**Title:** Sleep Disorder Trends, Epigenetic Markers, and Genetic Variation of Circadian Genes in Adenomatous Polyp Formation

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**Introduction:** Sleep disturbances and sleep quality have allegedly increased and decreased over time, respectively, in the United States (US). Virtually every physiological process follows a circadian pattern, and cellular processes (i.e., cell proliferation, DNA damage response, and apoptosis) are often disrupted by sleep disturbances and other factors, potentially giving rise to adverse health outcomes such as cancer. Additionally, both genetic variation and epigenetic patterns within clock genes, which may impact sleep quality, may also increase one’s risk for developing cancer. The role of sleep disturbances in the formation of adenomas, precursor lesions to colorectal cancer (CRC), has been previously explored; however, little is known regarding the influences of genetic variation in the PERIOD3 (PER3) variable number tandem repeat (VNTR) and epigenetic patterns of clock genes on adenoma formation. Furthermore, few studies have attempted to fully characterize sleep disorder trends in a national sample over time, as it has been alleged that sleep quality has declined over time. Examination of sleep disorder trends is of public health significance because individuals affected by sleep disturbances may be at an increased risk for cancer development, particularly CRC. **Materials and Methods:** In order to examine sleep disorder trends over time and to determine the genetic and epigenetic influences of adenoma formation, two studies were conducted. The first study utilizes data from a national sample of US Veterans across an eleven year span to carry out a serial cross-sectional study to produce annual prevalences
of sleep disorders, which were further stratified by additional factors. Cases were defined as patients with at least two outpatient sleep disorder diagnoses at least 30 days apart based on ICD-9 codes and American Academy of Sleep Medicine (AASM) criteria. Additional analyses were carried out to determine factors that were associated with the development of new sleep disorders in the last year of the study using multivariable unconditional logistic regression models to produce odds ratios (OR) and 95% confidence intervals (95% CI). The second study (Epigenetics and Diet in the Carcinogenesis Process, EDCaP) provided information on epigenetic markers and PER3 VNTR variation on participants recruited from a local endoscopy center. The epigenetic marker study was carried out at local endoscopy center (South Carolina Medical Endoscopy Center, Site 1 [n=107]). For the PER3 VNTR arm of the study, data from Site 1 (n = 93) and an additional endoscopy center were pooled (WJB Dorn Veterans Administration Medical Center [Site 2, n=53]). Cases were defined as individuals with at least one histologically confirmed adenoma, and controls were subjects with a normal colonoscopy, or a normal biopsy not requiring heightened surveillance (e.g., hyperplastic polyp). To determine if cases had differential promoter methylation in selected genes or an increased odds of possessing at least 5-repeat allele in the PER3 VNTR compared to controls, unconditional logistic regression analyses were used to calculate ORs and 95% CIs, while adjusting for confounders.

Results: In the study using a national sample of US Veterans, apneas and insomnias were the first and second most common sleep disorders diagnosed among Veteran patients, respectively. Total sleep disorder prevalence rose six-fold across the study period. Additional analyses indicated that several comorbid diseases may influence the development of sleep disorders. With regard to the methylation study, compared to controls, cases were more likely to be hypomethylated in the MINT1, PER1, and PER3 promoters. In the PER3 VNTR study, cases
were more likely to possess the 5-repeat PER3 genotype relative to controls. **Discussion:** Given the evidence that diagnosed sleep disorder prevalence has risen over time in the national sample study, a subset of individuals may be increasing their risk for cancer, potentially through genetic or epigenetic mechanisms. Epigenetic and genetic variations in clock genes appear to influence the development of adenomas, which presents an opportunity to develop novel biomarkers in accessible tissues (e.g., blood). It is also important to note that these genetic and epigenetic markers may influence sleep disturbances, which have also been previously linked to cancer formation.