Influences of the Interactions of Genetic Variations of Seven Core Circadian Clock Genes with Lifestyle Factors on Metabolic Parameters

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Keywords
Circadian rhythms · Circadian clock gene · Lifestyle factor · Metabolic parameter

Abstract

Introduction: In mammals, circadian rhythms regulate many behavioral and physiological processes. Genetic and epidemiological studies have shown that dysregulation of the circadian rhythm induces chronic metabolic diseases, such as obesity, diabetes, and dyslipidemia. We aimed to know the interactions of genetic variations of seven core circadian clock genes with lifestyle factors on the determination of metabolic parameters.

Methods: We have analyzed the impacts of genotype of seven core circadian clock genes (i.e., \textit{CLOCK}, \textit{BMAL1}, \textit{PER1}, \textit{PER2}, \textit{PER3}, \textit{CRY1}, and \textit{CRY2}) and lifestyle factors (i.e., physical activity and sleep duration) in 575 Japanese males on the determination of metabolic parameters (i.e., body mass index [BMI], serum glucose, glycated hemoglobin [HbA1c], low-density lipoprotein cholesterol [LDL-C], and high-density lipoprotein cholesterol [HDL-C] levels).

Results: We have detected the associations between genotypes of \textit{PER3} and serum HbA1c level and genotypes of \textit{CRY1} and serum LDL-C level. Additionally, the interactions of the genotypes of \textit{PER1} and \textit{PER3} with physical activity for determining BMI, the genotypes of \textit{CLOCK} with physical activity for determining serum HbA1c levels were observed. Furthermore, for determining serum HDL-C levels, the interactions of the genotypes of \textit{CRY2} with physical activity or sleep duration were observed.

Discussion/Conclusion: Our findings indicate that the interactions of genotypes for core circadian clock genes and lifestyle factors (i.e., physical activity and sleep duration) are important for determining metabolic parameters.

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Introduction

Almost all living organisms from prokaryotes to mammals have circadian rhythms adapted to the 24-h rotation period of Earth. In mammals, circadian rhythms regulate many behavioral and physiological processes, including the sleep-wake cycle, feeding/fasting rhythm, hormonal axes regulation, immune responses, and metabolic homeostasis [1–5]. Genetic and epidemiological studies have shown that dysregulation of the circadian rhythm induces chronic metabolic diseases, such as obesity, dia-
Interactions of Genetic Variations of Clock Genes with Lifestyle Factors

Introduction

The circadian system is programmed by the master clock in the suprachiasmatic nuclei of the hypothalamus and many secondary clocks in the brain and peripheral organs [2, 8]. In mammals, the main circadian clocks are driven by transcription/translation feedback loops composed of positive transcriptional activators, including aryl hydrocarbon receptor nuclear translocator-like protein (ARNTL), which is also known as brain and muscle ARNT-like protein 1 (BMAL1), and circadian locomotor output cycle kaput (CLOCK), and negative repressors, such as cryptochromes (CRYs) and periods (PERs). CRYs form complexes with PERs that bind to the BMAL1/CLOCK complex and repress E-box-driven transcription of many genes associated with circadian rhythms [2, 5, 9]. Therefore, BMAL1, CLOCK, CRYs, PERs are regarded as products of the core circadian clock genes. The roles of core circadian clock genes in metabolic homeostasis have been extensively studied using mouse models of targeted gene deletions, which are regarded as useful makers for early detection and monitoring of metabolic disorders such as obesity, type 2 diabetes, and dyslipidemia. Furthermore, we analyzed the interactions of genotypes of seven core circadian clock genes with lifestyle factors (i.e., physical activity and sleep duration) for determining metabolic parameters in 575 apparently healthy Japanese male.

Subjects

The subjects in this study were 575 Japanese males (age range, 45–65 years; mean age, 54.2 ± 5.6 years). They were recruited from the participants in routine medical checkups at a medical center near the University of Shizuoka. The majority (71.3%) of participants in the medical checkups were male, and since some of the metabolic parameters are modulated sex hormones, we decided to analyze only males in this study.

A blood sample was collected from each subject after overnight fasting. The clinical characteristics of subjects are shown in Table 1. The fifty-five subjects with medications for diabetes and/or dyslipidemia were excluded from analyses concerning for various metabolic parameters (i.e., serum glucose, HbA1c, HDL-C, and LDL-C levels). Written informed consent was obtained from all subjects before participation in this study, and the study protocol was approved by the Ethics Committee of the University of Shizuoka (Approval No. 17-1).

Table 1. Characteristics of the study subjects

| Parameter                     | Mean ± SD or Percentage |
|-------------------------------|-------------------------|
| n                             | 575                     |
| Age, years                    | 54.2±5.6                |
| BMI, kg/m²                    | 23.6±2.8                |
| Glucose, mg/dL                | 103.1±21.8              |
| HbA1c, %                      | 5.77±0.81               |
| LDL-cholesterol, mg/dL        | 129.7±28.8              |
| HDL-cholesterol, mg/dL        | 56.1±15.1               |
| Medication for type 2 diabetes, % | 7.3                     |
| Medication for dyslipidemia, % | 2.4                     |
| Physical activity (≥1 h/week), % | 52.3                   |
| Sleep duration (6 ± hours/day), % | 49.6                   |

Data are expressed as mean ± SD or percentage.

DNA Analysis

Genomic DNA was isolated from peripheral blood leukocytes by the phenol extraction method. We selected single nucleotide polymorphisms (SNPs) of seven core circadian clock genes (PER1-rs3027178, PER2-rs2304672, PER3-rs228697, CRY1-rs2287161, CRY2-rs7945565, CLOCK-rs1801260, and BMAL1-rs7950226) based on previous reports because these SNPs were used in multiple association studies, and their minor allele frequencies in the Japanese population were above 0.10 [10, 16, 18, 19]. The genotypes of each SNP were determined by polymerase chain reaction (PCR)-restriction fragment length polymorphisms methods.

Lifestyle Factor Assessment

Self-reported data obtained from the subjects were used to assess lifestyle factors (i.e., physical activity and sleep duration). Physical activity was classified as active (≥1 h/week of moderate to intense exercise) or inactive (<1 h/week of moderate to intense exercise). Sleep duration was classified as short (habitual sleep duration ≤6 h/day) or other (habitual sleep duration >6 h/day).

Statistical Analyses

Multiple linear regression analysis was used to assess the relationships among SNPs of seven core circadian clock genes and metabolic parameters (i.e., serum glucose, HbA1c, LDL-C, and HDL-C levels) by incorporating age and BMI as covariates and to assess the relationships among SNPs and BMI by incorporating age as covariate. The interactions of genotypes with lifestyle factors

References

[10, 16, 18, 19]
Table 2. Relationships between genotypes of seven SNPs in core circadian clock genes and metabolic parameters

| Gene   | N   | BMI, kg/m² | Glucose, mg/dL | HbA1c, % | LDL-C, mg/dL | HDL-C, mg/dL |
|--------|-----|------------|----------------|----------|--------------|--------------|
| PER1   |     |            |                |          |              |              |
| rs3027178 | AC  | 243        | 23.7±2.9       | 221      | 99.5±11.5    | 220          |
|         | CC  | 75         | 23.6±3.1       | 72       | 97.1±9.6     | 72           |
|         |     | p = 0.60   | p = 0.12       | p = 0.18 | p = 0.018    | p = 0.10     |
| PER2   |     |            |                |          |              |              |
| rs2304672 | CC  | 500        | 23.6±2.8       | 456      | 99.8±14.9    | 451          |
|         | CG + GG | 67       | 23.7±3.3       | 57       | 99.9±10.0    | 57           |
|         |     | p = 0.77   | p = 0.88       | p = 0.67 | p = 0.86     | p = 0.58     |
| PER3   |     |            |                |          |              |              |
| rs228697 | CC  | 489        | 23.6±2.8       | 440      | 99.2±11.4    | 435          |
|         | CG + GG | 72       | 23.7±3.0       | 68       | 102.8±26.9   | 68           |
|         |     | p = 0.75   | p = 0.055      | p = 0.0069 | p = 0.029    | p = 0.029    |
| CRY1   |     |            |                |          |              |              |
| rs2287161 | CC  | 389        | 23.5±2.8       | 349      | 99.9±15.7    | 344          |
|         | CG + GG | 179       | 23.7±2.8       | 166      | 99.2±10.9    | 166          |
|         |     | p = 0.48   | p = 0.48       | p = 0.48 | p = 0.0060   | p = 0.52     |
| CRY2   |     |            |                |          |              |              |
| rs7945565 | AG + GG | 195       | 23.8±2.9       | 173      | 100.4±16.1   | 172          |
|         |     | p = 0.33   | p = 0.048      | p = 0.83 | p = 0.28     | p = 0.08     |
| CLOCK  |     |            |                |          |              |              |
| rs1801260 | TT  | 400        | 23.7±2.9       | 363      | 99.8±15.3    | 359          |
|         | TC + CC | 169       | 23.4±2.7       | 152      | 99.2±11.6    | 151          |
|         |     | p = 0.36   | p = 0.74       | p = 0.18 | p = 0.26     | p = 0.012    |
| BMAL1  |     |            |                |          |              |              |
| rs7950226 | AG  | 242        | 23.8±2.8       | 222      | 100.3±17.6   | 221          |
|         | GG  | 103        | 23.5±3.1       | 94       | 98.7±11.8    | 94           |
|         |     | p = 0.31   | p = 0.78       | p = 0.70 | p = 0.31     | p = 0.26     |

Values are shown as mean±SD. p values for BMI were calculated by multiple linear regression analysis incorporating age as covariate. p values for glucose, HbA1c, LDL-C, and HDL-C were calculated by multiple linear regression analyses incorporating age and BMI as covariates. Statistically significant p values (p < 0.05; Bonferroni correction) are indicated in bold. * The fifty-five subjects with medication for diabetes and/or dyslipidemia were excluded in analysis for glucose, HbA1c, LDL-C, and HDL-C.

(i.e., physical activity and sleep duration) for determining metabolic parameters were also calculated using multiple linear regression analysis.

Statistical analyses were performed using JMP software (SAS Institute, Cary, NC, USA). A p value of <0.007 (0.05/7 SNPs; Bonferroni correction for multiple testing) was considered significant for association studies, while a p value of <0.05 was considered nominally significant for interaction analysis.

Results

The relationships between SNPs of seven core circadian clock genes (PER1-rs3027178, PER2-rs2304672, PER3-rs228697, CRY1-rs2287161, CRY2-rs7945565, CLOCK-rs1801260, BMAL1-rs7950226) and metabolic parameters (BMI, serum glucose, HbA1c, LDL-C, and HDL-C levels) were examined in 575 Japanese males (age range, 45–65 years; mean age, 54.2 ± 5.6 years). In the five SNPs (PER2, PER3, CRY1, CRY2, CLOCK), we classified the subjects into two groups, as homozygotes for major alleles or carriers of minor alleles due to low minor alleles frequencies (<0.20). We have detected significant associations between genotypes of PER3 and serum HbA1c level (p = 0.0069) and genotypes of CRY1 and serum LDL-C level (p = 0.0060) after Bonferroni correction after adjusting for age and BMI, while serum levels of glucose, HDL-C, and BMI were not associated with the genotypes of these seven core circadian clock genes (Table 2).

Next, the effects of interactions of genotypes of seven core circadian clock genes with lifestyle factors (i.e., physical activity and sleep duration) on metabolic parameters (i.e., BMI, serum glucose, HbA1c, LDL-C, and HDL-C levels) were assessed using multiple linear regression analysis. Individually, physical activity and sleep duration were not associated with any of the metabolic parameters (Tables 3, 4). For determining BMI, we observed the in-
### Table 3. Multiple linear regression analysis examining the interactions of genotypes of seven core circadian clock genes with physical activity for determining BMI, HbA1c, HDL-C levels

| Determinant variables | BMI | HbA1c | HDL-C |
|-----------------------|-----|-------|-------|
| R²                    | 0.049 | 0.076 | 0.18 |
| Intercept             | 24.7 | 5.06  | 90.3  |

| Determinant variables | β-coefficient | SE   | p value | β-coefficient | SE   | p value | β-coefficient | SE   | p value |
|-----------------------|---------------|------|---------|---------------|------|---------|---------------|------|---------|
| Age                   | -0.017        | 0.022 | 0.43    | 0.0005        | 0.0046 | 0.91    | 0.21          | 0.11 | 0.065   |
| BMI                   | -              | -     | -       | 0.025         | 0.0093 | 0.071   | -1.94         | 0.23 | <0.0001 |
| Physical activity     | -0.16         | 0.27  | 0.55    | 0.026         | 0.036  | 0.64    | -1.22         | 1.37 | 0.37    |
| PER1 genotype         | -0.087        | 0.18  | 0.63    | 0.083         | 0.037  | 0.027   | 0.34          | 0.91 | 0.71    |
| PER2 genotype         | -0.056        | 0.19  | 0.77    | 0.027         | 0.040  | 0.50    | -0.57         | 1.00 | 0.57    |
| PER3 genotype         | -0.097        | 0.19  | 0.60    | -0.12         | 0.038  | 0.0028  | 1.71          | 0.94 | 0.072   |
| CRY1 genotype         | -0.091        | 0.13  | 0.49    | 0.020         | 0.028  | 0.48    | 0.68          | 0.84 | 0.40    |
| CRY2 genotype         | -0.15         | 0.13  | 0.27    | 0.0035        | 0.028  | 0.90    | 0.12          | 0.68 | 0.86    |
| CLOCK genotype        | 0.066         | 0.14  | 0.63    | 0.030         | 0.028  | 0.29    | -1.46         | 0.70 | 0.037   |
| BMAL1 genotype        | -0.04         | 0.18  | 0.82    | 0.0016        | 0.037  | 0.97    | -0.015        | 0.91 | 0.99    |
| PER1 genotype X physical activity | 0.40          | 0.18  | 0.025  | -0.0099       | 0.037  | 0.79    | 0.99          | 0.91 | 0.28    |
| PER2 genotype X physical activity | 0.11          | 0.19  | 0.57    | 0.038         | 0.040  | 0.35    | 0.18          | 1.00 | 0.99    |
| PER3 genotype X physical activity | -0.39         | 0.19  | 0.035  | -0.037        | 0.038  | 0.34    | 0.49          | 0.95 | 0.61    |
| CRY1 genotype X physical activity | -0.17         | 0.13  | 0.19    | 0.049         | 0.028  | 0.074   | 0.54          | 0.68 | 0.43    |
| CRY2 genotype X physical activity | 0.047         | 0.13  | 0.72    | -0.044        | 0.027  | 0.11    | -1.35         | 0.67 | 0.046   |
| CLOCK genotype X physical activity | -0.13         | 0.14  | 0.35    | 0.061         | 0.029  | 0.033   | 0.23          | 0.70 | 0.74    |
| BMAL1 genotype X physical activity | 0.22          | 0.18  | 0.21    | -0.023        | 0.037  | 0.53    | 0.11          | 0.91 | 0.91    |

β-coefficient, standardized coefficient; SE, standard error of coefficient. Nominally significant p values (p < 0.05) for interaction are indicated in bold.

### Table 4. Multiple linear regression analysis examining the interactions of genotypes of seven core circadian clock genes with sleep duration for determining serum HDL-C levels

| Determinant variables | β-coefficient | SE   | p value |
|-----------------------|---------------|------|---------|
| R²                    | 0.18          |      |         |
| Intercept             | 89.7          |      |         |

| Determinant variables | β-coefficient | SE   | p value |
|-----------------------|---------------|------|---------|
| Age                   | 0.21          | 0.11 | 0.060   |
| BMI                   | -1.93         | 0.22 | <0.0001 |
| Sleeping duration     | 0.86          | 1.37 | 0.53    |
| PER1 genotype         | -0.090        | 0.91 | 0.92    |
| PER2 genotype         | -0.52         | 1.00 | 0.61    |
| PER3 genotype         | 1.46          | 0.92 | 0.12    |
| CRY1 genotype         | 0.38          | 0.68 | 0.58    |
| CRY2 genotype         | 0.25          | 0.67 | 0.70    |
| CLOCK genotype        | -1.31         | 0.70 | 0.064   |
| BMAL1 genotype        | 0.060         | 0.90 | 0.95    |
| PER1 genotype X sleep duration | -0.008       | 0.91 | 0.99    |
| PER2 genotype X sleep duration | 1.25         | 1.00 | 0.21    |
| PER3 genotype X sleep duration | -0.65        | 0.93 | 0.49    |
| CRY1 genotype X sleep duration | -0.020       | 0.68 | 0.98    |
| CRY2 genotype X sleep duration | -1.66        | 0.67 | 0.014   |
| CLOCK genotype X sleep duration | 0.055        | 0.70 | 0.94    |
| BMAL1 genotype X sleep duration | -0.13        | 0.90 | 0.88    |

β-coefficient, standardized coefficient; SE, standard error of coefficient. Nominally significant p values (p < 0.05) for interaction are indicated in bold.
interactions of PER1 or PER3 genotypes with physical activity (β ± SE = 0.40 ± 0.18, p = 0.025; β ± SE = −0.39 ± 0.19, p = 0.035, respectively). For determining serum HbA1c levels, we observed the interactions of CLOCK genotypes with physical activity (β ± SE = 0.061 ± 0.029, p = 0.033). Furthermore, we observed the interactions of CRY2 genotypes with physical activity or sleep duration for the determination of HDL-C levels (β ± SE = −1.35 ± 0.67, p = 0.046; β ± SE = −1.66 ± 0.67, p = 0.014, respectively), although the associations for each individual genotype of PER1 and PER3 to BMI, CLOCK genotypes to HbA1c, CRY2 genotypes to HDL-C were not observed (Table 2). These data indicate that the genetic effects due to PER1, PER3, CLOCK, or CRY2 genotypes are modified by physical activity or sleep duration. The remaining results after multiple linear regression analysis for interactions of each genotype and physical activity or sleeping duration are shown in online supplementary Tables 3 and 4 (see www.karger.com/doi/10.1159/000525859 for all online suppl. material), in which the interactions for each genotype and physical activity or sleeping duration were not observed.

Discussion

The prevalence of common chronic metabolic diseases, such as obesity, type 2 diabetes, and dyslipidemia, continues to increase worldwide. Such chronic metabolic diseases are complex multifactorial disorders that result from the interactions of genetic and environmental factors, including lifestyle habits [20–22]. It is necessary to understand how genetic and lifestyle factors interact to modulate the development of metabolic diseases. Many studies for finding the gene-lifestyle interactions for chronic metabolic diseases have been conducted; however, it is difficult to elucidate the precise biological mechanisms underlying gene-lifestyle interactions [20–24].

The internal circadian clock and lifestyle factors are important to maintain circadian rhythms and metabolic homeostasis [10, 17]. Therefore, combined analyses of variations of circadian clock genes and lifestyle factors may be a valuable model to analysis the gene-lifestyle interactions. In this study, we have detected the interactions of the genotypes of PER1 and PER3 with physical activity for determining BMI, that of the genotypes of CLOCK with physical activity for determining serum HbA1c (Table 3), and that of the genotypes of CRY2 with physical activity or sleep duration for determining serum HDL-C levels by multiple linear regression analysis (Tables 3, 4), although each individual genotype was not associated with such metabolic parameters (Tables 3, 4). At present, there is no evidence that physical activity or sleep duration directly affects the expression or function of these genes. In contrast, the genetic effects of PER3 and CRY1 for determining serum HbA1c or LDL-C levels were not modified by physical activity or sleep duration (online suppl. Tables 1, 2).

The associations of many SNPs in core circadian clock genes with metabolic diseases such as obesity, type 2 diabetes mellitus, and hyperlipidemia in various populations with different ethnicities or ages have been extensively studied, although all results have not always been consistent [10, 15, 16, 25]. On the other hand, the studies for interactions of genetic variations of circadian clock genes with lifestyle factors have been defined [26, 27]. Dashti et al. [26] reported the results of meta-analysis using 15 cohort studies of European descent for gene-environment interactions of circadian-related genes; in this meta-analysis, they found an interaction between CRY2 genotype and sleep duration for serum HDL-C levels, which was consistent with our findings.

Although our study population was small, we have detected some interactions of the genotypes of core circadian clock genes with lifestyle factors (i.e., physical activity and sleep duration) on determining metabolic parameters containing BMI, serum HbA1c, and HDL-C levels. This may be due to our study population consisting of relatively uniform population (Japanese males, age range, 45–65 years). We need to study the larger and varied populations to confirm the interactions of genetic and lifestyle factors in which we have detected in this study.

In addition, there are concerns that modern unhealthy lifestyles, such as inadequate physical activity, chronic sleep insufficiency, inappropriate sleep/wake schedules, and poor eating habits, contribute in numerous ways to disruptions of circadian rhythms and maintaining metabolic homeostasis. We need further studies to reveal a convincing link between circadian rhythms and human health.

Conclusions

In this study, we have detected the associations between genotypes of PER3 and serum HbA1c level and genotypes of CRY1 and serum LDL-C level. In addition, the interactions of the genotypes of PER1, PER3, or CLOCK with physical activity were observed for deter-
mining BMI or serum HbA1c levels, and for determining serum HDL-C levels, the interactions of the genotypes of CRY2 with physical activity or sleep duration were observed. These findings indicate that the interactions of genetic variations in circadian clock genes with lifestyle factors are important for maintaining metabolic homeostasis and human health.

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**Statement of Ethics**

The study protocol was approved by the Ethics Committee of the University of Shizuoka (Shizuoka, Japan; approval No. 17-1) and conducted in accordance with the Ethical Principles for Medical Research Involving Human Subjects described in the Declaration of Helsinki. Written informed consent was obtained from each subject prior to participation in this study.

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**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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**Author Contributions**

All authors contributed to the design of the study. Kimiko Yamakawa-Kobayashi, Sayaka Ishikawa, and Nagi Miyake carried out the genetic analysis. Kimiko Yamakawa-Kobayashi and Yuya Ohhara performed the statistical analysis. Toshinoda Goda conducted the recruitment for study subjects. Kimiko Yamakawa-Kobayashi drafted the manuscript. All authors read and approved the final manuscript.

**Data Availability Statement**

All the datasets generated or analyzed during this study are shown in online supplementary Table 3. Further inquiries can be directed to the corresponding author.
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