Introduction
Night shift work is associated with increased cancer risk, but the molecular mechanisms are not well-understood. It is hypothesized that melatonin suppression due to night shift work could impact DNA methylation in circadian genes, although this has been evaluated by few studies.

Objectives
This study explored the relationship between night shift work parameters and patterns of melatonin secretion on methylation in circadian genes among women.

Methods
A cross-sectional study was conducted in 2019–2020 among 74 female healthcare employees who participated in a previous study in which urinary melatonin levels were evaluated over a 48-hour period. Participants provided information on demographics, lifestyle behaviors, and night shift work such as current night shift work pattern, duration in years, and intensity (consecutive nights). The Illumina Infinium MethylationEPIC beadchip was applied to DNA extracted from new blood samples to measure methylation at 1150 CpG loci across 22 circadian genes. Multiple linear regression was used to examine the association between night shift work, melatonin parameters and methylation levels at each CpG site, while accounting for the false-discovery rate (q = 0.2).

Results
Compared to day workers, night shift workers had hypermethylation in the promoter region of CSNK1E (q = 0.15). Women who worked night shifts for ≥ 10 years exhibited hypomethylation in the body of NR1D1 (q = 0.08) compared to those with < 10 years of history. Hypermethylation in the body of ARNTL was also apparent for those who worked ≥ 3 consecutive night shifts a week compared to < 3 nights (q = 0.18). Among night shift workers, melatonin patterns (24-hr concentrations, peak timing) were associated with methylation at three loci (RORA, MTNR1A, PER3) (q ≤ 0.20). No association between melatonin and methylation was identified among day workers.

Conclusion
These findings suggest that circadian misalignment among night shift workers is associated with differential methylation in several circadian genes, but larger studies are needed to confirm.