermined. The transcriptome offers important information about gene structure, expression and regulation. Understanding these processes may be essential for interpreting the elements of the genome and the role these elements may play in disease processes such as insomnia.

Multiple approaches were developed for measuring the transcriptome; in recent years, however, RNA sequencing was recognized as an optimal method for capturing the transcriptome dynamics across different tissue types.[19] More specifically, RNA-seq supports both the discovery and quantification of transcripts using a single high-throughput sequencing assay with high specificity and sensitivity,[20] yet few studies have used this method to determine gene-activity pathways that relate to sleep disturbance in clinical samples.

The aim of this study was to identify transcriptomic profiles by comparing RNA sequencing in the whole blood of individuals with and without insomnia. We hypothesized that there would be distinct, measurable differences in gene expression levels between individuals with and without insomnia.

2.2 Methods

To conduct this study, samples were acquired from a completed case-control study comparing 15 subjects with primary insomnia and 15- age and gender- matched "good sleeper" controls.[21] Participants between the ages of 25 and 50 and free of significant medical or psychiatric comorbidity were recruited from the community. Witnessed written informed consent was obtained from each participant at the University of Pennsylvania Clinical and Translational Research Center. Research diagnostic criteria for primary insomnia were as follows: self-reported complaint of difficulty initiating or maintaining sleep, waking up too early or nonrestorative sleep; daytime consequences as a result of the poor sleep; duration of at least 1 month; sleep disturbance is not secondary to a medical or psychiatric condition. Exclusion criteria included: significant medical or psychiatric illness (e.g. major depression or chronic pain) based on clinical history of psychiatric interview, evidence or diagnosis of sleep disorder other than insomnia as determined by clinical history or polysomnography screening, current shiftwork (working during the

evening or night shift), current use medications or over the counter products, or body mass index (BMI) >27. Questionnaires were be collected at the first visit for information on demographics, sleep quality, and psychological functioning including the Insomnia Severity Index (self-report estimate of sleep disturbance) [22], the Trait portion of the State-Trait Anxiety Inventory [23], and the Quick Inventory of Depressive Symptomatology – self-report version.[24] A detailed description of the parent case control study has been previously published.[25]

2.2.1 Sample Processing

Blood samples from study participants were collected in PAX gene blood RNA tubes at 0900 via an indwelling intravenous catheter. Blood tubes were stored at -80^{o}C until all samples had been collected and then were processed as a batch. RNA was extracted from blood samples using the PAXgene Blood miRNA kit (PreAnalytiX). Purified RNA samples were prepared with TruSeq Stranded Total RNA with Ribo-Zero Globin Kit (Qiagen). The library was sequenced with the 150 bp paired-ends on the Illumina NovaSeq-6000 platform.

2.2.2 Statistical Analysis

Quality control for the raw reads were performed with FASTQC version 0.11.8, and adapter trimming was done with BBDUK in BBTOOLS version 38.42. Spliced alignment for the reads to the human reference genome hg38 using STAR version 2.7.2a with default parameters. The percentage of mapped reads ranged from 86.2% to 93.1%. Differential gene expression analysis was performed using DESeq2 version 1.24.0. DESeq2 provides methods to test for differential expression by use of the negative binomial distribution and a shrinkage estimator for the distribution's variance. P-values are generated using a modified Fisher's exact test provided within DESeq2 and further corrected for multiple hypothesis testing using the Benjamini-Hochberg correction method to decrease the false discovery rate (FDR). Significant differential expressed genes were yielded at a FDR of 5% and a minimum fold-change of 1.3x. Pathway analysis was performed using QIAGEN's Ingenuity® Pathway Analysis (IPA) software (build version 389077M, released 2019-08-30, content version 27821452, Qiagen, Redwood City, CA).

2.3 Results

2.3.1 Demographic and Clinical Characteristics

Table I provides a summary of the demographic and clinical characteristics of the sample. Participants with insomnia (n=15) and good sleepers (n=15) were between the ages of 25-50 years (insomnia group Mean = 39.20, SD = 9.59 years; control group Mean = 36.07, SD = 7.96 years). Each group had 5 males and 10 females with participants identifying as white (66.67%), African American (26.67%), or Asian (6.67%). The insomnia group's mean (SD) Insomnia Severity Index (ISI) was 15.14 (2.37) and the good sleeper group's mean (SD) ISI was 1.73 (4.72). The Quick Inventory of Depressive Symptomatology mean (SD) was 5.67 (2.95) for individuals with insomnia and 1.27 (1.34) for good sleepers, and the State-Trait Anxiety Inventory (trait portion) was 30.6 (8.47) and 26.2 (4.84) for insomnia and good sleepers respectively. Both the ISI and the Quick Inventory of Depressive Symptomatology mean scores significantly differed between the insomnia group ($p < .001$, $r = .815$) and control group ($p < .001$, $r = .699$).

2.3.2 RNA-Seq and Network Analysis

We obtained an average of 31.2 million 150 base pair (bp) paired end reads per sample. Of those, an average of 89.7% of the read pairs mapped to the reference genome (hg38). We examined differential expression of aligned reads using DESeq2, and a gene was identified as differentially expressed if it showed a log fold change (LFC) ±0.50 and had a FDR p-adjusted < 0.05. The majority of the differentially expressed genes were identified as protein coding genes (Figure 2). Out of the 45,233 genes with nonzero total read count, there were 288 significantly differentially expressed genes between individuals with insomnia and normal controls (Figure 3). Top 3 significantly upregulated genes included LINC02224 (FC_{lg2}=5.289, P_{adj}= 0.012), DUX4L9 (FC_{lg2}=3.404, P_{adj}= 0.005) and TUSC3 (FC_{lg2}=3.352, P_{adj}= 0.033). Highest significantly down regulated genes were CTXN2 (FC_{lg2}=-2.861, P_{adj}= 0.031), CSMD1 (FC_{lg2}=-2.377, P_{adj}= 0.001) and SLC12A1(FC_{lg2}=-2.140, P_{adj}= 0.009).

To examine interactions of the differentially regulated genes with other gene products, we formed molecular networks in IPA using functional relationships. IPA illustrates non-deregulated genes with no color, up-regulated genes in red and down-regulated genes in green. 143 up regulated and 145 down regulated genes were used to generate IPA networks. Five significant networks were identified between participants with insomnia versus controls; however only we chose to focus only on the top 3 networks, which had IPA network scores above 40 (Table II). In this study, score of >40 was deemed as significant, which is equivalent to the p-value of 10^-40. Genes reported in the network are reported in Table III (upregulated) and Table IV (downregulated).

1 *Network 1:* The highest scoring candidate network centered on hematological diseases, hereditary disorders, organismal injuries and abnormalities (Figure 4). The top three up regulated genes were HBD (Log 2 Fold Change [FC_{lg2}] = 2.207, p_{adj} = 0.001), HBG1 (FC_{lg2} = 2.015, p_{adj} = 0.001) and HBB (FC_{lg2} = 1.838, p_{adj} = 0.004). The top downregulated gene was LY96 (FC_{lg2} = -0.731, p_{adj} = 0.017). This network consisted of multiple upregulated hub genes (IgG, HBA1/HBA2, IGHG2 and hemoglobin) and non-deregulated hubs (Immunoglobulin, Ige, Fc gamma receptor, BCR complex and NFkB complex).

2 *Network 2*: Network 2 genes were related to developmental disorder, metabolic disease and hereditary disorders (Figure 5). Of the 22 genes identified in the network, there were 8 mitochrondrial genes identified. The highest fold changes were observed in MUC5AC (FC_{lg2} = -1.550, p_{adj} = 0.024) and MUC2 (FC_{lg2} = 2.021, p_{adj} = 0.024). UTS2 (FC_{lg2} = -0.655, p_{adj} = 0.014) was identified in this network and has been previously associated with insomnia in military populations [26]. Key hub genes in this network include: growth hormone, cytokine, TACR1, STAT5a/b, ERK1/2 and Rock.

3 *Network 3*: This network encompassed genes related to cell cycle, cell mediated immune response and cellular development (Figure 6). Three genes had a fold change > 2.0: SIAH2 (FC_{lg2} = -2.140, p_{adj} = 0.009), PTPRU (FC_{lg2} = -2.138, p_{adj} = 0.002) and NCAPG (FC_{lg2} = 2.178, p_{adj} = 0.013). Eleven nonregulating complexes were identified including two histone complexes (Histone h3 and h4), TCR,

ERK, CD3, caspase, 26s Proteasome, Ubiquitin, Hdac, Rb, and E2f. SQSTM1, PRKDC and TCF3 were downregulated hub genes; CDC6 and CCNA2 were upregulated hubs.

2.4 Discussion

We determined that insomnia was associated with differential gene expression by comparing insomnia patients to matched controls who were good sleepers. Our study identified unique gene-pathways, such as inflammatory, ubiquitin and oxidative stress pathways, that relate to insomnia. These novel findings provide insight into the transcriptomic changes associated with insomnia and may shed light alterations of biological process in insomnia and mechanisms underlying the disorder. Network analysis identified 5 candidate networks with the top 3 networks further examined. The differentially expressed genes identified within the IPA networks are also known to be related to inflammatory, metabolic disease, immune function and mitochondrial dysfunction.

In network 1, nuclear factor- kB (NF-kB) complex was a hub gene with indirect associations to multiple dysregulated genes (e.g. TNFSF13B and LY96) and complexes (e.g., IgG and Ige), all of which play a role in inflammatory response (Figure 3). Representing a family of inducible transcription factors, NF-kB regulates a large array of genes involved in immune and inflammatory responses. [27] Sleep loss has been shown to enhance cortical NF-kB activation in mouse brains.[28] Additionally, NF-kB has been observed to be greater in the morning after sleep loss.[29] Indirectly associated with NF-kB complex in Network 1, upregulated gene LY96 encodes a protein that is associated with toll-like receptor 4 and confers interaction with lipopolysaccharides (LPS) on the surface of immune cells, causing the secretion of pro-inflammatory cytokines.[30] Reduced sleep time and fragmentation has been linked to increases in LPS signaling, increases in permeability of the epithelial barriers and can also cause alterations in gut microorganisms. [31] Research indicates that the immune and inflammatory pathways are implicated in the regulation of homeostatic functions, including sleep.[32] Additionally, sleep loss has been associated with the activation NF-kB molecular pathway resulting in inflammatory gene expression and the increased risk of inflammation-related diseases.[29] Thus, these outcomes support the finding of an association between

insomnia and inflammation. Longitudinal tracking of people at risk for insomnia is needed to determine the direction of this effect.

Previous research has associated mitochondrial dysfunction and oxidative stress to insomnia, an association that was further supported by Network 2. In this analysis, a smaller up-regulated network of mitochondrial genes (MT-ND1,2,4,5,6, MT-RNR1, MT-CYB) were associated with insomnia. Specifically, in this network, there was an upregulated Mitochondrial complex 1 and nicotinamide adenine dinucleotide (NADH) hydrogenase (Figure 4). Mitochondrial complex 1 is responsible for the first step in the electron transport process, the electron transfer from NADH to ubiquinone and then through several over complexes to provide energy for ATP synthesis.[33] The genes identified in this network are implicated in oxidative stress. Oxidative stress occurs when there is an imbalance in the oxidative-redox system, either because the production has increased or decreased, and this process plays an important role in the development of diseases such as autoimmune disorders, cancer, aging, cardiovascular, neurodegenerative diseases and psychiatric disorders.[34-37] Gulec et al., found that poor sleep quality led to higher levels of oxidative stress factors in individuals with primary insomnia.[38] Additionally, previous research has consistently concluded both that sleep may have a protective role against oxidative damage and that sleep loss is an oxidative challenge.[38,39] The STAT (signal transducer and activator of transcription) protein family cross talks with major cytokine signaling pathways like NF-kB. STAT 5a/b (nonregulatory, Network 2) is associated with growth hormone, cytokines and mitochondrial complex. In 24 healthy participants who underwent partial sleep loss (0300-0700 hours) there was an activation of STAT family proteins after sleep recovery.[40] Moreover, previous research has shown that STAT proteins act as a key signaling cascade mediating cytokine receptor derived signals and growth factors.[41]

UTS2 (upregulated, Network 2) encodes for the neuropeptides orexin A and B, which are involved in the regulation of sleep. Gill et al. (2017) identified 44 transcripts and 43 genes comparing military personnel with and without insomnia using microarray technology. Among the identified genes,

urotensin 2, which is involved in the regulation of rapid eye movement sleep, was downregulated 6-fold in individuals in insomnia.[26] Taken together, our findings in Network 2 support previous studies linking insomnia to mitochondrial dysfunction and suggests that interventions to address these biological markers may provide new avenues to treat insomnia. Protein ubiquitination regulates various biological processes protein, such as kinase activation and DNA repair, not only through targeting degradation of proteins by proteasome but also by regulation of protein function.[42] For example, protein ubiquitination is required for the removal of oxidized or misfolded proteins that accumulate as a result of neuronal function during wakefulness. Lack of clearance and removal of these oxidized or misfolded proteins has been linked to cognitive impairment in individuals with insomnia[26] and Alzheimer's disease.[43] Additionally, ubiquitination has been shown to play a role in insomnia phenotype through the activity of E3 ubiquitin ligase Cul3[44] and may disrupt circadian rhythm via Ubiquitin C-terminal hydrolase L1.[45] In Network 3, the ubiquitin complex has a direct association with SQSTM1 (also known as Ubiquitin-Binding Protein P62) which has been shown to play a regulatory function in ubiquitin-mediated proteolysis (Figure 7). SQSTM1 has also been associated with protein aggregation in neurodegenerative disorders of aging including Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and related tauopathies.[46,47] Comorbid insomnia and sleep disturbances are common in individuals with neurodegenerative conditions.[6,48] Although insomnia may be a secondary factor associated with the condition such as pain, depression or side effects of medication, insomnia may also be a direct consequence of the disease. FANCL (upregulated, Network 3) is a ubiquitin ligase that is a member of the Fanconi anemia complementation group. Fanconi anemia is a genetic disorder that is characterized by hypersensitivity to DNA crosslinking agents, increased chromosomal breakage and defective DNA repair [49]. The current findings in Network 3 extend previous research in the role of ubiquitination as a mediator of sleep-related gene activity in insomnia.

This study's limitations, including a relatively small samples size and a lack of diversity found in the general population of people with insomnia, may limit generalizability of the findings. However,

given the case control study design, we were able to examine age- and sex- matched group of individuals with and without insomnia, who did not use medications nor have any additional comorbidities. This enabled us to investigate the potential role of multiple genes in insomnia while limiting potential confounding factors. An important strength of the present study was the use of a novel and high throughput RNA-seq technology for the analysis of gene expression at the level of the whole transcriptome. Additionally, whole blood samples have been recognized to be a useful surrogate of gene expression in the peripheral and central nervous system and can be collected in a minimally invasive manner that is amenable for potential future diagnostic test development.[50] Therefore, while the present study provides important initial findings of differentially expressed genes for insomnia, subsequent investigations should aim to recruit larger groups of subjects representing the diverse demographic characteristics of the U.S. population. Our novel methodological approach can be used to understand the genetic mechanisms and dysregulation underlying insomnia.

2.5 Conclusion

To our knowledge, this was the first study to analyze transcriptomic gene expression variation using peripheral blood in individuals with insomnia. Our study identified potential differences in gene expression that distinguished subjects with insomnia from controls indicating differences in inflammation/immune, mitochondrial, metabolic, and inflammatory signaling pathways. Identification of dysregulated pathways in insomnia may provide novel insight to the pathophysiology of the disorder and the mechanisms through which insomnia negatively affects an individual's physical and mental health. Further research into the transcriptomics of insomnia is warranted to further understand the different pathways associated with insomnia and so that new avenues of treatment can be explored.

CITED LITERATURE

1. Taylor DJ, Mallory LJ, Lichstein KL, Durrence HH, Riedel BW, Bush AJ. Comorbidity of chronic insomnia with medical problems. *Sleep.* 2007;30(2):213-218.

2. Buysse DJ, Angst J, Gamma A, Ajdacic V, Eich D, Rossler W. Prevalence, course, and comorbidity of insomnia and depression in young adults. *Sleep.* 2008;31(4):473-480.

3. Johnson EO, Roth T, Breslau N. The association of insomnia with anxiety disorders and depression: exploration of the direction of risk. *Journal of psychiatric research.* 2006;40(8):700-708.

4. Ohayon MM, Roth T. Place of chronic insomnia in the course of depressive and anxiety disorders. *Journal of psychiatric research.* 2003;37(1):9-15.

5. George C. Perspectives on the management of insomnia in patients with chronic respiratory disorders. *Sleep.* 2000;23:S31-35; discussion S36-38.

6. Dauvilliers Y. Insomnia in patients with neurodegenerative conditions. *Sleep medicine.* 2007;8:S27-S34.

7. Malhotra RK. Neurodegenerative disorders and sleep. *Sleep medicine clinics.* 2018;13(1):63-70.

8. Wilson KG, Eriksson MY, Joyce L, Mikail SF, Emery PC. Major depression and insomnia in chronic pain. *The Clinical journal of pain.* 2002;18(2):77-83.

9. Javaheri S, Redline S. Insomnia and risk of cardiovascular disease. *Chest.* 2017;152(2):435-444.

10. Sofi F, Cesari F, Casini A, Macchi C, Abbate R, Gensini GF. Insomnia and risk of cardiovascular disease: a meta-analysis. *European journal of preventive cardiology.* 2014;21(1):57-64.

11. Morin CM, Drake CL, Harvey AG, et al. Insomnia disorder. *Nat Rev Dis Primers.* 2015;1:15026.

12. Vgontzas AN, Liao D, Pejovic S, Calhoun S, Karataraki M, Bixler EO. Insomnia with objective short sleep duration is associated with type 2 diabetes: a population-based study. *Diabetes care.* 2009;32(11):1980-1985.

13. Daley M, Morin CM, LeBlanc M, Gregoire JP, Savard J. The economic burden of insomnia: direct and indirect costs for individuals with insomnia syndrome, insomnia symptoms, and good sleepers. *Sleep.* 2009;32(1):55-64.

14. Wickwire EM, Shaya FT, Scharf SM. Health economics of insomnia treatments: The return on investment for a good night's sleep. *Sleep Med Rev.* 2016;30:72-82.

15. Hammerschlag AR, Stringer S, de Leeuw CA, et al. Genome-wide association analysis of insomnia complaints identifies risk genes and genetic overlap with psychiatric and metabolic traits. *Nat Genet.* 2017;49(11):1584-1592.

16. Lane JM, Liang J, Vlasac I, et al. Genome-wide association analyses of sleep disturbance traits identify new loci and highlight shared genetics with neuropsychiatric and metabolic traits. *Nat Genet.* 2017;49(2):274-281.

17. Lane JM, Jones SE, Dashti HS, et al. Biological and clinical insights from genetics of insomnia symptoms. *Nature Genetics.* 2019;51(3):387-393.

18. Saus E, Soria V, Escaramis G, et al. Genetic variants and abnormal processing of pre-miR-182, a circadian clock modulator, in major depression patients with late insomnia. *Human molecular genetics.* 2010;19(20):4017-4025.

19. Hrdlickova R, Toloue M, Tian B. RNA-Seq methods for transcriptome analysis. *Wiley Interdiscip Rev RNA.* 2017;8(1).

20. Wang B, Kumar V, Olson A, Ware D. Reviving the Transcriptome Studies: An Insight Into the Emergence of Single-Molecule Transcriptome Sequencing. *Frontiers in genetics.* 2019;10:384.

21. Gehrman P, Sengupta A, Harders E, Ubeydullah E, Pack AI, Weljie A. Altered diurnal states in insomnia reflect peripheral hyperarousal and metabolic desynchrony: a preliminary study. *Sleep.* 2018;41(5).

22. Bastien CH, Vallières A, Morin CM. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. *Sleep medicine.* 2001;2(4):297-307.

23. Kendall PC, Finch Jr A, Auerbach SM, Hooke JF, Mikulka PJ. The State-Trait Anxiety Inventory: a systematic evaluation. *Journal of consulting and clinical psychology.* 1976;44(3):406.

24. Rush AJ, Trivedi MH, Ibrahim HM, et al. The 16-Item Quick Inventory of Depressive Symptomatology (QIDS), clinician rating (QIDS-C), and self-report (QIDS-SR): a psychometric evaluation in patients with chronic major depression. *Biological psychiatry.* 2003;54(5):573-583.

25. Gilbert KS, Kark SM, Gehrman P, Bogdanova Y. Sleep disturbances, TBI and PTSD: Implications for treatment and recovery. *Clin Psychol Rev.* 2015;40:195-212.

26. Gill JM, Lee H, Baxter T, et al. A Diagnosis of Insomnia Is Associated With Differential Expression of Sleep-Regulating Genes in Military Personnel. *Biol Res Nurs.* 2015;17(4):384-392.

27. Liu T, Zhang L, Joo D, Sun SC. NF-kappaB signaling in inflammation. *Signal transduction and targeted therapy.* 2017;2.

28. Chen Z, Gardi J, Kushikata T, Fang J, Krueger JM. Nuclear factor-κB-like activity increases in murine cerebral cortex after sleep deprivation. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology.* 1999;276(6):R1812-R1818.

29. Irwin MR, Wang M, Ribeiro D, et al. Sleep loss activates cellular inflammatory signaling. *Biological psychiatry.* 2008;64(6):538-540.

30. McAleer JP, Vella AT. Understanding how lipopolysaccharide impacts CD4 T-cell immunity. *Critical reviews in immunology.* 2008;28(4):281-299.

31. Wu T, Yang L, Jiang J, et al. Chronic glucocorticoid treatment induced circadian clock disorder leads to lipid metabolism and gut microbiota alterations in rats. *Life sciences.* 2018;192:173-182.

32. Irwin MR, Opp MR. Sleep Health: Reciprocal Regulation of Sleep and Innate Immunity. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.* 2017;42(1):129-155.

33. Lenaz G, Fato R, Genova ML, Bergamini C, Bianchi C, Biondi A. Mitochondrial Complex I: structural and functional aspects. *Biochimica et biophysica acta.* 2006;1757(9-10):1406-1420.

34. Islam MT. Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. *Neurological research.* 2017;39(1):73-82.

35. Herken H, Uz E, Ozyurt H, Sogut S, Virit O, Akyol O. Evidence that the activities of erythrocyte free radical scavenging enzymes and the products of lipid peroxidation are increased in different forms of schizophrenia. *Mol Psychiatry.* 2001;6(1):66-73.

36. Savaş HA, Herken H, Yürekli M, et al. Possible role of nitric oxide and adrenomedullin in bipolar affective disorder. *Neuropsychobiology.* 2002;45(2):57-61.

37. Tsaluchidu S, Cocchi M, Tonello L, Puri BK. Fatty acids and oxidative stress in psychiatric disorders. *BMC psychiatry.* 2008;8(1):S5.

38. Gulec M, Ozkol H, Selvi Y, et al. Oxidative stress in patients with primary insomnia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry.* 2012;37(2):247-251.

39. Reimund E. The free radical flux theory of sleep. *Medical hypotheses.* 1994;43(4):231-233.

40. Irwin MR, Witarama T, Caudill M, Olmstead R, Breen EC. Sleep loss activates cellular inflammation and signal transducer and activator of transcription (STAT) family proteins in humans. *Brain, behavior, and immunity.* 2015;47:86-92.

41. Miklossy G, Hilliard TS, Turkson J. Therapeutic modulators of STAT signalling for human diseases. *Nature reviews Drug discovery.* 2013;12(8):611-629.

42. Sun L, Chen ZJ. The novel functions of ubiquitination in signaling. *Current opinion in cell biology.* 2004;16(2):119-126.

43. Swomley AM, Butterfield DA. Oxidative stress in Alzheimer disease and mild cognitive impairment: evidence from human data provided by redox proteomics. *Archives of toxicology.* 2015;89(10):1669-1680.

44. Stavropoulos N, Young MW. insomniac and Cullin-3 regulate sleep and wakefulness in Drosophila. *Neuron.* 2011;72(6):964-976.

45. Pfeffer M, Plenzig S, Gispert S, Wada K, Korf H-W, Von Gall C. Disturbed sleep/wake rhythms and neuronal cell loss in lateral hypothalamus and retina of mice with a spontaneous deletion in the ubiquitin carboxyl-terminal hydrolase L1 gene. *Neurobiology of aging.* 2012;33(2):393-403.

46. Boland B, Yu WH, Corti O, et al. Promoting the clearance of neurotoxic proteins in neurodegenerative disorders of ageing. *Nature reviews Drug discovery.* 2018;17(9):660-688.

47. Kuusisto E, Salminen A, Alafuzoff I. Ubiquitin-binding protein p62 is present in neuronal and glial inclusions in human tauopathies and synucleinopathies. *Neuroreport.* 2001;12(10):2085-2090.

48. Wulff K, Gatti S, Wettstein JG, Foster RG. Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. *Nature Reviews Neuroscience.* 2010;11(8):589.

49. Chaugule VK, Walden H. Specificity and disease in the ubiquitin system. *Biochemical Society Transactions.* 2016;44(1):212-227.

50. Dorsey SG, Renn CL, Griffioen M, et al. Whole blood transcriptomic profiles can differentiate vulnerability to chronic low back pain. *PLoS One.* 2019;14(5):e0216539.

TABLE I

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

	Insomnia (n = 15)	**Controls (n = 15)**	**Significance**
Age	39.20 (9.59)	36.07 (7.96)	r = .181, p = .339
Gender	66.7% Female	66.7% Female	N/A
Race & Ethnicity			χ^2 = 1.053, p = .789
White	60%	73.3%	
African American	33.3%	20%	
Asian	6.7%	6.7%	
Ethnicity			χ^2 = 1.154, p = .283
Hispanic	20%	6.7%	
Non-Hispanic	80%	93.3%	
ISI	11.00 (7.65)	5.60 (7.07)	r = .815, p < .001*
STAI	29.73 (6.50)	27.07 (7.55)	r = .321, p = .084
QIDS	4.53 (3.15)	2.40 (3.02)	r = .699, p < .001*

ISI- Insomnia Severity Index; STAI- Trait portion of the State-Trait Anxiety Inventory; QIDS- Quick Inventory of Depression Symptomatology- self-report version.

*Denotes significant differences from the control group p <.05. It is noted that there is no significant difference between insomnia and control group for STAI score.

TABLE II

NETWORK SCORES IDENTIFIED THROUGH IPA ANALYSIS

Insomnia vs. Control	
Network	**IPA Network Score**
Hematological Disease, Hereditary Disorder, Organismal Injury and Abnormalities	**46**
Developmental Disorder, Hereditary Disorder, Metabolic Disease	**43**
Cell Cycle, Cell mediated Immune Response, Cellular Development	**43**
Carbohydrate Metabolism, Post-Translational Modification, Protein Synthesis	33
Cell Cycle, Cellular Development, Cellular Growth and Proliferation	27

Note. Bolded font identifies the candidate networks selected for further analysis.

TABLE III

UPREGULATED GENES

Gene Name	HGNC ID	Chromosome	Gene Start (bp)	Gene End (bp)	Log2 Fold Change	p (adj)
LINC00676	HGNC:44394	13	109728282	109730007	5.288885353	0.012036143
DUS2	HGNC:26014	16	67987746	68079320	3.40491483	0.005308602
TRBV7-9	HGNC:12243	7	142529290	142529762	3.352342752	0.03341794
HIST1H3G	HGNC:4772	6	26269405	26271815	2.830062112	0.004518189
GMDS	HGNC:4369	6	1623806	2245605	2.445602431	0.006830877
AC010307.3*					2.426204622	0.043533771
AC245100.7*					2.318136821	0.0463067
HBA2	HGNC:4824	16	172876	173710	2.291195628	0.000390909
GRM4	HGNC:4596	6	34018645	34155622	2.259419138	0.046330676
HBD	HGNC:4829	11	5232678	5243657	2.206690521	0.000792763
SEMA6B	HGNC:10739	19	4542593	4559684	2.191788352	0.035254457
NCAPG	HGNC:24304	4	17810979	17844865	2.178374165	0.012633071
AC079325.1*					2.096430821	0.002422908
MUC2	HGNC:7512	11	1074875	1110511	2.02142334	0.023686481
HBG1	HGNC:4831	11	5248079	5249859	2.015283896	0.000673333
MT-ND6	HGNC:7462	MT	14149	14673	1.899302898	0.001687489
MTATP6P1	HGNC:44575	1	633696	634376	1.856745524	0.043535983
CD177	HGNC:30072	19	43353686	43363172	1.838370908	0.005777926
HBB	HGNC:4827	11	5225464	5229395	1.837845887	0.003569678
PTGES3P1	HGNC:43824	1	89104285	89104767	1.813186937	0.005280524
UBE2Q2P1	HGNC:37439	15	84526781	84571216	1.778350322	0.002432419
AL450405.1*					1.692562416	0.005192987
CD109	HGNC:21685	6	73695785	73828316	1.642233112	0.03492413

IGHG4	HGNC:5528	14	105620506	105626066	1.62177224	0.013602931
IGHG2	HGNC:5526	14	105639559	105644790	1.570021142	0.004683521
TCF3	HGNC:11633	19	1609290	1652615	1.511166166	0.047413972
FBN2	HGNC:3604	5	128257909	128659185	1.442788253	0.005811243
IGLV1-36	HGNC:5876	22	22431958	22432465	1.402047826	0.001399866
CXCR4	HGNC:2561	2	136114349	136118165	1.398616804	0.022126891
C1QA	HGNC:1241	1	22636628	22639678	1.387372516	0.00529848
MT-ND5	HGNC:7461	MT	12337	14148	1.373363842	0.001979008
ALDH5A1	HGNC:408	6	24494867	24537207	1.350050905	0.002702823
KRT8P39	HGNC:39873	12	68705634	68707066	1.321925723	0.000161469
AHRR	HGNC:346	5	321759	435110	1.306683599	0.010266167
AC025809.2*					1.297091564	0.046089458
AC244453.2*					1.24791336	0.005395399
C1QL3	HGNC:19359	10	16513734	16521879	1.234106416	0.032247412
SDC3	HGNC:10660	1	30869466	30908758	1.158953592	0.008708151
NPRL3	HGNC:14124	16	84271	138860	1.141127697	0.005719207
AC079325.2*					1.11877402	5.54E-05
AC109347.2*					1.101141783	0.038589091
GTF2I	HGNC:4659	7	74650231	74760692	1.064847027	0.048287782
MIR1244-2	HGNC:38321	5	118974586	118974670	1.055391357	0.010511323
TESPA1	HGNC:29109	12	54948015	54984762	1.039312324	0.00049107
MID1IP1	HGNC:20715	X	38801432	38806537	0.995466509	0.017026142
SAMD9L	HGNC:1349	7	93130056	93148385	0.994229979	5.62E-05
AC064805.1*					0.993150821	5.05E-05
CCNB2P1	HGNC:50850	7	7909300	7910449	0.981050166	0.014159408
SCOC	HGNC:20335	4	140257286	140385726	0.961200885	0.048287782
C1D	HGNC:29911	2	68041130	68110948	0.95694642	0.001687489
OR2A7	HGNC:8234	7	144257663	144264792	0.931209442	0.004261005

IGF2R	HGNC:5467	6	159969099	160113507	0.925848215	0.033526884
PCSK5	HGNC:8747	9	75890644	76362339	0.924298892	0.032623171
COMT	HGNC:2228	22	19941607	19969975	0.895514779	0.007004419
CALML4	HGNC:18445	15	68190705	68206110	0.88515094	0.049077709
CHMP7	HGNC:28439	8	23243637	23262000	0.855044193	0.014253924
DPY19L1P1	HGNC:22395	7	32580949	32761787	0.852704079	0.000555828
DFFA	HGNC:2772	1	10456522	10472529	0.850381052	0.005395399
CEP57L1	HGNC:21561	6	109095110	109163932	0.849149271	0.04023288
AC245060.5*					0.838563312	0.016121195
MS4A3	HGNC:7317	11	60056587	60071115	0.834782637	0.034521973
MT-RNR1	HGNC:7470	MT	648	1601	0.831256892	0.027632169
AP000240.1*					0.823549906	0.013324274
SLC25A53	HGNC:31894	X	104099214	104157009	0.815210933	0.013535901
ITPKB-IT1	HGNC:41349	1	226656640	226675067	0.813882848	0.005395399
SNORA73B	HGNC:10116	1	28508559	28508762	0.792706463	0.021387541
AL390728.6*					0.787384584	0.006619097
HPGD	HGNC:5154	4	174490175	174523154	0.770431864	0.023178748
HMOX2	HGNC:5014	16	4474690	4510347	0.766786355	0.000391895
TMEM110-MUSTN1*					0.754631818	0.005395399
HMGB1P5	HGNC:4997	3	22381819	22382929	0.740900181	0.027860156
GBP1	HGNC:4182	1	89052319	89065230	0.732797023	0.016459221
CLEC12A	HGNC:31713	12	9951316	9995694	0.729658307	0.031376721
HMCN2	HGNC:21293	9	130265882	130434123	0.725044236	0.028707796
BCAT1	HGNC:976	12	24810024	24949101	0.72357633	0.046183627
PPP2R5B	HGNC:9310	11	64917553	64934475	0.720103572	0.012034242
AC051619.8*					0.714607803	0.038381259
PRKDC	HGNC:9413	8	47773108	47960183	0.711979649	0.049077709
MT-ND2	HGNC:7456	MT	4470	5511	0.71194756	0.001387977

CLEC12B	HGNC:31966	12	10010627	10018796	0.705246662	0.006107333
MT-ND1	HGNC:7455	MT	3307	4262	0.703290417	0.009845316
GAMT	HGNC:4136	19	1397026	1401570	0.692132668	0.046089458
C17orf107	HGNC:37238	17	4899418	4902934	0.690820513	0.000139229
AC246787.2*					0.682018716	0.021263799
AC092794.1*					0.679478545	0.005395399
MT-ND4	HGNC:7459	MT	10760	12137	0.67583431	0.001839374
NBEA	HGNC:7648	13	34942287	35673022	0.671988154	0.007822616
AL022067.1*					0.662316482	0.010142861
SQSTM1	HGNC:11280	5	179806398	179838078	0.636309876	0.009223276
AL357054.4*					0.635731084	0.002339947
AC108866.1*					0.634894049	0.019431431
LRRC37A4P	HGNC:25479	17	45506741	45551537	0.633677437	0.04055775
ACAP2-IT1	HGNC:41426	3	195280723	195282741	0.629454906	0.004729187
AC132872.2*					0.624519099	0.036214538
RGCC	HGNC:20369	13	41457550	41470871	0.618275643	0.001566582
SNORA79B	HGNC:52222	14	20323179	20323326	0.616181426	0.048287782
AC063965.1*					0.616118035	0.003389908
AC004241.5*					0.61371773	0.024285468
MT-ATP8	HGNC:7415	MT	8366	8572	0.61365574	0.000792763
ATP6V0E2-AS1	HGNC:44180	7	149867697	149880610	0.613000912	0.001387977
ABRA	HGNC:30655	8	106759483	106770244	0.610687375	0.037105293
SNORA71D	HGNC:32657	20	38433865	38433998	0.608949231	0.01757152
HORMAD1	HGNC:25245	1	150698060	150720895	0.607557726	0.00049107
AL121603.2*					0.601805099	0.001829816
7SK*					0.589701046	0.023186228
HNRNPA1P48	HGNC:48778	16	51553436	51647132	0.58564543	0.02009259
GEMIN6	HGNC:20044	2	38751534	38785002	0.585311079	0.007308639

MIR4742	HGNC:41565	1	224398227	224398311	0.583629434	0.046089458
HIST1H2BB	HGNC:4751	6	26043277	26043657	0.577799705	0.036317257
MRTO4	HGNC:18477	1	19251805	19260128	0.575987839	0.02384503
ASXL3	HGNC:29357	18	33578219	33751195	0.575082915	0.012114193
AC007336.1*					0.571922579	0.00828741
AC008555.8*					0.571488394	0.021228821
AC055733.2*					0.568713444	0.023862362
RNU6-1177P	HGNC:48140	1	61852499	61852602	0.566670761	0.035320552
TMIGD2	HGNC:28324	19	4292232	4302431	0.559414716	0.028401822
CD300LD	HGNC:16848	17	74579365	74592283	0.556821087	0.045721415
C1QB	HGNC:1242	1	22652762	22661637	0.553203415	0.012482076
RN7SL333P	HGNC:46349	1	169859756	169860052	0.548765881	0.047491504
PRRG4	HGNC:30799	11	32829927	32858120	0.547600561	0.027774697
AC024475.1*					0.54321363	0.032229171
RN7SL674P	HGNC:46690	2	11584773	11585047	0.542773652	0.024909913
SOD1P3	HGNC:45136	8	125951861	125952314	0.541665808	0.001491342
LRRC75A	HGNC:32403	17	16441577	16492193	0.539763551	0.025005447
BX539320.1*					0.53818529	0.020920427
SUSD3	HGNC:28391	9	93058688	93085133	0.538098545	0.043195674
RGS18	HGNC:14261	1	192158462	192185815	0.537719672	0.032761781
CARMIL1	HGNC:21581	6	25279078	25620530	0.534979852	0.001566582
HIGD1A	HGNC:29527	3	42784298	42804531	0.531186135	0.046089458
SASH1	HGNC:19182	6	148272304	148552048	0.52870275	0.00724422
UBE2M	HGNC:12491	19	58555712	58558954	0.5279074	0.020920427
NT5C3AP1	HGNC:18530	4	117574512	117576174	0.5233739	0.010266167
THYN1	HGNC:29560	11	134248279	134253370	0.520967447	8.37E-05
CLSTN3	HGNC:18371	12	7129698	7158945	0.517799811	1.74E-05
SCARNA6	HGNC:32562	2	233288676	233288940	0.512093368	0.000699574

MT-CYB	HGNC:7427	MT	14747	15887	0.511531957	0.049077709
RTN4IP1	HGNC:18647	6	106570771	106629498	0.511439083	0.027115413
RAP1GAP	HGNC:9858	1	21596215	21669363	0.511324062	9.91E-06
C9orf24	HGNC:19919	9	34379019	34397810	0.508458982	0.009682448
FAM118A	HGNC:1313	22	45308968	45341955	0.507453725	0.003902482
PFKP	HGNC:8878	10	3066333	3137718	0.505769552	0.000997589
HBG2	HGNC:4832	11	5253188	5505605	0.503366223	0.000132159
CENPBD1P1	HGNC:28421	19	58573503	58605223	0.501244621	0.023224584

* Denotes non-protein coding gene

TABLE IV

DOWNREGULATED GENES

Gene Name	HGNC ID	Chromosome	Gene Start (bp)	Gene End (bp)	Log2 Fold Change	p (adj)
CTBP2P8	HGNC:45200	1	68161761	68163090	-2.681457155	0.030981129
CROCCP3	HGNC:29405	1	16467436	16499257	-2.376831555	0.000783625
SIAH2	HGNC:10858	3	150741125	150763477	-2.140316641	0.008639573
PTPRU	HGNC:9683	1	29236516	29326813	-2.137746803	0.002203225
RNU4-2	HGNC:10193	12	120291763	120291903	-2.054274646	0.019805623
LINC00402	HGNC:42732	13	74231457	74259976	-1.698477251	0.012633071
AP000812.2*					-1.672457758	0.026500088
LINC00243	HGNC:30956	6	30798654	30830659	-1.659169192	0.035729394
MUC5AC	HGNC:7515	11	1157953	1201138	-1.550476271	0.024129318
ARHGAP45	HGNC:17102	19	1065923	1086628	-1.452720984	0.046330676
FLCN	HGNC:27310	17	17212212	17237188	-1.383173537	0.017186599
MYOM2	HGNC:7614	8	2045046	2165552	-1.369036503	0.000364103
AC023355.1*					-1.300916472	0.005765746
RNU7-13P	HGNC:34109	1	184821428	184821489	-1.286007431	0.000831891
RHOBTB3	HGNC:18757	5	95713522	95824383	-1.226284067	0.010203666
ACHE	HGNC:108	7	100889994	100896974	-1.212529668	0.001703244
AC068775.1*					-1.100577984	0.017609415
AC008440.3*					-1.098168642	0.001829816
LYPD8	HGNC:44208	1	248739415	248755759	-0.969329872	0.040390809
NOSIP	HGNC:17946	19	49555468	49590262	-0.937835098	0.024612042
TRAV19	HGNC:12115	14	22007512	22008181	-0.878035544	0.011959826
LINC00540*					-0.811776007	0.025005447
AC244196.4*					-0.80601474	0.011475689

LY96	HGNC:17156	8	73991392	74029079	-0.730919384	0.016955712
AC123912.2*					-0.714143689	0.014410298
OPA1-AS1	HGNC:40421	3	193618609	193627337	-0.659240592	0.001387977
UTS2	HGNC:12636	1	7843083	7853512	-0.655321471	0.014253924
AC008739.5*					-0.637518321	0.011303215
OR2A42	HGNC:31230	7	144228244	144239605	-0.613772502	0.044353808
AC004889.1*					-0.609321253	0.024417665
AC012358.2*					-0.602735711	0.019153117
RFLNB	HGNC:28705	17	439978	445939	-0.592813586	5.63E-05
SFTPD-AS1	HGNC:51589	10	79968213	79973213	-0.589390656	0.031916962
CCNA2	HGNC:1578	4	121816444	121823933	-0.587753434	0.003888074
ESRP2	HGNC:26152	16	68229033	68238102	-0.587371744	0.017189934
LINC02224	HGNC:53093	5	44495099	44658569	-0.57766205	0.008708151
MAMSTR	HGNC:26689	19	48712725	48719725	-0.567301233	0.012130625
STX2	HGNC:3403	12	130789600	130839266	-0.557966452	0.048067446
PIH1D1	HGNC:26075	19	49446298	49453497	-0.529042417	0.042405333
AC004076.2*					-0.520470896	0.007428505
PPFIBP2	HGNC:9250	11	7513298	7657127	-0.513973574	0.01757152
CSMD1	HGNC:14026	8	2935353	4994972	-0.501157702	0.04178043
TRBV20-1	HGNC:12196	7	142626649	142627399	-0.499413222	0.03287649
SSPN	HGNC:11322	12	26121991	26299290	-0.493205307	0.019210241
OR10AA1P	HGNC:14989	1	158808399	158809335	-0.4916091	0.013253616
GALNT6	HGNC:4128	12	51351247	51392867	-0.487783631	0.018737658
FANCL	HGNC:20748	2	58159243	58241372	-0.472160415	0.036691724
AK5	HGNC:365	1	77282019	77559966	-0.463309725	0.016955712
AL031708.1*					-0.463284714	0.024657524
TRBV7-4	HGNC:12238	7	142455174	142455635	-0.452801703	0.038376638
OR2A1	HGNC:8229	7	144312464	144322668	-0.450972004	0.030000464

TRBV10-2	HGNC:12178	7	142424965	142425465	-0.44789044	0.045960243
SNORD3B-2	HGNC:33190	17	19063346	19064136	-0.439683051	0.043261017
TRAJ36	HGNC:12066	14	22505110	22505167	-0.438003071	0.030603316
C17orf51*					-0.437576403	0.012124162
WIPI2	HGNC:32225	7	5190196	5233840	-0.434302012	0.032229171
SWT1	HGNC:16785	1	185157080	185291781	-0.431690337	0.001387977
TMEM187	HGNC:13705	X	153972754	153983194	-0.427032145	0.010355041
ATP23	HGNC:29452	12	57906039	57959148	-0.425839989	0.002496885
RN7SL19P	HGNC:46035	8	70654578	70654883	-0.42535539	0.030123453
CTD-2201I18.1*					-0.42482123	0.005719207
SYNJ2	HGNC:11504	6	157981863	158099176	-0.414182905	0.005460987
PKP2	HGNC:9024	12	32790745	32896840	-0.412278433	0.009181728
AC022167.2*					-0.410517842	0.020402157
FOLR3	HGNC:3795	11	72114869	72139892	-0.40925929	0.004683521
LINC00189	HGNC:18461	21	29193480	29288205	-0.407993321	0.023611123
SULT1A2	HGNC:11454	16	28591943	28597050	-0.40588172	0.000550224
TRAC	HGNC:12029	14	22547506	22552156	-0.405299308	0.032604947
AL133406.2*					-0.383901331	0.043433506
LINC00399	HGNC:42728	13	109400696	109401641	-0.382834854	0.011797381
TNRC6C-AS1	HGNC:44360	17	78107398	78111799	-0.382409641	0.043069155
JCHAIN	HGNC:5713	4	70655541	70681817	-0.381616714	0.040905297
TRBV10-3	HGNC:12179	7	142544212	142544685	-0.381249021	0.036691724
ACTG1P20	HGNC:51500	1	27325329	27325796	-0.377480793	0.003888074
AL136038.4*					-0.370142985	0.002905047
AC245427.1*					-0.369091967	0.001258635
TMED7	HGNC:24253	5	115613210	115632992	-0.368468497	0.017186599
WLS	HGNC:30238	1	68098473	68233120	-0.365576074	0.007590411
DUX4L9	HGNC:33855	4	190021407	190022665	-0.357683006	0.036691724

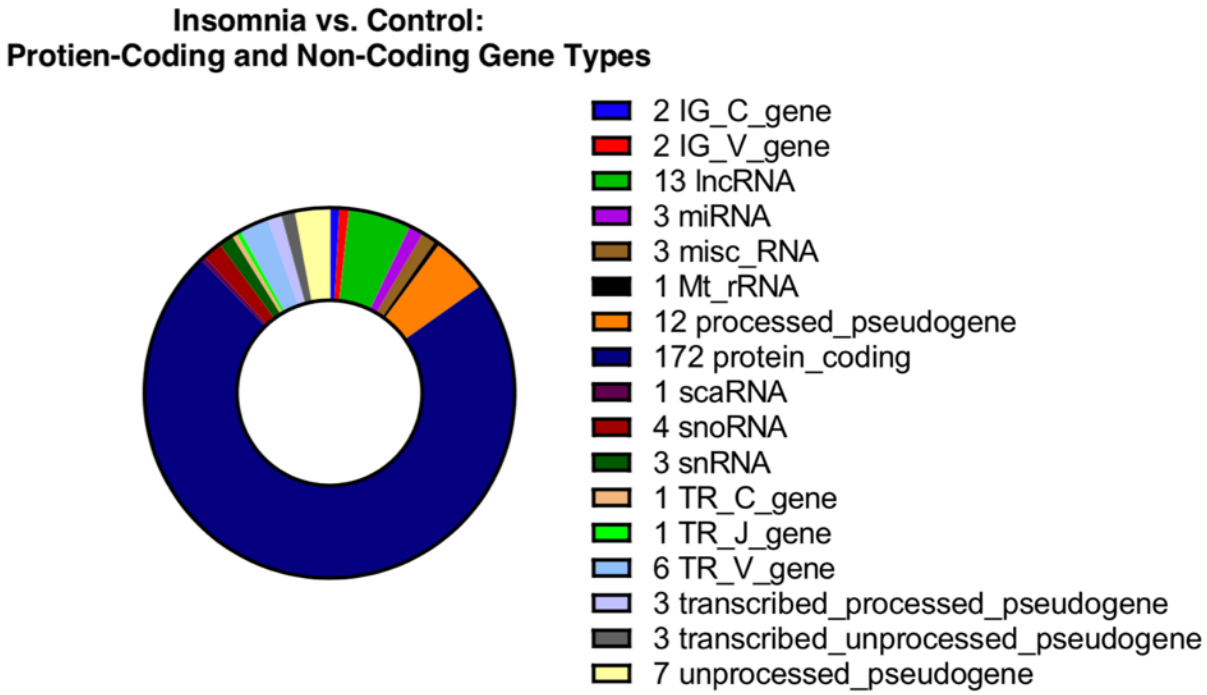
TACR1	HGNC:11526	2	75046463	75199520	-0.349886449	0.049077709
THAP11	HGNC:23194	16	67842320	67844195	-0.34639538	0.02019248
KAT8	HGNC:17933	16	31114489	31131393	-0.345990299	0.036938795
VN1R108P	HGNC:37432	20	25734264	25735093	-0.338820658	0.000920675
RAP2C	HGNC:21165	X	132203024	132219480	-0.337637701	0.037712556
NAV3	HGNC:15998	12	77324641	78213010	-0.331397756	0.030397274
CFAP43	HGNC:26684	10	104129888	104232362	-0.325965358	0.019683993
KLF17P1	HGNC:41521	12	62234390	62235515	-0.324121654	0.030518778
ALDOC	HGNC:418	17	28573115	28576948	-0.322334003	0.015305696
AL357054.1*					-0.317161704	0.04232214
HSPA7	HGNC:5240	1	161606291	161608217	-0.311781124	0.046638167
CARHSP1	HGNC:17150	16	8852942	8869012	-0.308717913	0.030045872
NEBL	HGNC:16932	10	20779973	21174187	-0.304574018	0.040390809
ALDH7A1	HGNC:877	5	126531200	126595362	-0.293952095	0.048573956
ZNF749	HGNC:32783	19	57435325	57447101	-0.291157605	0.037444765
ZRANB1	HGNC:18224	10	124942123	124988189	-0.2911576	0.03744476
P2RY14	HGNC:16442	3	151212117	151278542	-0.290525004	0.012940819
TPRA1	HGNC:30413	3	127571232	127598267	-0.288340687	0.015571056
IRF2BP1	HGNC:21728	19	45883608	45886141	-0.288103958	0.012077284
TUSC3	HGNC:30242	8	15417215	15766649	-0.278100943	0.012130625
AL596202.1*					-0.277094843	0.031127907
CLEC1B	HGNC:24356	12	9985642	10013424	-0.272899351	0.024909913
FKRP	HGNC:17997	19	46746046	46776988	-0.268940463	0.003902482
MGME1	HGNC:16205	20	17969018	17991122	-0.260632884	0.001387977
AC010978.1*					-0.260219396	0.011979684
LRRC63	HGNC:34296	13	46211943	46277366	-0.25662183	0.031916962
CDC6	HGNC:1744	17	40287879	40304657	-0.248142782	0.018530233
PDLIM4	HGNC:16501	5	132257696	132273454	-0.245709178	0.041267561

TNFSF13B	HGNC:11929	13	108251240	108308484	-0.243616492	0.000627092
FCGR1CP	HGNC:3615	1	143874793	143883575	-0.23789847	0.016308044
CYP51A1P3	HGNC:41991	6	148478693	148480763	-0.235821753	0.000253443
WDR25	HGNC:21064	14	100376418	100530303	-0.234515025	0.01320419
SSTR3	HGNC:11332	22	37204237	37212477	-0.231683276	0.041267561
TECPR1	HGNC:22214	7	98214624	98252232	-0.229081405	0.028988803
AL662795.2*					-0.22820981	0.02682473
DSCC1	HGNC:24453	8	119833976	119855894	-0.220103749	0.009279519
EPHX2	HGNC:3402	8	27490781	27545564	-0.219494337	0.04599398
IL27RA	HGNC:17290	19	14031762	14053218	-0.215650827	0.037798342
TPM3P7	HGNC:39170	2	25809925	25810656	-0.215351317	0.026567688
RPS23	HGNC:10410	5	82273320	82278396	-0.215009727	0.036381979
CHMP4BP1	HGNC:43616	14	55298644	55299231	-0.214360503	0.01507168
CARD17	HGNC:33827	11	105092469	105101431	-0.213071247	0.046089458
SLC12A1	HGNC:10910	15	48178438	48304078	-0.205359426	0.005308602
NINJ2	HGNC:7825	12	564296	663779	-0.204062752	0.012302969
MDS2	HGNC:29633	1	23581495	23640568	-0.203630019	0.012057807
COMMD8	HGNC:26036	4	47450787	47463702	-0.19779119	0.011617357
RPL7P11	HGNC:35667	1	8750430	8751087	-0.192092184	0.03492413
CTXN2	HGNC:31109	15	48178122	48203758	-0.189526034	0.046330676
GBP5	HGNC:19895	1	89258950	89272804	-0.182084627	0.025592842
TCTEX1D1	HGNC:26882	1	66752459	66779047	-0.179708045	0.01395624
THBS4	HGNC:11788	5	79991311	80083287	-0.176809627	0.004626251
DDX31	HGNC:16715	9	132592997	132670401	-0.175880147	0.02682473
TEX9	HGNC:29585	15	56244009	56445997	-0.175468637	0.03341794
GSG1L	HGNC:28283	16	27787528	28063714	-0.174278717	0.044216181
CPT1B	HGNC:2329	22	50568861	50578465	-0.174196501	0.043322702
IGLV3-21	HGNC:5905	22	22711689	22713203	-0.17337868	0.005559554

SPTSSA	HGNC:20361	14	34432788	34462240	-0.171618281	0.03341794
MIR548E	HGNC:35275	10	110988926	110989013	-0.168922775	0.033366223
ANXA9	HGNC:547	1	150982249	150995634	-0.168723751	0.018086962
DCTN5	HGNC:24594	16	23641466	23677472	-0.168139628	0.019805623
DEF6	HGNC:2760	6	35297818	35321771	-0.165894959	0.002420874
JUND	HGNC:6206	19	18279694	18281622	-0.164550697	0.006647673
PRDM5	HGNC:9349	4	120684919	120922870	-0.163076693	0.019210241
HMGB1P6	HGNC:4998	15	71164770	71165415	-0.161659546	0.01757152
PI4K2B	HGNC:18215	4	25160663	25279204	-0.160411246	0.037345748
GGACT	HGNC:25100	13	100530164	100589528	-0.159573389	0.047048603

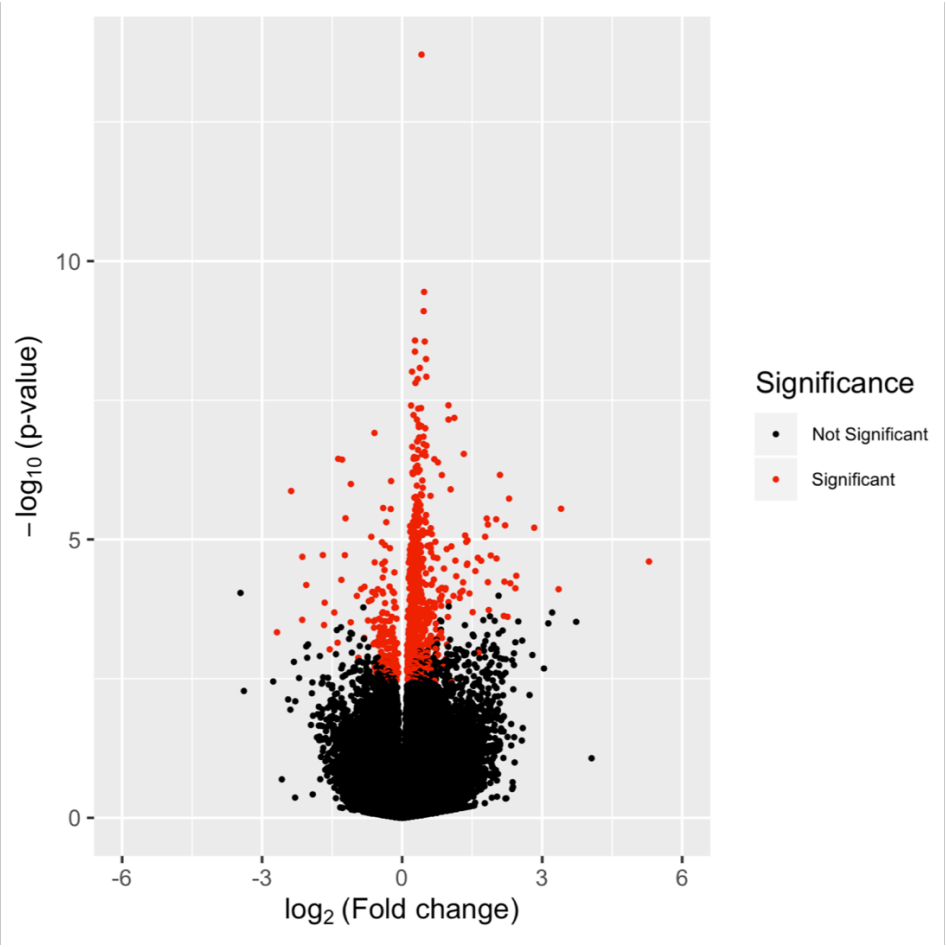
* Denotes non-protein coding gene

Figure 2. Pie chart of protein coding and non-protein coding gene.



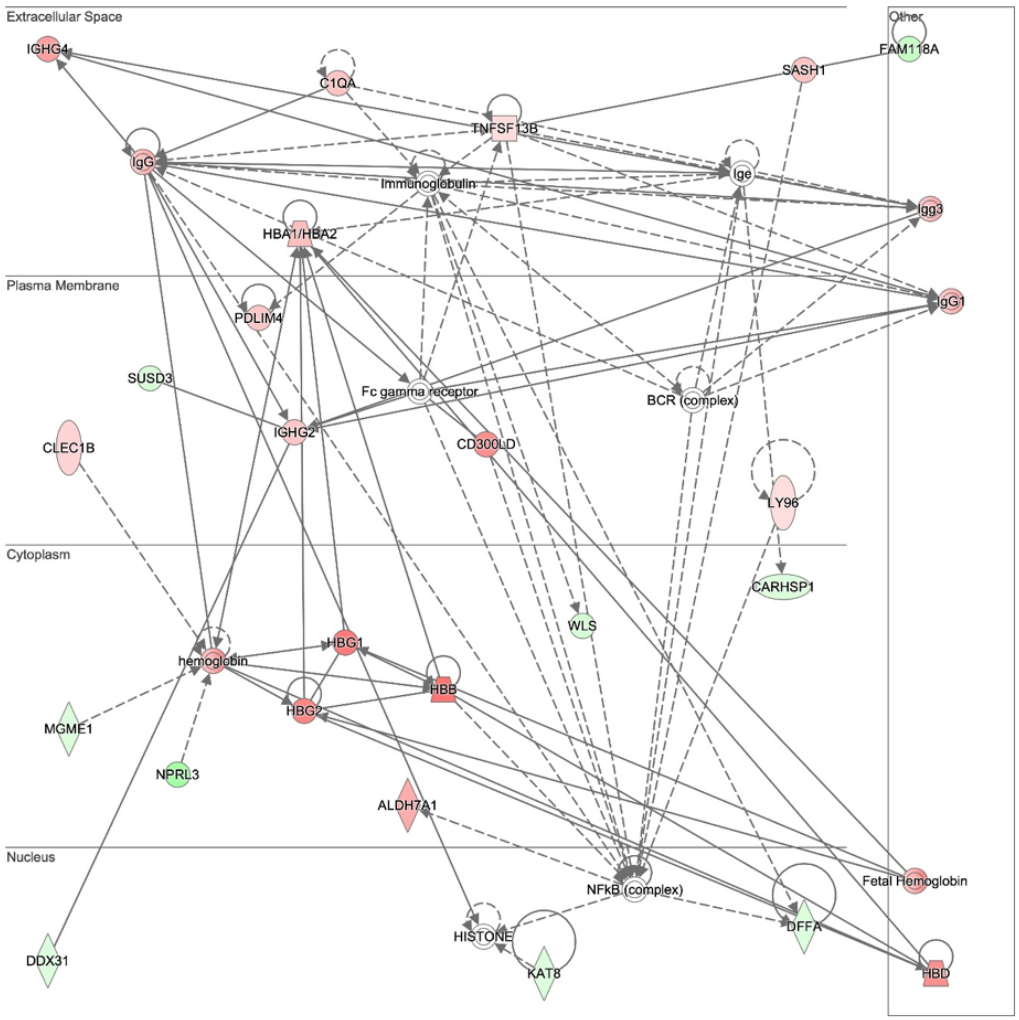
Pie chart depicting protein coding and non-protein coding gene types for indivudals with insomnia and controls.

Figure 3. Volcano plot of dysregualted genes identifyed by RNA-sequencing.



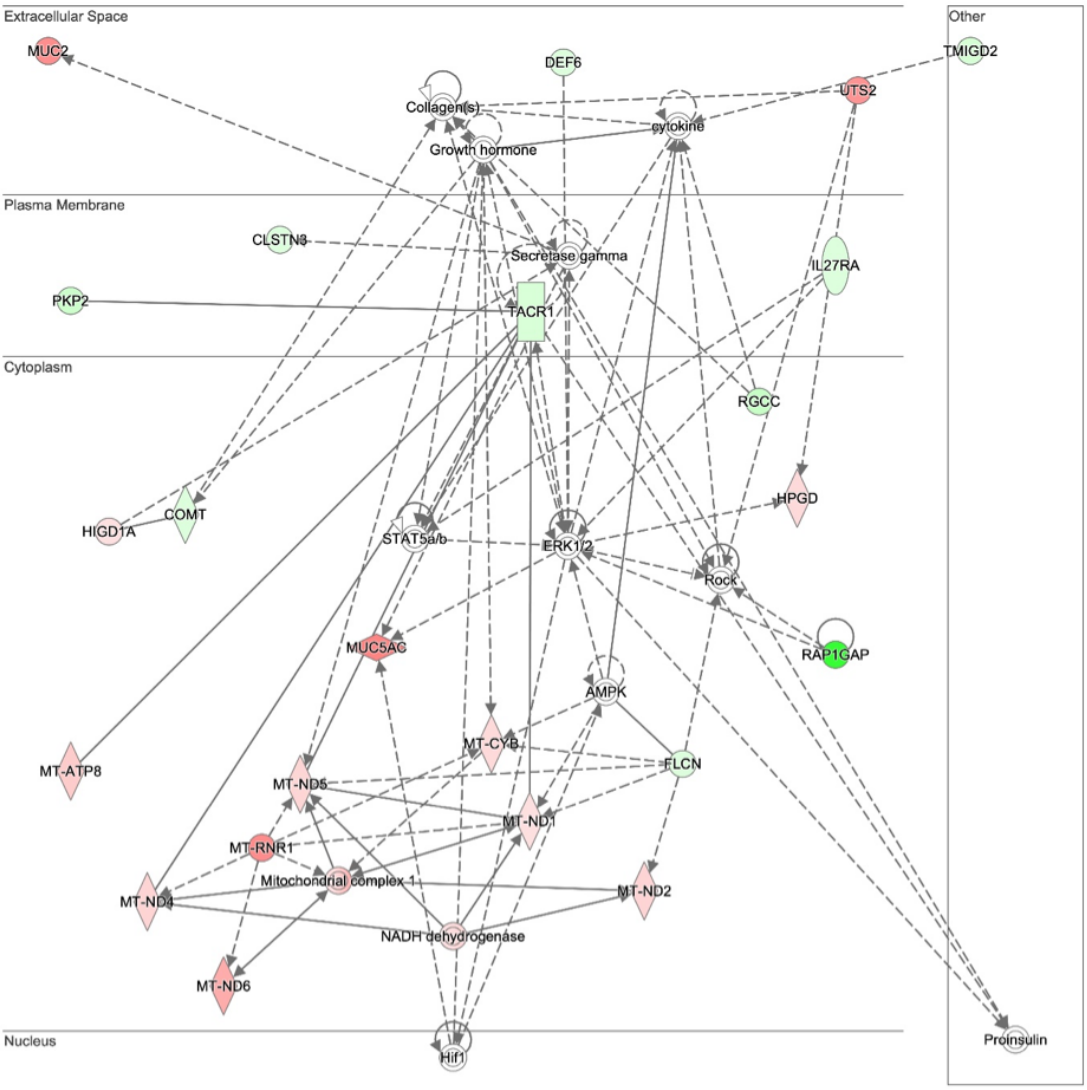
Black dot indicates non-significant gene and red indicates signficant gene yielded at a false discovery rate of 5% and a minimum fold-change of 1.3x.

Figure 4. IPA Network 1



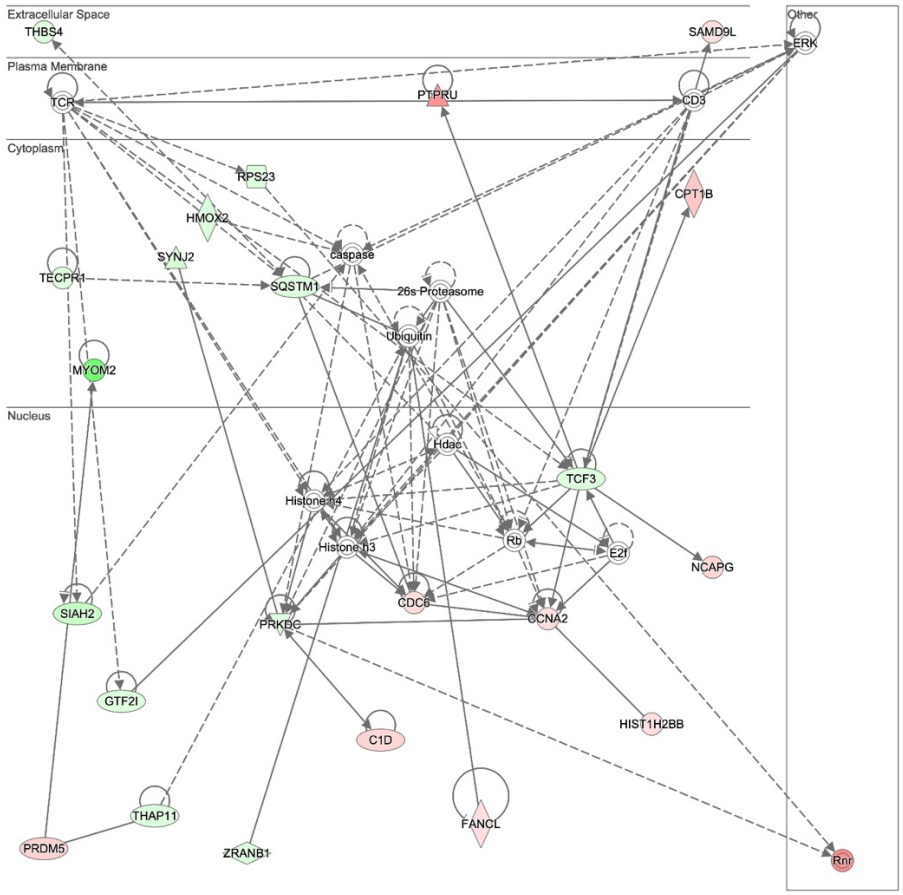
Green indicates that the gene is downregulated. Red indicates that the gene is upregulated, with increased color saturation representing more extreme measurement in the dataset. Solid lines represent interactions, non-targeting interactions, or correlations between chemicals, proteins, or RNA. Arrowed lines represent activation, causation, expression, localization, membership, modification, molecular cleavage, phosphorylation, protein-DNA interactions, protein-TNA interaction, regulation of binding, transcription. Shapes represent molecule type (double circle=complex/group; square=cytokine; diamond=enzyme; inverted triangle=kinase; triangle=phosphatase; oval=transcription regulator; trapezoid=transporter; circle=other).

Figure 5. IPA Network 2.



Green indicates that the gene is downregulated. Red indicates that the gene is upregulated, with increased color saturation representing more extreme measurement in the dataset. Solid lines represent interactions, non-targeting interactions, or correlations between chemicals, proteins, or RNA. Arrowed lines represent activation, causation, expression, localization, membership, modification, molecular cleavage, phosphorylation, protein-DNA interactions, protein-TNA interaction, regulation of binding, transcription. Shapes represent molecule type (double circle=complex/group; square=cytokine; diamond=enzyme; inverted triangle=kinase; triangle=phosphatase; oval=transcription regulator; trapezoid=transporter; circle=other).

Figure 6. IPA Network 3.



Green indicates that the gene is downregulated. Red indicates that the gene is upregulated, with increased color saturation representing more extreme measurement in the dataset. Solid lines represent interactions, non-targeting interactions, or correlations between chemicals, proteins, or RNA. Arrowed lines represent activation, causation, expression, localization, membership, modification, molecular cleavage, phosphorylation, protein-DNA interactions, protein-TNA interaction, regulation of binding, transcription. Shapes represent molecule type (double circle=complex/group; square=cytokine; diamond=enzyme; inverted triangle=kinase; triangle=phosphatase; oval=transcription regulator; trapezoid=transporter; circle=other).

3. A Review of the Glymphatic System and its Implications for Alzheimer's Disease and Brain Injury Research

3.1 Introduction

Sleep is a vital function, necessary for immunity, tissue growth and repair, and memory.[1] During sleep, the brain remains metabolically active. The brain lacks lymphatic vessels, and therefore metabolites and proteins, that are the waste products of brain metabolism, are transported into the interstitial fluid (ISF) and cerebrospinal fluid (CSF). A clearance pathway unique to the central nervous system—the glymphatic system—was first defined by Iliff et al. in 2012.[2] The glymphatic pathway is more active during slow wave sleep periods compared with wakefulness. This observation suggests that conditions that disrupt sleep (e.g., insomnia, brain injuries, neurodegeneration) could adverse effect glymphatic processes and impair brain function. Dysfunction of the glymphatic system may play a role in pathophysiology of neurodegenerative diseases, such as Alzheimer's disease (AD), in which the accumulation of amyloid beta (Aβ) contributes to progressive deficits.[3,4] Traumatic brain injury (TBI) pathophysiology may also impair glymphatic mechanisms, and it remains unknown whether impaired clearance of metabolites via the glymphatic pathway contributes to the deficits affecting patients with TBI.

A understanding of the glymphatic clearance of metabolic waste products is critical for achieving an improved understanding of conditions, such as AD and TBI, and for identifying novel biomarkers that measure disease development and progression. The purpose of this review was to examine knowledge about the functions of the glymphatic system during sleep versus wakefulness and define the implications for AD and TBI research.

3.2 What is the glymphatic system?

The glymphatic system, is a highly polarized and organized system of continuous cerebrospinal fluid (CSF) and interstitial fluid (ISF) interchange. Blood is separated from the CSF and ISF by the blood-brain-barrier (BBB) and the blood-CSF barrier. The BBB is comprised of blood vessel endothelial cells coupled by tight junctions, restricting macromolecules to move freely between the blood into the

brain parenchyma. The blood-CSF barrier is formed by tight junctions between the choroid plexus epithelial cells.[5,6] This continuous interchange of fluids is facilitated by CSF moving from the subarachnoid space to the perivascular space, also known as the Virchow-Robin space.[3,7] The influx of CSF is facilitated by a combination of arterial pulsatility, slow vasomotion, and the CSF pressure gradient. The CSF from the Virchow-Robin space continues to flow into the perivascular space around the arterioles, capillaries and venules. Astrocytic vascular endfeet expressing aquaporin-4 (AQP4), expressed highly in the brain vasculature and form the boundary of the perivascular space, transport the CSF from the perivascular space into the brain parenchyma. Glial AQP4 channels play an important role in the macroscopic waste clearance by enabling fluid exchanges between the CSF and the brain parenchyma interstitial fluid (Figure 7).[2,3,5,6] Each of these components make up the glymphatic system and enable the clearance of waste products from the CNS.

One of the first hallmark studies of the glymphatic system completed by Iliff and colleagues in 2012[2] demonstrated that a substantial amount of subarachnoid CSF cycles through the interstitial space in the brain using in-vivo two photon imaging of small florescent tracers. They further demonstrated in transgenic mouse models that when APQ4 channels in astrocytes are removed from the brain, there is an approximate 70% reduction in interstitial solute clearance, suggesting that APQ4 channels play a key role in the fluid flow of CSF and ISF in the brain. Moreover, fluorescently tagged amyloid beta protein, a metabolic waste product, was shown to be transported from the ISF an and then out of the brain, suggesting that the glymphatic flow plays a role in the clearance of metabolic products whose buildup is associated with neurodegenerative conditions. This then lead to a studies that showed brain glymphatic functions increase as much as two-fold times after sleep than during wakefulness. Xie et al., conducted in-vivo 2 photon imaging of glymphatic influx in the mouse cortex.[8] CSF influx in the wake state was reduced by 90% compared to a natural sleep and anesthetized state.[8] Additionally, sleep had a greater difference in glymphatic influx correlated with the volume fraction of interstitial space compared to wakefulness, from 22-24% to 13-15% respectively.[8] This observation indicates that the interstitial space volume increases in sleep, allowing for CSF-ICF exchange to clear metabolites and neurotoxic waste

products produced during wakefulness (Figure 8). When looking at these findings together, this information suggest that sleep enhances the metabolic waste clearance in the brain and that dysregulation of the glymphatic flow can lead to aggregation of waste products in the brain thus increasing risk of neurodegenerative conditions.

3.3 Neurodegeneration: Alzheimer's Disease

One disease that has shown an association with glymphatic is Alzheimer's disease, which is a neurodegenerative disorder characterized by loss of memory, difficulty completing familiar tasks, mood and behavioral changes.[9] Although progressive memory loss is a hallmark clinical symptom of AD, physiological alterations in the brain can be found as part of the normal aging process. Approximately 25 million individuals suffer from dementia,[10] of whom most suffer from Alzheimer's disease, with physiological alterations attributed to age-related accumulation of fibrillary protein aggregates such as extracellular senile plaques (amyloid B-Aβ) and intracellular neurofibrillary tangles (hyper-phosphorylated tau).[4] Aβ, which plays a physiologic role in synaptic regulation, neuronal growth and repair, is degraded and removed through multiple pathways. [11,12]

3.3.1 Sleep/wake disruption in AD

There is growing evidence that sleep disturbances may contribute to AD by facilitating accumulation of Aβ in the brain. Complaints of sleep changes and circadian disruption are common in AD and severity of disruption is parallel to the severity and progression of the disease.[13] Individuals experience difficulties falling asleep, maintaining sleep, fragmentation of sleep and endure excessive sleepiness during the day.[14] Sundowning syndrome, a sleep-wake cycle alteration, is a symptom in AD that manifests with increments of neuropsychiatric conditions in the late afternoon, evening or at night. The disruption in the circadian process alters sleep patterns, core body temperature[15] and imbalance in hormone secretion. In addition to physiologic alterations, exogenous factors, such as pain, pharmacologic drugs, cardiac, respiratory and gastrointestinal distress, may be responsible for sleep disturbances in patient with AD, especially when looking at long-term care.[16] In a study with 20 healthy individuals who

underwent one night of sleep deprivation, PET scans revealed significant Aβ after one night of sleep deprivation compared to rested sleep.[17]

3.3.2 Role of glymphatic system in AD

Prior research has proposed that the glymphatic pathway could play a substantial role in the net clearance of Aβ.[2] Transgenic mouse models exist that mimic a range of AD-related pathologies allowing researchers to investigate pathophysiology of the disease process. In a AQP4 knockout mouse model, impaired glymphatic function resulted in a 55% reduction in the clearance of radiolabeled $^{125}IA\beta_{1-40}$ compared with clearance in wildtype mice.[2] However, in 12-month old AQP4 gene knockout (AQP4$^{-/-}$) Aβ precursor protein/presenilin 1 (APP/PS1) transgenic mice, the deletion of AQP4 had a tendency to reduce neuroinflammation but exacerbate brain Aβ accumulation and cognitive dysfunction.[18] Moreover, paravascular Aβ clearance is regulated by sleep-wake cycle, which is often disrupted in patients with AD. Burfiend et al. conducted a human genetics study with 634 individuals with and without AD, five AQP4 single-nucleotide polymorphisms (SNPs) were associated with cognition and functional decline in AD.[19] Four of the five SNPs identified, rs3763043, rs3763040, rs9951307, and rs3875089, were associated with slow to rapid decline in cognitive function and functioning. Conversely, Rs335929 was associated in slower cognitive decline but faster functional decline.

Rainey-Smith and colleagues conducted a cross-sectional observational study in older adults with normal cognitive functioning where genetic variation of AQP4 (measured by SNP data from the AQP4 genomic region), was compared to overall sleep quality (measured by the Pittsburg Sleep Quality Index), sleep latency (time it takes to fall asleep), sleep duration and brain Aβ burden (measured via positron emission tomography (PET) using one of the following radiolabeled Aβ tracers).[20] The study revealed that several APQ4 SNPs moderated the effect of sleep latency (rs491148, rs9951307, rs7135406, rs3875089, rs151246) and duration(rs72878776, rs491148 and rs2339214) on Aβ burden. Findings suggest that variation in APQ4 moderates the associated between sleep and Aβ burden. Although further mechanistic studies are needed to understand the role of AQP4 and the glymphatic pathway in AD, these findings suggest that variations in the APQ4 gene can alter the clinical presentation and course of AD.

3.3.3 Biomarkers in AD

In AD, CSF biomarkers of Aβ plaque pathology and as well as neuronal phosphorylated-tau (p-tau) are associated with neurodegeneration and tau pathology. Biomarkers of Aβ plaque are measured with reduced concentration of CSF Aβ42 or Aβ42/40 ratio due to the selective retention of Aβ in brain tissue.[21] Moreover, total tau (T-tau) and p-tau can be measured in CSF. Although CSF based biomarkers for AD have been part of clinical practice, CSF collection (through lumbar puncture) is an invasive process, undoubtably making blood based testing the preferred method of biomarker measurement.

Several independent studies suggest that blood plasma Aβ42/40 ratio reflects Aβ pathology in the brain. Immunoassays for measurement of Aβ42 in plasma are based on the ultrasensitive digital enzyme-linked sandwich immunoassay (ELISA)[22,23] or the Luminex[24] techniques. Single-cell molecular (Simoa) Aβ42 assay breakthrough provides highly sensitive, precise and reproducible Aβ42 measurement in blood plasma and serum.[25] Simoa technology has also identified ultrasensitive measures for T-tau in plasma.[26] In addition, neurofilament light chain (NfL) is a biomarker that can be measured with immunoassays with CSF, plasma and serum. NfL reflect axonal damage in a variety of neurodegenerative diseases including AD, Huntington's disease and frontotemporal dementia.[27] Ashton et al., (2019) conducted a longitudinal study where blood plasma NfL (collected at two time points) was compared to participants post-mortem brain tissue neurofibrillary tangles.[28] 12 cognitively health individuals and 57 individuals with AD who participated in the study. Plasma NfL levels were significantly higher in AD compared to cognitively healthy subjects at both time points ($p<0.01$). Additionally, plasma NfL was elevated with increasing severity of AD, which was classified by post-mortem pathology and plasma NfL increased over time independent of post-mortem tangle pathology. Overall, these studies provide evidence that blood plasma measurement of Aβ and NfL may be an accessible clinical biomarker for neurodegeneration.

3.4 Neurovascular: Traumatic Brain Injury

Approximately 1.7 million individuals suffer a traumatic brain injury (TBI) each year.[29] Military personnel have seen an increase in TBI and it has been classified as the signature injury of troops serving in Iraq and Afghanistan.[30] TBI may be caused by a bump, blow or jolt to the head or a penetrating head

injury that disrupts normal brain function.[31] Clinical presentation and symptoms vary by severity of injury, ranging from loss of consciousness, headache, dizziness, nausea and even death. Furthermore, many initial features of the injury do not transfer into long-term consequences making biomarkers beneficial for diagnosis and treatment of the injury.[32]

3.4.1 Sleep/wake disruption in TBI

TBI is strongly associated with several sleep–wake disturbances with the most common being insomnia and hypersomnia.[33] Characterized by difficulty maintaining or initiating sleep, insomnia is reported in 30-60% of individuals following TBI of all severity.[34] In a study with 452 participants who suffered from mild to severe TBI, 50.2% of participants reported insomnia symptoms and 29.4% of individuals met the criteria for a insomnia diagnosis.[35] In deployed military personnel who completed self-reported measures of insomnia and clinical interviews, compared to controls who reported insomnia, individuals with single TBI were four times more likely and individuals with multiple TBI were 10 times more likely to exceed the threshold for clinical insomnia compared to military personnel with no history of TBI.[36] Hypersomnia, which refers to either excessive daytime sleepiness or excessive time spent sleeping, has been significantly prevalent in the acute period following TBI.[37,38] Individuals with more severe TBI injuries have a greater association with sleepiness. Although, sleepiness may improve in many patients overtime, it may persist in up to a quarter of patients 1 year after injury.[39] There is a large body of literature now that describes sleep-wake disturbances and disorders associated with TBI however there is limited research that provides clues to the underlying pathophysiology of changes.

3.4.2 Role of glymphatic system in TBI

Previous research has given insight that TBI disrupts the BBB resulting in diffusion of biomarkers into blood circulation.[40,41] Brain injury causes disruption of the tight junction complexes and the integrity of membranes resulting in increased paracellular permeability.[42] This causes oxidative stress and increases the production of proinflammatory mediators and there is an increase inflammatory cells to the injured brain parenchyma.[43] In a closed-skull moderate TBI model in adult male mice, glymphatic pathway function was reduced by 60% after TBI, with impairments persisting for at least 1 month after

injury.[44] Additionally, there was a loss of perivascular AQP4 polarization resulting in impairment of glymphatic pathway which is a key feature of astrocytes reacting to the injury.[44]

3.4.3 Biomarkers in TBI

Potential biomarkers for TBI have been well researched in in hopes that the cellular factors identified in the blood may be used to measure the severity of brain injury although limited biomarkers have advanced to the clinical setting. Some of the most widely studied blood biomarkers for TBI include protein biomarkers for neuronal cell body injury (Ubiquitin C-terminal hydrolase-L1 (UCH-L1), Neuron specific enolase (NSE)),[45-47] astroglial injury (glial fibrillary acidic protein (GFAP), S100B),[48-50] neuronal cell death (αII-spectrin breakdown products), axonal injury (Neurofilament (NF) proteins), white matter injury (Myelin basic protein (MBP)), post-injury neurodegeneration (total Tau and phospho-Tau) and post-injury autoimmune response (brain antigen-targeting autoantibodies).[51]

A new 4 marker panel, including copeptin, galectin-3, matrix metalloproteinases-9 (MMP-9), and occluding, was recently developed to aid in the diagnosis of mTBI and concussion. Copeptin, a glycopeptide, is a part of a precursor peptide of arginine vasopressin and neurophysin II produced by hypothalamic neurons and can be used as a marker for individual hemodynamic stress response.[52] The measurement of copeptin has been useful in diagnosis of diabetes insipidus[53] and monitoring of cardiovascular disease[54] and sepsis.[55] Galectin-3, a pro-inflammatory lectin family member, plays an important role in numerous biological activities including cell growth, apoptosis, pre-mRNA splicing, transformation, angiogenesis, inflammation, fibrosis and host defense. Previous studies have found galectin-3 as a potential diagnostic and prognostic biomarkers for certain types of heart disease (heart failure,[56] coronary artery disease, atrial fibrillation and hypertension),[57,58] renal disease[59] and cancer.[60] MMP-9, a collagenase enzyme, directly degrades extracellular matrix and activates cytokines and chemokines to regulate tissue remodeling.[61] Recent advances in research has highlighted MMP-9 as a potential biomarker for certain cancers[62] and cardiac remodeling.[63] Occludin, an integral tight junction protein, is a key structural component of the blood-brain-barrier.[64] Rapid loss of occluding contributes to BBB disruption and is frequently seen with ischemic stroke.[65,66] The 4 candidate plasma biomarker was

tested in adults who sustained a concussion within 24 hours of enrollment and controls, which comprised of uninjured patients and patients with orthopedic injury. All 4 markers had a significant increase when comparing injured individuals to healthy controls. Galectin-3 and occluding levels correlated positively in patients with mTBI but not in patients with orthopedic injury or uninjured controls, which may aid in the diagnosis of suspected mTBI.[67]

In addition to protein biomarkers, miRNA profiling may provide valuable information into the mechanisms of normal cellular function and disease process. miRNA, found in extracellular vesicles, are small non-coding RNAs that regulate gene expression at the post-transcriptional level.[68] They are highly expressed in the brain, are stable in peripheral biofluids such as blood, saliva, urine and can cross the BBB. miRNA profiling and detection provide valuable information on their essential roles in normal cellular function and disease, projecting their use in the clinical practice for the diagnosis and prognosis of several pathologies. Recent reports indicate a host of miRNA candidates that may be representative of CNS injury across a spectrum of severity and time. For example, in a cohort of male athletes who suffered a sports-related mTBI, five salivary miRNA based biomarkers (miR-27b-3p, let-7i-5p, miR-142-3p, miR-107, miR-135b-5p) were found to be significantly up related ($p < 0.05$) in athletes with mTBI.[69] Although candidate miRNA have been identified for TBI, it is critical to acknowledge that larger sample sizes, keeping in consideration of confounding factors such as injury severity, comorbid conditions and age, are needed for more robust and specific outcomes.

3.5 Conclusion and Future Direction

This review describes the evidence about the functions of the glymphatic symptom during sleep versus wakefulness and defines the implications for AD and TBI research. The structural and biophysiological components of the glymphatic system have brought new understanding to the highly organized transport and clearance system in the brain. The pathway entails a continuous CSF and ISF exchange that is facilitated by AQP4 channels on astrocytic end foot. Additionally, during sleep, the pathway is especially active, with a clearance rate of pathogenic aggregates nearly doubling. A clearer understanding of these underlying mechanisms can aid not only in the identification of individuals at risk

for sleep disorders but may also assist clinicians in providing preventive care for those at increased risk of dysregulated sleep following neurodegeneration and/or brain injury. Additional research is needed to elucidate the details of the human glymphatic system, identify factors that enhance or impair its function and pathological changes in the glymphatic function during human disease development and progression. These innovations could give rise to novel therapeutic targets, such as through biomarker detection, to further improve prognostic and diagnostic tools available in clinical settings. Since the time of ancient Greek philosophers, sleep has been the subject of numerous theories and with the building literature and technology, understanding the glymphatic system may be able to answer some of the fundamental questions of our brain mechanisms.

CITED LITERATURE

1. Saper CB. The neurobiology of sleep. *Continuum (Minneapolis, Minn).* 2013;19(1 Sleep Disorders):19-31.

2. Iliff JJ, Wang M, Liao Y, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Science translational medicine.* 2012;4(147):147ra111-147ra111.

3. Jessen NA, Munk AS, Lundgaard I, Nedergaard M. The Glymphatic System: A Beginner's Guide. *Neurochemical research.* 2015;40(12):2583-2599.

4. Wang J, Gu BJ, Masters CL, Wang Y-J. A systemic view of Alzheimer disease—insights from amyloid-β metabolism beyond the brain. *Nature reviews neurology.* 2017;13(10):612.

5. Iliff JJ, Thrane AS, Nedergaard M. Chapter 3 - The Glymphatic System and Brain Interstitial Fluid Homeostasis. In: Caplan LR, Biller J, Leary MC, et al., eds. *Primer on Cerebrovascular Diseases (Second Edition).* San Diego: Academic Press; 2017:17-25.

6. Plog BA, Nedergaard M. The Glymphatic System in Central Nervous System Health and Disease: Past, Present, and Future. *Annual review of pathology.* 2018;13:379-394.

7. Mestre H, Kostrikov S, Mehta RI, Nedergaard M. Perivascular spaces, glymphatic dysfunction, and small vessel disease. *Clinical science (London, England : 1979).* 2017;131(17):2257-2274.

8. Xie L, Kang H, Xu Q, et al. Sleep drives metabolite clearance from the adult brain. *Science.* 2013;342(6156):373-377.

9. Hyman BT, Phelps CH, Beach TG, et al. National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimer's & dementia.* 2012;8(1):1-13.

10. Qiu C, Kivipelto M, von Strauss E. Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. *Dialogues in clinical neuroscience.* 2009;11(2):111-128.

11. J Baranello R, L Bharani K, Padmaraju V, et al. Amyloid-beta protein clearance and degradation (ABCD) pathways and their role in Alzheimer's disease. *Current Alzheimer Research.* 2015;12(1):32-46.

12. Parihar MS, Brewer GJ. Amyloid-β as a modulator of synaptic plasticity. *Journal of Alzheimer's Disease.* 2010;22(3):741-763.

13. Peter-Derex L, Yammine P, Bastuji H, Croisile B. Sleep and Alzheimer's disease. *Sleep medicine reviews.* 2015;19:29-38.

14. Weldemichael DA, Grossberg GT. Circadian rhythm disturbances in patients with Alzheimer's disease: a review. *International journal of Alzheimer's disease.* 2010;2010.

15. Klegeris A, Schulzer M, Harper DG, McGeer PL. Increase in core body temperature of Alzheimer's disease patients as a possible indicator of chronic neuroinflammation: a meta-analysis. *Gerontology.* 2007;53(1):7-11.

16. Martin JL, Ancoli-Israel S. Sleep disturbances in long-term care. *Clinics in geriatric medicine.* 2008;24(1):39-50.

17. Shokri-Kojori E, Wang G-J, Wiers CE, et al. β-Amyloid accumulation in the human brain after one night of sleep deprivation. *Proceedings of the National Academy of Sciences.* 2018;115(17):4483-4488.

18. Xu Z, Xiao N, Chen Y, et al. Deletion of aquaporin-4 in APP/PS1 mice exacerbates brain Aβ accumulation and memory deficits. *Molecular neurodegeneration.* 2015;10(1):58.

19. Burfeind KG, Murchison CF, Westaway SK, et al. The effects of noncoding aquaporin-4 single-nucleotide polymorphisms on cognition and functional progression of Alzheimer's disease. *Alzheimer's & Dementia: Translational Research & Clinical Interventions.* 2017;3(3):348-359.

20. Rainey-Smith SR, Mazzucchelli GN, Villemagne VL, et al. Genetic variation in Aquaporin-4 moderates the relationship between sleep and brain Aβ-amyloid burden. *Translational psychiatry.* 2018;8(1):1-11.

21. Zetterberg H. Applying fluid biomarkers to Alzheimer's disease. *American Journal of Physiology-Cell Physiology.* 2017;313(1):C3-C10.

22. Vanderstichele H, Kerschaver EV, Hesse C, et al. Standardization of measurement of β-amyloid (1-42) in cerebrospinal fluid and plasma. *Amyloid.* 2000;7(4):245-258.

23. Mehta PD, Pirttilä T, Mehta SP, Sersen EA, Aisen PS, Wisniewski HM. Plasma and cerebrospinal fluid levels of amyloid β proteins 1-40 and 1-42 in Alzheimer disease. *Archives of neurology.* 2000;57(1):100-105.

24. Hansson O, Zetterberg H, Vanmechelen E, et al. Evaluation of plasma Aβ40 and Aβ42 as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment. *Neurobiology of aging.* 2010;31(3):357-367.

25. Zetterberg H, Mörtberg E, Song L, et al. Hypoxia due to cardiac arrest induces a time-dependent increase in serum amyloid β levels in humans. *PloS one.* 2011;6(12):e28263.

26. Randall J, Mörtberg E, Provuncher GK, et al. Tau proteins in serum predict neurological outcome after hypoxic brain injury from cardiac arrest: results of a pilot study. *Resuscitation.* 2013;84(3):351-356.

27. Zetterberg H. Blood-based biomarkers for Alzheimer's disease—an update. *Journal of neuroscience methods.* 2019;319:2-6.

28. Ashton NJ, Leuzy A, Lim YM, et al. Increased plasma neurofilament light chain concentration correlates with severity of post-mortem neurofibrillary tangle pathology and neurodegeneration. *Acta Neuropathologica Communications.* 2019;7(1):5.

29. Faul M, Wald MM, Xu L, Coronado VG. Traumatic brain injury in the United States; emergency department visits, hospitalizations, and deaths, 2002-2006. 2010.

30. Roozenbeek B, Maas AI, Menon DK. Changing patterns in the epidemiology of traumatic brain injury. *Nature Reviews Neurology.* 2013;9(4):231.

31. Blennow K, Hardy J, Zetterberg H. The neuropathology and neurobiology of traumatic brain injury. *Neuron.* 2012;76(5):886-899.

32. McKee AC, Daneshvar DH. The neuropathology of traumatic brain injury. *Handbook of clinical neurology.* 2015;127:45-66.

33. Sandsmark DK, Elliott JE, Lim MM. Sleep-Wake Disturbances After Traumatic Brain Injury: Synthesis of Human and Animal Studies. *Sleep.* 2017;40(5).

34. Ouellet M-C, Beaulieu-Bonneau S, Morin CM. Sleep-wake disturbances after traumatic brain injury. *The Lancet Neurology.* 2015;14(7):746-757.

35. Ouellet M-C, Beaulieu-Bonneau S, Morin CM. Insomnia in patients with traumatic brain injury: frequency, characteristics, and risk factors. *The Journal of head trauma rehabilitation.* 2006;21(3):199-212.

36. Bryan CJ. Repetitive traumatic brain injury (or concussion) increases severity of sleep disturbance among deployed military personnel. *Sleep.* 2013;36(6):941-946.

37. Baumann CR, Werth E, Stocker R, Ludwig S, Bassetti CL. Sleep-wake disturbances 6 months after traumatic brain injury: a prospective study. *Brain : a journal of neurology.* 2007;130(Pt 7):1873-1883.

38. Sommerauer M, Valko PO, Werth E, Baumann CR. Excessive sleep need following traumatic brain injury: a case–control study of 36 patients. *Journal of sleep research.* 2013;22(6):634-639.

39. Watson NF, Dikmen S, Machamer J, Doherty M, Temkin N. Hypersomnia following traumatic brain injury. *Journal of Clinical Sleep Medicine.* 2007;3(04):363-368.

40. Plog BA, Dashnaw ML, Hitomi E, et al. Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2015;35(2):518-526.

41. Dadas A, Janigro D. The role and diagnostic significance of cellular barriers after concussive head trauma. *Concussion (London, England).* 2018;3(1):Cnc53.

42. Chodobski A, Zink BJ, Szmydynger-Chodobska J. Blood–brain barrier pathophysiology in traumatic brain injury. *Translational stroke research.* 2011;2(4):492-516.

43. Neuwelt E, Abbott NJ, Abrey L, et al. Strategies to advance translational research into brain barriers. *The Lancet Neurology.* 2008;7(1):84-96.

44. Ren Z, Iliff JJ, Yang L, et al. 'Hit & Run'model of closed-skull traumatic brain injury (TBI) reveals complex patterns of post-traumatic AQP4 dysregulation. *Journal of Cerebral Blood Flow & Metabolism.* 2013;33(6):834-845.

45. Olivecrona Z, Bobinski L, Koskinen LO. Association of ICP, CPP, CT findings and S-100B and NSE in severe traumatic head injury. Prognostic value of the biomarkers. *Brain Inj.* 2015;29(4):446-454.

46. Rodriguez-Rodriguez A, Egea-Guerrero JJ, Gordillo-Escobar E, et al. S100B and Neuron-Specific Enolase as mortality predictors in patients with severe traumatic brain injury. *Neurol Res.* 2016;38(2):130-137.

47. Woertgen C, Rothoerl RD, Holzschuh M, Metz C, Brawanski A. Comparison of serial S-100 and NSE serum measurements after severe head injury. *Acta neurochirurgica.* 1997;139(12):1161-1164; discussion 1165.

48. Thelin EP, Johannesson L, Nelson D, Bellander BM. S100B is an important outcome predictor in traumatic brain injury. *J Neurotrauma.* 2013;30(7):519-528.

49. Goyal A, Failla MD, Niyonkuru C, et al. S100b as a prognostic biomarker in outcome prediction for patients with severe traumatic brain injury. *J Neurotrauma.* 2013;30(11):946-957.

50. Mercier E, Boutin A, Lauzier F, et al. Predictive value of S-100beta protein for prognosis in patients with moderate and severe traumatic brain injury: systematic review and meta-analysis. *BMJ (Clinical research ed).* 2013;346:f1757.

51. Gan ZS, Stein SC, Swanson R, et al. Blood Biomarkers for Traumatic Brain Injury: A Quantitative Assessment of Diagnostic and Prognostic Accuracy. *Front Neurol.* 2019;10:446.

52. Morgenthaler NG, Struck J, Jochberger S, Dunser MW. Copeptin: clinical use of a new biomarker. *Trends in endocrinology and metabolism: TEM.* 2008;19(2):43-49.

53. Fenske W, Refardt J, Chifu I, et al. A Copeptin-Based Approach in the Diagnosis of Diabetes Insipidus. *N Engl J Med.* 2018;379(5):428-439.

54. Giannopoulos G, Deftereos S, Panagopoulou V, et al. Copeptin as a biomarker in cardiac disease. *Current topics in medicinal chemistry.* 2013;13(2):231-240.

55. Struck J, Morgenthaler NG, Bergmann A. Copeptin, a stable peptide derived from the vasopressin precursor, is elevated in serum of sepsis patients. *Peptides.* 2005;26(12):2500-2504.

56. Amin HZ, Amin LZ, Wijaya IP. Galectin-3: a novel biomarker for the prognosis of heart failure. *Clujul medical.* 2017;90(2):129.

57. Zhong X, Qian X, Chen G, Song X. The role of galectin-3 in heart failure and cardiovascular disease. *Clinical and experimental pharmacology & physiology.* 2019;46(3):197-203.

58. Suthahar N, Meijers WC, Silljé HH, Ho JE, Liu F-T, de Boer RA. Galectin-3 activation and inhibition in heart failure and cardiovascular disease: an update. *Theranostics.* 2018;8(3):593.

59. Chen S-C, Kuo P-L. The role of galectin-3 in the kidneys. *International journal of molecular sciences.* 2016;17(4):565.

60. Dong R, Zhang M, Hu Q, et al. Galectin-3 as a novel biomarker for disease diagnosis and a target for therapy (Review). *International journal of molecular medicine.* 2018;41(2):599-614.

61. Yabluchanskiy A, Ma Y, Iyer RP, Hall ME, Lindsey ML. Matrix metalloproteinase-9: Many shades of function in cardiovascular disease. *Physiology (Bethesda, Md).* 2013;28(6):391-403.

62. Huang H. Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. *Sensors (Basel, Switzerland).* 2018;18(10).

63. Halade GV, Jin Y-F, Lindsey ML. Matrix metalloproteinase (MMP)-9: a proximal biomarker for cardiac remodeling and a distal biomarker for inflammation. *Pharmacology & therapeutics.* 2013;139(1):32-40.

64. Pan R, Yu K, Weatherwax T, Zheng H, Liu W, Liu KJ. Blood Occludin Level as a Potential Biomarker for Early Blood Brain Barrier Damage Following Ischemic Stroke. *Sci Rep.* 2017;7:40331.

65. Liu J, Jin X, Liu KJ, Liu W. Matrix metalloproteinase-2-mediated occludin degradation and caveolin-1-mediated claudin-5 redistribution contribute to blood–brain barrier damage in early ischemic stroke stage. *Journal of Neuroscience.* 2012;32(9):3044-3057.

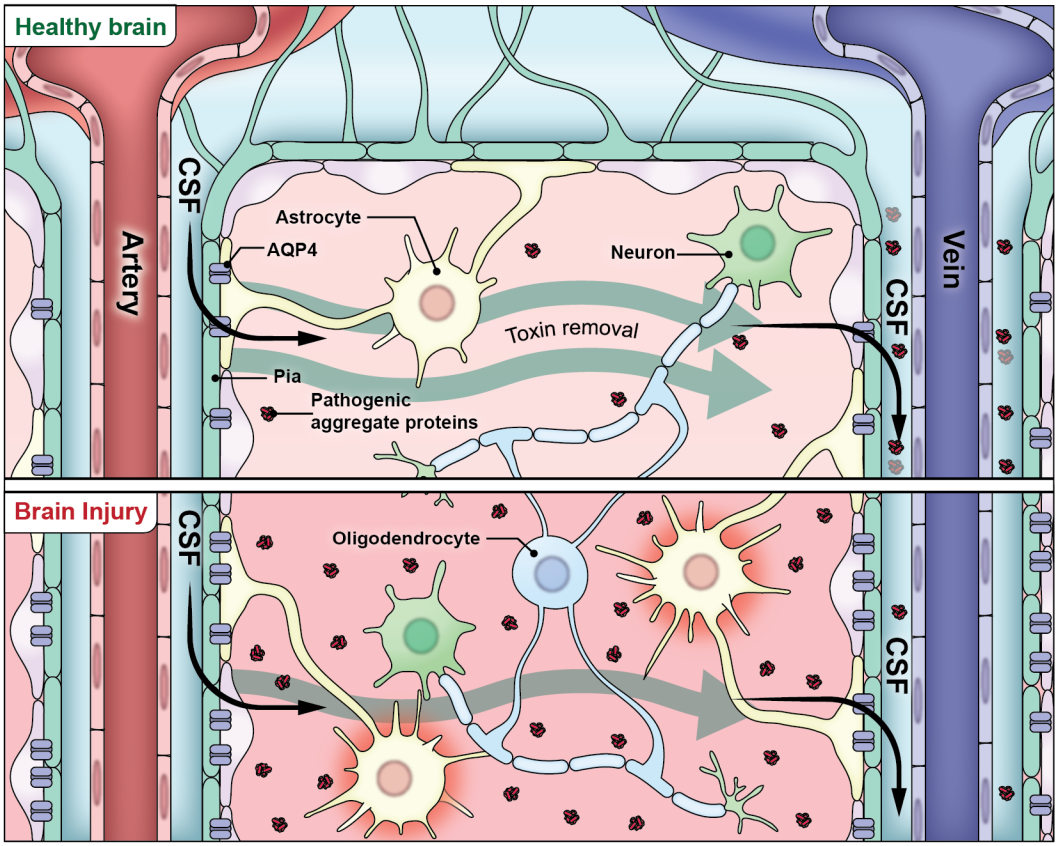
66. Ren C, Li N, Wang B, et al. Limb ischemic perconditioning attenuates blood-brain barrier disruption by inhibiting activity of MMP-9 and occludin degradation after focal cerebral ischemia. *Aging and disease.* 2015;6(6):406.

67. Shan R, Szmydynger-Chodobska J, Warren OU, Mohammad F, Zink BJ, Chodobski A. A New Panel of Blood Biomarkers for the Diagnosis of Mild Traumatic Brain Injury/Concussion in Adults. *J Neurotrauma.* 2016;33(1):49-57.

68. Perron MP, Provost P. Protein interactions and complexes in human microRNA biogenesis and function. *Frontiers in bioscience: a journal and virtual library.* 2008;13:2537.

69. Di Pietro V, Porto E, Ragusa M, et al. Salivary MicroRNAs: Diagnostic Markers of Mild Traumatic Brain Injury in Contact-Sport. *Frontiers in molecular neuroscience.* 2018;11:290.

Figure 7. Glymphatic flow of healthy and injured brain.

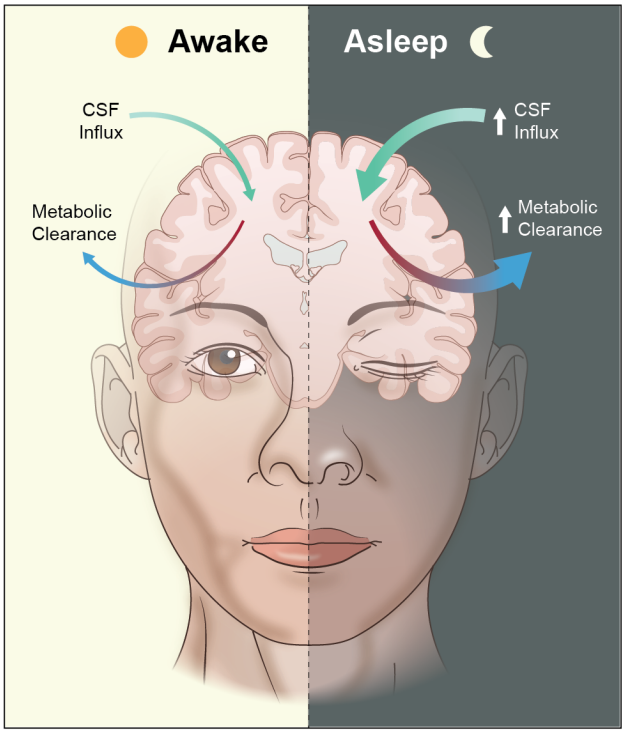


The blood brain barrier and blood-CSF separate the CSF and interstitial fluid. The CSF flows into the perivascular space via the astrocytic vascular endfeet expressing AQP4. AQP4 water channels plays a key role in the clearance of pathogenic aggregate protein (e.g. amyloid beta, tau protein) by fluid exchange between the brain parenchyma interstitial fluid and CSF. In the injured brain, astrocytic activation increases AQP4 activation to increase. Additionally, after brain injury there may be increase in pathogenetic aggregate proteins around the interstitial that needs to be cleared.

AQP4, aquaporin-4; CSF, cerebrospinal fluid.

Illustration created by Sara Mithani and Erina He from National Institutes of Health Medical Arts

Figure 8. Metabolic clearance during wakefulness and sleep.



During sleep, cerebrospinal fluid (CSF) influx increases two-fold more than wakefulness causing increased metabolic clearance.

Illustration created by Sara Mithani and Erina He from National Institutes of Health Medical Arts

APPENDIX

APPENDIX A. HUMAN RESEARCH PROTECTIONS PROGRAM

HRPP HUMAN RESEARCH PROTECTIONS PROGRAM
Intramural Research Program

Your Answers for OHSRP Determination '18-NINR-00362' (11/20/2017)

You may click here to return to OHSRP's Determinations website at any time.

SHOULD I STOP OR GO FORWARD WITH AN OHSRP SUBMISSION?

Question
"If this is a new request (i.e. not an amendment to a previously approved project), have you already started or completed your research activity? (If amending your project select "No" below) "

Your Answer
• No

Question
"Are the specimens/data that will be used in this project coming from an active, NIH IRB-approved protocol; or is the proposed activity a component of an active, NIH IRB-approved protocol, (e.g. the results of this activity will be used in support of the protocol)?"

Your Answer
• No

Question
"Does the NIH investigator or any other collaborator at NIH know the identity of (have identifiers associated with) the living human sources of the specimens/data that will be used on this project? (If the specimens/data are not individually identifiable, but the identity of the subjects may readily be ascertained by the investigator, e.g. because of a small sample size, please answer "Yes" below. Answer "No" below if interacting with subjects to conduct survey, interview or focus group research. Answer "No" below if conducting a retrospective chart review and will have no way to link the data to identifiers after completion)."

Your Answer
• No

Question
"Does this activity involve prisoners?"

Your Answer
• No

Question
"For this project, is NIH conducting a research activity that is part of an FDA-regulated protocol approved by an IRB, e.g. the protocol is studying the safety or efficacy of unapproved drugs/devices or new uses of approved drugs/devices?"

Your Answer
• No

Question
"* If yes, has the collaborator confirmed that the planned research activity, which will occur at NIH, is included in the IRB/ethics committee-approved protocol and consent form at his/her institution?"

Your Answer
** DID NOT ANSWER **

SECTION I - GENERAL INFORMATION

Question
"Project Title:"

Your Answer
RNA Expression and Insomnia

Question
"Project Description:"

Your Answer
We will examine the gene expression of participants with insomnia compared to participants classified as good sleepers. Samples will be analyzed from 30 subjects enrolled in the Metabolomics of Insomnia-Related Hyperarousal Study at the University of Pennsylvania, who have contributed blood samples for RNA sequencing analysis. The purpose of this study is to compare patterns of gene expression between people with insomnia versus good sleepers.

Question
"Upload Additional Project Information or Narrative Template (More Information):"

Your Answer
** DID NOT ANSWER **

Question
"Proposed Start Date:"

Your Answer
12/18/2017

Question
"Proposed Completion Date:"

Your Answer
12/18/2020

Question
"Search for Requestor (your name):"

Your Answer
Cassandra Lee Pattinson

Question
"If you are not the Senior Investigator (SI), what is your role?"

Your Answer
• Other, specify:

Question
"Other - please enter additional information here"

Your Answer

Post-doctoral visiting fellow who will oversee the study

Question
"Search for Senior Investigator*:"

Your Answer
Jessica M Gill

Question
"Search for Supervisor**:"

Your Answer
Ann King Cashion

Question
"Are there any additional NIH Investigators on your team conducting this research (e.g. junior investigator, contractor, fellow, student, etc...)?"

Your Answer
• Yes

Question
"Search for Additional Investigator #1:"

Your Answer
Chen Lai

Question
"Search for Additional Investigator #2:"

Your Answer
Hyung Suk Bruce Kim

Question
"Search for Additional Investigator #3:"

Your Answer
** DID NOT ANSWER **

Question
"Search for Additional Investigator #4:"

Your Answer
** DID NOT ANSWER **

Question
"Search for Additional Investigator #5:"

Your Answer
** DID NOT ANSWER **

SECTION II - SPECIAL CATEGORIES

Question
"Only select the applicable activities or materials below, if they are involved in the project. PLEASE NOTE: The majority of projects do not involve any of the activities listed below. Simply select "Involves other types of specimens/data or research activities" unless you are sure your activities are listed below. (Select all that apply.)"

Your Answer
** DID NOT ANSWER **

Question
"I (the Senior Investigator) certify that the proposed project:"

Your Answer
• Involves other types of specimens/data or research activities than those listed above. Please continue completing the request for determination form.

SECTION III - COLLABORATORS AND OTHER ENTITIES

Question
"Collaborator #1:"

Your Answer
Philip Gehrman

Question
"Institution/IC Name:"

Your Answer
University of Pennsylvania

Question
"FWA #:"

Your Answer
FWA00004028

Question
"City/State/Country:"

Your Answer
Philadelphia, PA

Question
"Email Address:"

Your Answer
gehrman@upenn.edu

Question
"Collaborator Actions"

Your Answer
• Both

Question
"Additional Collaborator Details:"

Your Answer
Dr. Gehrman is an assistant professor of psychology in the Department of Psychiatry at the University of Pennsylvania and will aid in obtaining samples and related clinical and demographic data. He will also be involved in reviewing the data generated.

Question
"Collaborator #2:"

Your Answer
Lai Chen

SECTION III - COLLABORATORS AND OTHER ENTITIES

Question
"Collaborator #1:"

Your Answer
Philip Gehrman

Question
"Institution/IC Name:"

Your Answer
University of Pennsylvania

Question
"FWA #:"

Your Answer
FWA00004028

Question
"City/State/Country:"

Your Answer
Philadelphia, PA

Question
"Email Address:"

Your Answer
gehrman@upenn.edu

Question
"Collaborator Actions"

Your Answer
• Both

Question
"Additional Collaborator Details:"

Your Answer
Dr. Gehrman is an assistant professor of psychology in the Department of Psychiatry at the University of Pennsylvania and will aid in obtaining samples and related clinical and demographic data. He will also be involved in reviewing the data generated.

Question
"Collaborator #2:"

Your Answer
Lai Chen

"FWA #:"

Your Answer
FWA00005897

Question
"City/State/Country:"

Your Answer
Bethesda, MD

Question
"Email Address:"

Your Answer
kimhy@mail.nih.gov

Question
"Collaborator Actions"

Your Answer
• Both

Question
"Additional Collaborator Details:"

Your Answer
Samples will also be analyzed under the supervision of Dr. Kim.

Question
"Upload Additional Collaborator Information:"

Your Answer
** DID NOT ANSWER **

SECTION IV - RESEARCH WITH SPECIMENS AND DATA

Question
"Does this activity include any of the following? (Select all that apply)"

Your Answer
- Research with specimens and/or data

Question
"Other - please enter additional information"

Your Answer
** DID NOT ANSWER **

Question
"* If you selected Program Evaluation or QA/QI above, does the activity only involve interview or survey procedures?"

Your Answer
** DID NOT ANSWER **

Question
"What role(s) will the NIH Investigator(s) have on this research project? (Select all that apply)"

Your Answer
- Receiving data/specimens from a collaborator to conduct research
- Analyzing specimens or data
- Running laboratory assays for research
- Sending data/specimens to a collaborator to conduct research

Question
"Other - please enter additional information here"

Your Answer
** DID NOT ANSWER **

Question
"Identify the types of data or specimen involved in this project (Select all that apply)"

Your Answer
- Specimens, specify below:

Question
"Medical Records - specify source"

Your Answer
** DID NOT ANSWER **

Question
"Data - specify type"

Your Answer

** DID NOT ANSWER **

Question
"Specimens - specify type"

Your Answer
whole blood

Question
"Imaging - specify type"

Your Answer
** DID NOT ANSWER **

Question
"Other - please enter additional information here"

Your Answer
** DID NOT ANSWER **

Question
"Biorepository - specify name"

Your Answer
** DID NOT ANSWER **

Question
"Database or website - Enter name and URL"

Your Answer
** DID NOT ANSWER **

Question
"Select attachment to upload:"

Your Answer
De-Identification_Agreement_Template_Gill_and_Gehrman.pdf

SECTION IV - RESEARCH WITH SPECIMENS AND DATA - Continued

Question
"Do all the specimens/data or information already exist?"

Your Answer
• Yes

Question
"If Yes, were the specimens/data for this project originally collected for:"

Your Answer
• research purposes (even if also collected for clinical purposes)

Question
"Is/was there IRB/Ethics Committee approval for the collection or use of the specimens/data at your collaborator's site (if applicable)?"

Your Answer
• Yes

Question
"Please select the response that best describes the specimens/data utilized for this activity: (Please confirm this with your collaborator, if applicable, prior to submitting this form.)"

Your Answer
• Specimens/data will be coded, however that code cannot be used by anyone to identify specific individuals.

Question
"If an existing identifiable specimens/data set will be de-identified before the research commences, please indicate who will conduct the de-identification:"

Your Answer
• Collaborator(s) or provider(s) of the specimens/data

Question
"NIH SENIOR INVESTIGATOR CERTIFICATION FOR RESEARCH INVOLVING CODED SPECIMENS/DATA Answer below only when the other party can identify the human source(s) directly, through a code-key or when using an Honest Broker:
I (the Senior Investigator) certify that I will not be able to re-identify the human source(s) of the specimens/data in this project, and I have obtained one or more of the following to confirm this. Further, I promise to retain this documentation and provide it upon request. (Select all that apply)"

Your Answer
• An email confirmation between the collaborator and the NIH investigator that contains the de-identification agreement (Click here to read the OHRP regulatory guidance and click here to download the De-identification Agreement Email Template)

Question
"If applicable, will the recipient of the specimens/data be returning results to the sender? (Select all that apply)"

Your Answer
• Yes, only aggregate results will be returned (e.g. summary statistics, not individual line-item data)

Question
"* If coded results are being returned to a collaborator who has the code-key to re-identify the human sources of the specimens/data, is there IRB/ethics committee approval for this research collaboration at the collaborator's institution?"

Your Answer
** DID NOT ANSWER **

Question
"Additional Info:"

Your Answer
** DID NOT ANSWER **

SECTION V - RESEARCH INVOLVING EDUCATIONAL RESEARCH OR TESTING, SURVEY OR INTERVIEW PROCEDURES, OR OBSERVATION OF PUBLIC BEHAVIOR

Question
"Specify the types of research or procedures involved in this project: (Select all that apply)"

Your Answer
** DID NOT ANSWER **

Question
"Other - please enter additional information here"

Your Answer
** DID NOT ANSWER **

Question
"How will data collection be conducted? (Select all that apply)"

Your Answer
** DID NOT ANSWER **

Question
"In-person at my collaborator's institution(s) or research site(s) - please enter additional information here"

Your Answer
** DID NOT ANSWER **

Question
"In-person at another site(s) - please enter additional information here"

Your Answer
** DID NOT ANSWER **

Question
"Other - please enter additional information here"

Your Answer
** DID NOT ANSWER **

Question
"Who will be conducting the data collection? (Select all that apply)"

Your Answer
** DID NOT ANSWER **

Question
"Off-site contractor - please enter additional information here"

Your Answer
** DID NOT ANSWER **

Question
"Online survey tool - please enter additional information here"

Your Answer
** DID NOT ANSWER **

Question
"Other - please enter additional information here"

Your Answer
** DID NOT ANSWER **

Question
"What is the age range of subjects involved in the research?"

Your Answer
** DID NOT ANSWER **

Question
"* If children aged less than 18 years and the project involves observation of public behavior, will the NIH investigator(s) participate in the activities being observed?"

Your Answer
** DID NOT ANSWER **

Question
"Does your project fall into any of the categories of 'clinical research' as defined by the NIH? (See NIH Glossary & Acronym List for the full NIH definition of 'clinical research'.)"

Your Answer
** DID NOT ANSWER **

Question
"If the Planned Enrollment Report is required per above, confirm that it has been approved by the IC approver as scientifically valid: (This step must be completed before uploading)"

Your Answer
** DID NOT ANSWER **

Question
"What is the z-number for this project?"

Your Answer
** DID NOT ANSWER **

Question
"Upload Planned Enrollment Report"

Your Answer
** DID NOT ANSWER **

Question
"Online survey tool - please enter additional information here"

Your Answer
** DID NOT ANSWER **

Question
"Other - please enter additional information here"

Your Answer
** DID NOT ANSWER **

Question
"What is the age range of subjects involved in the research?"

Your Answer
** DID NOT ANSWER **

Question
"* If children aged less than 18 years and the project involves observation of public behavior, will the NIH investigator(s) participate in the activities being observed?"

Your Answer
** DID NOT ANSWER **

Question
"Does your project fall into any of the categories of 'clinical research' as defined by the NIH? (See NIH Glossary & Acronym List for the full NIH definition of 'clinical research'.)"

Your Answer
** DID NOT ANSWER **

Question
"If the Planned Enrollment Report is required per above, confirm that it has been approved by the IC approver as scientifically valid: (This step must be completed before uploading)"

Your Answer
** DID NOT ANSWER **

Question
"What is the z-number for this project?"

Your Answer
** DID NOT ANSWER **

Question
"Upload Planned Enrollment Report"

Your Answer
** DID NOT ANSWER **

VITA

Sara M. Mithani, BSN, RN

EDUCATION

Institution	Degree	Major	Year
Case Western Reserve University	BSN	Nursing	2009

EDUCATION IN PROGRESS

Institution	Degree	Major	Year
University of Illinois Chicago	PhD	Nursing	Expected 05/2020

ADDITIONAL TRAINING

2019	Certificate in Sleep Health; University of Illinois at Chicago, Chicago IL
2019	Applied Machine Learning; 1 semester course at National Institutes of Health; Grade: A
2019	Introduction to Python; 1 semester course at National Institutes of Health; Audit
2019	SciPhD Certificate Program: The Business of Science; National Institutes of Health
2019	Translational Science Training Program; 2-day intensive at National Institutes of Health
2019	Bioinformatic for Analysis of Next Generation Sequencing; 1 semester course at National Institutes of Health; Audit
2018	Summer Genetics Institute; 4-week intensive at National Institutes of Health
2017	Graduate Summer Opportunity to Advance Research; 3 month summer fellowship at National Institutes of Health with focus on translational research

EMPLOYMENT AND TEACHING

2016-2019	Maxim Healthcare Registered Nurse, Chicago, Illinois
2016-2019	Research Assistant, Laboratory of Sleep Neurobiology, Chicago, Illinois Lab Principal Investigator- Dr. Anne Fink
2017-2018	Teaching Assistant; NURS 534: Advanced Physiology
2016-2017	Teaching Assistant; NURS 397: Issues in Nursing Practice
2014-2016	Research Assistant, Neuroscience Laboratory, Cleveland, Ohio Lab Principal Investigator- Dr. Michael Decker
2012-2014	Research Assistant, Heart ABC-Adherence, Behavior, Cognition Study Principal Investigator- Dr. Mary Dolansky

HONORS AND AWARDS

2020	Scholarship and Acceptance to attend American Academy of Sleep Medicine Foundation Young Investigators Research Forum
2020	Podium Presentation at National Institutes of Health Graduate Symposia
2019-2020	Graduate Partnership Program at the National Institutes of Health- National Institutes of Nursing Research

2018-2020	Jonas Healthcare Scholar, 2018-2020 Cohort
2019	Invited International Presentation at Aga Khan University, Karachi, Pakistan. "From Bedside to Bench: The Future of Nursing Science"
2018-2019	W. E. Van Doren Scholarship
2017	University of Illinois College of Nursing Travel Funding for Midwest Nursing Research Society
2018	Kappa Alpha Theta Foundation Scholarship
2016-2018	Ph.D. Deans Teaching Assistant Award
2016	Case Western Reserve University School of Nursing Travel Funding for Midwest Nursing Research Society
2015	2nd Place Poster Presentation Award, Case Western Reserve University Research Symposium
2015	Summer Research Program Funding Recipient ($3,500)
2015-2016	Deans High Honors- Case Western Reserve University
2015-2016	Outstanding Campus Partner, Spartans for Special Olympics
2014-2015	Dorothy Pijan Outstanding Establishment Student Event, Spartans for Special Olympics
2014	2nd Place Poster Presentation Award, Case Western Reserve University Research Symposium
2014	Case Western Reserve University School of Nursing Travel Funding for Midwest Nursing Research Society

PUBLICATIONS

Pattinson, C., Guedes, V., Edwards, K., **Mithani, S.**, Yun, S., Taylor, P., Dunbar, K., Kim, HS., Chen, L., Roy, M., & Gill, J. (In Review). Sleep problems alter gene expression in military personnel and veterans with post-traumatic stress disorder: An RNA sequencing study. *SLEEP*.

Kanefsky, R., Motamedi, V., **Mithani, S.**, Mysliwiec, V., Gill, J. M., & Pattinson, C. L. (2019). Mild traumatic brain injuries with loss of consciousness are associated with increased inflammation and pain in military personnel. *Psychiatry research*, *279*, 34-39.

Mithani, S., & Fink, A. M. (2019). Mathematical Models of Sleep and Circadian Rhythms: A Case for Using the 2-Process Model in Neuroscience Nursing. *Journal of Neuroscience Nursing*, *51*(1), 48-53.

Motamedi, V., Kanefsky, R., Matsangas, P., **Mithani, S.**, Jeromin, A., Brock, M. S., ... & Gill, J. (2018). Elevated tau and interleukin-6 concentrations in adults with obstructive sleep apnea. *Sleep medicine*, *43*, 71-76.

DeBaz, C., Shamia, H., Hahn, J., **Mithani, S.**, Sadeghi, G., & Palomo, L. (2015). Periodontitis impacts quality of life in postmenopausal women. *Climacteric*, *18*(4), 637-642

Williams, K., **Mithani, S.**, Sadeghi, G., & Palomo, L. (2018). Effectiveness of Oral Hygiene Instructions Given in Computer-Assisted Format versus Self-Care Instructor. *Dentistry journal*, *6*(1), 2.

SELECTED PUBLISHED ABSTRACTS

Mithani, S., Kim, H., Yun, S., Gehrman, P., Fink, A., Gil, J. (2019, October). RNA sequencing reveals transcriptomic changes in individuals with insomnia. American Society of Human Genetics.

Mithani, S., Pattinson, C., Guedes, V., Edwards, K., Devoto, C., Turtzo, C., ... & Gill, J. (2019, July). Acute Postconcussive Symptom Domains And MRI Following Mild Traumatic Brain Injury. In Journal Of Neurotrauma (Vol. 36, No. 13, pp. A89-A89).

Pattinson, C., Guedes, V. A., Edwards, K., **Mithani, S.**, Yun, S., Taylor, P., ... & Roy, M. J. (2019, July). Sleepiness Alters Gene Expression In Military Personnel And Veterans With TBI And PTSD. In Journal Of Neurotrauma (Vol. 36, No. 13, pp. A147-A147).

Fink, A. M., **Mithani, S**., & Carley, D. W. (2017). Systolic blood pressure is increased during non-REM sleep after light-phase sleep fragmentation in rats. Sleep.

SELECTED POSTER PRESENTATIONS

Mithani, S., Pattinson, C., Guedes, V., Edwards, K., Devoto, C., Turtzo, C., ... & Gill, J. (2019, July). Acute Postconcussive Symptom Domains And MRI Following Mild Traumatic Brain Injury. National Neurotrauma Society, Pittsburgh, Philadelphia.

Mithani, S., Maki, K., Fink, A. (2018, April). Sleep Restriction Increases Blood Pressure in Rats: Implications for Developing a Pre-Clinical Model to Measure Cardiovascular Responses to Shift Work Schedule. Midwest Nursing Research Society Conference, Cleveland, Ohio.

Mithani, S., Cho, Y., Rusch H., Gill, J. (2017, August). Gene Expression in Military Veterans with Sleep Disturbances. National Institutes of Health, Summer Poster Day, Bethesda, Maryland.

Mithani, S., Kodoma, N., Barbado, E., Damato, E., Galan, R., Decker, M. (2017, April). High-Density Electroencephalography to Quantify Cortical Neural Network Activity. Midwest Nursing Research Society Conference, Milwaukee, Wisconsin and Case Western Reserve University Research Symposium, Cleveland, Ohio

Mithani, S., Strohl, K. (2015, December). Sleep Hygiene Education to Improve Quality of Life in Veteran Population. Case Western Reserve University Research Symposium, Cleveland, Ohio.

Mithani, S., Dolansky, M., Givens, S., Hughes, J. (2015, March). Heart Failure Self-Management: Differences in Young and Old. Midwest Nursing Research Society Conference, St. Louis, Missouri.

PROFESSIONAL ORGANIZATIONS

2020-present	Society for Neuroscience
2020-present	Western Institute of Nursing Society
2019-present	American Academy of Sleep Medicine
2019-present	American Society for Human Genetics
2019-present	National Neurotauma Society

2018-present	American Health Association
2014-2020	Midwest Nursing Research Society

LEADERSHIP AND SERVICE ORGANIZATION

2018-present	Sigma Theta Tau, National Nursing Honor Society Alpha Lambda Chapter
2014-present	Kappa Alpha Theta, Eta Pi Chapter

LEADERSHIP AND SERVICE ACTIVITIES

2019-present	International Student Mentor, Aga Khan University, Karachi, Pakistan
2019-present	National Team, Program Manager, Aga Khan Economic Planning Board; Business and Professionals Alliance
2009-2019	Aga Khan Foundation-Aga Khan Development Network; Midwest Region Education and Development Team Lead (2017-2018), Partnership Walk, Village in Action Lead Coordinator (2016-2017)
2018	Ismaili Professional Network; Logistics Team Lead- National Alliance Conference
2013-2016	Spartans for Special Olympics; Founding and Executive Member