0024

ARC GENOTYPE MODULATES EEG SPECTRAL POWER FOLLOWING TOTAL SLEEP DEPRIVATION

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Introduction: Sleep homeostasis is manifested by a robust increase in slow wave sleep (SWS) following acute total sleep deprivation (TSD), with concomitant changes in spectral power of the non-REM (NREM) sleep EEG marked by substantial interindividual differences. We previously found that a single nucleotide polymorphism of the activity-regulated cytoskeleton associated protein (ARC) gene modulates SWS rebound following TSD. Here we sought to determine whether ARC genotype is also associated with interindividual differences in spectral power of the NREM sleep EEG.

Methods: 50 healthy adults (27.3±4.9 years; 28 females) participated in one of two in-laboratory studies. Each participant had a 10h baseline sleep opportunity (22:00–08:00), 38h TSD, and a 10h recovery sleep opportunity (22:00–08:00). Sleep periods were recorded polysomnographically and visually scored according to AASM criteria. Genomic DNA was assayed for the ARC c.*742 + 58C>T non-coding SNP, rs35900184. Log-transformed NREM sleep EEG spectral power (C3-M3 derivation) over 0.2 Hz frequency bins in each of four frequency bands – delta (0.8–4.0 Hz), theta (4.2–8.0 Hz), alpha (8.2–12.0 Hz), and beta (12.2–16.0 Hz) – was analyzed by band using mixed-effects ANOVA with fixed effects for ARC genotype, night (baseline, recovery), frequency bin, and their interactions. Analyses included study and age as covariates and a random effect over subjects on the intercept.

Results: The genotype distribution in this sample was 33 C/C homozygotes, 11 C/T heterozygotes, and 6 T/T homozygotes. There was a significant ARC by night interaction in the theta ($F_{2,1833}=5.94$, $p=0.003$) and alpha ($F_{2,1833}=8.58$, $p<0.001$) bands. Compared to baseline sleep, during recovery sleep C/C homozygotes had 18.9% more theta power and 8.7% more alpha power, C/T heterozygotes had 17.9% more theta power and 7.6% more alpha power, and T/T homozygotes had 20.0% more theta power and 15.1% more alpha power.

Conclusion: Our results show that ARC genotype mediates the NREM sleep EEG response to TSD; compared to C allele carriers, homozygosity for the T allele is associated with a much more pronounced increase in alpha power, as well as a larger increase in theta power. The functional implications of this ARC effect remain to be determined.

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0025

CIRCADIAN DYSREGULATION OF HUMAN DNA REPAIR GENES AND ELEVATED DNA DAMAGE IN SIMULATED NIGHT SHIFT SCHEDULE

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Introduction: Circadian misalignment from night shift (NS) work is associated with increased risk of cancer. In a simulated NS study, we sought to investigate the potential role of circadian disruption of cancer hallmark pathway genes.

Methods: N=14 healthy adults (aged 22-34) participated in a laboratory study. Seven were assigned to a simulated day shift (DS) schedule involving 3 days of daytime wakefulness (06:00-22:00); the other seven were assigned to a simulated NS schedule involving 3 days of nighttime wakefulness (18:00-10:00). Subjects then underwent a 24-hour constant routine protocol, during which blood was collected at 3-hour intervals. Leukocytes extracted from blood were subjected to transcriptomics using the NanoString nCounter PanCancer Pathways panel augmented with canonical clock genes. Statistical analysis involved mixed-effects cosinor analysis followed by functional enrichment analysis of rhythmic genes. Leukocytes were also subjected to endogenous DNA damage assessment through alkaline comet and immunofluorescence assays. Furthermore, exogenous DNA damage from exposure to ionizing radiation was investigated for blood collected at opposite times of day (07:30 and 19:30) based on DNA damage biomarkers assessed with immunofluorescence and immunoblot assays.

Results: Transcriptomics data showed that the simulated NS schedule, as compared to the simulated DS schedule, significantly altered the endogenous circadian rhythmicity of genes involved in cancer hallmark pathways, as measured under constant routine. A DNA repair pathway showed enrichment of rhythmic genes following the DS schedule ($P<0.05$), but not following the NS schedule. Functional assessments revealed that the NS schedule was associated with increased endogenous DNA damage, as evidenced by alkaline comet assay ($P<0.001$) and increased BRCA1 foci ($P<0.01$) and γH2AX foci by immunofluorescence assay ($P<0.001$). After exposure to ionizing radiation, there were increased BRCA1 foci ($P<0.01$) and γH2AX foci by immunofluorescence assay ($P<0.005$) and elevated DNA damage response signaling biomarkers by immunoblot assay, especially in the samples collected at 19:30.

Conclusion: These results suggest that a NS schedule causes circadian dysregulation of DNA repair genes and increases DNA damage – a primary hallmark of carcinogenesis – which may underlie the elevated cancer risk in NS workers.
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0026

TARGETED GENOME SEQUENCING IDENTIFIES MULTIPLE RARE VARIANTS IN CAVEOLIN-1 ASSOCIATED WITH OBSTRUCTIVE SLEEP APNEA

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Introduction: Obstructive sleep apnea (OSA) is a common disorder associated with increased risk for cardiovascular disease, diabetes, and premature mortality. There is strong clinical and epidemiologic evidence supporting the importance of genetic factors influencing OSA, but limited data implicating specific genes.
Methods: Leveraging high depth genomic sequencing data from the NHLBI Trans-Omics for Precision Medicine (TOPMed) program and imputed genotype data from multiple population-based studies, we performed linkage analysis in the Cleveland Family Study followed by multi-stage gene-based association analyses in independent cohorts to search for rare variants contributing to OSA severity as assessed by the apnea-hypopnea index (AHI) in a total of 7,708 European-Americans.
Results: We identified 21 non-coding rare variants in Caveolin-1 (CAV1) associated with lower AHI after accounting for multiple comparisons (P = 7.4×10-8). These non-coding variants together significantly contributed to the linkage evidence. Follow-up analysis revealed significant associations between increased CAV1 expression with lower AHI (P=0.024) and higher minimum overnight oxygen saturation (P=0.007).
Conclusion: Caveolin-1 is a membrane scaffolding protein that is essential in the formation of plasma membrane lipid rafts and mediates cholesterol trafficking; regulates several signaling molecules including transforming growth factor β (TGF-beta), Toll Like Receptor 4 (TLR4) and endothelial nitric oxide synthase (eNOS); with mutations implicated in disorders associated with OSA: pulmonary hypertension, diabetes, atherosclerosis, endothelial and cardiac dysfunction, and inflammation. Our results indicate that caveolin-1 also plays a significant role in OSA, with rare variants and higher CAV1 expression associated with lower AHI.
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0027

RECIPROCAL MODULATION OF CORTICAL EXCITATORY AND INHIBITORY SYNAPSES BY WAKE-SLEEP HOMEOSTATIC STATE

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Introduction: A widely debated function of sleep involves a homeostatic program of down-regulation of excitatory synaptic strength following an overall increase during the preceding waking period, preserving however the previously existing synaptic weights associated with newly acquired memories. We tested this hypothesis by applying thorough statistical analysis of parameters of excitatory and inhibitory miniature postsynaptic currents (mEPSC/mIPSC) recorded ex vivo in mouse cortical pyramidal neurons at three characteristic wake/sleep stages.
Methods: Cingulate cortex coronal slices were obtained at fixed Zeitgeber time (ZT6, to control for circadian clock) from control C57BL6 (MEF2C f/f) mice subjected to 6h acute sleep deprivation (SD), recovery sleep =4hSD+2h(RS), or 6h control sleep (CS). mEPSCs and mIPSCs were recorded from functionally identified whole-cell patch-clamped pyramidal neurons in cortical layer 2/3 (L2/3). Statistical analysis of frequencies, amplitudes, and charge transfer rates of mEPSCs and mIPSCs was done using non-parametric Kruskal-Wallis multiple comparison test and K-means clustering test.
Results: mEPSC frequency (F) and charge transfer (CT) were significantly reduced for RS and CS compared to SD (F: -57%, -47%; CT: -64%, -55%). mEPSC amplitude (A) was significantly reduced for CS compared to SD (-15%). Two-centroid clustering test revealed that analyzed parameters of F, A and CT for SD condition were approximately evenly split between upper and lower range clusters, while the same parameters for RS and CS conditions revealed a pronounced redistribution (>75% lower-, <25% upper ranges). Wake/sleep state related changes of mIPSC parameters showed opposite pattern compared to excitatory synapses. All three parameters were increased in RS vs. SD (F: +63%, A: +7%, CT: +42%) and this difference reached significance levels in CS vs. SD (F: +88%, A: +24%, CT: +109%). Clustering analysis of mIPSC parameters revealed mostly stable distribution pattern between upper and lower ranges for all wake/sleep states.
Conclusion: Significant changes in excitatory/inhibitory balance in the frontal cortex is part of the homeostatic response upon transition from wakefulness to various phases of sleep. The excitatory component prevails during wakefulness, while the inhibitory component peaks during sleep.
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0028

NETWORK ANALYSIS OF ADIPOKINES IN SLEEP DISORDERS AND METABOLIC DYSREGULATION

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Introduction: Adipokines are a growing group of secreted proteins that play important roles in metabolism. Accumulating evidence suggests that adipokines may mediate the close association between sleep disturbance and metabolic derangements. Due to the extensive crosstalk between adipokines and metabolic pathways, an integrated approach is required to better understand the significance of adipokines in sleep disorders and associated metabolic dysregulation. In the current study, we employed network analysis, a set of concepts and methods derived from graph theory, to obtain novel insights into the roles of adipokines in sleep disorders and associated metabolic dysregulation.
Methods: A network of six adipokines and their molecular targets is constructed based on current understanding of their roles in sleep and metabolic disorders using an adjacency matrix. The network is then visualized and analyzed using an open source platform Gephi to derive network-level metrics, including degree and centrality measures. These metrics are used to explore the relationship between sleep disturbance and associated metabolic dysregulation in several disease processes.