involved in neurocognitive functioning (CALN1), internalizing disorders (MAD1L1) and blood pressure (ZNF618).
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OBJECTIVE AND SUBJECTIVE MEASURES OF SLEEP INITIATION ARE DIFFERENTIALLY ASSOCIATED WITH DNA METHYLATION IN ADOLESCENTS

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Introduction: The onset of puberty is associated with a shift in the circadian timing of sleep leading to delayed sleep initiation [i.e., later sleep onset time (SOT)] driven by later bedtimes and/or longer sleep onset latency (SOL). Subjective sleep initiation, as per self-reports, and objective sleep initiation, as per actigraphy (ACT) or polysomnography (PSG), assess equally relevant but different domains of the same physiological process. Several GWAS have identified genes associated with sleep and circadian phenotypes; however, little is known regarding the epigenetic basis and significance of delayed sleep initiation in adolescence, a critical developmental period.
Methods: We analyzed data from 263 adolescents from the Penn State Child Cohort follow-up study who had complete subjective (self-reported questionnaires), at-home 7-night ACT, and in-lab 9-hour PSG data for bedtime, SOL and SOT. Epigenome-wide single-nucleotide resolution of DNA methylation in cytosine-phosphate-guanine (CpG) sites and surrounding regions were obtained from peripheral leukocytes. Linear regression assessed the association between bedtime, SOL and SOT with site-specific methylation levels, adjusting for sex, age, race/ethnicity, body mass index, and psychotropic medication use. P-values were adjusted using the Benjamini & Hochberg method to correct for false discovery rate and, thus, q-values are reported.
Results: Exome-wide analysis showed differential methylation in 1450 unique genes across the 9 sleep measurements, while GWAS-informed analysis yielded 57 genes. Gene hits were identified for bedtime (PSG), SOL (subjective, ACT and PSG) and SOT (subjective and PSG): 14 genes were associated with both subjective and PSG-measured SOL, 34 with both ACT- and PSG-measured SOL, and one (TBC1D22A) with subjective, ACT- and PSG-measured SOL. One gene (ABTB2) was associated with subjective and PSG-measured SOT.
Conclusion: Objective and subjective sleep initiation in adolescents is associated with altered DNA methylation in genes previously identified in adult GWAS of sleep and circadian phenotypes. Our data provides evidence for a potential epigenetic link between habitual (subjective and ACT) SOL and in-lab SOT and DNAm in genes involved in circadian regulation (i.e., RASD1, RAI1), metabolism (i.e., FADS1, WNK1, SLC5A6), and neuropsychiatric disorders (i.e., PRR7, SDK1, FAM172A).
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DYSREGULATED SLEEP AND NREM SLEEP ELECTROENCEPHALOGRAM DELTA POWER INDUCED BY INTERMITTENT HYPOXIA EXPOSURE ARE ATTENUATED IN NLRP3 KNOCKOUT MICE

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Introduction: The nucleotide-binding domain leucine rich family pyrin containing 3 (NLRP3) inflammasome protein complex activates caspase-1 to convert the pro-forms of IL-18 and IL-1 beta (IL-1β) into their active forms and is involved in homeostatic sleep and sleep responses to pathogenic stimuli. Intermittent hypoxia (IH) is a hallmark of sleep disordered breathing (SDB) and both IL-18 receptors are associated with SDB in humans. Thus, we hypothesized that NLRP3 inflammasomes are involved in SDB-related sleep disturbances.
Methods: Sleep architecture was assessed by polysomnography in NLRP3 knockout (KO) and wild-type (WT) mice (N = 8 per group). A gas exchange mixer delivered house air serving as a control or chronic IH that involved 90 second episodic oxygen reductions that consisted of ambient oxygen (21%) with brief hypoxic conditions (~10%) that lasted for 3 seconds for the first 10 h of the light period for 5 consecutive days. Gene expression and protein levels in the brain and lungs were assayed using real-time polymerase chain reaction and enzyme-linked immunosorbent assays, respectively.
Results: In WT mice, significant increased non-rapid-eye movement (NREM) sleep amounts and NREM sleep electroencephalogram delta power (0.5-4 Hz) were found after 1 day of IH compared to control conditions ($p < 0.001$). After 5 days of IH, WT mice showed a significant attenuation in NREM sleep amounts and NREM sleep delta power ($p < 0.001$) and increased wake bout frequency ($p = 0.006$) when compared to control conditions. However, the IH-induced NREM sleep and NREM sleep delta power enhancements and reductions were attenuated (21-35%) in NLRP3 KO mice compared to WT mice. In WT mice, NLRP3, IL-1β, IL-18 and caspase-1 gene expression, IL-1β and IL-18 protein levels, and caspase-1 activity were significantly increased in the somatosensory cortices, NTS, and lungs after both 1 and 5 days of IH when compared to control conditions ($p < 0.05$ for all), although NLRP3 KO mice did not exhibit significant differences in molecules downstream of NLRP3 inflammasome activation.
Conclusion: Our findings indicate that altered NLRP3 inflammasome activation contributes to dysregulated sleep occurring from IH and likely is involved in sleep disturbances in SDB.
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