



Original article

Scand J Work Environ Health 2013;39(2):187-194

doi:10.5271/sjweh.3303

Circadian gene expression in peripheral blood leukocytes of rotating night shift nurses

by [Reszka E](#), [Peplonska B](#), [Wieczorek E](#), [Sobala W](#), [Bukowska A](#), [Gromadzinska J](#), [Lie J-A](#), [Kjuus H](#), [Wasowicz W](#)

Affiliation: Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, 8 Teresy St., 91-348 Lodz, Poland. edyta@imp.lodz.pl

Key terms: [cancer](#); [chronotype](#); [circadian gene expression](#); [gene](#); [leukocyte](#); [night shift](#); [night work](#); [nurse](#); [shift work](#); [shift worker](#); [sleep](#)

This article in PubMed: www.ncbi.nlm.nih.gov/pubmed/22552736



This work is licensed under a [Creative Commons Attribution 4.0 International License](http://creativecommons.org/licenses/by/4.0/).

Circadian gene expression in peripheral blood leukocytes of rotating night shift nurses

by Edyta Reszka, PhD,¹ Beata Peplonska, MD, PhD,² Edyta Wieczorek, MSc,¹ Wojciech Sobala, MSc,² Agnieszka Bukowska, MSc,² Jolanta Gromadzinska, PhD,¹ Jenny-Anne Lie, PhD,³ Helge Kjuus, MD, PhD,³ Wojciech Wasowicz PhD¹

Reszka E, Peplonska B, Wieczorek E, Sobala W, Bukowska A, Gromadzinska J, Lie J-A, Kjuus H, Wasowicz W. Circadian gene expression in peripheral blood leukocytes of rotating night shift nurses. *Scand J Work Environ Health*. 2013;39(2):187–194. doi:10.5271/sjweh.3303

Objective It has been hypothesized that the underlying mechanism of elevated breast cancer risk among long-term, night-working women involves circadian genes expression alteration caused by exposure to light at night and/or irregular work hours. The aim of the present study was to determine the effect of rotating night shift work on expression of selected core circadian genes.

Methods The cross-sectional study was conducted on 184 matched nurses and midwives, who currently work either day or rotating night shifts, to determine the effect of irregular work at night on circadian gene expression in peripheral blood leukocytes. Transcript levels of *BMAL1*, *CLOCK*, *CRY1*, *CRY2*, *PER1*, *PER2*, and *PER3* were determined by means of quantitative real-time polymerase chain reaction (PCR).

Results After adjusting for hour of blood collection, there were no statistically significant changes of investigated circadian genes among nurses and midwives currently working rotating night shifts compared to nurses working day shifts. The highest expression of *PER1* messenger ribonucleic acid (mRNA) was observed for women currently working shifts who had worked >15 years in rotating night shift work. *PER1* gene expression was associated with the lifetime duration of rotating night shift work among women currently working night shifts ($P=0.04$). *PER1* and *PER3* transcript levels in blood leukocytes were significantly down-regulated in the later versus early hours of the morning between 06.00–10.00 hours (β -coefficient -0.226, $P=0.001$ and β -coefficient -0.181, $P<0.0001$, respectively).

Conclusions These results suggest that current rotating night shift work does not affect circadian gene expression in human circulating leukocytes. In analysis of the peripheral clock in human studies, the hour of blood collection should be precisely specified.

Key terms cancer; chronotype; night work; nurse; shift work; shift worker; sleep.

Circadian rhythm, observed in various metabolic and physiological processes, is governed by the endogenous clock found in the suprachiasmatic nucleus (SCN) of the hypothalamus and by peripheral clocks. The circadian system of the organism acts independently of external cues, but it can adapt to environmental conditions, such as light, temperature, meals/feeding etc. The circadian clock in the SCN is synchronized to external cues, and it coordinates the peripheral clock by sending signals to peripheral tissues (1). The molecular mechanism of the

self-sustained circadian system of the organism involves circadian genes. Circadian genes presenting oscillatory transcriptional-translational feedback loops have been found not only in the SCN, but also in peripheral tissues, including human peripheral leukocytes. The biological clock is controlled by several genes, including *BMAL1*, *CLOCK*, *Period (PER)* and *Cryptochrome (CRY)* (2).

Some epidemiological studies have found that long-term, night-working women have a higher risk of breast cancer than women who do not work at night (3). One

¹ Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, Lodz, Poland.

² Department of Environmental Epidemiology, Nofer Institute of Occupational Medicine, Lodz, Poland.

³ National Institute of Occupational Health, Oslo, Norway.

Correspondence to: Edyta Reszka, Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, 8 Teresy St., 91-348 Lodz, Poland. [E-mail: edyta@imp.lodz.pl]

of several proposed mechanisms underlying cancer risk among individuals exposed to light at night involves circadian genes expression alteration (4). Circadian genes may play an important role in the control of multiple biological processes (5), including DNA repair, oxidative stress, maintenance of genomic stability, cell proliferation, and apoptosis (6, 7). Therefore, they may have important relevance to the carcinogenic process. Alteration of circadian genes was found to be associated with various disorders in humans, including sleep and mental disorders, cardiovascular diseases, obesity, impaired glucose tolerance, alcohol abuse, and cancer (8–12).

It has been widely observed that a molecular feedback loop exists for specific clock genes that act as positive and as well negative regulators of the biological clock. Moreover, circadian genes expression among humans is reported to be similar to that observed in rodent peripheral tissue, although tissue-specific clock genes expression patterns were also observed (1). The daily variations in expression of clock genes associated with different rhythmicity and maximal or minimal expression in various peripheral tissues of the human organism are the strongest for peripheral blood leukocytes, probably due to the presence of various cell populations in that tissue. However, blood leukocytes subpopulations may be useful for the investigation of human circadian rhythms as circadian genes are expressed with the peak level occurring during the habitual time of activity (13). In leukocytes, including peripheral blood mononuclear cells (PBMC), the acrophase of *PER* messenger ribonucleic acid (mRNA) was observed in the early morning and morning (13–19). *CRY1* and *CRY2* mRNA expression level peaked also in the morning in circulating leukocytes (20), while the positive regulator of circadian rhythm – *BMAL1* mRNA – presented maximal expression in PBMC and leukocytes in the evening or midnight, as it is in approximate antiphase with the other negative regulators of circadian rhythm (15, 18, 20–22). Interestingly, in the majority of studies, various populations of blood leukocytes presented lack of rhythmicity of *CLOCK* mRNA expression (17, 20).

Humans are well synchronized to the external light–dark cycles. However, disturbances of these cycles by irregular sleep hours, shift work, or light at night exposure may induce alteration of the SCN and peripheral circadian rhythms. Therefore, the aim of our analysis, which forms part of a cross-sectional epidemiological study of nurses and midwives (23), was to determine the effect of rotating night shift work on the expression of selected core circadian genes: *BMAL1*, *CLOCK* (positive regulators), *CRY1*, *CRY2*, *PER1*, *PER2*, and *PER3* (negative regulators) as indicators of peripheral clock.

Methods

Study population

A cross-sectional study of 354 nurses and midwives, all female, currently working rotating night shifts and 370 nurses who work only during the day was carried out in Lodz, Poland in 2008–2011 (23). Women were selected from the Local Registry of the Chamber of Nurses and Midwives. The questionnaire data and biological samples were collected in 2008–2010. The inclusion criteria for the nurses were age 40–60 years and current employment. Gene expression analysis was conducted among 92 pairs of nurses and midwives. The required number of rotating night shift workers were sampled from the study population. For each rotating night shift worker, one non-night shift nurse was randomly selected matched for age (within two years) and sampling season (March–September and October–February).

The study participants completed a structured questionnaire during in-person interviews. Data included information on demographics, occupational history, the current and previous jobs, physical activity [according to the International Questionnaire on Physical Activity (IPAQ)], sleep quality [according to the Pittsburgh Sleep Quality Index (PSQI)], chronotype (according to self-reported preference), tobacco smoking, and alcohol use. Characteristics for the two groups are shown in table 1.

The Ethics Committee for Scientific Research at the Nofer Institute approved the study protocol, and a written informed consent was obtained from each study participant.

Gene expression analysis

Blood samples were collected into S-Monovette® heparinized test tubes (Sarstedt AG & Co, Nümbrecht, Germany) in the morning (06:00–10:00 hours). Transcript levels of *BMAL1*, *CLOCK*, *CRY1*, *CRY2*, *PER1*, *PER2*, and *PER3* in peripheral blood leukocytes were determined by means of quantitative real-time polymerase chain reaction (PCR). Primers were designed with Beacon Designer 7.01 (PREMIER Biosoft International, Palo Alto, CA, USA). Amplicon sequence complied with exon-exon boundaries (table 2). Total RNA was isolated from whole blood, immediately after delivery to the lab, by means of QIAamp RNA Blood Mini Kit (Qiagen). cDNA was synthesized on 250 ng RNA with Quantitect Kit (Qiagen, Syngen Biotech, Hilden, Germany). Expression of circadian genes was quantified with FastStart SYBR Green Master (Roche, Basel, Switzerland) and using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the endogenous control, which presents stable expression levels in RNA isolated from peripheral blood leukocytes.

Table 1. Selected characteristics of nurses and midwives under different work shifts. [BMI=body mass index; MET=metabolic equivalents of task; PSQI=Pittsburgh Sleep Quality Index; SD=standard deviation]

	Day workers (N=92)				Rotating shift workers (N=92)				P-value
	Mean	SD	N	%	Mean	SD	N	%	
Age (years)	48.1	4.7			47.7	4.9			0.62
BMI (kg/m ²)	26.7	4.8			27.3	4.7			0.41
Job duration (years)	27.0	5.5			26.7	6.4			0.72
Total night shifts work (years)	12.0	8.1			24.2	7.0			0.00001
Total night shifts work (categories)									
<5 years			19	20.7			0		0.0001
6–15 years			38	41.3			8	8.7	
>15 years			35	38.0			84	91.3	
Time since quitting night shift work (years)									
<5 years	24.6	15.4							
6–15 years	17.8	8.6							
>15 years	7.3	1.1							
Menopausal status									
Premenopausal			57	62.0			61	66.3	0.54
Postmenopausal			35	38.0			31	33.7	
Physical activity (MET hours per week) ^a	199.9	89.0			254.2	82.6			<0.0001
Quality of sleep (PSQI score) ^{a, b}	6.8	3.7			6.1	3.1			0.142
Chronotype									
Morning type			51	55.4			41	48.9	0.56
Evening type			47	44.6			45	51.1	
Smoking									
Never smokers			36	39.1			38	41.3	0.49
Former smokers			29	31.5			22	23.9	
Current smokers			27	29.4			32	34.8	
Alcohol consumption ^a									
No			70	76.9			90	97.8	<0.0001
Yes			21	23.1			2	2.2	
Antidepressants use ^a									
No			74	91.4			87	100	0.005
Yes			7	8.6			0		
Fasting ^a									
No			62	68.1			74	80.4	0.06
Yes			29	31.9			18	19.6	
Blood sampling									
March–September				58	63.0		57	62.0	0.88
October–February				34	37.0		35	38.0	
Hour	08:30	0.50			07:15	0.37			<0.0001

^a Missing quality of sleep data for 1 woman, physical activity data for 11 women (1 day and 2 shift workers), alcohol consumption data for 1 day shift worker, antidepressants use for 16 woman (11 day and 5 shift workers), fasting status data for one day shift worker.

^b 0=best quality of sleep.

Reference gene-normalized relative quantification with efficiency correction for target and reference gene (Pfaffl method) was calculated using Q-Gene, a Microsoft Excel®-based software application (Microsoft, Redmond, WA, USA) coded in Visual Basic (24). PCR efficiencies were calculated using dilutions of five pooled cDNA samples, which were reverse transcribed from total human RNA isolated from leukocytes. All the samples were amplified in duplicate. Randomly selected pairs of samples were simultaneously amplified on the same plate for the eight genes examined. To determine inter-assay variability, 12 samples (6 pairs) were run over 2 separate occasions for ≤30 days of the experiment. Inter- and intra-assays coefficients of variability (CV) for the examined eight genes (calculated using relative expression values) were <15% and <10%, respectively.

Statistical analysis

Arithmetic means with standard deviations (SD) and frequencies of the basic characteristics were calculated. The linear regression was used to compare means of the continuous variables, and the Pearson Chi-square test was calculated to compare the frequencies distribution among women working on rotating night shifts and day workers. The associations between rotating night shift work measures and circadian genes expression were computed by linear regression. All expression variables were log transformed except *BMAL1* and *CRY2*. The list of covariates included age, current smoking status (yes/no), antidepressants use (yes/no), hour of blood collection, quality of sleep (expressed as PSQI), physical activity [expressed in metabolic equivalents of task (MET) hours per week], season of the year of blood

Table 2. Oligonucleotides for gene expression analysis. [F=forward; R=reverse; NM=messenger RNA transcripts accession number; nt=nucleotide; bp=base pairs]

Gene	Size	NM number	Primers ^a	Amplicon size and position	nt
<i>BMAL1</i>	2757 nt	NM_001030272	F GCCGAATGATTGCTGAGG R CACTGGAAGGAATGTCTGG	168 bp	1837–2004
<i>CLOCK</i>	5801 nt	NM_004898	F ACGACGAGAAGCTGGCATTG R TCCGAGAAGAGGCAGAAGG	152 bp	1493–1644
<i>CRY1</i>	3273 nt	NM_004075	F TGTGTTCCAGATTCCTTGG R TACCTTCATTCTCTTCC	198 bp	1802–1999
<i>CRY2</i>	4202 nt	NM_021117	F TCTTCCAGCAGTTCTTCC R GTAGTCCACCAATGATG	185 bp	1356–1540
<i>PER1</i>	4656 nt	NM_002616	F CAATGGTTCAAGTGGCAATG R GGAGGCTGTAGGCAATGG	182 bp	334–515
<i>PER2</i>	6342 nt	NM_022817	F AGCCAATGAAGAGTATTACCG R GCCACCGCAACATATCG	141 bp	684–824
<i>PER3</i>	6203 nt	NM_016831	F CCATCCTACTGCCACTGTTCTG R ATATACTGCTGCCGCTGCTTCC	98 bp	3286–3383

^a Primer 5' -3'.

collection (March–September/October–February), body mass index (BMI), menopausal status (self-reported pre-/postmenopausal), alcohol drinking the day before blood collection (yes/no), and fasting (<6 hours of blood collection/≥6 hours of blood collection). Of these covariates in the final models for each gene expression, only significant confounders were retained (the list of confounders is provided in tables 3 and 4). Stepwise selection method was used with the Akaike information criterion (AIC) to include variables. The value of $P < 0.05$ for group characteristics was considered to represent statistical significance, and the statistical tests were two-sided. To account for multiple comparison (7 clock genes) we applied Bonferroni correction method with significance level of $P < 0.05/7 = 0.007$.

Statistical analyses were performed with STATA11 (StataCorp LP, College Station, TX, USA) software.

Results

The average total shift work duration among women currently working rotating night shifts was significantly longer (24.2 years) than among current day workers (12.0 years). All of the women and midwives who were current day workers had previously worked rotating night shifts. The majority (91.3%) of current rotating night shift workers had been working night shifts for >15 years. The proportion of women currently working day shifts who had previously worked >15 years on a rotating night shift schedule was 38.0%, with an average of 7.3 years since quitting night shift work (table 1).

Rotating shift nurses and midwives drank alcohol beverages the day before collection significantly less frequently than day working women ($P < 0.0001$). None of the rotating night shift nurses used antidepressants and women in this group were more physically active

than day workers ($P < 0.0001$).

Among rotating night shift women, blood samples were collected at the end of night shift at 07:15 (SD 0.37) hours and at the beginning of work [08.30 (SD 0.50) hours] for day working nurses ($P < 0.0001$).

All subjects showed detectable transcript levels in blood leukocytes, however with large interindividual variation. For all analyzed circadian genes, the mRNA level of the particular gene presented positive correlation with the mRNA level of other genes in the whole study group and subgroups defined by rotating work status, age, BMI, menopausal status, smoking status, or chronotype.

PER1 and *PER3* mRNA expression was associated with the hour of blood collection. Transcripts level was significantly down-regulated in the late versus early hours of the morning (06:00–10:00 hours) (β -coefficient -0.226, $P = 0.001$ for *PER1* and β -coefficient -0.181, $P < 0.0001$ for *PER3*).

An elevated circadian gene expression was observed among rotating night shift compared with day workers. However, we did not observed statistically significant changes of investigated genes expression among nurses working night compared to day shifts, when confounding factors that significantly influenced particular gene expression were included in the analysis (table 3). In case of *PER1*, we observed a strong effect of collinearity with time of blood sampling, as both variables were of borderline significance but the joint test was very significant ($P = 0.00008$).

Circadian gene expression was not associated with the duration of rotating night shift work, specified as three categories of total night shift work (<5, 6–15, and >15 years) among women currently and previously (current day workers) working night shifts (table 4). The highest expression of *PER1* mRNA was observed among current rotating shift women ($N = 84$) who had worked a rotating night shift work schedule >15 years compared

Table 3. Circadian gene relative expression among nurses and midwives under different work shifts and correlation coefficients between gene expression and rotating night shift work.

	Day workers (N=92)		Rotating shift workers (N=92)		P-value	β -coefficient	P-value ^a
	Mean ^a	SD	Mean ^a	SD			
<i>BMAL1</i>	0.282	0.078	0.296	0.085	0.39	0.018	0.13
<i>CLOCK</i>	0.411	0.134	0.444	0.148	0.08	0.061	0.21
<i>CRY1</i>	0.483	0.174	0.506	0.216	0.43	0.007	0.90
<i>CRY2</i>	0.346	0.122	0.378	0.150	0.18	0.007	0.72
<i>PER1</i>	0.976	0.754	1.452	1.114	0.0001	0.264	0.03
<i>PER2</i>	0.242	0.082	0.256	0.083	0.25	0.041	0.37
<i>PER3</i>	0.112	0.049	0.134	0.053	0.004	0.129	0.09

^a Adjusted for tobacco smoking, quality of sleep, antidepressants use (*BMAL1*); physical activity (*CLOCK*); age, tobacco smoking, physical activity, quality of sleep (*CRY1*); physical activity (*CRY2*); hour of blood collection; season of the year of blood collection (*PER1*); age (*PER2*); hour of blood collection, quality of sleep, antidepressants use (*PER3*).

Table 4. Circadian gene relative expression and different categories of total night shift work in nurses and midwives under different work shifts. [SD=standard deviation]

	6–15 years (N=46)				P-value ^a	>15 years (N=119)				P-value ^a
	Day workers (N=38)		Rotating shift workers (N=8)			Day workers (N=35)		Rotating shift workers (N=84)		
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
<i>BMAL1</i>	0.275	0.065	0.291	0.104	0.37	0.282	0.068	0.296	0.084	0.54
<i>CLOCK</i>	0.400	0.141	0.448	0.087	0.29	0.437	0.125	0.44	0.153	0.98
<i>CRY1</i>	0.482	0.174	0.482	0.230	0.96	0.511	0.165	0.522	0.208	0.57
<i>CRY2</i>	0.348	0.116	0.338	0.131	0.86	0.344	0.105	0.351	0.119	0.90
<i>PER1</i>	1.031	0.608	0.863	0.512	0.72	0.943	0.902	1.509	1.141	0.01
<i>PER2</i>	0.257	0.093	0.252	0.069	0.96	0.247	0.070	0.257	0.084	0.79
<i>PER3</i>	0.117	0.055	0.138	0.040	0.19	0.116	0.080	0.134	0.054	0.31

^a Adjusted for tobacco smoking, quality of sleep, antidepressant drug use (*BMAL1*); physical activity (*CLOCK*); age, tobacco smoking, physical activity, quality of sleep (*CRY1*); physical activity (*CRY2*); hour of blood collection; season of the year of blood collection (*PER1*); age (*PER2*); hour of blood collection, quality of sleep, antidepressants use (*PER3*).

with day workers who had previously worked rotating shifts >15 years (N=35) (β -coefficient 0.264, P=0.03) (table 4). Moreover, the *PER1* transcripts level was associated with the lifetime duration of rotating night shift work among current night shift workers (β -coefficient 0.026, P=0.04), while we did not observe such an association among current day workers (β -coefficient -0.006, P=0.50) (figure 1).

Discussion

Circadian gene expression patterns may be useful as peripheral clock biomarkers in circulating leukocytes. In the presented gene expression study, we analyzed circadian gene expression in peripheral blood leukocytes of nurses and midwives who worked irregular night shifts. To exclude the majority of factors and conditions that may possibly influence leukocyte gene expression (25), we analyzed randomly selected nurses matched for age (within 2 years) and sampling season (March–September and October–February). In the total study group and subgroups of current day workers and

rotating night workers, significant correlations were observed between expression of positive and negative regulators of peripheral clock, regardless of menopausal status, smoking, alcohol use, chronotype, age, BMI, sleep quality or physical activity. It may confirm dependence of circadian genes in peripheral tissue as they are linked to the same process of transcriptional-translational feedback loop.

Further, we used a whole population of white blood cells, which includes mononuclear and polymorphonuclear cells, as it was found that there is no difference in the expression of *PER1* mRNA between these two populations of white blood cells (16). However, it should be pointed out that different rhythmicity of clock gene expression was observed for peripheral blood leukocytes, probably due to the presence of various cell populations in that tissue (14–22). In the majority of in vivo studies, daily monitoring of the expression of clock genes in human peripheral leukocyte populations has been extensively investigated. In human peripheral leukocytes, clock genes have demonstrated expression rhythmicity with *PER* and *CRY* mRNA maximal expression in the early morning or morning and *BMAL1* mRNA maximal expression in the evening or midnight, while

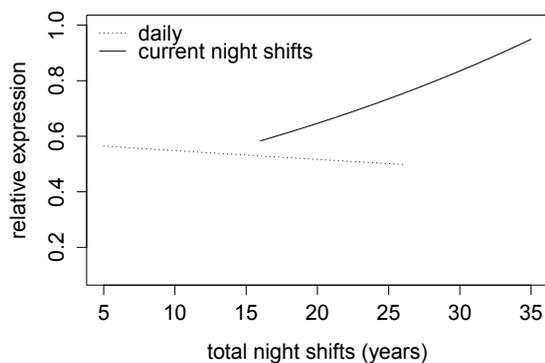


Figure 1. Association between *PER1* gene expression and the lifetime duration of rotating night shift work among nurses and midwives currently working night (β -coefficient 0.026, $P=0.04$) and day shifts (β -coefficient -0.006 , $P=0.50$) (adjusted for hour of blood collection).

CLOCK mRNA expression shows lack of rhythmicity (15, 17, 20). In the present study, the venous blood was collected once, in the morning between 06:00–10:00 hours, and we were, therefore, able to assess *PER1*, *PER2*, *PER3*, *CRY1*, and *CRY2* mRNA expression at their approximate acrophase. At the same time, *BMAL1* mRNA expression was in an antiphase to these genes. In addition, the highest transcript level in circulating blood leukocytes, measured in the morning, was observed for *PER1*, while the lowest was observed for *PER3*.

In the crude analysis of circadian gene expression in circulating leukocytes, we observed significant up-regulation of constitutive *PER1* and *PER3* mRNA expression in circulating leukocytes of nurses and midwives, who currently work rotating night shifts, compared to day workers. When we adjusted gene expression for significant confounding factors, the association between rotating shift work and circadian gene expression disappeared. However, the highest expression of *PER1* mRNA was observed for women currently working shifts who had worked rotating night shift schedule >15 years compared with day workers who had previously worked rotating shifts >15 years. Moreover, the results of our study suggested that up-regulation of *PER1* mRNA expression may be associated with the lifetime duration of night shift work among nurses and midwives who currently work rotating night shifts. These findings could result from a cumulative effect of night shift work on *PER1* gene expression, associated with phase delay or advance of maximal circadian gene expression or direct effect of artificial light at night exposure. It is widely known that resetting of the peripheral clocks can occur through regulation of the expression of clock genes caused by internal or external cues. Generally, light exposure in the morning advances and light exposure in the evening delays circadian systems in organ-

isms, which may cause serious effects due to irregular and disturbed wake–sleep cycle of humans (26). It was found that shift work caused a delay of the clock gene's acrophase in human peripheral tissues. Results of a study on circadian gene mRNA expression (*NR1D1*, *NR1D2*, *PER2*, and *PER3*) in head hair follicle cells of rotating shift workers showed circadian gene expression phase delay after the second week of late shift work (27). Under short-term acute sleep deprivation (40 hours) and light conditions, *PER2* mRNA expression in PBMC presented delayed phase shift and loss of circadian rhythmicity, while *BMAL1* mRNA expression did not show any changes (28). James et al (22) found that a shifted sleep–wake schedule with light at night exposure significantly affects circadian expression of *PER1* and *PER2* peripheral circadian oscillators in PBMC and centrally-driven melatonin secretion. Blue light reduced *BMAL1* mRNA expression in PBMC of jaundiced neonates under light therapy with covered eyes (29), but short wavelength light at 460 nm (blue light) induced *PER2* expression in oral mucosa during a 2-hour exposure of human subjects in the evening (30).

Circadian gene expression may be also regulated by both photic and circadian inducers because it has been widely observed that other clues, such as dietary and feeding habits (31), alcohol use (10), depression (32), age (33), chronotype (18), may also influence peripheral circadian rhythm measured in peripheral leukocytes. In our cross-sectional study, no association has been observed between BMI, fasting status, alcohol drinking, and circadian gene expression.

Interestingly, we observed that *PER1* and *PER3* transcripts level was significantly down-regulated in the late versus early hours of the morning. The analysis of time of blood collection in the morning indicated that blood samples from rotating shift women were collected at 07:15 (SD 0.37) hours and 08:30 (SD 0.50) hours for day workers. This 1.15-hour difference in blood sampling of women under two work schedules may result from observed differences in *PER1* and *PER3* gene expression in the crude analysis. Based on rhythmic expression of circadian genes in various peripheral tissues, we might have observed increasing expression or acrophase of these two genes in the leukocytes of rotating shift women at 07:15 hours, while – among day workers – measured expression could have been attributable to decreasing phase at 08:30 hours. Due to highly significant collinearity of night shift work status and time of blood collection, we were unable to distinguish whether the effect we saw was attributed to the former or the latter. In comparison, the results from the only study on circadian genes mRNA expression analyzed in peripheral blood cells (collected at approximately 09:00 hours) of 25 Japanese shift workers and 45 participants who kept regular hours showed that the *PER3* transcripts

level were higher among shift working women compared to day workers (β -coefficient 0.31, $P < 0.05$) (29).

In summary, in our study, we did not observe a significant association between circadian gene expression and rotating shift work; time of blood collection was found to be an important covariate in the analyses of *PER1* and *PER3* mRNA morning expression. Further studies with daily monitoring of circadian genes expression are needed to (i) confirm the impact of irregular working hours and light at night exposure on peripheral circadian clocks in humans and (ii) elucidate the molecular mechanisms of light-induced circadian gene alteration in human circulating leukocytes. Importantly, when analysing the peripheral clock in human studies, the hour of blood collection should be precisely specified.

Acknowledgments

This project is supported by a grant from the Polish-Norwegian Research Fund (PNRF 243-AI-1/07) and ECNIS2 (7PR/2011/266198). The authors declare they have no actual or potential competing financial interests. Part of this work was presented at 47th Congress of the European Societies of Toxicology, Eurotox 2011 in Paris.

References

- Lamont EW, James FO, Boivin DB, Cermakian N. From circadian clock gene expression to pathologies. *Sleep Med.* 2007;8:547–56. <http://dx.doi.org/10.1016/j.sleep.2006.11.002>.
- DeBruyne JP, Hogenesch JB. A CRY in the Night. *Dev Cell.* 2011;20:144–5. <http://dx.doi.org/10.1016/j.devcel.2011.01.014>.
- Wang XS, Armstrong ME, Cairns BJ, Key TJ, Travis RC. Shift work and chronic disease: the epidemiological evidence. *Occup Med (Lond).* 2011;61:78–89. <http://dx.doi.org/10.1093/occmed/kqr001>.
- Stevens RG. Circadian disruption and breast cancer: from melatonin to clock genes. *Epidemiology.* 2005;16:254–8. <http://dx.doi.org/10.1097/01.ede.0000152525.21924.54>.
- Garault M, Madrid JA. Chronobiological aspects of nutrition, metabolic syndrome and obesity. *Adv Drug Deliv Rev.* 2010;62:967–78. <http://dx.doi.org/10.1016/j.addr.2010.05.005>.
- Blask DE, Hill SM, Dauchy RT, Xiang S, Yuan L, Duplessis T, et al. Circadian regulation of molecular, dietary, and metabolic signaling mechanisms of human breast cancer growth by the nocturnal melatonin signal and the consequences of its disruption by light at night. *J Pineal Res.* 2011;51:259–69. <http://dx.doi.org/10.1111/j.1600-079X.2011.00888.x>.
- Hoffman AE, Zheng T, Ba Y, Stevens RG, Yi CH, Leaderer D, et al. Phenotypic effects of the circadian gene Cryptochrome 2 on cancer-related pathways. *BMC Cancer.* 2010;10:110. <http://dx.doi.org/10.1186/1471-2407-10-110>.
- Ando H, Ushijima K, Kumazaki M, Eto T, Takamura T, Irie S, et al. Associations of metabolic parameters and ethanol consumption with messenger RNA expression of clock genes in healthy men. *Chronobiol Int.* 2010;27:194–203. <http://dx.doi.org/10.3109/07420520903398617>.
- Cuninkova L, Brown SA. Peripheral circadian oscillators: interesting mechanisms and powerful tools. *Ann N Y Acad Sci.* 2008;1129:358–70. <http://dx.doi.org/10.1196/annals.1417.005>.
- Huang MC, Ho CW, Chen CH, Liu SC, Chen CC, Leu SJ. Reduced expression of circadian clock genes in male alcoholic patients. *Alcohol Clin Exp Res.* 2010;34:1899–904. <http://dx.doi.org/10.1111/j.1530-0277.2010.01278.x>.
- Sookoian S, Gianotti TF, Burgueño A, Pirola CJ. Gene-gene interaction between serotonin transporter (SLC6A4) and CLOCK modulates the risk of metabolic syndrome in rotating shiftworkers. *Chronobiol Int.* 2010;27:1202–18. <http://dx.doi.org/10.3109/07420528.2010.496913>.
- Woon PY, Kaisaki PJ, Bragança J, Bihoreau MT, Levy JC, Farrall M, et al. Aryl hydrocarbon receptor nuclear translocator-like (BMAL1) is associated with susceptibility to hypertension and type 2 diabetes. *Proc Natl Acad Sci U S A.* 2007;104:14412–7. <http://dx.doi.org/10.1073/pnas.0703247104>.
- Boivin DB, James FO, Wu A, Cho-Park PF, Xiong H, Sun ZS. Circadian clock genes oscillate in human peripheral blood mononuclear cells. *Blood.* 2003;102:4143–5. <http://dx.doi.org/10.1182/blood-2003-03-0779>.
- Ackermann K, Sletten TL, Revell VL, Archer SN, Skene DJ. Blue-light phase shifts PER3 gene expression in human leukocytes. *Chronobiol Int.* 2009;26:769–79. <http://dx.doi.org/10.1080/07420520902929045>.
- Archer SN, Viola AU, Kyriakopoulou V, von Schantz M, Dijk DJ. Inter-individual differences in habitual sleep timing and entrained phase of endogenous circadian rhythms of BMAL1, PER2 and PER3 mRNA in human leukocytes. *Sleep.* 2008;31:608–17.
- Kusanagi H, Mishima K, Satoh K, Echizenya M, Katoh T, Shimizu T. Similar profiles in human period1 gene expression in peripheral mononuclear and polymorphonuclear cells. *Neurosci Lett.* 2004;365:124–7. <http://dx.doi.org/10.1016/j.neulet.2004.04.065>.
- Kusanagi H, Hida A, Satoh K, Echizenya M, Shimizu T, Pendergast JS, et al. Expression profiles of 10 circadian clock genes in human peripheral blood mononuclear cells. *Neurosci Res.* 2008;61:136–42. <http://dx.doi.org/10.1016/j.neures.2008.01.012>.
- Teboul M, Barrat-Petit MA, Li XM, Claustrat B, Formento JL, Delaunay F, et al. Atypical patterns of circadian clock gene expression in human peripheral blood mononuclear cells. *J Mol Med.* 2005;83:693–9. <http://dx.doi.org/10.1007/s00109-005-0697-6>.

19. Viola AU, Archer SN, James LM, Groeger JA, Lo JC, Skene DJ, et al. PER3 polymorphism predicts sleep structure and waking performance. *Curr Biol*. 2007;17:613–8. <http://dx.doi.org/10.1016/j.cub.2007.01.073>.
20. Yang MY, Yang WC, Lin PM, Hsu JF, Hsiao HH, Liu YC, et al. Altered expression of circadian clock genes in human chronic myeloid leukemia. *J Biol Rhythms*. 2011;26:136–48. <http://dx.doi.org/10.1177/0748730410395527>.
21. James FO, Boivin DB, Charbonneau S, Bélanger V, Cermakian N. Expression of clock genes in human peripheral blood mononuclear cells throughout the sleep/wake and circadian cycles. *Chronobiol Int*. 2007;24:1009–34. <http://dx.doi.org/10.1080/07420520701800736>.
22. James FO, Cermakian N, Boivin DB. Circadian rhythms of melatonin, cortisol, and clock gene expression during simulated night shift work. *Sleep*. 2007;30:1427–36.
23. Peplonska B, Bukowska A, Gromadzinska J, Sobala W, Reszka E, Lie J-A, et al. Night shift work characteristics and sulfatoksymelatonin (MT6s) in rotating night shift nurses and midwives. *Occup Environ Med*. 2012 May;69(5):339–46. <http://dx.doi.org/10.1136/oemed-2011-100273>.
24. Muller PY, Janovjak H, Miserez AR, Dobbie Z. Processing of gene expression data generated by quantitative real-time RT-PCR. *Biotechniques*. 2002;2:1372–9.
25. Dumeaux V, Olsen KS, Nuel G, Paulssen RH, Børresen-Dale AL, Lund E. Deciphering normal blood gene expression variation. The NOWAC postgenome study. *PLoS Genet*. 2010;6:e1000873. <http://dx.doi.org/10.1371/journal.pgen.1000873>.
26. Schwartz WJ, Tavakoli-Nezhad M, Lambert CM, Weaver DR, de la Iglesia HO. Distinct patterns of Period gene expression in the suprachiasmatic nucleus underlie circadian clock photoentrainment by advances or delays. *Proc Natl Acad Sci U S A*. 2011;108:17219–24. <http://dx.doi.org/10.1073/pnas.1107848108>.
27. Akashi M, Soma H, Yamamoto T, Tsugitomi A, Yamashita S, Yamamoto T, et al. Noninvasive method for assessing the human circadian clock using hair follicle cells. *Proc Natl Acad Sci U S A*. 2010;107:15643–8. <http://dx.doi.org/10.1073/pnas.1003878107>.
28. Kavcic P, Rojic B, Dolenc-Groselj L, Claustrat B, Fujs K, Poljak M. The impact of sleep deprivation and nighttime light exposure on clock gene expression in humans. *Croat Med J*. 2011;52:594–603. <http://dx.doi.org/10.3325/cmj.2011.52.594>.
29. Chen A, Du L, Xu Y, Chen L, Wu Y. The effect of blue light exposure on the expression of circadian genes: bmal1 and cryptochrome 1 in peripheral blood mononuclear cells of jaundiced neonates. *Pediatr Res*. 2005;58:1180–4. <http://dx.doi.org/10.1203/01.pdr.0000183663.98446.05>.
30. Cajochen C, Jud C, Münch M, Kriebitzsch S, Wirz-Justice A, Albrecht U. Evening exposure to blue light stimulates the expression of the clock gene PER2 in humans. *Eur J Neurosci*. 2006;23:1082–6. <http://dx.doi.org/10.1111/j.1460-9568.2006.04613.x>.
31. Ando H, Ushijima K, Kumazaki M, Takamura T, Yokota N, Sait T, et al. Influence of age on clock gene expression in peripheral blood cells of healthy women. *J Gerontol A Biol Sci Med Sci*. 2010;65:9–13. <http://dx.doi.org/10.1093/geron/65.1.9>.
32. Gouin JP, Connors J, Kiecolt-Glaser JK, Glaser R, Malarkey WB, Atkinson C, et al. Altered expression of circadian rhythm genes among individuals with a history of depression. *J Affect Disord*. 2010;126:161–6. <http://dx.doi.org/10.1016/j.jad.2010.04.002>.
33. Hida A, Kusanagi H, Satoh K, Kato T, Matsumoto Y, Echizenya M, et al. Expression profiles of PERIOD1, 2, and 3 in peripheral blood mononuclear cells from older subjects. *Life Sci*. 2009;84:33–37. <http://dx.doi.org/10.1016/j.lfs.2008.10.012>.

Received for publication: 9 December 2011