SLEEP AND BIOLOGICAL RHYTHMS
THE JAPANESE SOCIETY OF SLEEP RESEARCH

Sleep and Biological Rhythms 2015; **13**: 342–347 doi:10.1111/sbr.12123

ORIGINAL ARTICLE

PER1 polymorphism associated with shift work disorder

Yukari TANIYAMA, Takenori YAMAUCHI, Shouhei TAKEUCHI and Yoshiki KURODA
Department of Public Health, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

Abstract

Workers with shift work schedules often experience shift work disorder (SWD). However, all shift workers do not necessarily develop SWD. The aim of this cross-sectional study was to investigate the association between susceptibility to SWD and clock gene polymorphisms. A total of 257 male workers from a semiconductor factory were recruited in this study. Participants completed questions about age, body mass index (BMI), shift work history, lifestyle, living conditions, the Epworth Sleepiness Scale (ESS) score, the Pittsburgh Sleep Quality Index (PSQI) score and SWD, and were genotyped with regard to the five clock gene polymorphisms. Workers were divided into two groups, namely, shift workers with SWD ($n = 172$) and those without SWD ($n = 85$). ESS and PSQI scores were significantly different between the two groups. The result of multiple logistic regression analysis showed that the *PER1* G/G (OR: 0.29; 95% CI: 0.08–0.97) and C/G (OR: 0.43; 95% CI: 0.19–0.96) genotype were associated with SWD. The present study findings suggest that there is an association between the *PER1* polymorphism rs3027188 and susceptibility to SWD in Japanese shift workers.

Key words: circadian rhythms, clock genes, genetic polymorphism, PER1, shift work disorder.

INTRODUCTION

In modern society, shift work is common among employees in the public service and factory settings. It has been reported that shift work can cause or perpetuation of health problems such as cardiovascular and gastrointestinal disease,[1] metabolic syndrome,[2] and an increased risk of cancer.[3]

Moreover, many shift workers experience excessive sleepiness while working at night and insomnia during the day when attempting to sleep after working at night. This sleep disorder was named shift work disorder (SWD),[4] and it is diagnosed on the basis of the International Classification of Sleep Disorders-2 (ICSD-2) criteria. SWD is a member of the class of circadian rhythm sleep disorders (CRSD). A misalignment between the endogenous circadian rhythms and sleep–wake cycles, known as internal desynchronization, is considered the primary cause of CRSD.[5] However, all shift workers do not necessarily develop SWD. It is thought to be important for shift workers to be able to predict their risk for developing SWD. However, SWD risk cannot be predicted correctly yet. We were interested in investigating tolerance or susceptibility to SWD (individual difference to SWD) as per the individual's shift work schedule.

It was shown that diurnal preference is related to shift work tolerance.[6] In addition, this diurnal preference was reported to be related to variations in clock genes, such as a single nucleotide polymorphism (SNP) in *PER2*,[7] and a variable number tandem repeat (VNTR) in *PER3*.[8] Also, a relationship between CRSD and clock genes has been reported.[8–10] For example, *PER3* VNTR was associated with delayed sleep phase.[8,9] A missense mutation in *PER2* was linked to familial advanced sleep phase syndrome.[10] Therefore, we hypothesized that polymorphisms in clock genes could contribute to SWD susceptibility. However, to our knowledge, few studies have investigated the relationship between SWD and

Correspondence: Dr Yukari Taniyama, Department of Public Health, Faculty of Medicine, University of Miyazaki, 5200 Kiyotakecho Kihara, Miyazaki-city 889-1692, Japan.
Email: yukari_taniyama@med.miyazaki-u.ac.jp
Accepted 23 March 2015.

clock gene polymorphisms. We analyzed the relationship between polymorphisms in clock genes (*PER1*, *PER2, PER3, CRY1*, and *CRY2*) and SWD.

MATERIALS AND METHODS

Subjects

A total of 257 male employees, working rotating shifts in the manufacturing unit of base materials for the semiconductor industry in Japan were recruited as participants during the period of August 2010 to July 2011. The mean age was 39.5 ± 8.4 years (range: from 20 to 59 years). The experience of shift work varied from 0.4 to 35 years (mean: 16.4 ± 7.3).

There were two different work schedules (A and B) at the factory, including two cycles. One cycle consisted of working hours from 08.30 to 17.45 hours for three consecutive days, followed by three consecutive night shifts from 20.30 to 05.45 hours (workers did not work for 27 h between day and night shifts), followed by 3 days off. The other cycle involved working for three consecutive days from 10.45 to 20.30 hours, followed by working from 22.45 to 08.30 hours for three consecutive days, followed by 3 days off. Schedule A consisted of the two cycles repeating alternately, while Schedule B consisted of only the first cycle. The Ethics Committee of the Miyazaki Medical College approved this study and a written informed consent was obtained from each participant.

Questionnaires

Background data of participants

A questionnaire was filled out by the participants regarding years of shift work experience, present smoking habits (yes: every day and sometimes, no: never), drinking habits (yes: every day and sometimes, no: never), caffeine intake per day, and living conditions (alone, with family). Medical history and body mass index (BMI; kg/m^2) details were obtained from the most recent medical examination data.

SWD

Shift work disorder was defined on the basis of the four ICSD-2 criteria:[4] (i) complaint of insomnia or excessive sleepiness temporally associated with a recurring work schedule that overlaps with the usual time for sleep; (ii) symptoms associated with the shift work schedule over the course of at least 1 month; (iii) circadian and sleep-time misalignment as demonstrated by sleep log or actigraphy for 7 days or more; and (iv) sleep disturbance that cannot be explained by another sleep disorder, a medical or neurological disorder, mental disorder, medication use, or substance use disorder. We asked the participants three questions as described by Waage *et al*.[11] to diagnose SWD: (i) do you experience difficulties in sleeping or experience excessive sleepiness? (yes or no); (ii) is the sleep or sleepiness problem related to your work schedule where you have to work when you normally would sleep? (yes or no); and (iii) have you had this sleep or sleepiness problem related to the work schedule for at least 1 month? (yes or no). Participants who responded "yes" to all three questions were classified as the SWD group. These questions were also used by Flo *et al*.[12] who confirmed their reliability for assessing SWD in epidemiological studies. In addition, we excluded those individuals who were under medical treatment for sleep apnea syndrome; neurological, psychiatric, and urological disorders; or chronic allergies.

Evaluation of sleep status

We used the Japanese version of the Pittsburgh Sleep Quality Index (PSQI)[13] to evaluate the quality of sleep conditions and identify sleep disorders during the previous month. The PSQI was not suitable for evaluation of workers following a rotating shift schedule; therefore, we asked shift workers to complete this questionnaire with regard to only their night shift. The Japanese version of the PSQI consisted of 19 self-rated questions concerning sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbance, use of sleep medications, and daytime insomnia. Each question was scored from 0 to 3, with total scores ranging from 0 to 21. A score of >5 on the Japanese version of the PSQI indicated a sleep disorder. Validity and reliability was verified to be the same as the original PSQI.

Evaluation of sleepiness

The sleepiness of subjects during the daytime was measured by the Japanese version of the Epworth Sleepiness Scale (ESS).[14] The ESS consisted of right questions and each question was scored from 0 to 3. The total scores were between 0 and 24, where a score of ≥11 would indicate excessive daytime sleepiness.

Genotyping

Genomic DNA was extracted from hair samples using a DNA Extractor FM Kit (Wako Pure Chemical Industries,

Table 1 Clock genes polymorphisms analyzed in the shift workers

Gene	dbSNP	Region	Location	Major allele†	Minor allele	Major homo (%)	Hetero (%)	Minor homo (%)
PER1	rs3027188	Intron	17p13.1	C	G	55.8	34.8	9.4
PER2	rs934945	Exon	2q37.3	G	A	48.9	37.1	14.0
PER3	–	Exon	1p36.23	4-repeat	5-repeat	66.5	29.1	4.4
CRY1	rs55794336	Intron	12q23-q24.1	A	G	32.5	50.6	16.9
CRY2	rs2292912	Intron	11p11.2	G	C	41.8	42.4	15.8

†Ancestral allele of PER1 and CRY2 is G and C, although major allele of PER1and CRY2 in Asian is C and G.

Osaka, Japan). We detected genotypes of *PER1, PER2, CRT1*, and *CRY2* using real-time polymerase chain reaction (real-time PCR) and the restriction fragment length polymorphism method for *PER3*. The probes for *PER1* (rs3027188), *PER2* (rs934945), and *CRY2* (rs2292912) as well as the primers for *PER3* VNTR polymorphism were used, as described previously.[7,9,15,16] Additionally, we sourced the SNP site on *CRY1* (rs55794336) from The National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/snp; Table 1).

For real-time PCR, we used the TaqMan SNP genotyping assay kit (Applied Biosystems, Foster City, CA, USA) as per the manufacturer's protocol with a real time thermocycler (Applied Biosystems 7300 Real-time PCR System). For *PER3*, the forward and reverse primers used were 5'-AAAATTTTATGACACTACCA GAATGGCTGAC-3' and 5'-AACCTTGTACTTCCACAT CAGTGCCTGG-3', respectively. The 50 μL PCR mix was comprised of 1 μL genomic DNA, 0.25 μL of each primer (100 μM), 10 μL 10 × buffer, 7 μL $MgCl_2$ (25 mM), 1.5 μL dNTPs (10 mM), and 0.25 μL KAPA Taq EXTRA. We performed 40 cycles using Taq polymerase (Genetics, Japan) under the following conditions: denaturation at 95°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 1 min. The resultant PCR products were separated on 2% agarose gel.

Statistical analysis

A χ^2 test was used to determine the discrepancies of distribution (Hardy–Weinberg equilibrium). Results for continuous variables are presented as the mean ± standard deviation. The categorical variables are expressed as frequencies. A *t*-test for continuous variables and χ^2 tests for categorical variables were used to investigate differences between data from the SWD group and the non-SWD group. Odds ratios (OR) and 95% confidence intervals (95% CI) were derived using multiple logistic regression analysis in order to explore the associated factors for SWD. Age, BMI, shift work experience, smoking habits, drinking habits, caffeine intake per day, living conditions, and clock gene polymorphisms were included as independent variables in the multiple logistic regression analysis. We considered *P*-values less than 0.05 as statistically significant. All analyses were conducted using R for Windows software (version R-2.15.1).

RESULTS

We divided the participants into shift workers with SWD (SWD group) and shift workers without SWD (non-SWD group) on the basis of their responses to the three questions described earlier. Table 2 summarizes the characteristics of both groups. The SWD group consisted of 172 participants, while the non-SWD group had 85. There was no difference between the two groups in terms of age, BMI, shift work experience, smoking habits, drinking habits, and caffeine intake per day. The rate of living alone was statistically higher in the non-SWD group (16.7%) than that in the SWD group (5.9%) ($P = 0.01$). The ESS and PSQI scores in the SWD group were significantly higher than that in the non-SWD group.

All genotypes evaluated were consistent with the Hardy–Weinberg equilibrium. Multiple logistic regression analysis was conducted with SWD at dependent variable (Table 3). The OR suggested no significant association between SWD and a number of variables: age, BMI, shift work experience, smoking habits, drinking habits, caffeine intake per day, living conditions, as well as the genotypes *PER2*, *PER3*, *CRY1*, and *CRY2*. However, the *PER1* G/G (OR: 0.29; 95% CI: 0.08–0.97) and C/G (OR: 0.43; 95% CI: 0.19–0.96) genotype showed associations with SWD.

DISCUSSION

It is well accepted that shift work is important in our present industrial society. According to the survey on the

Table 2 Comparison between SWD group and non-SWD group

	SWD group (n = 172) (mean ± sd)	non-SWD group (n = 85) (mean ± sd)	P value
Age (years)	40.0 ± 8.1	38.4 ± 8.9	0.17†
BMI (kg/m^2)	22.7 ± 2.9	22.5 ± 3.2	0.60†
Shift work experience (years)	17.0 ± 6.8	14.9 ± 8.1	0.05†
Smorkers (%)	55.3	58.8	0.69‡
Drinkers (%)	71.6	68.2	0.68‡
Caffeinated drink (cups/day)	3.6 ± 2.1	3.7 ± 2.1	0.81†
Living alone (%)	5.9	16.7	0.01‡
ESS score	9.2 ± 4.5	6.8 ± 3.8	<0.01†
PSQI score	6.6 ± 2.5	4.6 ± 2.2	<0.01†

†t-test. ‡χ^2 test.

Table 3 Results of multiple logistic regression analysis for variables associated with shift work disorder

Variable	OR	95%CI	P-value
Age	1.02	0.96–1.08	0.539
Body mass index	1.08	0.95–1.23	0.232
Shift year	1.05	0.98–1.12	0.165
Living alone – no	1.00		
Living alone – yes	0.87	0.22–3.63	0.839
Smoker – no	1.00		
Smoker – yes	0.92	0.42–1.99	0.828
Drinker – no	1.00		
Drinker – yes	1.31	0.56–3.04	0.536
Amount of caffeine intake	1.06	0.89–1.26	0.529
PER1 – C/C	1.00		
PER1 – C/G	0.43	0.19–0.96	0.043
PER1 – G/G	0.29	0.08–0.97	0.045
PER2 – G/G	1.00		
PER2 – G/A	2.01	0.87–4.82	0.108
PER2 – A/A	0.71	0.23–2.18	0.552
PER3 – 4r/4r	1.00		
PER3 – 4r/5r	0.74	0.32–1.71	0.482
PER3 – 5r/5r	0.73	0.08–16.25	0.801
CRY1 – A/A	1.00		
CRY1 – A/G	0.86	0.36–2.04	0.735
CRY1 – G/G	1.40	0.45–4.52	0.565
CRY2 – G/G	1.00		
CRY2 – G/C	0.67	0.30–1.48	0.319
CRY2 – C/C	2.47	0.78–9.10	0.144

CI, confidence intervals; OR, odds ratio.

state of employee's health by the Ministry of Health, Labour and Welfare, around 5.2 million people, representing about 9.2% of all workers in Japan, are engaged in rotating shifts including night shifts (http://www.mhlw.go.jp/toukei/list/49-19.html%20). Shift work causes SWD. Previous studies indicate that about 26.1% to 44.3% of rotating-shift workers were diagnosed with SWD.[11,12] We surmised that susceptibility to SWD is related to clock gene polymorphisms. However, to our knowledge, there are no reports on an association between SWD and clock gene polymorphisms. Therefore, we investigated the relationship between SWD and a few clock gene polymorphisms (*PER* and *CRY*).

Circadian oscillation in mammals is driven by a negative feedback loop involving the Period (*PER1*, *PER2*, and *PER3*) and Cryptochrome (*CRY1* and *CRY2*) genes. The translated proteins form heteropolymers with each other and with casein kinase 1ε (CK1ε),[17] which contributes to the phosphorylation of specific sites that influence protein stability and nuclear translocation.[18] In the nucleus, these heteropolymers inhibit the positive transcription factors (CLOCK/NPAS2 and BMAL1/2) that stimulate *PER/CRY* transcription, thereby closing the circadian cycle. The genetic basis of sleep homeostasis is currently not well understood.[19] However, in the previous studies of clock gene polymorphisms, Gamble *et al*. reported that nurses with the *PER3* and *NPAS2* polymorphisms experienced reduced dozing during work timings and that the *PER2* polymorphism was associated with long sleep duration during the day shift-work timings.[20] It was reported that the *PER3* VNTR polymorphism has been associated with disrupted sleep architecture and compromised cognitive performance.[21] On the other hand, the *CRY1* and *CRY2* polymorphisms have been reported to be associated with shift work and a psychiatric disorder that may be affected by the biological rhythm, although the relation with sleep disorder has not been described.[22,23] The ratio of *CRY1* gene polymorphisms was different between day workers and night workers in nurses and midwives,[22] while *CRY2* gene polymorphism was related to seasonal affective disorder or winter depression.[23] In the current study, we were not able to show the relevance of *PER3*, *PER2*, *CRY1*, and *CRY2* polymorphisms in SWD. However, the mutant *PER3* is quite uncommon in the Asian

population;[24] therefore, the effect of these polymorphisms requires further investigation within a larger population.

Our study has, for the first time, revealed that the *PER1* intronic polymorphism rs3027188 is associated with susceptibility to SWD. Although a recent study investigated the interaction between the *PER1* polymorphism rs3027188 and shift work history with respect to breast cancer,[15] no significant interaction was detected. In shift work, the mechanism by which *PER1* influences cancer and SWD may be different.

The *PER1* intronic polymorphism rs3027188 is located in the non-coding region of the CK1ε binding domain; CK1ε binding is necessary for phosphorylation of the PER/CRY protein. Phosphorylation of PER by CK1ε affects the timing of the circadian cycle.[10] SNPs in introns are known to influence the regulation of transcription, splicing, and other aspects of RNA processing or stability.[25,26] For instance, the intronic SNPs in FTO[27] affect primary transcript levels and the risk allele of the intronic SNP in the XPC DNA repair gene influences splicing efficiency.[28] Therefore, the polymorphism in the intron of *PER1* is likely to induce a functional deficit because of decreased mRNA transcription levels or aberrant splicing of mRNA. In addition, it was reported that *PER1* and *PER2* are linked to different downstream pathways.[29] Thus, it is possible that downstream gene clusters regulated by each clock gene are different and only the *PER1* rs3027188 polymorphism might be directly associated with SWD. In addition, it was reported that *PER1* transcriptionally suppresses *CRY2* as well as the basic circadian mechanism.[30] Therefore, it is also likely that each clock gene regulates other transcription factors or transcription regulatory factors and that the regulation of gene clusters associated with SWD is disturbed by the PER1 polymorphism indirectly. However, it is currently unclear whether the SWD-associated intronic SNP from the current study exerts a direct effect on *PER1* expression, or whether it is in linkage disequilibrium with another functional SNP.

In this study, the ratio of SWD was very high compared with the rates found by other studies using the same criteria for SWD. Asaoka *et al*. reported that the prevalence of SWD among nurses in a Japanese population working shift schedules was 24.4%.[31] However, these nurses were all female, did not engage in continuous night work, and could take a nap during night shifts. Therefore, sex and shift schedules are different from those in our study. In a study involving subjects with a similar shift schedule to our study, involving two or three consecutive night shifts per week, Flo *et al*. reported that the prevalence of SWD was 37.6% among nurses.[12] However, 90% of the subjects were female, and they reported that the prevalence of SWD in females was 0.57-fold that in males. Furthermore, according to the international HapMap project, the PER1 genotype distribution in Asian populations is *C*/*C*:*C*/*G*:*G*/*G* = 0.56:0.40:0.04, while that in Europeans is 0.03:0.32:0.65. Thus, the *C*/*C* genotype was much more common in Asians than in Europeans, and the *C*/*C* genotype was associated with SWD in this study. This may explain the high ratio of SWD noted in this study.

Our study has some limitations. First, our findings were limited to male rotating shift workers from a single factory in Japan. Therefore, this may limit the ability to generalize our results to female workers, other shift schedules, other factories, and other ethnicities. Second, we were not able to evaluate the influence of the amount of work performed, including weekly work hours and overtime hours. Third, only three questions were used to define SWD, which may be a limitation. Three questions are not capable of detecting the severity of SWD. Therefore, subjects with mild SWD who do not need treatment may be mixed with a severely SWD-affected subject who needs treatment. Finally, because a polysomnography or a multiple sleep latency test was not performed, we cannot evaluate objective sleep. Thus, it is not entirely clear whether this association with SWD is genuine. Further larger sample size study and replication study are required to clarify an association between genetic polymorphism and SWD for prophylactic measures against SWD.

CONCLUSION

The present study findings suggest that there is an association between the *PER1* intronic polymorphism rs3027188 and susceptibility to SWD in Japanese shift workers, and that our findings may explain why susceptibility to SWD varies among shift workers. Further studies on larger populations and in vitro functional analyses are required to determine the effects of such polymorphism on SWD pathogenesis and susceptibility.

CONFLICT OF INTEREST

None of the authors have a conflict of interest to declare in relation to this study.

ACKNOWLEDGMENTS

The authors are grateful to all those who participated in this study. This study was made possible by subsidized

projects of the Occupational Health Promotion Foundation salary award.

REFERENCES

1 Drake CL, Roehrs T, Richardson G, Walsh JK, Roth T. Shift work sleep disorder: prevalence and consequences beyond that of symptomatic day workers. *Sleep* 2004; **27**: 1453–62.
2 Karlsson B, Knutsson A, Lindahl B. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. *Occup. Environ. Med.* 2001; **58**: 747–52.
3 Costa G, Haus E, Stevens R. Shift work and cancer – considerations on rationale, mechanisms, and epidemiology. *Scand. J. Work Environ. Health* 2010; **36**: 163–79.
4 American Academy of Sleep Medicine. *The International Classification of Sleep Disorders: Diagnostic and Coding Manual*, 2nd edn. American Academy of Sleep Medicine: Westchester, 2005.
5 Hida A, Kitamura S, Katayose Y *et al*. Screening of clock gene polymorphisms demonstrates association of a PER3 polymorphism with morningness-eveningness preference and circadian rhythm sleep disorder. *Sci. Rep.* 2014; **4**: 6309.
6 Sack RL, Auckley D, Auger RR *et al*. Circadian rhythm sleep disorders: part I, basic principles, shift work and jet lag disorders. An American Academy of Sleep Medicine review. *Sleep* 2007; **30**: 1460–83.
7 Lee H-J, Kim L, Kang S-G *et al*. PER2 variation is associated with diurnal preference in a Korean young population. *Behav. Genet.* 2011; **41**: 273–7.
8 Archer SN, Robilliard DL, Skene DJ *et al*. A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep* 2003; **26**: 413–15.
9 Ebisawa T, Uchiyama M, Kajimura N *et al*. Association of structural polymorphisms in the human period3 gene with delayed sleep phase syndrome. *EMBO Rep.* 2001; **2**: 342–6.
10 Toh KL, Jones CR, He Y *et al*. An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science* 2001; **291**: 1040–3.
11 Waage S, Moen BE, Pallesen S *et al*. Shift work disorder among oil rig workers in the North Sea. *Sleep* 2009; **32**: 558–65.
12 Flo E, Pallesen S, Magerøy N *et al*. Shift work disorder in nurses – assessment, prevalence and related health problems. *PLoS ONE* 2012; **7**: e33981.
13 Doi Y, Minowa M, Uchiyama M. Development of the Japanese version of the Pittsburgh Sleep Quality Index. *Seishinka Chiryougaku* 1998; **13**: 755–63. (In Japanese.)
14 Takegami M, Suzukamo Y, Wakita T *et al*. Development of a Japanese version of the Epworth Sleepiness Scale (JESS) based on item response theory. *Sleep Med.* 2009; **10**: 556–65.
15 Grundy A1, Schuetz JM, Lai AS *et al*. Shift work, circadian gene variants and risk of breast cancer. *Cancer Epidemiol.* 2013; **37**: 606–12.
16 Zhu Y, Stevens RG, Hoffman AE *et al*. Testing the circadian gene hypothesis in prostate cancer: a population-based case-control study. *Cancer Res.* 2009; **69**: 9315–22.
17 Reppert SM, Weaver DR. Coordination of circadian timing in mammals. *Nature* 2002; **418**: 935–41.
18 Walton KM, Fisher K, Rubitski D *et al*. Selective inhibition of casein kinase 1 epsilon minimally alters circadian clock period. *J. Pharmacol. Exp. Ther.* 2009; **330**: 430–9.
19 Franken P, Dijk DJ. Circadian clock genes and sleep homeostasis. *Eur. J. Neurosci.* 2009; **29**: 1820–9.
20 Gamble KL, Motsinger-Reif AA, Hida A *et al*. Shift work in nurses: contribution of phenotypes and genotypes to adaptation. *PLoS ONE* 2011; **6**: e18395.
21 Viola AU, Archer SN, James LM *et al*. PER3 polymorphism predicts sleep structure and waking performance. *Curr. Biol.* 2007; **17**: 613–18.
22 Reszka E, Peplonska B, Wieczorek E *et al*. Rotating night shift work and polymorphism of genes important for the regulation of circadian rhythm. *Scand. J. Work Environ. Health* 2013; **39**: 178–86.
23 Partonen T. Clock gene variants in mood and anxiety disorders. *J. Neural Transm.* 2012; **119**: 1133–45.
24 Barbosa AA, Pedrazzoli M, Koike BD, Tufik S. Do Caucasian and Asian clocks tick differently? *Braz. J. Med. Biol. Res.* 2010; **43**: 96–9.
25 Birney E, Lieb JD, Furey TS, Crawford GE, Iyer VR. Allele-specific and heritable chromatin signatures in humans. *Hum. Mol. Genet.* 2010; **19**: 204–9.
26 Cheung VG, Spielman RS. Genetics of human gene expression: mapping DNA variants that influence gene expression. *Nat. Rev. Genet.* 2009; **10**: 595–604.
27 Berulava T, Horsthemke B. The obesity-associated SNPs in intron 1 of the FTO gene affect primary transcript levels. *Eur. J. Hum. Genet.* 2010; **18**: 1054–6.
28 Khan SG, Muniz-Medina V, Shahlavi T *et al*. The human XPC DNA repair gene: arrangement, splice site information content and influence of a single nucleotide polymorphism in a splice acceptor site on alternative splicing and function. *Nucleic Acids Res.* 2002; **30**: 3624–31.
29 Liu Z, Huang M, Wu X *et al*. PER1 phosphorylation specifies feeding rhythm in mice. *Cell Rep.* 2014; **7**: 1509–20.
30 Richards J, All S, Skopis G *et al*. Opposing actions of Per1 and Cry2 in the regulation of Per1 target gene expression in the liver and kidney. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2013; **305**: R735–47.
31 Asaoka S, Aritake S, Komada Y *et al*. Factors associated with shift work disorder in nurses working with rapid-rotation schedules in Japan: the nurses' sleep health project. *Chronobiol. Int.* 2013; **30**: 628–36.