Published in final edited form as:
Chronobiol Int. 2010 July ; 27(5): 1093–1104. doi:10.3109/07420528.2010.490065.

EPIGENETIC EFFECTS OF SHIFTWORK ON BLOOD DNA METHYLATION

Valentina Bollati[1], **Andrea Baccarelli**[1,2], **Samantha Sartori**[1,3], **Letizia Tarantini**[1], **Valeria Motta**[1], **Federica Rota**[1], and **Giovanni Costa**[1,2]

[1]Dipartimento di Medicina del Lavoro, Università degli Studi di Milano, Milan, Italy

[2]Foundation IRCCS Ca' Granda–Ospedale Maggiore Policlinico, Milan, Italy

[3]Institute of Medical Statistics and Biometrics, "G.A. Maccacaro," Università degli Studi di Milano, Milan, Italy

Abstract

In the present study, the authors investigated the effects of shiftwork exposure on DNA methylation using peripheral blood DNA from subjects working in two chemical plants in Northern Italy. The investigation was designed to evaluate (a) DNA methyl- ation changes in Alu and long interspersed nuclear element-1 (LINE-1) repetitive elements as a surrogate of global methylation and (b) promoter methylation of gluco- corticoid receptor (GCR), tumor necrosis factor alpha (TNF-α), and interferon- gamma (IFN-γ). One hundred and fifty white male workers (mean ± SD: 41.0 ± 9 yrs of age) were examined: 100 3 × 8 rotating shiftworkers (40.4 ± 8.7 yrs of age) and 50 day workers (42.2 ± 9.4 yrs of age). The authors used bisulfite-pyrosequencing to esti- mate repetitive elements and gene-specific methylation. Multiple regression analysis, adjusted for age, body mass index (BMI), and job seniority, did not show any signifi- cant association between the five DNA methylation markers and shiftwork. However, job seniority, in all subjects, was significantly associated with Alu (β = −0.019, $p = .033$) and IFN-γ (β = −0.224, $p < .001$) methylation, whereas TNF-α methylation was inversely correlated with age (β = −0.093, $p < .001$). Considering only shiftworkers, multiple regression analysis, adjusted for age, BMI, and job seniority, showed a sig- nificant difference between morning and evening types in TNF-α methylation (mean morning type [MT] 11.425 %5mC versus evening type [ET] 12.975 %5mC; β = 1.33, $p = .022$). No difference was observed between good and poor tolerance to shiftwork. Increasing job seniority (<5, 5–15, >15 yrs) was associated with significantly lower Alu (β = −0.86, $p = .006$) and IFN-γ methylation (β = −6.50, $p = .007$) after adjust- ment for age, BMI, and morningness/eveningness. In addition, GCR significantly increased with length of shiftwork (β = 3.33, $p = .05$). The data showed alterations in blood DNA methylation in a group of shiftworkers, including changes in Alu repeti- tive elements methylation and gene-specific methylation of IFN-γ and TNF-α promoters. Further studies are required to determine the role of such alterations in mediating the effects of shiftwork on human health.

Address correspondence to Prof. Giovanni Costa, Dipartimento di Medicina del Lavoro "Clinica del Lavoro L. Devoto," Via S. Barnaba 8, 20122 Milano, Italy. Tel.: ++39 0250320151; Fax: ++39 0250320150; giovanni.costa@unimi.it.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

This paper was presented at the 19th International Symposium on Shiftwork and Working Time, August 2–6, 2009, San Servolo Island, Venice, Italy.

Keywords

Cancer; DNA methylation; Epigenetic changes; Night work; Shiftwork

INTRODUCTION

According to several epidemiological studies, shiftwork that requires work at night can have a negative impact on the health and well-being due to a mismatch between the endogenous circadian system and environmental synchronizers (e.g., light/dark cycle). Besides the short-term effects, which can be summarized as a form of "jet-lag" syndrome, the most reported long-term effects are chronic sleep, gastrointestinal, neuropsychological, metabolic, and cardiovascular disorders, as well as negative interference on pregnancy (Akerstedt, 1998; Knutsson, 2003; Biggi et al., 2008; Esquirol et al., 2009; Lin et al., 2009; Suwazono et al., 2009). More recently, the International Agency on Research on Cancer (IARC) has classified "shiftwork that involves circadian disruption" as "probably carcinogenic to humans" (Group 2A) on the basis of "limited evidence in humans for the carcinogenicity of shift-work that involves nightwork," and "sufficient evidence in experimental animals for the carcinogenicity of light during the daily dark period (biological night)" (Straif et al., 2007; Kloog et al., 2009; Kantermann & Roenneberg, 2009).

In mammals, physiological and hormonal processes as well as behavioral reactions follow circadian rhythms that are driven by an endogenous master clock (Chen et al., 2005; Duguay & Cermakian, 2009). Light exposure and melatonin secretion are considered the links between night work and health problems in workers (Erren et al., 2008; Haus, 2007; Stevens, 2009).

An emerging area of interest is in epigenetic reprogramming of cir- cadian genes, such as by promoter methylation (Sahar & Sassone-Corsi, 2007; Zhu et al., 2006). There is evidence that breast tumor cells have an altered expression than the surrounding normal cells (Chen et al., 2005), although this may be a result of the disease process and not its cause. Some environmental factors have been linked to aberrant changes in methylation both in experimental and epidemiological studies (Baccarelli et al., 2009; Bollati et al., 2007, 2009; Pavanello et al., 2009; Rusiecki et al., 2008; Tarantini et al., 2009; Wright et al., 2010). Because these changes are small and potentially cumulative, it may be difficult to establish the cause-effect relationships among environmental factors, epigenetic changes, and diseases (Baccarelli & Bollati, 2009). In fact, there is still some debate as to whether methylation of the genome,which is largely determined in utero or early life, stays constant through life, or whether, how much, and in which loci it changes during life due to environmental or internal factors (Christensen et al., 2009; Feinberg, 2007).

Disruption of the circadian system may be a risk factor in the devel- opment of cancer, but the molecular mechanisms that are involved are far from unclear (Haus, 2007; Haus & Smolensky, 2006). Aberrant DNA methylation patterns, including global hypomethylation and gene- specific hyper/hypomethylation, are commonly observed in cancer tissues. Methylation of repetitive sequences, such as Alu and long inter- spersed nuclear element-1 (LINE-1), tend to decrease in each step of the progression from normal to cancer cells. The human genome comprises ~1.4 million copies of Alu repetitive elements and a half a million copies of LINE-1 elements (Lander et al., 2001; Yang et al., 2004), and repetitive element methylation has been shown to correlate with total genomic methylation content (Weisenberger et al., 2005; Yang, et al., 2004). Decreases in global DNA methylation content have been associ- ated with widespread alterations in gene expression and chromatin packaging control, as well as with higher genomic instability (Dean et al., 2005).

The glucocorticoid receptor (GCR) gene is involved in hypothalamic- pituitary-adrenal axis (HPA) functioning through negative feedback inhi- bition and is susceptible to stress-related programming. The human GCR promoter region is extensively methylated with diverse methylation profile demonstrated in normal donors (Meaney & Szyf, 2005). The tumor necrosis factor alpha (TNF-α) is a potent cytokine that plays important roles in diverse cellular events, such as cell survival, proliferation, differentiation, death, and also regulates inflammation in in vitro and in vivo animal systems, via the induction of adhesion molecules and chemokine expression (Sullivan et al., 2007). TNF-α is commonly elevated in a variety of human diseases, including cancer, and TNF-α promoter is susceptible to regulation by cytosine methylation (Sullivan, et al., 2007). Interferon-gamma (IFN-γ) is a cytokine able to trigger the formation and release of reactive oxygen species. It is critical for innate and adaptive immunity and tumor control and aberrant IFN- γ expression has been observed in many cancers (Schoenborn & Wilson, 2007).

In the present study, we investigated the effects of shiftwork exposure on DNA methylation using peripheral blood DNA from subjects working in two chemical plants in Northern Italy. The investigation was designed to evaluate (a) DNA methylation changes in Alu and long interspersed nuclear element-1 (LINE-1) repetitive elements as a surrogate of global genomic methylation content and (b) promoter methylation of GCR, TNF-α, and IFN-γ.

MATERIALS AND METHODS

Subjects

Our study population consisted of 150 white males (mean ± SD: 41 ± 9 yrs of age) free of cancer, cardiovascular, and pulmonary disease employed in two chemical plants in Northern Italy. One hundred partici- pants (40.4 ± 8.7 yrs of age) worked a backward rotating 8-h schedule, and 50 participants were day workers (42.2 ± 9.4 yrs of age). The start and end of shifts were morning shift: 06:00–14:00 h, afternoon shift: 14:00–22:00 h, and night shift: 22:00–06:00 h. Participants worked 4 days and then had 2 rest days. The participants were engaged in the same production departments and exposed to the same risk factors. The exposure to environmental concentrations of all chemical substances was <25% of Threshold Limit Values (ACGIH, 2008). Job seniority was shorter in shift (mean ± SD: 11.6 ± 8.6 yrs) than in day workers (16.6 ± 9.8 yrs). The average (SD) body mass index (BMI) of shiftworkers was 24.8 kg/m^2 (± 2.9), whereas the BMI of day workers was 25.1 kg/m^2 (± 3.2); 19.5% shiftworkers and 26.2% day workers were current smokers.

Detailed information on lifestyle, use of medication, past and recent medical conditions, and residential history were collected through per- sonal interview as part of an annual medical screening of employees. Morningness/eveningness was assessed by the Horne-Östberg questionnaire (1976), and “poor tolerance” to shifwork was defined via the pres- ence of at least one of the following conditions: persistent gastrointestinal, sleep, or anxiety/depression disorders that required the administration of medication, e.g., antiacids, hypnotics, sedatives (Reinberg & Ashkenazi, 2008). Data collection and analyses in the present study were conducted in accordance with the ethical standards of this journal (Portaluppi et al., 2008).

Blood Collection

Seven milliliters of whole blood were collected by venous phlebotomy in EDTA tubes. Blood samples were shipped to the Center of Molecular and Genetic Epidemiology in Milan and processed within 3 h. Buffy coat was extracted and stored in cell lyses solution until DNA extraction. All samples were coded and frozen at −20°C.

DNA Extraction and Bisulfite Treatment of the DNA

DNA was extracted from stored frozen buffy coat of 7 mL of whole blood, using the Maxwell® 16 System (Promega, Madison, Wisconsin). Five hundred nanograms of DNA (concentration 25 ng/μL) were treated using EZ DNA Methylation-Gold™ Kit (Zymo Research, Orange, CA, USA) according to the manufacturer's protocol. Final elution was per- formed with 30 μL of M-Elution Buffer.

Repetitive Element PCR and Pyrosequencing

Analysis of repetitive element DNA methylation was performed using previously published methods (Bollati et al., 2007; Yang et al., 2004), with minor modifications. Polymerase chain reaction (PCR) primers were designed towards a consensus Alu or LINE-1 sequence and allowed for the amplification of a representative pool of repetitive elements to serve as a surrogate for global DNA methylation changes. A 50-μLPCR was carried out in 25 μL of GoTaq Green Master mix (Promega), 1 pmol of the forward primer, 1 pmol of the reverse primer, 50 ng of bisulfite- treated genomic DNA, and water. One of the two primers was biotin- labeled to purify the final PCR product using Sepharose beads. The PCR product was bound to Streptavidin Sepharose HP (Amersham Biosciences, Uppsala, Sweden), and the Sepharose beads containing the immobilized PCR product were purified, washed, denatured using a 0.2 M NaOH solution, and washed again using the Pyrosequencing Vacuum Prep Tool (Pyrosequencing, Inc., Westborough, MA), as recommended by the manufacturer. Then, 0.3 μM pyrosequencing primer was annealed to the purified single-stranded PCR product, and pyrosequencing was performed using the PyroMark MD (Pyrosequencing, Inc.). The degree of methylation was expressed for both Alu and LINE-1 as the percentage of methylated cytosines divided by the sum of methylated and unmethy- lated cytosines (%5mC). We used built-in controls to verify bisulfite con- version efficiency. Every sample was tested three times for each marker to confirm reproducibility and increase the precision of our results. The average of the three replicates was used in statistical analyses. A similar method was used to measure gene-specific methylation in glococorticoid receptor (GCR), tumor necrosis factor alpha (TNF-α), and interferon- gamma (IFN-γ) promoters.

Statistical Analysis

Two-sample t tests with equal variances, analysis of variance (ANOVA), and multiple regression analysis, adjusted for age, BMI, and job seniority, were carried out using the Stata 10 (StataCorp, 2007) stat- istical package.

RESULTS

DNA Methylation in Shift- and Day Workers

By univariate regression analysis, a significant difference in Alu methylation was observed between shift- and day workers (25.4 %5mC versus 25.1 %5mC; $p = .04$), whereas no significant differences were detected in LINE-1, GCR, TNF-α, and IFN-γ (Table 1). The result of a multiple regression analysis, adjusted for age, BMI, and job seniority, found no significant association between DNA methylation markers and shiftwork (Table 2). In all subjects, job seniority was significantly associ- ated with Alu ($\beta = -0.019$, $p = .033$) and IFN-γ ($\beta = -0.224$, $p = .001$) decreased methylation, whereas TNF-α was inversely correlated with age ($\beta = -0.093$, $p = .001$) (see Table 2).

DNA Methylation in Morning- and Evening-Type Shiftworkers

Considering only the shiftworkers, multiple regression analysis adjusted for age, BMI, and job seniority found a significant difference in TNF-α methylation between morning (MT, 35 persons) and evening (ET, 25 persons) types (mean MT = 11.42 %5mC, mean ET = 12.97

%5mC; $p = .006$) (see Table 3). Among shiftworkers, the association between job seniority and Alu and IFN-γ methylation was confirmed, and GCR showed a significant association with job seniority ($\beta = 0.152$, $p = .05$) (see Table 2).

DNA Methylation and Job Seniority in Shiftworkers

We divided the shiftworkers into the following job seniority categories (<5, 5–15, >15 yrs). On average, we found a significant trend for Alu methylation ($p = .03$), TNF-α methylation ($p = .01$), and IFN-γ methyl- ation ($p = .004$); these concentrations decreased as seniority increased (see Table 4). Using multiple regression adjusted for age, BMI, and morningness/ eveningness (see Table 5), we found a significant difference in Alu methylation for workers with job seniority >15 yrs ($\beta = -0.86$, $p = .006$) and in IFN-γ methylation for workers with job seniority between 5 and 15 yrs ($\beta = -4.28$, $p = .02$) and for workers with job seniority >15 yrs ($\beta = -6.50$, $p = .007$).

DNA Methylation and Tolerance to Shiftwork

No differences in methylation was found in subjects with good (n = 40) or poor (n = 35) tolerance to shiftwork.

DISCUSSION

In this study, we investigated the effects of shiftwork exposure on DNA methylation using peripheral blood DNA from subjects working in two chemical plants in Northern Italy. Although multivariate analysis revealed no significant differences between day and shiftworkers for the five markers of methylation (Alu, LINE-1, GCR, TNF-α, and IFN-γ), some differences were found when we examined the workshift subgroups. In particular, we observed a significant influence of job seniority on hypo- methylation of Alu and IFN-γ and a significant influence of morningness type on TNF-α hypomethylation. Age may not explain these results because after controlling for age, we obtained a significant correlation between Alu hypomethylation and job seniority.

We used DNA methylation analyses of Alu and LINE-1 repetitive elements to evaluate global methylation. Due to the heavy methylation of repetitive elements, these assays, which are easier to carry out than pre- vious methods employed to quantify total genomic 5-mC, can detect decreases in DNA methylation and serve as a surrogate for global methyl- ation (Yang, et al., 2004). Global DNA hypomethylation is generally associated with chromosomal instability, reactivation of retrotransposable elements, and expression of genes that would normally be silenced by methylation (Jones & Baylin, 2002; Smith & Crocitto, 1999).

Job seniority was significantly correlated with IFN-γ hypomethylation. Epigenetic methylation status of IFN-γ may play a mechanistic role in the modulation of cytokine secretion, and an altered IFN-γ expression has been found in disease conditions that have also been associated with shift- work, such as cardiovascular disease and cancer. Because IFN-γ is a prime proinflammatory cytokine in inflammation (Zhang, 2007), this hypomethylation may lead to an increase in inflammation.

Deregulation in a complex and well-coordinated response to inflam- mation can lead to unresolved inflammation and a protumorigenic micro- environment (Hussain & Harris, 2007). Mediators of inflammation, such as cytokines, can produce genetic alteration, including point mutations in tumor suppressor genes, changed gene-expression profile, and induced post-translational modifications leading to genetic and physiological instability and cancer. These alterations have been described in cancer- prone chronic inflammatory diseases before the development of cancer (Hussain & Harris, 2007). Therefore, this

regulation of epigenetic control may represent a mechanism linking shiftwork with increased cancer risk that has been observed in some epidemiological studies.

Shiftworkers defined as “morning type” showed TNF-α hypomethyla- tion, a change that is potentially related to an increased gene expression. TNF-α is a proinflammatory cytokine that induces neutrophil prolifer- ation during inflammation (Murray et al., 1997). The low levels of the cytokine may aid in maintaining homeostasis by regulating the body’s cir- cadian system (Cavadini et al., 2007; Tracey & Cerami, 1990; Duguay & Cermakian, 2009). Because TNF-α hypomethylation could result in decreased expression in shiftworkers defined as morning types, the increase of TNF-α in morning subjects may indicate reduction in circa- dian system regulation.

The results from this study should be considered as preliminary; thus, we recommend future studies to confirm our findings. Although several other genes might have been included in our study, we selected GCR, TNF-α, and IFN-γ for DNA methylation analysis because of their known role in inflammatory and cancer pathways and because they are expressed in many human tissues, including blood, which was the source of DNA for our study. Our results showed alterations in blood DNAmethylation in a population of shiftworkers, including changes in Alu repetitive elements methylation and gene-specific methylation of IFN-γ and TNF-α promoters. Because it has been shown by Bonsch and co-workers (2007) that methylation exhibits circadian rhythmicity, one limit- ation of our study is that we measured DNA methylation in one sample obtained from subject, although all the samples were taken approximately at the same time. Further studies comparing full 24-h patterns, rather than one point sampling, are required to better assess whether changes in methylation are due to a phase shift rather than a total increase or decrease in DNA methylation. Moreover, it would be interesting in the future to assess methylation level in a group of former long-time shiftworkers, after ceasing shiftwork, to assess if these effects are transient or permanent. In addition, further studies will also be needed to determine the role of such alterations in mediating the effects of shiftwork on human health.

REFERENCES

ACGIH. Documentation of the TLVs and BEIs. 7th ed. ACGIH Worldwide; Cincinnati: 2008.

Åkerstedt T. Shift work and disturbed sleep/wakefulness. Sleep Med. Rev. 1998; 2:117–128. [PubMed: 15310506]

Baccarelli A, Bollati V. Epigenetics and environmental chemicals. Curr. Opin. Pediatr. 2009; 21:243–251. [PubMed: 19663042]

Baccarelli A, Wright RO, Bollati V, Tarantini L, Litonjua AA, Suh HH, Zanobetti A, Sparrow D, Vokonas PS, Schwartz J. Rapid DNA methylation changes after exposure to traffic par- ticles. Am. J. Respir. Crit. Care Med. 2009; 179:572–578. [PubMed: 19136372]

Biggi N, Consonni D, Galluzzo V, Sogliani M, Cosat G. Metabolic syndrome in permanent night workers. Chronobiol. Int. 2008; 25:443–454. [PubMed: 18484373]

Bollati V, Baccarelli A, Hou L, Bonzini M, Fustinoni S, Cavallo D, Byun HM, Jiang J, Marinelli B, Pesatori AC, Bertazzi PA, Yang AS. Changes in DNA methylation patterns in subjects exposed to low-dose benzene. Cancer Res. 2007; 67:876–880. [PubMed: 17283117]

Bollati V, Schwartz J, Wright R, Litonjua A, Tarantini L, Suh H, Sparrow D, Vokonas P, Baccarelli A. Decline in genomic DNA methylation through aging in a cohort of elderly subjects. Mech. Ageing Dev. 2009; 130:234–239. [PubMed: 19150625]

Bonsch D, Hothorn T, Krieglstein C, Koch M, Nehmer C, Lenz B, Reulbach U, Kornhuber J, Bleich S. Daily variations of homocysteine concentration may influence methylation of DNA in normal healthy individuals. Chronobiol. Int. 2007; 24:315–326. [PubMed: 17453850]

Cavadini G, Petrzilka S, Kohler P, Jud C, Tobler I, Birchler T, Fontana A. TNF-alpha sup- presses the expression of clock genes by interfering with E-box-mediated transcription. Proc. Natl. Acad. Sci. U. S. A. 2007; 104:12843–12848. [PubMed: 17646651]

Chen ST, Choo KB, Hou MF, Yeh KT, Kuo SJ, Chang JG. Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. Carcinogenesis. 2005; 26:1241–1246. [PubMed: 15790588]

Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR, Wiemels JL, Nelson HH, Karagas MR, Padbury JF, Bueno R, Sugarbaker DJ, Yeh RF, Wiencke JK, Kelsey KT. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. PLoS Genet. 2009; 5:pe1000602.

Dean W, Lucifero D, Santos F. DNA methylation in mammalian development and disease. Birth Defects Res. C Embryo Today. 2005; 75:98–111. [PubMed: 16035040]

Duguay D, Cermakian N. The crosstalk between physiology and circadian clock proteins. Chronobiol. Int. 2009; 26:1479–1513. [PubMed: 20030537]

Erren TC, Pape HG, Reiter RJ, Piekarski C. Chronodisruption and cancer. Naturwissenschaften. 2008; 95:367–382. [PubMed: 18196215]

Esquirol Y, Bongard V, Mabile L, Jonnier B, Soulat J-M, Perret B. Shift work and metabolic syndrome: respective impacts of job strain, physical activity and dietary rhythms. Chronobiol. Int. 2009; 26:544–559. [PubMed: 19360495]

Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. Nature. 2007; 447:433–440. [PubMed: 17522677]

Haus E. Circadian disruption in shiftwork is probably carcinogenic to humans. Chronobiol. Int. 2007; 24:1255–1256. [PubMed: 18075812]

Haus E, Smolensky M. Biological clocks and shift work: circadian dysregulation and potential long-term effects. Cancer Causes Control. 2006; 17:489–500. [PubMed: 16596302]

Horne JA, Östberg O. A self-assessment questionnaire to determine morningness–eveningness in human circadian systems. Int. J. Chronobiol. 1976; 4:97–110. [PubMed: 1027738]

Hussain SP, Harris CC. Inflammation and cancer: an ancient link with novel potentials. Int. J. Cancer. 2007; 121:2373–2380. [PubMed: 17893866]

Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat. Rev. Genet. 2002; 3:415–428. [PubMed: 12042769]

Kantermann T, Roenneberg T. Is light-at-night a health risk or a risk predictor? Chronobiol. Int. 2009; 26:1069–1074. [PubMed: 19731106]

Kloog I, Haim A, Stevens RG, Portnov BA. Global co-distribution of light at night (LAN) and cancers of prostate, colon, and lung in men. Chronobiol. Int. 2009; 26:108–125. [PubMed: 19142761]

Knutsson A. Health disorders of shift workers. Occup. Med. (Lond). 2003; 53:103–108. [PubMed: 12637594]

Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blocker H,

Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ. Initial sequencing and analysis of the human genome. Nature. 2001; 409:860–921. [PubMed: 11237011]

Lin Y-C, Hsiao T-J, Chen P-C. Persistent shift-work exposure accelerates development of metabolic syndrome among middle-aged female employees: a five-year follow-up. Chronobiol. Int. 2009; 26:740–755. [PubMed: 19444753]

Meaney MJ, Szyf M. Environmental programming of stress responses through DNA methyl- ation: life at the interface between a dynamic environment and a fixed genome. Dialog. Clin. Neurosci. 2005; 7:103–123.

Murray J, Barbara JA, Dunkley SA, Lopez AF, Van Ostade X, Condliffe AM, Dransfield I, Haslett C, Chilvers ER. Regulation of neutrophil apoptosis by tumor necrosis factor-alpha: require- ment for TNFR55 and TNFR75 for induction of apoptosis in vitro. Blood. 1997; 90:2772–2783. [PubMed: 9326245]

Pavanello S, Bollati V, Pesatori AC, Kapka L, Bolognesi C, Bertazzi PA, Baccarelli A. Global and gene-specific promoter methylation changes are related to anti-B[a]PDE-DNA adduct levels and influence micronuclei levels in polycyclic aromatic hydrocarbon-exposed individuals. Int. J. Cancer. 2009; 125:1692–1697. [PubMed: 19521983]

Portaluppi F, Touitou Y, Smolensky MH. Ethical and methodological standards for laboratory and medical biological rhythm research. Chronobiol. Int. 2008; 25:999–1016. [PubMed: 19005901]

Reinberg A, Ashkenazi I. Internal desynchronization of circadian rhythms and tolerance to shift work. Chronobiol. Int. 2008; 25:625–643. [PubMed: 18622820]

Rusiecki JA, Baccarelli A, Bollati V, Tarantini L, Moore LE, Bonefeld-Jorgensen EC. Global DNA hypomethylation is associated with high serum-persistent organic pollutants in Greenlandic Inuit. Environ. Health Perspect. 2008; 116:1547–1552. [PubMed: 19057709]

Sahar S, Sassone-Corsi P. Circadian clock and breast cancer: a molecular link. Cell Cycle. 2007; 6:1329–1331. [PubMed: 17534151]

Schoenborn JR, Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. Adv. Immunol. 2007; 96:41–101. [PubMed: 17981204]

Smith SS, Crocitto L. DNA methylation in eukaryotic chromosome stability revisited: DNA methyltransferase in the management of DNA conformation space. Mol. Carcinog. 1999; 26:1–9. [PubMed: 10487516]

Stevens RG. Light-at-night, circadian disruption and breast cancer: assessment of existing evi- dence. Int. J. Epidemiol. 2009; 38:963–970. [PubMed: 19380369]

Straif K, Baan R, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Altieri A, Benbrahim-Tallaa L, Cogliano V. Carcinogenicity of shift-work, painting, and fire-fighting. Lancet Oncol. 2007; 8:1065–1066. [PubMed: 19271347]

Sullivan KE, Reddy AB, Dietzmann K, Suriano AR, Kocieda VP, Stewart M, Bhatia M. Epigenetic regulation of tumor necrosis factor alpha. Mol. Cell Biol. 2007; 27:5147–5160. [PubMed: 17515611]

Suwazono Y, Dochi M, Oishi M, Tanaka K, Kobayashi E, Sakata K. Shiftwork and impaired glucose metabolism: a 14-year cohort study on 7104 male workers. Chronobiol. Int. 2009; 26:926–941. [PubMed: 19637051]

Tarantini L, Bonzini M, Apostoli P, Pegoraro V, Bollati V, Marinelli B, Cantone L, Rizzo G, Hou L, Schwartz J, Bertazzi PA, Baccarelli A. Effects of particulate matter on genomic DNA methylation content and iNOS promoter methylation. Environ. Health Perspect. 2009; 117:217–222. [PubMed: 19270791]

Tracey KJ, Cerami A. Metabolic responses to cachectin/TNF. A brief review. Ann. N. Y. Acad. Sci. 1990; 587:325–331.

Weisenberger DJ, Campan M, Long TI, Kim M, Woods C, Fiala E, Ehrlich M, Laird PW. Analysis of repetitive element DNA methylation by MethyLight. Nucleic Acids Res. 2005; 33:6823–6836. [PubMed: 16326863]

Wright RO, Schwartz J, Wright RJ, Bollati V, Tarantini L, Park SK, Hu H, Sparrow D, Vokonas P, Baccarelli A. Biomarkers of lead exposure and DNA methylation within retrotranspo- sons. Environ. Health Perspect. 2010; 118:790–795. doi:101289/ehp.0901429. [PubMed: 20064768]

Yang AS, Estecio MR, Doshi K, Kondo Y, Tajara EH, Issa JP. A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. Nucleic Acids Res. 2004; 32:e38. doi:10.1093/nar/gnh032. [PubMed: 14973332]

Zhang J. Yin and yang interplay of IFN-gamma in inflammation and autoimmune disease. J. Clin. Invest. 2007; 117:871–873. [PubMed: 17404615]

Zhu Y, Zheng T, Stevens RG, Zhang Y, Boyle P. Does “clock” matter in prostate cancer? Cancer Epidemiol. Biomarkers Prev. 2006; 15:3–5. [PubMed: 16434577]

TABLE 1

Differences in methylation of Alu, LINE-1, GCR, TNF-α, and IFN-γ between day workers and shiftworkers

	Day workers (n = 50)		Shiftworkers (n = 100)		
	Average	**SE**	**Average**	**SE**	***p* value**
Global DNA methylation markers					
Alu (%5mC)	25.1	0.09	25.4	0.07	**.04**
LINE-1 (%5mC)	82.5	0.17	82.3	0.12	.29
Gene-specific methylation markers					
GCR (%5mC)	44.7	0.51	44.9	0.40	.72
TNF-α (%5mC)	11.7	0.39	12.1	0.24	.42
IFN-γ (%5mC)	82.1	0.69	81.4	0.55	.75

TABLE 2

Multiple regression analysis for shiftworker condition adjusting for age, body mass index, and job seniority

	Shiftworkers/ day workers	**Age**	**Body mass index**	**Job seniority**
Global DNA methylation markers				
Alu (%5mC)	β = 0.162	β = 0.008	β = 0.001	**β = −0.019**
	p = .210	*p* = .346	*p* = .951	***p* = .033**
LINE-1 (%5mC)	β = −0.212	β = −0.010	β = −0.032	β = 0.008
	p = .339	*p* = .470	*p* = .345	*p* = .603
Gene-specific methylation markers				
GCR (%5mC)	β = 0.622	β = −0.036	β = −0.110	β = 0.092
	p = .380	*p* = .437	*p* = .312	*p* = .064
TNF-α (%5mC)	β = 0.027	**β = −0.093**	β = −0.012	β = −0.029
	p = .950	***p* = .001**	*p* = .850	*p* = .333
IFN-γ (%5mC)	β = −0.705	β = 0.069	β = 0.068	**β = −0.224**
	p = .452	*p* = .254	*p* = .635	***p* = .001**

TABLE 3

Multiple regression analysis in shiftworkers, for morning- versus evening-type workers, adjusting for age, body mass index, and job seniority

	Morning type/ evening type	**Age**	**Body mass index**	**Job seniority**
Global DNA methylation markers				
Alu (%5mC)	β = 0.274	β = 0.013	β = 0.009	**β = −0.041**
	p = .132	*p* = .340	*p* = .767	***p* = .005**
LINE-1 (%5mC)	β = −0.216	β = −0.009	β = −0.011	β = 0.008
	p = .459	*p* = .675	*p* = .825	*p* = .725
Gene-specific methylation markers				
GCR (%5mC)	β = 0.916	β = −0.024	β = −0.241	**β = 0.152**
	p = .359	*p* = .745	*p* = .154	***p* = .053**
TNF-α (%5mC)	**β = 1.312**	β = −0.042	β = −0.034	β = −0.066
	***p* = .022**	*p* = .311	*p* = .722	*p* = .135
IFN-γ (%5mC)	β = −0.387	β =0.116	β = 0.287	**β = −0.343**
	p = .777	*p* = .246	*p* = .212	***p* = .002**

TABLE 4

Mean (and SD) values of methylation markers in shiftworkers with different job seniority (< 5, 5–15, >15 yrs)

	Job seniority				
	<5 yrs (N = 24)	**5–15 yrs (N = 54)**	**>15 yrs (N = 22)**	***p* value (ANOVA)**	***p* trend**
Global DNA methylation markers					
Alu (%5mC)	25.52 (0.68)	25.44 (0.78)	25.04 (0.71)	0.057	**0.032**
LINE-1 (%5mC)	82.05 (1.30)	82.52 (1.26)	82.00 (0.97)	0.130	0.931
Gene-specific methylation markers					
GCR (%5mC)	43.87 (4.48)	44.92 (3.81)	46.21 (3.88)	0.145	**0.049**
TNF-α (%5mC)	12.53 (2.09)	12.43 (2.56)	10.68 (2.11)	**0.009**	**0.012**
IFN-γ (%5mC)	85.15 (3.89)	81.86 (5.61)	80.61 (5.85)	**0.011**	**0.004**

TABLE 5

Multiple regression analysis in shiftworker, for morning- versus evening-type workers adjusting for age, body mass index, and job seniority

	Job seniority			
	5–15 yrs	**>15 yrs**	**Morning type/ evening type**	**Age**
Global DNA methylation markers				
Alu (%5mC)	β = −0.30	**β = −0.86**	β = 0.23	β = 0.01
	p = .20	***p* = .006**	*p* = .21	*p* = .44
LINE-1 (%5mC)	β = 0.38	β = 0.03	β = −0.211	β = −0.012
	p = .31	*p* = .94	*p* = .47	*p* = .56
Gene-specific methylation markers				
GCR (%5mC)	β = 1.39	**β = 3.33**	β = 1.10	β = 0.03
	p = .28	***p* = .05**	*p* = .28	*p* = .65
TNF-α (%5mC)	β = 0.55	β = −0.32	**β = 1.33**	β = −0.064
	p = .45	*p* = .73	***p* = .02**	*p* = .11
IFN-γ (%5mC)	**β = −4.28**	**β = −6.50**	β = −0.66	β = 0.07
	***p* = .02**	***p* = .007**	*p* = .63	*p* = .46