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Circadian Clock Gene Polymorphisms and Sleep-Onset Problems in a Population-Based Cohort Study: The Yamagata Study

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A number of genome-wide association studies have investigated sleep phenotypes and disorders in humans. However, the contribution of genetic variation to sleep problems in Japanese populations has remained unclear. Sleep-onset problems are the most common symptom of insomnia. Here, we examined the relationship between single nucleotide polymorphisms (SNPs) of *BMAL1* (*ARNTL1*), *CLOCK*, *CRY1*, *CRY2*, and *PER2*, which are genes involved in the clock mechanism, and sleep-onset problems in a Japanese general population. This study included 1,397 subjects aged ≥ 40 years who participated in an annual health check-up in Yamagata Prefecture. A total of 80 SNPs of 5 circadian clock genes were analyzed. Multivariate logistic regression analyses identified variant rs11113179 in *CRY1* and variants rs1026071 and rs1562438 in *BMAL1* as genetic risk factors for sleep induction disorder. These findings suggest that *CRY1* and *BMAL1* polymorphisms are related to sleep-onset problems in a Japanese general population. However, none of the SNPs remained significant at a stringent level of multiple correction.

Keywords: circadian clock gene; Japanese general population; single nucleotide polymorphism; sleep-onset problems; Yamagata study
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Introduction

The molecular circadian clock is now understood to consist of a negative feedback loop involving the *Period* (*PER1, PER2, PER3*) and *Cryptochrome* (*CRY1, CRY2*) genes. Other genes involved in the molecular generation of circadian rhythms include *Casein Kinase 1* (CK1), *Circadian Locomotor Output Cycles Kaput Protein* (*CLOCK*), and *Brain and Muscle ARNT-like Protein* (*BMAL1* and *BMAL2*) (Reppert and Weaver 2002; Lowrey and Takahashi 2011).

Several reports have described the association of circadian clock gene polymorphisms with sleep traits and insomnia. In the HUNT3 study, Bragantini et al. (2019) reported that 16 SNPs were significantly associated ($p < 0.05$) with at least 1 symptom of insomnia. In particular, they detected a significant association between rs10861688 in *CRY1* and early awakening. Three recent large-scale genome-wide association studies (GWASs) of chronotypes were conducted on subjects of European ancestry from the 23andME cohort and the UK biobank (Hu et al. 2016; Jones et al. 2016; Lane et al. 2016). Three genetic variants of the *PER2* gene were identified in all three GWASs. However, few studies have investigated these associations in Asian populations, including Japanese. Mishima et al. (2005) reported that *CLOCK* 3111T/C(rs1801260) was associated with evening preference and delayed sleep timing in a Japanese population. Miyagawa et al. (2019) reported that a missense variant in *PER2* was associated with delayed sleep-awake phase disorder in a case-control study. However, both of these studies involved a relatively small number of young people and did not examine the association of

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genetic factors with sleep in a general population.

Here, we examined the relationship between polymorphisms of *BMAL1*, *CLOCK*, *CRY1*, *CRY2*, and *PER2* and sleep-onset problems in a Japanese general population.

Materials and Methods

The Yamagata Study was a community-based prospective cohort study performed as part of a molecular epidemiological study which utilized regional aspects of the Global Center of Excellence (COE) program in Japan (Otaki et al. 2016; Kon et al. 2019; Sakurada et al. 2020). This study was approved by the ethics committee of Yamagata University School of Medicine (2019-292). All participants provided written informed consent prior to enrollment, and the procedures were performed in accordance with the Declaration of Helsinki.

The study subjects were participants in a community-based annual health check-up in which residents aged ≥ 40 years of 7 cities (Yamagata, Sakata, Kaminoyama, Sagae, Higashine, Yonezawa, and Tendo) in Yamagata Prefecture, Japan, were invited to participate. The study had no exclusion criteria. From 2009 to 2019, a total of 20,969 subjects (8,558 men and 12,411 women) were enrolled. Of these, we selected 1,397 subjects (693 men and 704 women) who were not missing any information from interview or laboratory tests in the first year of the baseline survey to undergo genotyping. Individuals were selected for inclusion if sleep measures were available and their genotype had been collected. A total of 70 SNPs of 5 genes [*BMAL1* (*ARNTL1*), *CLOCK*, *CRY1*, *CRY2*, and *PER2*] were analyzed. Multivariate logistic regression analyses were performed to identify the genetic polymorphisms associated with sleep-onset problems. After excluding 15 subjects from analysis due to incomplete data, data from 1,382 subjects were finally entered into the statistical analysis.

Measurements

At baseline, the survey subjects were mailed a self-reported questionnaire to document their medical history, current medications and clinical symptoms, blood pressure, sleep habits, alcohol drinking status, smoking status, physical activity, education level, marital status, level of perceived mental stress, and social participation.

Sleep habits were assessed via two questions, as follows: (1) “Currently, how long do you sleep on average each night?”; and (2) “Currently, do you sleep regularly?” (possible answers of “yes” or “no”). Sleep onset problem was assessed via one question, as follows: “Currently, do you usually fall asleep easily?” (possible answers of “yes” or “no”, with responders of “no” considered to have sleep onset problem).

Alcohol drinking status was classified into the three categories of current drinker, past drinker, or nondrinker. Smoking status was classified into the three categories of current smoker, past smoker, or nonsmoker. Frequency of physical activity was classified into three categories of none, rarely, or ≥ 1 time/week. Perceived mental stress was evaluated using a single question: “Have you felt mental stress in the past year?”, and classified into the three categories of severe/high, moderate, or low.

Laboratory parameters were obtained at the health check-up site. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or the use of antihypertensive medications. Presence of diabetes was defined as a plasma glucose level ≥ 126 mg/dL, HbA1c ≥ 6.5% (National Glycohemoglobin Standardization Program), or the use of antidiabetic medications. Dyslipidemia was defined as total cholesterol ≥ 220 mg/dL, triglyceride ≥ 150 mg/dL, or anti-hyperlipidemic medicine use.

Genotyping

Genotyping was performed using an Illumina 660W-Quad array containing 550K common tag-SNPs (Illumina, San Diego, CA, USA). Genomic DNA (1 μg) extracted from sera by the magnet-bead method was digested using a restriction enzyme mixture for fragmentation, and purified nucleotide fragments were hybridized on a BeadChip array with locus-specific hybridization probes overnight. After hybridization, SNP nucleotide fluorescence intensity was measured using a Beadstation 500GX instrument (Illumina). SNP genotyping was performed using GenomeStudio genotyping modules with the B-allele frequency and population SNP signal intensity data. Linkage disequilibrium was determined according to the D’ calculation using the LDheatmap package with the R statistical language. The Hardy-Weinberg equilibrium for genotype distributions was assessed using the genetics packages included with the R statistical language.

Statistical analyses

Data are expressed as the mean ± standard deviation (SD) for continuous values and as percentages of the total number of subjects for categorical variables. Analysis of variance was performed to evaluate differences in mean values, and the chi-squared test was used to evaluate differences in proportions. Multivariate logistic regression analyses were performed to identify the genetic polymorphisms associated with sleep induction disorder. In the multivariate-adjusted model, the odds ratio (OR) was adjusted for age and sex. Eleven cases were excluded from multivariate analyses due to a lack of clinical parameters. P values < 0.05 were considered statistically significant. All statistical analyses were performed using JMP, version 14 (SAS Institute, Cary, NC, USA).

Results

The characteristics of the participants are shown in Table 1. Participants comprised 686 men (49.6%) and 696 women (50.4%). A total of 231 (16.7%) participants were classified as having sleep induction disorder. Their mean age ± SD at baseline data collection was 60.4 ± 8.8 years.

Table 1. Characteristics of study subjects.

	Sleep onset problem		
	No	Yes	P value
Number of subjects, n (% of the total subjects)	1151 (83.3)	231 (16.7)	
Male sex, n (%)	566 (49.1)	120 (51.9)	0.442
Age (years), mean (SD)	60.8 (8.9)	60.4 (8.8)	0.502
Sleep duration (hours), mean (SD)	6.8 (1.0)	6.9 (1.1)	0.330
Sleep regularity, n (%)			0.677
regular	876 (89.7)	172 (88.7)	
irregular	101 (10.3)	22 (11.3)	
Obesity, n (%)	222 (26.1)	44 (25.1)	0.782
BMI, kg/m^2, mean (SD)	23.3 (3.3)	23.0 (3.3)	
Medical history, n (%)			
Diabetes	101 (10.4)	20 (10.6)	0.937
Hypertension	254 (26.1)	57 (30.2)	0.253
Dyslipidemia	584 (56.2)	105 (49.3)	0.065
Drinker, n (%)			0.780
Current	533 (56.7)	106 (57.6)	
Past	17 (1.8)	2 (1.1)	
Never	390 (41.5)	76 (41.3)	
Smoking, n (%)			0.030*
Current	121 (13.1)	32 (17.7)	
Past	237 (25.7)	57 (31.5)	
Never	566 (61.3)	92 (50.8)	
Education, n (%)			0.619
Primary or junior high school	133 (14.2)	31 (16.9)	
High school	520 (55.3)	97 (52.7)	
college or higher education	287 (30.5)	56 (30.4)	
Physical activity, n (%)			0.038*
none	157 (17.4)	21 (11.7)	
rarely	106 (11.8)	31 (17.3)	
≥ 1 time/week	638 (70.8)	127 (71.0)	
Perceived mental stress, n (%)			0.105
high/severe	668 (72.5)	115 (64.6)	
moderate	220 (23.9)	55 (30.9)	
low	34 (3.7)	8 (4.5)	
Marital status, n (%)			0.490
living with spouse	775 (92.4)	147 (90.7)	
single, divorced, or widowed	64 (7.6)	15(9.3)	

Data are shown as mean (SD) or n (%).
*$p < 0.05$

Their mean sleep duration ± SD was 6.9 ± 1.1 h. About 90% of participants reported regular sleep habits. Among subjects with sleep onset problem, current smokers were significantly more frequent than among subjects with no sleep-onset problems.

A total of 80 single nucleotide polymorphisms (SNPs) were analyzed (Table 2). Of these, 70 were detected in more than 10 individuals. We examined the association of these 70 SNPs with sleep-onset problems.

The associations between SNPs and sleep-onset problems were evaluated using logistic regression analysis (Table 3). After adjustment for age and sex, variant rs11113179 in *CRY1* was found to be significantly associated with an increased odds of sleep-onset problems [aOR of CT/TT vs. CC 1.51, 95% confidence interval (CI) 1.06-2.14, p = 0.022], and variants rs1026071 and rs1562438 in *BMAL1* were also found to be significantly associated with sleep-onset problems (aOR of GG vs. AG/AA 1.54, 95% CI 1.05-2.28, p = 0.027; aOR of TT vs. TC/CC 1.60, 95% CI 1.06-2.41, p = 0.026). Finally, we performed logistic regression analyses with smoking status and physical activity as confounding factors, as smoking and physical activity

Table 2. List of analyzed single nucleotide polymorphisms (SNPs).

GENE	Chr	NCBI SNP Reference	SNP type	Genotype	Frequency	P-HWE
CLOCK	4	rs1056547	3 Prime UTR Variant	CC/CA/AA	0.38/0.45/0.17	0.16
CLOCK	4	rs11931061	Intron Variant	AA/AG/GG	0.59/0.35/0.05	0.82
CLOCK	4	rs12649507	Intron Variant	AA/AG/GG	0.38/0.45/0.17	0.02
CLOCK	4	rs13132420	Intron Variant	AA/AG/GG	0.38/0.45/0.17	0.02
CLOCK	4	rs17722979	Intron Variant	GG/GA/AA	0.70/0.27/0.03	0.56
CLOCK	4	rs1801260	3 Prime UTR Variant	AA/AG/GG	0.70/0.27/0.03	0.56
CLOCK	4	rs3817444	Intron Variant	CC/CA/AA	0.59/0.35/0/05	0.82
CLOCK	4	rs6855837	3 Prime UTR Variant	CC	1	NA
CRY1	12	rs10047525	Intron Variant	AA	1	NA
CRY1	12	rs1056560	3 Prime UTR Variant	AA/AC/CC	0.53/0.41/0.06	0.19
CRY1	12	rs10778528	Intron Variant	AA/AC/CC	0.53/0.40/0.06	0.21
CRY1	12	rs10861688	Intron Variant	GG/GA/AA	0.50/0.42/0.08	0.70
CRY1	12	rs11113179	Intron Variant	GG/GA/AA	0.75/0.23/0.02	0.82
CRY1	12	rs3809235	5 Prime UTR Variant	AA/AG/GG	0.53/0.41/0.07	0.24
CRY1	12	rs7138365	Intron Variant	GG/GA/AA	0.53/0.41/0.06	0.19
CRY1	12	rs7303842	Intron Variant	GG/GA/AA	0.63/0.34/0.03	0.13
CRY1	12	rs8192440	Synonymous Variant	GG/GA/AA	0.88/0.11/0	0.64
CRY2	11	rs7945565	Intron Variant	AA/AG/GG	0.68/0.29/0.03	0.63
CRY2	11	rs10838524	Intron Variant	AA/AG/GG	0.68/0.29/0.03	0.63
CRY2	11	rs10838527	3 Prime UTR Variant	AA	1	NA
CRY2	11	rs11038689	Intron Variant	AA/AG/GG	0.73/0.25/0.02	0.66
CRY2	11	rs11038695	Intron Variant	GG	1	NA
CRY2	11	rs11605924	Intron Variant	AA/AC/CC	0.68/0.29/0.03	0.63
CRY2	11	rs12281674	Intron Variant	AA/AG/GG	0.9/0.1/0	0.78
CRY2	11	rs2292910	3 Prime UTR Variant	AA/AC/CC	0.58/0.37/0.05	0.15
CRY2	11	rs2292913	Intron Variant	AA/AG/GG	0.38/0.48/0.14	0.68
CRY2	11	rs4755345	Intron Variant	AA/AG/GG	0.38/0.48/0.14	0.68
CRY2	11	rs4756035	Intron Variant	GG/GA/AA	0.38/0.48/0.14	0.60
CRY2	11	rs7123390	Intron Variant	GG/GA/AA	0.73/0.24/0.02	0.58
PER2	2	rs10462023	Intron Variant	GG/GA/AA	0.63/0.33/0.04	0.51
PER2	2	rs11892306	Intron Variant	CC/CA/AA	0.45/0.45/0/10	0.50
PER2	2	rs11894535	Intron Variant	GG/GA/AA	0.54/0.40/0.07	0.53
PER2	2	rs13391269	Intron Variant	GG	1	NA
PER2	2	rs2304669	Synonymous Variant	AA/AG/GG	0.74/0.24/0.02	0.36
PER2	2	rs2304670	Synonymous Variant	GG/GA/AA	0.89/0.11/0	0.45
PER2	2	rs2304673	Intron Variant	AA/AC/CC	0.76/0.23/0.01	0.54
PER2	2	rs3739064	Intron Variant	AA/AG/GG	0.67/0.28/0.05	0.004
PER2	2	rs6431590	Intron Variant	AA/AG/GG	0.44/0.45/0.11	0.55
PER2	2	rs744837	Intron Variant	AA/AG/GG	0.78/0.21/0.02	0.31
PER2	2	rs7570188	Intron Variant	AA/AC/CC	0.75/0.23/0.03	0.07
PER2	2	rs880140	Intron Variant	GG/GA/AA	0.79/0.20/0.02	0.35
PER2	2	rs934945	Missense Variant	GG/GA/AA	0.49/0.42/0.09	0.90
BMAL1	11	rs1026071	Intron Variant	AA/AG/GG	0.34/0.47/0.20	0.11
BMAL1	11	rs10741615	Intron Variant	AA	1	NA
BMAL1	11	rs10741616	Intron Variant	AA/AG/GG	0.25/0.5/0.24	0.82
BMAL1	11	rs10766077	Intron Variant	AA/AG/GG	0.41/0.47/0.12	0.33
BMAL1	11	rs10832020	Intron Variant	AA/AG/GG	0.58/0.36/0.05	0.82
BMAL1	11	rs10832027	Intron Variant	GG/GA/AA	0.50/0.41/0.10	0.32
BMAL1	11	rs10832030	Intron Variant	GG/GA/AA	0.32/0.49/0.20	0.70
BMAL1	11	rs11022765	Intron Variant	CC/CA/AA	0.51/0.41/0.08	0.68

BMAL1	11	rs11022775	Intron Variant	GG/GA/AA	0.86/0.13/0	0.83
BMAL1	11	rs11022778	Intron Variant	AA/AC/CC	0.70/0.28/0.02	0.26
BMAL1	11	rs11022780	Intron Variant	GG/GA/AA	0.57/0.36/0.06	0.71
BMAL1	11	rs11022781	Intron Variant	AA/AG/GG	0.57/0.37/0.06	0.76
BMAL1	11	rs11022783	Intron Variant	GG/GA/AA	0.49/0.42/0.09	0.74
BMAL1	11	rs11600996	Intron Variant	AA/AG/GG	0.57/0.37/0.06	0.76
BMAL1	11	rs12290622	Intron Variant	GG/GA/AA	0.26/0.52/0.22	0.27
BMAL1	11	rs1562437	Intron Variant	GG/GA/AA	0.53/0.39/0.09	0.13
BMAL1	11	rs1562438	Intron Variant	GG/GA/AA	0.35/0.47/0.17	0.49
BMAL1	11	rs16912563	Intron Variant	CC	1	NA
BMAL1	11	rs16912751	Intron Variant	AA/AG/GG	0.69/0.28/0.03	0.29
BMAL1	11	rs1868049	Intron Variant	GG/GA/AA	0.25/0.50/0.24	0.91
BMAL1	11	rs1982350	Intron Variant	AA/AG/GG	0.41/0.47/0.12	0.33
BMAL1	11	rs2290037	Intron Variant	AA/AG/GG	0.71/0.27/0.02	0.60
BMAL1	11	rs3789327	Intron Variant	AA/AG/GG	0.47/0.44/0.09	0.36
BMAL1	11	rs4757143	Intron Variant	AA/AG/GG	0.60/0.36/0.04	0.13
BMAL1	11	rs4757144	Intron Variant	GG/GA/AA	0.26/0.50/0.24	1.00
BMAL1	11	rs4757145	Intron Variant	AA/AG/GG	0.26/0.50/0.24	1.00
BMAL1	11	rs4757151	Intron Variant	AA/AG/GG	0.35/0.47/0.18	0.42
BMAL1	11	rs6486121	Intron Variant	GG/GA/AA	0.76/0.21/0.02	0.17
BMAL1	11	rs7107287	Intron Variant	AA/AC/CC	0.26/0.52/0.22	0.24
BMAL1	11	rs7123257	Intron Variant	GG	1	NA
BMAL1	11	rs7126796	Intron Variant	AA/AG/GG	0.57/0.39/0.05	0.02
BMAL1	11	rs7131131	Intron Variant	GG	1	NA
BMAL1	11	rs7924734	Intron Variant	AA/AG/GG	0.36/0.48/0.16	0.69
BMAL1	11	rs7937060	Intron Variant	GG/GA/AA	0.75/0.22/0.02	0.08
BMAL1	11	rs7949336	Intron Variant	AA/AG/GG	0.29/0.50/0.21	0.82
BMAL1	11	rs7950226	Intron Variant	AA/AG/GG	0.33/0.49/0.18	0.91
BMAL1	11	rs895685	Intron Variant	GG	1	NA
BMAL1	11	rs969485	Intron Variant	GG/GA/AA	0.28/0.48/0.23	0.32

Chr, chromosome; P-HWE, P-value of Hardy-Weinberg equilibrium; NA, no variant alleles observed.

rates differed among subjects with sleep-onset problems. After adjustment for age, sex, smoking status, and physical activity, variant rs1026071 in *BMAL1* was found to be significantly associated with increased odds of sleep-onset problems (aOR of GG vs. AG/AA 1.55, 95% CI 1.00-2.51, $p = 0.048$).

Linkage disequilibrium (LD) between the 10 SNPs examined in the *BMAL1* gene among the study participants is presented in Fig. 1. A strong LD relationship was detected between the rs1026071 and rs1562438 SNPs.

Using Bonferroni correction, we found no association between the selected SNPs in the clock genes and sleep-onset problems. However, on applying a less conservative threshold ($p < 0.05$), we observed a weak association between the three SNPs and sleep-onset problems.

Discussion

Insomnia disorder is the second-most prevalent mental disorder, with an estimated prevalence or approximately 20% in a Japanese population (Kim et al. 2000). Sleep-onset problems are the most common symptom of insomnia, and the diagnostic criteria for insomnia according to the DSM-5 include sleep induction disorder. A number of GWASs have been conducted on sleep phenotype and sleep disorders in humans. However, most of those studies targeted subjects of European ancestry. The contribution of genetic variation to sleep disorders in Asian populations has remained unclear, especially in Japanese populations. In the present study, we identified three SNPs in the *CRY1* and *BMAL1* genes as being genetic risk factors for sleep-onset problems in a Japanese general population. Rs11113179 in the *CRY1* gene and the SNPs rs1026071 and rs1562438 in the *BMAL1* gene were associated with sleep-onset problems, even after adjustment for age and sex.

In our study, rs11113179 in *CRY1* showed an association with sleep-onset problems. Grundy et al. (2013) reported that rs1113179 in *CRY1* showed an association with breast cancer risk. However, this association lost significance following adjustment for the false discovery rate.

We found that rs1026071 and rs1562438 in *BMAL1* were significantly associated with sleep-onset problems. Dmitrzak-Weglarz et al. (2015) reported that carriers of

Table 3. Results from the logistic regression analyses of association between single nucleotide polymorphisms and sleep onset problem.

Gene (number of markers)	Associated markers (position; minor allele)	Unadjusted		Model 1		Model 2	
		P value	Odds ratio [95% CI]	P value	Odds ratio [95% CI]	P value	Odds ratio [95% CI]
CLOCK (8)	-						
CRY1 (9)	rs11113179 (chr12:107059007; T)	0.021	1.51 [1.06-2.14]	0.022	1.51 [1.06-2.14]	0.055	1.49 [0.99-2.24]
CRY2 (12)	-						
PER2 (13)	-						
BMAL1 (38)	rs1026071 (chr11: 13343165; G)	0.027	1.54 [1.05-2.27]	0.027	1.55 [1.05-2.28]	0.048	1.59 [1.00-2.51]
	rs1562438 (chr11: 13342653; T)	0.025	1.60 [1.06-2.41]	0.026	1.60 [1.06-2.41]	0.062	1.58 [0.98-2.57]

Model 1: adjusted for age and sex.
Model 2: adjusted for age, sex, smoking, and physical activity.

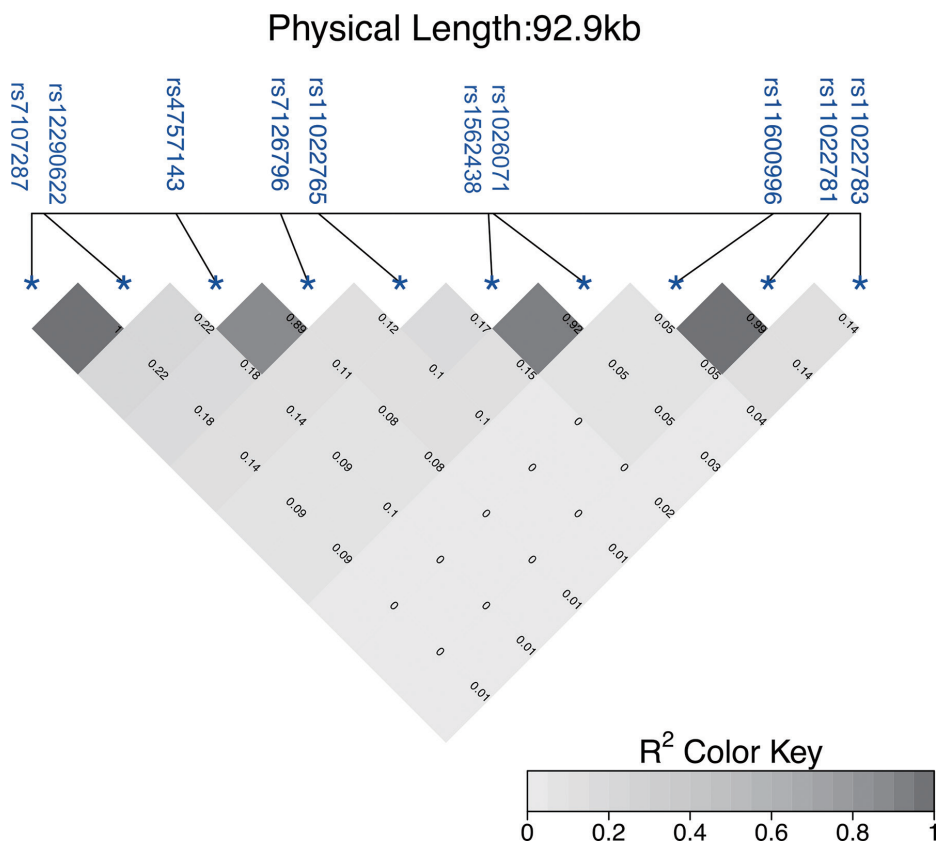

Fig. 1. Linkage disequilibrium for the 10 single nucleotide polymorphisms (SNPs) in the BMAL1 gene. A strong linkage disequilibrium relationship was detected between the rs1026071 and rs1562438 SNPs.

homozygote variants of rs1562438TT in *BMAL1* more frequently had difficulty falling asleep than other genotypes carriers with bipolar disorder in Poland. Using Bonferroni correction, we found no association between the selected SNPs and sleep-onset problems. Of note, similar results were obtained for different ancestries. Further studies are thus needed to assess the interactions between *BMAL1* polymorphisms and sleep disorders.

Few reports have described the association between sleep disorder and genetic factors in Asians, including Japanese populations. Mishima et al. (2005) reported that the 3111C allele of the *CLOCK* gene was associated with evening preference and delayed sleep timing in 421 healthy Japanese adults. Matsuo et al. (2007) showed that bp2114G/A *hPER2* was associated with diurnal preference in 71 healthy Japanese subjects. Song et al. (2016) revealed that the *PER2* rs2278749 genotype was associated with diurnal preference in a Korean population. Unfortunately, we were unable to examine these associations in the present study because genotyping for these variants was not performed. However, we observed no association between variants in the *CLOCK* and *PER2* genes and sleep-onset problems in our study. We suggest two possible reasons for these discrepant findings. First, the study population of the

present study was older than those described in other studies. Second, we examined the association between these genes and sleep-onset problem, while the other studies mainly examined the association between chronotype and genetic polymorphisms.

Although none of the three SNPs of the *CRY1* and *BMAL1* genes was predicted to have any functional relevance, our results indicate that sleep disorder in a healthy Japanese general population is associated with SNPs of the *CRY1* and *BMAL1* genes. As the SNPs identified in this study are intronic and not known to affect splicing, they may be in LD with another functional SNP that may instead underlie this association.

Several limitations associated with this study should also be mentioned. First, while the inclusion of a significant number of genetic markers to investigate possible associations between clock genes and sleep induction disorder was considered a strength, the inclusion of so many markers incurred a risk of false positive (type 1) error. Caution must therefore be practiced when interpreting individual significant results. Second, the sample size may not have been large enough to detect weak associations. Third, as mentioned above, we assessed sleep-onset problems in this study using a single, simple question. In future studies, the use of questionnaires concerning sleep, insomnia, and chronotype, such as the Pittsburgh sleep quality index, Insomnia Severity Index, or Morningness-Eveningness questionnaire, may improve the classification of participants according to symptoms by providing greater attention to clinical and environmental factors. Fourth, we did not obtain information on any diagnosis of insomnia or the use of hypnosis. Fifth, the study subjects were participants in a community-based annual health check-up. As such, they might have been more health-conscious and had a higher level of social activity than the general population, so some degree of selection bias might also be present.

In conclusion, despite the abovementioned limitations, our results suggest that genetic variations in genes with circadian expression are associated with sleep-onset problems in a Japanese general population. The present findings are consistent with several prior studies that detected circadian gene involvement in sleep disorders. Further studies are needed to determine the specific roles played by individual circadian genes.

Conflict of Interest

The authors declare no conflict of interest.

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