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Shift work, *hCLOCK* T3111C polymorphism, and endometriosis risk

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Abstract

Background—Endometriosis, a dysplastic disease affecting approximately 5-10% of U.S. reproductive-age women, has been linked in epidemiologic studies to exposures indicating high circulating estrogen levels. One such exposure may be night shift work, which has been associated with menstrual disruption and increased risk of two other estrogen-influenced diseases, breast cancer and adverse coronary events.

Methods—In this population-based case-control study, 235 health maintenance organization enrollees aged 18-49 first diagnosed with surgically-confirmed endometriotic disease between 4/1/1996 and 3/31/2001 were frequency-matched on age with 545 randomly selected female GH enrollees without history of endometriosis. Participants were asked about night shift work in all paid fulltime or part-time jobs worked from age 18 to the reference date. Genotypes for T3111C *hClock* were determined for a subset of 218 cases and 456 controls.

Results—Any night shift work was associated with a 50% increase in risk of endometriosis (odds ratio (OR) 1.48, 95% confidence interval (CI) 0.96, 2.29), and working more than half of shifts on a job at night was associated with a nearly doubled disease risk (OR 1.98, 95% CI 1.01, 3.85.) Changing sleep patterns on days off was associated with further increases in disease risk. T3111C *hClock* polymorphism was unrelated to endometriosis status and did not modify the effect of shift work on endometriosis.

Conclusions—These findings suggest that some aspect of night shift work may influence the development of endometriosis.

Introduction

Endometriosis, the presence of functioning endometrial glands and stroma outside the uterine cavity, affects 5 -10% of United States women of reproductive age[1] and is the third leading cause of gynecologic hospitalization in the United States. The stimulation of endometriosis tissue growth is thought to be related to estrogen synthesis and metabolism [2]. Some evidence suggests that serum estrogen may have a circadian secretion pattern in premenopausal women after adjustment for the monthly ovarian rhythm [3, 4], raising the possibility that estrogen may be vulnerable to circadian disruption. One cause of circadian disruption, night shift work, has been found to affect estrogen secretion and metabolism and has been associated with increased risk of several estrogen-influenced conditions, including menstrual cycle changes [5, 6], breast cancer [7-9], and adverse coronary events [10]. Sensitivity to circadian disruption may have genetic

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components in humans. A common single-nucleotide polymorphism (T3111C) in *hClock*, the human counterpart of a circadian rhythm gene (Circadian Locomotor Output Cycles Kaput) found in most mammals, has been associated with diurnal preference [11], insomnia in mood disorders [12], and seasonal affective disorder [13]. A deletion mutation of the transcriptional activation region of the homologous gene in mice causes irregular, lengthy estrous cycles [14]. We conducted this population-based case-control study to test these hypotheses: 1) that shift work is associated with endometriosis risk, and 2) that *hClock* T3111C polymorphism may modify the effect of shift work on endometriosis.

Methods and Materials

Parent study

In the parent study, Women's Risk of Endometriosis (WREN), all women 18 to 49 years of age enrolled a large health-maintenance organization in Washington State (Group Health Cooperative (GH)) who were first diagnosed with endometriosis (International Classification of Disease 9th Revision diagnostic codes 617.0-617.5, 617.8, and 617.9, excluding 617.0, adenomyosis without endometriosis) between April 1, 1996 and March 31, 2001 (n = 467) were invited to participate as cases. The reference date was the month and year a woman first visited GH reporting symptoms leading to a diagnosis of endometriosis. Controls (n = 1016) were randomly selected from a list of women enrolled in GH during the same time period, frequencymatched on five-year age group and assigned a reference date to correspond to the distribution of case reference dates. Women who did not speak English or who reported a hysterectomy or bilateral oophorectomy at initial telephone eligibility screening were excluded from participation. Participants were interviewed in person using a structured questionnaire that included questions regarding demographics, employment, prior medical conditions, menstrual history, pregnancy history, contraceptive methods, hormone use, cigarettes and alcohol use, and family history of endometriosis. In-patient and out-patient medical records of cases were reviewed and abstracted for symptom type (e.g. pelvic pain, abnormal bleeding) and severity, and endometriosis lesion characteristics (e.g. location, dimensions.)

This study was approved by the institutional review boards of Fred Hutchinson Cancer Research Center and GH. After exclusions, 341 (73.0%) cases and 742 (73.0%) controls participated in the study (see Figure 1). Each participant gave written informed consent to participate in the study, and was compensated $20 for her time.

Participants were asked about every paid job held for six months or longer from age 18 to the reference date. Occupational information collected included: job title and duties and industry (subsequently coded using 1980 Census-Occupation codes); hours worked per week; calendar years job started and ended; and detailed chemical and radiation exposures.

Follow-up study

At the time of the parent study, 330 cases and 684 controls consented to be re-contacted for future studies and provided contact information (see Figure 1 for full participation details.) In a follow-up study of disease recurrence (WRENSYR), cases who granted permission to be re-contacted were interviewed by telephone with a detailed questionnaire updating the items from the parent study to the present date, and inquiring about shift work in jobs worked before the original reference date as well as jobs between the reference date and the present date. WRENSYR participants were compensated $20 for their time. Controls granting consent for recontact and reporting jobs in WREN were interviewed by telephone, and were asked only about shift work in jobs before the original reference date. Fourteen controls reported no jobs during the parent study, and therefore did not require re-contact. Control participants were not compensated for the 10-minute interview. All participants gave verbal consent to be

interviewed. This study was approved by the institutional review boards of Fred Hutchinson Cancer Research Center and GH.

After medical record review, nine cases whose disease did not meet the Holt-Weiss standard for definite or possible disease [15] were excluded from the study. The final analysis group consisted of 235 cases and 545 controls.

Assessment of shift working status

A modification of the shift-work questions asked by Davis et al. in a study of breast cancer etiology [7] was used. For each job the participant reported in WREN, she was asked if she worked anything other than the day shift. If so, she was asked the percentage of the time she worked day shift, evening shift, or night shift, and whether, on days off, she usually got up and went to bed at roughly the same time, within 2 hours, as on workdays. Finally, she was asked whether, during a 4-week period at this job, she usually worked the same shift or rotated shifts. Day shifts were defined as starting after 05:00 and ending before 19:00; evening shifts as starting after 12:00 and ending before 02:00; and night shifts as starting after 19:00 and ending before 09:00.

Genetic assessment

Leukocytic DNA from venous blood samples (204 cases and 456 controls in this analysis) and buccal epithelium genomic DNA from oral rinses (14 cases in this analysis) was extracted by salt precipitation. The T to C transition at position 3111 of the 3' untranslated region of *hClock* was amplified using the primers described by Katzenberg et al. [11]. The thermal cycling conditions were: five cycles 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 1 minute; 30 cycles 95°C for 30 seconds,55°C for 30 seconds, 72°C for 1 minute; and a final extension of 5 minutes at 72°C. A fraction of the PCR products, including a negative control containing all reaction components except the genomic DNA, was run on a 1% agarose gel to ensure that the specific 221 base pair (bp) band was generated. Per the methods of Desan et al. [16], the PCR product was digested with Bsp1286I (New England Biolabs, Beverly, MA), which generates 95- and 126-bp bands in individuals homozygous for the C allele, 95-, 126- and 221-bp bands in heterozygous individuals, and a 221-bp band in individuals homozygous for the T allele. The restriction fragments were resolved by electrophoresis on a 3% agarose gel.

Analytic methods

Unconditional logistic regression was used to calculate odds ratios for the relationship between shift work and endometriosis and associated 95% confidence intervals (CIs) using STATA 8.0 (College Station, TX). All models were adjusted for the matching variables age and reference year as well as relevant confounders. Potential confounding factors, chosen as potentially related to both exposure and outcome but not on a plausible causal pathway, were race/ethnicity, household income, education, marital status, gravidity, parity and use of alcohol, cigarettes and oral contraceptives. Each potential confounder, treated categorically, was evaluated individually in a model containing the relevant shift and the match variables to determine which, if any, changed the estimate of the association between shift work and endometriosis by 10% or more. Nulliparity was treated as a binary variable, and gravidity as a four-level ordered categorical variable. Those confounders were then included in a multivariate model to determine a final, conservatively adjusted estimate of association.

For logistic regressions, those who had worked only days or never worked outside the home served as the reference group. Analyses of lifetime occupational shifts worked compared the reference group to those who had ever worked any non-day shifts; those who had ever worked evenings at any job; those who had worked more than 50% of shifts at any job during evenings; those who had ever worked nights at any job; and those who had worked more than 50% of

shifts at any job during nights. Those who changed sleep times on their days off were compared to those who had not, by shift worked, and compared to the reference group. Duration analyses compared the reference group to those who had worked each of the shifts examined for 2 or fewer years, between 2 and 5 years, and more than 5 years. Duration of shift worked was also examined by case status using Cuzick's nonparametric test for trend across ordered groups [17].

Logistic regression was also used to perform analyses of the risk of endometriosis associated with *hClock* T3111C polymorphism, using the T/T genotype as the reference group. Analyses treating T/C and C/C as separate groups and grouping them together as "any C" were conducted. The matching variables, age and reference year, were included in all models. Because there was no reason to believe that gene distribution was related to variables measured in the study, confounding was not further evaluated in these analyses. To determine whether the association between shift work and endometriosis varied by polymorphism status, we tested for interaction between polymorphism status and the types of shift work examined in the main analyses using logistic regression. Specifically, interaction was defined by a significant ($\alpha <= 0.05$) Wald test of the cross product term.

Because a previous study found a positive association between endometriosis and working night shifts was absent among nulliparous women [18], we performed subanalyses of this association in paras and nulliparas separately.

Results

Demographic characteristics are shown in Table 1. Cases and controls were similarly distributed in terms of age, race/ethnicity, marital status, education, income, and use of oral contraceptives. Cases had fewer pregnancies and fewer births than controls. Cases were more likely than controls to use alcohol and tobacco at the reference date.

Six participants, all cases, had never worked a paid job for six months or longer since the age of 18. Among those who did work outside the home, cases worked a lifetime mean of 17.9 years, with a standard deviation of 7.6 years, and controls worked a lifetime mean of 18.0 years, with a standard deviation of 7.6 years.

Compared with working only the day shift in all reported jobs, or having reported no jobs, ever working a job with any amount of evening or night shift work was modestly but not significantly associated with case status (odds ratio (OR), adjusted for age and reference year: 1.32, 95% confidence interval (CI) 0.94, 1.87.) No single variable made a difference of more than 10% in the point estimate, nor did using all the variables in a single model (OR 1.30, 95% CI 0.89, 1.88.) Results of the separate multivariate analyses of evening and night shift work, and of sleep changes during days off, are presented in Table 2. Confounders changing any model estimates more than 10% were race/ethnicity and education, which both altered only the smallest groups (those who worked night shifts more than 50% of the shifts worked in a given job.) Thus, all the adjusted models in Table 2 include, in addition to the matching variables age and reference year, both of these variables.

Endometriosis risk was moderately but not significantly increased among women who ever worked a job with any amount of evening shift work, who ever worked night shift work, or who worked a job with more than 50% of hours during evening shift (ever any evenings OR 1.27, 95% CI 0.90, 1.81; ever any nights OR 1.48, 95% CI 0.96, 2.29; more than 50% evenings OR 1.45, 95% CI 0.98, 2.15). Working more than 50% of job hours during night shift was associated with a doubling of endometriosis risk (OR 1.98, 95% CI 1.01, 3.85). In general, changing sleep patterns on days off further increased the elevations in endometriosis risk seen with shift work, with highest risk among women working more than 50% of hours during night

shifts and changing sleep patterns (OR 2.54, 95% CI 0.88, 7.35.) This association reached statistical significance among those who ever worked a job with more than 50% of hours during evening shifts and changed sleep patterns (OR 1.51, 95% CI 1.00, 2.28.)

As shown in Table 3, duration had differing effects on endometriosis risk associated with evening and night shift work. For night shift work, endometriosis risk elevations were higher with increasing duration of work (test for trend: any night shift work $\chi^2 = 2.69$, $p = 0.10$; >50% night shift work $\chi^2 = 3.74$, $p = 0.05$). Women who worked more than 50% of job hours during night shifts for more than five years had the highest risk of endometriosis (OR 5.32, 95% CI 1.21, 23.48.)

As shown in Table 4, women who reported ever working more than one type of shift in a four-week period appeared to have a modestly increased risk of endometriosis, but this increase was only slightly higher than that among women whose shift did not rotate, and neither reached statistical significance. The association did not vary depending on whether a job included evenings or nights in the shifts worked.

The T3111C polymorphism of *hClock* was not associated with disease (adjusted for age and reference year, T/T OR 1.00, reference category; T/C OR 0.87, 95% CI 0.62, 1.23; C/C OR 1.28 95% CI 0.65, 2.53; any C versus T/T OR 0.92, 95% CI 0.66, 1.28); there were no significant interactions between polymorphism and shift worked (Table 5.) Distribution of T3111C was in Hardy-Weinberg equilibrium among the controls (likelihood-ratio χ^2: 0.698, p =0.403.)

Among the controls, neither gravidity nor nulliparity was related to ever working evening or night shift. An analysis by parity showed results consistent with the main analyses after controlling for age and reference year (nulliparous women: any shift OR 1.21, 95% CI 0.71, 2.07; any evening shift OR 1.09, 95% CI 0.63, 1.88; any night shift OR 1.57, 95% CI 0.78, 3.15; parous women: any shift OR 1.49, 95% CI 0.93, 2.39; any evening shift OR 1.45, 95% CI 0.90, 2.33; any night shift OR 1.43, 95% CI 0.81, 2.52) (data not shown.)

Discussion

In this population-based case control study, night shift work was associated with a 50% increase in risk of endometriosis, and working more than 50% of shifts on a job at night was associated with a nearly twofold risk increase. Evening shift work was not significantly related to risk of endometriosis, but showed a similar pattern of elevated risk with more than 50% evening shifts. Changing sleep patterns on days off was associated with further increases in risk among those who worked more than 50% of evening or night shift. We found no relationship between *hClock* T3111C polymorphism and endometriosis, nor any effect of the polymorphism on the relationship of shift work to endometriosis.

A prior study of Norwegian women found that those who ever worked the night shift were 1.8 times as likely to report endometriosis as other women [18]. This small study did not adjust for potential confounders, but our current findings are consistent with theirs. Moen and Schei dismissed their finding, noting, “Among only nulliparous women shift work was not related to endometriosis (OR 0.79, 95% CI 0.10, 6.5). In general, women without children are probably more prone to irregular working terms.” [18, p. 561] This generalization is uncited and, although perhaps true for Norwegian women, does not hold true in the United States. In a multivariate analysis of 1991 U.S. Bureau of Labor Statistics (BLS) data, Presser found that women were more likely to work shifts other than days on their principal job if they had preschool children, and less likely if they had school-age children, than women without children or with children 14 or older [19]. As parity increases, so does the number of older children in a household; thus, the presence of only preschool children can be taken as representing lower parity and lower

maternal age. We adjusted for nulliparity and gravidity, without substantive changes to the relationships in endometriosis and shift, and analysis limited to nulliparous women revealed relationships very similar to those in the whole group. To our knowledge, no other studies have evaluated shift work in endometriosis.

In the present study, although changing sleep patterns on days off appeared to increase risk of endometriosis among shift workers, rotating shifts did not. Our study measured rotation in very broad terms, and was not adequately powered to look at the pattern of shift rotation (speed and direction.) Generally speaking, the shorter the rotation, the more severe the health effects, presumably because of loss of synchrony between external and internal circadian cues. Changing sleep patterns on days off was more common in our population than shift rotation, and may have captured loss of synchrony or maladaption to shift work that could not be measured with our shift rotation item.

An explanation advanced for the relationship between breast cancer and night shift work may relate to the present findings. Melatonin is a potential agent of oncostasis [2021], and is suppressed by light at night [22]. Melatonin may inhibit estradiol secretion [23], or melatonin and estradiol may be mutually inhibitory [24]. If melatonin inhibits cancer, it might also inhibit the inappropriate growth of ectopic endometriotic tissue.

A second potential explanation for the role of shift work in endometriosis is that working at night might cause sustained stress, in all such workers or in a subset whose vulnerability is defined by as-yet undetermined factors (such as job strain, adaptability to shift work, or family difficulties.) Although sustained stress suppresses gonadotrophin release[25], it may also exacerbate inflammatory disorders despite the anti-inflammatory effects of cortisol. Endometriosis has a significant chronic inflammatory component [26]

Family [27, 28], twin [29, 30] and genealogical database [31] studies suggest that endometriosis has a genetic basis, with one study showing the most parsimonious explanatory model has additive effects of individual-level environmental and genetic mechanisms [29]. At the time of our study design, the only well-characterized sequence variant of any of the human circadian protein genes common enough in general populations to be studied in our case-control population was the T3111C polymorphism (rs 1801260) in the 3' untranslated region of the *hClock* gene (4q12-GDB:9785615), which codes for a helix-loop-helix-PAS subunit of a transcriptional activator of the *Period* and *Cryptochrome* genes. We did not find evidence of an association between risk of endometriosis and this polymorphism, nor that this polymorphism modified the association between shift work and endometriosis, but it is possible that circadian genetics may still play a role in this relationship Additional work to evaluate the role of this gene in adaptation to shift work and diurnal preference may be of interest, as may explorations of variants of other components of the human circadian oscillator.

Strengths of this study include nearly complete case ascertainment in a defined population, such that the full spectrum of diagnosed disease was represented. The control population, drawn randomly from GH rolls, has a race, income, and educational profile very similar to that of western Washington [32]. Because cases and controls were GH members, there was no difference in the ability to seek care and hence no disparity of socioeconomic status introduced by access to care. This strength was attenuated by the loss of participants from the originally eligible pool through refusal to participate in the parent study, refusal to be re-contacted for subsequent studies, loss to follow-up, and refusal to participate in the present study. None of these steps individually lost many subjects, but the overall loss was considerable, though comparable to similar ancillary studies. Although controls reporting prior diagnoses of endometriosis were excluded from the study, the possibility exists that some controls had undiagnosed asymptomatic or mildly symptomatic endometriosis, potentially attenuating odds ratios.

One limitation is that cases were slightly more likely to agree to be re-contacted than controls. The final response rate was lower among cases, probably because of the more involved follow-up interview. To defray the possibility that shift workers would be less likely to participate, we attempted contact from 8 AM to 9 PM, and scheduled interviews at participant convenience. Given the relationship to shift work we found, if shift workers with endometriosis had been less likely to participate because of the more involved interview, the risk observed would have been an underestimate. We found strong, consistent relationships, and have no reason to believe that this was an important influence on the results.

Another limitation is that because we do not know the mechanism by which shift work may affect endometriosis, we could not determine the etiologically relevant time point at which to evaluate shift work. If exposure leads to disease only in a specific timeframe, then our estimates of risk based on ever/never working shift work underestimate the association between shift work and disease.

Individual response to shift work is variable, and may depend on whether the individual chooses to work the shift [33]. Even when an individual values the ability to work nonstandard hours, working nonstandard shifts disrupts social rhythms and presents challenges to sleep. We were not, unfortunately, able to consider the reasons for shift work in the present study, as lifetime recall of such variables is likely to be undependable, and cannot be validated.

Of the 5.3 million U.S. women who work shifts other than a regular daytime schedule, 1.1 million work a night shift [34]. This suggests that many women may be at substantive risk for endometriosis, if the results of this exploratory study are reproducible in other contexts. Moreover, the current findings add to the growing body of evidence suggesting that some aspect of shift work is disruptive to estrogen-influenced body functions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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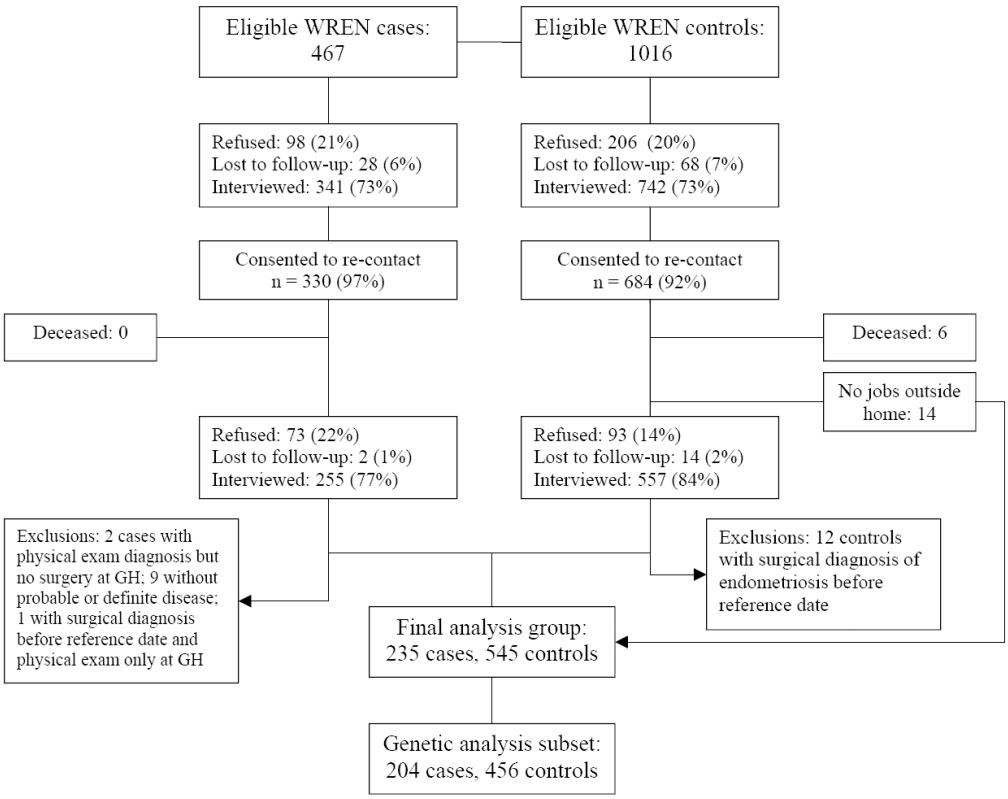

FIGURE 1.
Flow chart of women enrolled in the Women's Risk of Endometriosis Study who were included in this analysis.

Table 1

Sociodemiographic and health characteristics of case and control participants.

	Cases		Controls	
	Number	**Percent**	**Number**	**Percent**
Total	235	100	545	100
Age (years)				
<20	6	2.5	8	1.5
20-24	14	6.0	27	5.0
25-29	21	8.9	40	7.3
30-34	30	12.8	65	11.9
35-39	48	20.4	131	24.0
40-44	67	28.5	150	27.5
45-49	49	20.8	124	22.7
Race/Ethnicity				
Non-Hispanic (NH) Caucasian	195	83.0	463	84.9
NH African-American	7	3.0	25	4.6
NH Asian/Pacific Islander	12	5.1	30	5.5
NH Native American	2	0.9	2	0.4
NH Other	6	2.5	12	2.2
Hispanic Asian/Pacific Islander	4	1.7	1	0.2
Hispanic Non-Asian/Pacific Islander	9	3.8	12	2.2
Marital status				
Married	139	59.1	344	63.1
Single, never married	27	11.5	79	14.5
Divorced/Separated/Widowed	35	14.9	69	12.7
Living as married	34	14.5	53	9.7
Highest educational level completed				
< 12 years	8	3.4	8	1.5
12 years	40	17.0	99	18.2
Some college	73	31.1	198	36.3
College graduate	72	30.6	130	23.9
Post graduate	42	17.9	110	20.2
Family Income				
<$25,000	34	14.5	73	13.4
$25,000-<$35,000	31	13.2	67	12.3
$35,000-<$50,000	52	22.1	113	20.7
$50,000-<$70,000	57	24.3	121	22.2
$70,000-<$90,000	23	9.8	93	17.1
≥$90,000	28	11.9	67	12.3
Refused/unknown	10	4.3	11	2.0
Parity				
0 births	113	48.1	149	27.3
1 births	38	16.2	119	21.8

	Cases		Controls	
	Number	Percent	Number	Percent
2 births	34	27.2	171	31.4
3 births or more	20	8.5	106	19.5
Gravidity				
0 pregnancies	87	37.0	108	19.8
1 pregnancies	35	14.9	98	18.0
2 pregnancies	59	29.5	141	25.9
3 births or more	54	23.0	198	36.3
Tobacco use				
Never	133	56.6	328	60.2
Former	54	23.0	132	24.2
Current	48	20.4	85	15.6
Alcohol use				
Never	70	29.8	184	33.8
Former	43	18.3	126	23.1
Current	122	51.9	235	43.1
Oral contraceptive use				
Never	25	10.6	85	15.6
Former	182	77.5	394	72.3
Current	28	11.9	66	12.1

Table 2

Relationships between endometriosis and lifetime occupational shifts worked, and between endometriosis and sleep time change on non-working nights by shift.

	Cases	Controls	OR[1] (95% CI)	OR[2] (95% CI)
Days only/no job	61	168	1.0 (reference)	1.0 (reference)
Any evening shifts	160 (72.4)	357 (68.0)	1.25 (0.88, 1.77)	1.27 (0.90, 1.81)
Did not change sleep time	25 (11.3)	74 (14.1)	0.94 (0.55, 1.61)	1.00 (0.57, 1.73)
Changed sleep time	135 (61.1)	283 (53.9)	1.33 (0.93, 1.91)	1.34 (0.93, 1.93)
More than 50% evening shifts	97 (61.4)	193 (53.5)	1.41 (0.96, 2.07)	1.45 (0.98, 2.15)
Did not change sleep time	15 (9.5)	37 (10.3)	1.14 (0.58, 2.22)	1.20 (0.60, 2.37)
Changed sleep time	82 (51.9)	156 (43.2)	1.47 (0.99, 2.20)	1.51 (1.00, 2.28)
Any night shifts	65 (51.6)	133 (44.2)	1.40 (0.92, 2.14)	1.48 (0.96, 2.29)
Did not change sleep time	43 (34.1)	91 (30.2)	1.36 (0.85, 2.17)	1.45 (0.89, 2.35)
Changed sleep time	22 (17.5)	42 (13.9)	1.50 (0.82, 2.73)	1.55 (0.84, 2.85)
More than 50% night shifts	21 (25.6)	33 (16.4)	1.94 (1.02, 3.68)	1.98 (1.01, 3.85)
Did not change sleep time	13 (15.9)	24 (11.9)	1.63 (0.77, 3.47)	1.75 (0.80, 3.83)
Changed sleep time	8 (9.8)	9 (4.5)	2.81 (1.00, 7.84)	2.54 (0.88, 7.35)

OR: odds ratio; CI: confidence interval.

[1] Adjusted for age and reference year.

[2] Adjusted for age, reference year, race/ethnicity, and education

Table 3

Relationships between endometriosis and duration of shifts worked.

	Cases	Controls	OR[1] (95% CI)	OR[2] (95% CI)
Days only/no job	61 (26.0)	168 (30.8)	1.0 (reference)	1.0 (reference)
Any evening shifts				
<=2 years	85 (36.2)	203 (37.3)	1.15 (0.78, 1.69)	1.16 (0.77, 1.75)
2-5 years	61 (26.0)	105 (19.3)	2.63 (1.06, 2.52)	1.66 (1.04, 2.63)
>5 years	28 (11.9)	69 (12.7)	1.19 (0.70, 2.03)	1.39 (0.79, 2.44)
Days only/no job	61 (38.6)	168 (46.1)	1.0 (reference)	1.0 (reference)
More than 50% evening shifts				
<=2 years	28 (17.7)	64 (17.7)	1.10 (0.64, 1.89)	1.13 (0.64, 1.99)
2-5 years	43 (27.2)	72 (19.9)	1.71 (1.05, 2.76)	1.76 (1.04, 2.95)
>5 years	26 (16.5)	57 (15.8)	1.42 (0.81, 2.48)	1.64 (0.91, 2.96)
Days only/no job	61 (26.0)	168 (30.8)	1.0 (reference)	1.0 (reference)
Any night shifts				
<=2 years	157 (66.8)	350 (64.2)	1.25 (0.88, 1.77)	1.29 (0.89, 1.86)
2-5 years	9 (3.8)	20 (3.7)	1.27 (0.55, 2.95)	1.50 (0.62, 3.65)
>5 years	8 (3.4)	7 (1.3)	3.39 (1.17, 9.81)	3.79 (1.22, 11.69)
Days only/no job	61 (74.4)	168 (83.6)	1.0 (reference)	1.0 (reference)
More than 50% night shifts				
<=2 years	8 (9.8)	18 (9.0)	1.31 (0.53, 3.24)	0.89 (0.31, 2.56)
2-5 years	7 (8.5)	10 (5.0)	2.14 (0.76, 6.00)	2.18 (0.71, 6.62)
>5 years	6 (7.3)	5 (2.5)	4.01 (1.13, 14.19)	5.32 (1.21, 23.48)

OR: odds ratio; CI: confidence interval

[1] Adjusted for age and reference year.

[2] Adjusted for age, reference year, race/ethnicity, marital status, education, gravidity, nulliparity.

Table 4

Relationships between endometriosis and working more than one type of shift in an average 4-week period.

	Cases	Controls	OR[1] (95% CI)	OR[2] (95% CI)
Days only/no job	61 (26.0)	168 (30.8)	1.0 (reference)	1.0 (reference)
No shift rotation[3]	85 (36.2)	194 (35.6)	1.23 (0.83, 1.81)	1.17 (0.78, 1.76)
Any shift rotation[3]	89 (37.9)	183 (33.6)	1.35 (0.92, 1.99)	1.38 (0.92, 2.05)
Evening shift				
Without rotation	74 (33.5)	184 (35.1)	1.12 (0.75, 1.68)	1.17 (0.78, 1.76)
With rotation	86 (38.9)	173 (33.0)	1.39 (0.94, 2.05)	1.38 (0.92, 2.05)
Night shift				
Without rotation	33 (26.2)	71 (23.6)	1.37 (0.82, 2.30)	1.55 (0.91, 2.63)
With rotation	32 (25.4)	62 (20.6)	1.43 (0.85, 2.41)	1.37 (0.80, 2.34)

[1] Adjusted for age and reference year.

[2] Adjusted for age, reference year, and nulliparity.

[3] Includes three participants who remembered that they worked shifts other than days but not which shifts were worked. Two of these participants recalled working more than one type of shift in a four-week period, and one recalled not doing so. Inclusion did not change estimates. These participants are excluded from shift-specific analyses.

Table 5

Endometriosis risk and hCLOCK T3111C polymorphism.

	Cases	Controls	All participants OR[1] (95% CI)
T/T	127 (58.3)	257 (56.4)	1.0 (reference)
T/C	76 (34.9)	175 (38.4)	0.84 (0.59, 1.29)
C/C	15 (6.9)	24 (5.3)	1.16 (0.58, 2.31)
T/T	127 (58.3)	257 (56.4)	1.0 (reference)
any C	91 (41.7)	199 (43.6)	0.88 (0.63, 1.22)
T/T days only/no job	34 (16.6)	69 (15.6)	1.0 (reference)
T/T any evening shifts	85 (41.6)	179 (40.6)	0.98 (0.60, 1.60)
T/C days only/no job	20 (9.8)	61 (13.8)	0.66 (0.34, 1.26)
T/C any evening shifts	51 (25.0)	108 (24.4)	0.96 (0.57, 1.64)
C/C days only/no job	3 (1.5)	7 (1.6)	0.93 (0.22, 3.87)
C/C any evening shifts	11 (5.4)	17 (3.9)	1.34 (0.56, 3.18)
T/T days only/no job	34 (28.8)	69 (27.6)	1.0 (reference)
T/T any night shifts	32 (27.1)	61 (24.4)	1.10 (0.60, 1.99)
T/C days only/no job	20 (16.9)	61 (24.4)	0.64 (0.34, 1.26)
T/C any night shifts	25 (21.2)	51 (20.4)	1.01 (0.54, 1.90)
C/C days only/ no job	3 (2.5)	7 (2.8)	0.85 (0.20, 3.58)
C/C any night shifts	4 (3.4)	1 (0.4)	8.43 (0.90, 78.7)
T/T days only/no jobs	34 (16.7)	69 (15.6)	1.0 (reference)
T/T any evening shifts	85 (41.7)	179 (40.6)	0.98 (0.60, 1.60)
Any C days only/no jobs	23 (11.3)	68 (15.4)	0.68 (0.27, 1.28)
Any C any evening shifts	62 (30.4)	125 (28.3)	1.01 (0.61, 1.69)
T/T days only/no jobs	34 (28.8)	69 (27.6)	1.0 (reference)
T/T any night shifts	32 (27.1)	61 (24.4)	1.10 (0.60, 1.99)
Any C days only/no jobs	23 (19.5)	68 (27.2)	0.67 (0.36, 1.27)
Any C any night shifts	29 (24.6)	52 (20.8)	1.15 (0.62, 2.13)

OR: odds ratio; CI: confidence interval

[1]Main effects models adjusted for age, reference year, and race/ethnicity. Interaction models adjusted for age and reference year.

Table 6

(Electronic appendix.) Endometriosis risk and hCLOCK T3111C polymorphism among NonHispanic Caucasians.

	Cases	Controls	Odds Ratio[1] (95% CI)
T3111C T/T	104 (57.1)	217 (55.5)	1.0 (reference)
T3111C T/C	66 (36.3)	155 (39.6)	0.88 (0.61, 1.28)
T3111C C/C	12 (6.6)	19 (4.9)	1.38 (0.64, 2.97)
T3111C T/T	104 (57.1)	217 (67.6)	1.0 (reference)
T3111C any C	78 (30.9)	174 (44.5)	0.93 (0.65, 1.33)

[1] Adjusted for age and reference year.