Genetic variations in circadian rhythm genes and susceptibility for myocardial infarction

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Abstract

Disruption of endogenous circadian rhythms has been shown to increase the risk of developing myocardial infarction (MI), suggesting that circadian genes might play a role in determining disease susceptibility. We conducted a case-control study on 200 patients hospitalized due to MI and 200 healthy controls, investigating the association between MI and single nucleotide polymorphisms (SNPs) in four circadian genes (ARNTL, CLOCK, CRY2, and PER2). The variants of all four genes were chosen based on their previously reported association with cardiovascular risk factors, which have a major influence on the occurrence of myocardial infarction. Statistically significant differences, assessed through Chi-square analysis, were found in genotype distribution between cases and controls of the PER2 gene rs35333999 (p=0.024) and the CRY2 gene rs2292912 (p=0.028); the corresponding unadjusted odds ratios, also significant, were respectively OR=0.49 (95% CI 0.26-0.91) and OR=0.32 (95% CI 0.11-0.89). Our data suggest that genetic variability in the CRY2 and PER2 genes might be associated with myocardial infarction.

Keywords: cardiovascular diseases, circadian rhythm, myocardial infarction, polymorphisms.

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Cardiovascular diseases (CVD) are the world’s leading cause of death (WHO, 2015), and myocardial infarction (MI) is the third leading cause of mortality in Croatia (Hrabak Zerjavic et al., 2010). In the last few years, genome-wide association studies (GWAS) have identified many genetic variants that contribute to a higher risk of MI (Erdmann et al., 2010). Despite numerous studies conducted on MI, its etiology is still largely unknown.

Human physiological activities and diseases are under the control of circadian rhythm. Several physiological factors can cause MI, and several of these factors are known to oscillate with circadian rhythm (Kanth et al., 2013). Some of those are blood pressure (Woon et al., 2007; Englund et al., 2009; Dashti et al., 2014), glucose homeostasis (Dashti et al., 2014; Lipkova et al., 2014), vascular endothelial function, myocardial contractile function and metabolism (Ebisawa et al., 2001; Martino and Sole, 2009; Bonney et al., 2013a,b).

Epidemiological studies found daylight to be the leading regulator of the human circadian rhythm, and sunlight cycles are crucial for keeping a healthy cardiovascular system (Bonney et al., 2013a,b). In humans, relevant relationships exist between circadian clocks and the metabolic syndrome (Englund et al., 2009).

There is increasing evidence that circadian rhythms have an important role in preserving homeostasis and appropriate body function including cardiac metabolism (Woon et al., 2007; Englund et al., 2009; Bonney et al., 2013a,b). In the mutant mouse models, it is emphasized that mutations in the PER2 gene were associated with protection from myocardial ischemia (Bonney et al., 2013a,b).

Circadian clock network consists of molecular components where ARNTL, CLOCK, CRY2 and PER2 genes represent the central node in the network (Corella et al., 2016). Those core clock genes establish the internal clock and constitute negative and positive transcriptional and translational feedback loops. Heterodimers of the ARNTL/CLOCK proteins initiate the transcription of the CRY2, PER2, and other clock-related genes. Heterodimers of the CRY2/PER2 proteins assemble the negative feedback loop and inhibit the transcriptional activity of the ARNTL and CLOCK genes (Takeda and Maemura, 2010, 2016).

The aim of this study was to explore a possibility of association of the genetic variability of the ARNTL, CLOCK, CRY2 and PER2 genes with myocardial infarction.
in humans. We implemented a case-control study on a popu-
lation of patients with myocardial infarction in compari-
son with a control population. The study was conducted
from August 2012 to December 2013. Patients with myo-
cardial infarction were hospitalized at the Clinical Depar-
tment of Cardiovascular Diseases and Intensive Care at the
University Hospital Osijek, Croatia.

Myocardial infarction was defined as the presence of
at least two of the following: typical increase of biochemi-
cal marker of myocardial necrosis – cardiac troponin T
(above the 99\textsuperscript{th} percentile), ischemic chest pain symptoms
lasting more than 30 minutes, and electrocardiography
changes (ECG) indicative of ischemia (ST-segment eleva-
tion or depression) (Thygesen et al., 2012). Thirty-eight pa-
tients were excluded from this study because they did not
meet the criteria as mentioned above or they had a per-
cutaneous coronary intervention or coronary artery bypass
grafting. Fifty-three patients refused to participate in the
study, and nine patients drop out of the study.

The control group consisted of 200 healthy sex- and
age-matched participants, whose medical documentation
did not show any history of cardiovascular diseases. Their
primary care physician chose them in an ambulatory. We
excluded any patient’s relatives from the control group be-
cause of complex heritability of cardiovascular risk factors
shown in monozygotic twins (Elder et al., 2009).

Systematic information on the medical history was
collected from all participants. The questionnaire included
questions about age, history of smoking, hypertension, dys-
lipidemia, respiratory diseases, diabetes mellitus, kidney
and liver diseases. All given information was checked in
patient’s medical record.

This study was approved by the Ethics Committee of
the Faculty of Medicine, the Josip Juraj Strossmayer Uni-
versity of Osijek (No. 2158-61-07-12-21) and by the Ethics
Committee of the University Hospital Osijek (No. 25-
13160-3/2012). The study was conducted according to the
Declaration of Helsinki and its amendments. Written in-
formed consent was obtained from all participants in the
study.

In this study, genetic variants were genotyped in four
key circadian rhythm regulating genes, ARNTL, CLOCK,
CRY2, and PER2. Ten SNPs previously associated with
cardiovascular risk factors were investigated. Six single nu-
cleotide polymorphisms (SNPs) were chosen from ARNTL
and CLOCK genes. Of these, three SNPs, rs3789327,
rs4757144, and rs12363415 in the ARNTL gene, and three
SNPs, rs11932595, rs6811520, and rs13124436 were se-
lected in the CLOCK gene. Two SNPs, rs2292912 and
rs10838524 in the CRY2 gene, and two SNPs, rs35333999,
and rs934945 were selected in the PER2 gene.

Genomic DNA was extracted from the peripheral
blood lymphocyte using standard procedures (QIAamp
DNA Blood Mini Kit, Qiagen, Hilden, Germany). Geno-
typing was carried out by real-time PCR method performed
on 7500 Real-Time PCR System (Applied Biosystems,
Foster City, CA, USA) using TaqMan SNP genotyping
assays. The PCR reaction mix of 6.25 \textmu L final volume con-
sisted of 6 ng of genomic DNA, 3.13 \textmu L of TaqMan Uni-
versal PCR Master Mix 2X, 0.15 \textmu L Assay Mix 40X, and
2.17 \textmu L ddH\textsubscript{2}O. The protocol for PCR amplification was:
initial denaturation step at 95 °C for 10 min, then 40 cycles
denaturation at 92 °C for 15 s, followed by 60 °C for 1
min, and a final extension at 60 °C for 1 min. The allelic
discrimination analysis was performed using SDS 7500
Software Version 2.3 (Applied Biosystems, Foster City,
CA, USA).

Chi-square tests (\(\chi^2\)) on contingency tables were used
to compare allelic and genotype frequencies in controls and
cases. To further assess the presence of associations, we
calculated, for the indicated genetic risk factors, the odds
ratios (OR) and their respective 95% confidence intervals
(CI). Analyses were performed using SHEsis web tool (Shi
and He, 2005; Li et al., 2009). An additional level of geno-
typing quality control was performed using Chi-Square
goodness-of-fit test, by comparing our genotype distribu-
tion with those predicted by Hardy-Weinberg equilibrium.
Associations were considered significant when they
reached the \(p\)-value of equal to or less than 0.05. Appropriate
corrections of significance values were also applied using
the Benjamini-Hochberger correction method (false-dis-
covery rate – FDR values) because of multiple SNPs were
investigated. The \(q\) values of less than 0.05 were considered
to be significant. As the participants of the study were not
related, haplotypes and the pairwise linkage disequilibrium
(LD) were estimated using SNPStats web tool (Solé et al.,
2006).

The prevalence of cardiovascular risk factors among
all participants included in the study sample is summarized
in Table 1. The mean age of the study population was 64 ±

| Variables | Patients (n=200) | Controls (n=200) | \(p\)-value |
|-----------|----------------|----------------|----------|
| Age year mean (SD) | 66 ± 12 | 62 ± 13 | 0.086 |
| Gender: males | 114 (57%) | 104 (52%) | 0.317 |
| Smoking | | | |
| Non-smokers | 99 (49.5%) | 151 (75.5%) | < 0.001 |
| Smokers | 41 (20.5%) | 39 (19.5%) | |
| Former smokers | 60 (30%) | 10 (5%) | |
| Hypertension | 107 (53.5%) | 59 (29.5%) | < 0.001 |
| Dyslipidemia | 26 (13%) | 23 (11.5%) | 0.648 |
| Respiratory disease | 2 (1.8%) | 8 (4%) | 0.055 |
| Diabetes mellitus 2 | 44 (22%) | 0 | - |
| Kidney diseases | 15 (7.5%) | 12 (6%) | 0.550 |
| Liver disease | 10 (5%) | 9 (4.5%) | 0.814 |

Numerical variables are presented as mean (SD), while categorical vari-
ables as number (percentage).
13 years, and 54.5% were males. Genotype frequencies of investigated polymorphisms were predicted by the Hardy-Weinberg equilibrium in the study and the control group ($p > 0.05$), except for rs6811520, which was excluded from further analyses. Minor allele frequencies of the almost all investigated SNPs are consistent with a reference population of HapMap phase 3, CEU; the exception was rs35333999 in the PER2 gene and rs6811520 in the CLOCK gene (Table 2). Genotype and allelic distribution of the ARNTL, CLOCK, CRY2 and PER2 polymorphisms of the 200 MI patients and 200 healthy controls are shown in Table 2.

We did not find any significant associations between rs3789327, rs4757144, and rs12363415 in the ARNTL gene and MI, and there was no significant difference comparing the frequencies of four the most frequent haplotypes for the

### Table 2 - Allele and genotype distribution and frequencies of the ARNTL, CLOCK, CRY2 and PER2 polymorphisms.

| Gene  | SNP               | Minor allele | MAF* patients | MAF* controls | $p$-value | Genotype | Genotype frequency (%) |
|-------|-------------------|--------------|---------------|---------------|-----------|----------|------------------------|
|       | **Gene**          | **SNP**      | **Minor allele** | **MAF* patients** | **MAF* controls** | **$p$-value** | **Genotype** | **Genotype frequency (%)** | **$\chi^2$** | **q value** |
|       | **Patients with MI** | **Controls** |               |                   |           |          |                         |                   |            |            |
| ARNTL | rs3789327         | C            | 0.42          | 0.44            | 0.475     | CC       | 33.5                    | 34.0               | 0.301     | 2.34       | 0.443      |
|       |                   |              |               |                   |           | CT       | 50.0                    | 44.0               |           |            |            |
|       |                   |              |               |                   |           | TT       | 16.5                    | 22.0               |           |            |            |
|       | rs4757144         | A            | 0.62          | 0.59            | 0.385     | AA       | 39.5                    | 36.0               | 0.694     | 0.73       | 0.867      |
|       |                   |              |               |                   |           | AG       | 45.5                    | 46.5               |           |            |            |
|       |                   |              |               |                   |           | GG       | 15.0                    | 17.5               |           |            |            |
|       | rs12363415        | G            | 0.15          | 0.17            | 0.501     | AA       | 71.0                    | 71.0               | 0.120     | 4.24       | 0.300      |
|       |                   |              |               |                   |           | AG       | 27.5                    | 24.0               |           |            |            |
|       |                   |              |               |                   |           | GG       | 1.5                     | 5.0                |           |            |            |
| CLOCK | rs1193259         | G            | 0.39          | 0.39            | 0.942     | AA       | 36.5                    | 35.0               | 0.878     | 0.26       | 0.878      |
|       |                   |              |               |                   |           | AG       | 49.5                    | 52.0               |           |            |            |
|       |                   |              |               |                   |           | GG       | 14.0                    | 13.0               |           |            |            |
|       | rs6811520         | T            | 0.39          | 0.49            | 0.004     | CC       | 37.5                    | 31.0               | 0.005     | 10.55      | 0.050      |
|       |                   |              |               |                   |           | CT       | 47.0                    | 40.0               |           |            |            |
|       |                   |              |               |                   |           | TT       | 15.5                    | 29.0               |           |            |            |
|       | rs1312443         | A            | 0.33          | 0.28            | 0.143     | AA       | 11.0                    | 7.0                | 0.294     | 2.45       | 0.443      |
|       |                   |              |               |                   |           | AG       | 43.0                    | 41.5               |           |            |            |
|       |                   |              |               |                   |           | GG       | 46.0                    | 51.5               |           |            |            |
| CRY2  | rs2292912         | G            | 0.20          | 0.26            | 0.054     | CC       | 62.0                    | 55.5               | 0.056     | 5.78       | 0.197      |
|       |                   |              |               |                   |           | CG       | 35.5                    | 37.0               |           |            |            |
|       |                   |              |               |                   |           | GG       | 2.5                     | 7.5                |           |            |            |
|       | rs1083852         | A            | 0.45          | 0.49            | 0.230     | AA       | 19.0                    | 26.3               | 0.212     | 3.11       | 0.424      |
|       |                   |              |               |                   |           | AG       | 52.5                    | 46.5               |           |            |            |
|       |                   |              |               |                   |           | GG       | 28.5                    | 27.2               |           |            |            |
| PER2  | rs3533399         | T            | 0.05          | 0.09            | 0.032     | CC       | 91.5                    | 84.0               | 0.059     | 5.64       | 0.197      |
|       |                   |              |               |                   |           | CT       | 7.5                     | 15.0               |           |            |            |
|       |                   |              |               |                   |           | TT       | 1.0                     | 1.0                |           |            |            |
|       | rs934945          | T            | 0.17          | 0.19            | 0.644     | CC       | 69.0                    | 67.5               | 0.864     | 0.29       | 0.878      |
|       |                   |              |               |                   |           | CT       | 27.5                    | 28.0               |           |            |            |
|       |                   |              |               |                   |           | TT       | 3.5                     | 4.5                |           |            |            |

*MAF – minor allele frequency
Pearson Chi-square test
CLOCK SNP rs6811520 showed a departure from the Hardy-Weinberg equilibrium and was excluded
$p$-values shown in the table are corrected for the multiple comparisons.
three analyzed SNPs in the ARNTL gene in the patients and control groups.

The SNPs in the CLOCK gene, rs11932595, and rs13124436 did not show significant association of genotype or allelic distribution between the patients and control groups. Accordingly, we did not find any significant difference when comparing the frequencies of four most frequent haplotypes for the two analyzed SNPs in the CLOCK gene in the study and control groups.

No significant associations were found between the rs2292912 and rs10838524 SNPs of the CRY2 gene and MI. Under the recessive genotype model (GG versus CC + CG), OR of 0.32 was estimated for the CRY2 gene polymorphism rs2292912 \( (p=0.028, \text{OR}=0.32 \text{ with 95% CI 0.11-0.89}) \) (Table 3). We did not find any significant difference when comparing the frequencies of the three most frequent haplotypes for the two analyzed SNPs in the CRY2 gene in the patients and control groups.

A statistically significant difference was seen in the allelic distribution of rs35333999 \( (p=0.033) \) in the PER2 gene. However, we did not find any significant association between the rs934945 polymorphism of the PER2 gene and MI. Under the dominant genotype model (TT + CT versus CC), an OR of 0.49 was estimated for the PER2 gene polymorphism rs35333999 \( (p=0.024, \text{OR}=0.49 \text{ with 95% CI 0.26-0.91}) \) (Table 3).

We analyzed the completed haplotypes in the four investigated genes. Table 4 shows the frequencies of predicted haplotypes in the patients and the control group. A statistically significant difference in haplotype distributions was confirmed at the PER2 gene locus when comparing the frequency of haplotype TC \( (p=0.033) \) between participants with MI and control group.

Linkage disequilibrium calculated for the CLOCK gene SNPs (rs11932595 and rs13124436) were \( D'=0.06, r^2=0.001 \). SNPs in the CRY2 gene were in LD \( (D'=0.97, r^2=0.31) \). There was no LD between SNPs in the ARNTL gene, LD between rs4757144 and rs12363415 was \( D'=0.15, r^2=0.001, \) between rs3789327 and rs4757144 was \( D'=0.10, r^2=0.01, \) and between rs3789327 and rs12363415 was \( D'=0.61, r^2=0.09. \) LD for SNPs in the PER2 gene (rs35333999 and rs934945) was \( D'=0.001, r^2=0.01. \)

In this case-control study we found an association between MI and gene variants of the CRY2 and PER2 gene in a sample of 400 participants. The circadian clock is a 24-hour internal system that allows an organism to maintain environmental changes and acclimate to them. Therefore, circadian rhythms handle a broad diversity of physiological and metabolic functions, and any interruption of these rhythms may influence on human health.

Two feedback loops, ARNTL/CLOCK and CRY/PER control expression of downstream transcription factors which regulate downstream target genes involved in different biochemical pathways, such as metabolism of glucose and lipids, synthesis of cholesterol, and others (Staels, 2006). A small number of studies have considered the role of the circadian rhythm in MI. One suggested that gene expression of the cardiomyocyte circadian clock influences myocardial contractile function, metabolism and gene expression (Bray et al., 2008). Another showed that in the cardiomyocyte-specific circadian clock mutant mice, the clock is a direct regulator of triglyceride metabolism in the heart (Tsai et al., 2010), while deletion of ARNTL in mice adipocyte resulted in obesity (Paschos et al., 2012).

PER2 is involved in the regulation of fatty acid metabolism with increased oxygen consumption (Grimaldi et al., 2010). Lipolysis was markedly attenuate in circadian clock mutant mice hearts, and there is a potential explanation for accelerated metabolic pathologies, such as atherosclerosis which might lead to MI in patients (Tsai et al., 2010). PER2 knock-out mice had larger infarct sizes, and the cardiac PER2 have an important role in fatty acid me-

### Table 3 - Genotype models of the ARNTL, CLOCK, CRY2 and PER2 polymorphisms.

|                 | Dominant model | Reccessive model | Codominant model |
|-----------------|----------------|------------------|------------------|
| **ARNTL**       |                |                  |                  |
| rs3789327       | 0.916          | 0.98             | 0.65-1.48        |
| rs4757144       | 0.498          | 0.83             | 0.49-1.42        |
| rs12363415      | 0.780          | 1.07             | 0.69-1.66        |
| **CLOCK**       |                |                  |                  |
| rs11932595      | 0.770          | 1.09             | 0.61-1.95        |
| rs13124436      | 0.271          | 0.80             | 0.54-1.19        |
| **CRY2**        |                |                  |                  |
| rs2292912       | 0.187          | 0.76             | 0.51-1.14        |
| rs10838524      | 0.785          | 0.94             | 0.61-1.46        |
| **PER2**        |                |                  |                  |
| rs35333999      | 0.024          | 0.49             | 0.26-0.91        |
| rs934945        | 0.747          | 0.93             | 0.61-1.42        |

**p-values** shown in the table are corrected for the multiple comparisons.
abolism and inflammation during myocardial ischemia and reperfusion (Bonney et al., 2013b).

Depletion of glycogen stores leads to increased infarct sizes in PER2 mutated mice because of reduced glycolysis during myocardial ischemia (Eckle et al., 2012). It has been shown that the protein PER2 has a cardioprotective role during myocardial ischemia in mice (Bonney et al., 2013a), and mutation of the PER2 gene is associated with a shorter circadian period during constant darkness (Vukolic et al., 2010). The study of Suarez-Barrientos et al. (2011) found that infarct size was larger in the early morning, what is similar to the findings that light-dependent stabilization of PER2 had cardioprotection role in ischemia (Eckle et al., 2012).

Genetic variations in the PER2 gene are associated with abdominal obesity (Garaulet et al., 2010) and metabolic syndrome (Garcia-Rios et al., 2012) due to its part in the lipid metabolism. PER2 activation during ischemia regulates fatty acid beta-oxidation during ischemia and inflammation during reperfusion by increasing inflammatory cytokines, metabolism and inflammation are connected, and inflammation can be a consequence of pathologic metabolism (Bonney et al., 2013b). Thereby, patients who have metabolic syndrome and higher inflammatory markers are at greater risk to develop CVD (Haffner, 2006). Although the genetic variation rs35333999 in the PER2 gene and rs2292912 in the CRY2 gene were associated with MI in this study, they are not precise because of the broad 95% CI values.

Disruption of the circadian clock has been implicated in the pathogenesis of cardiovascular disease, for which hypertension is a major factor (Kovanen et al., 2015). Aortic endothelial dysfunction with decreased production of nitric oxide was found in the mice with the mutated PER2 gene, as well as, decreased vasodilatory prostaglandins and elevated the release of cyclooxygenase-1-derived vasocostricators (Scott, 2015). In endothelial hemostatic function CLOCK, thrombomodulin, and plasminogen activator inhibitor-1 are involved. A circadian clock controls those genes in endothelial cells (Scott, 2015), and disruption of those genes might lead to atherosclerosis and MI. Some genetic variants of the CLOCK gene are related to obesity (Bandin et al., 2013; Garcia-Rios et al., 2012), metabolic syndrome, and CVD (Garcia-Rios et al., 2012).

The links from genetic variants to physiologic functions are most likely less than predicted, a study identified increased weight and obesity and features of metabolic syndrome as characteristics of CLOCK-deficient mice (Turek et al., 2005). Studies on CLOCK mutant mouse indicate an important role of myocardial CLOCK gene in energy metabolism, myocardial contractility, and in the diurnal heart rate control (Scott, 2015). In response to a high-fat diet, mutations in BMAL1 and CLOCK genes adjust circadian variation in glucose and triglycerides levels and affect the progress of insulin resistance (Scott, 2015).

Circadian rhythm has a significant role in regulating glucose metabolism, and cryptochromes are critical components of the circadian system in regulating glucose homoeostasis (Kelly et al., 2012; Lipkova et al., 2014), dysregulation of which can lead to diabetes mellitus type 2. Genetic variations of the BMAL1 gene, the mouse analog of the human ARNTL gene, are associated with diabetes mellitus type 2 and hypertension, providing evidence for the role of ARNTL variants in the pathology of the metabolic syndrome in human (Woon et al., 2007).

| Gene   | rs3789327 | rs4757144 | rs12363415 | Frequency patients | Frequency controls | p-value |
|--------|-----------|-----------|------------|-------------------|--------------------|---------|
| ARNTL  | T         | G         | A          | 0.16              | 0.19               | 0.562   |
| ARNTL  | C         | A         | A          | 0.16              | 0.18               | 0.604   |
| ARNTL  | C         | G         | A          | 0.13              | 0.13               | 0.627   |
| ARNTL  | T         | A         | A          | 0.39              | 0.35               | 0.073   |
| CLOCK  | rs11932595| rs13124436|            |                   |                    |         |
| CLOCK  | A         | G         |            | 0.36              | 0.39               | 0.381   |
| CLOCK  | G         | G         |            | 0.32              | 0.33               | 0.820   |
| CLOCK  | A         | A         |            | 0.25              | 0.21               | 0.276   |
| CLOCK  | G         | A         |            | 0.07              | 0.06               | 0.776   |
| CRY2   | rs2292912 | rs10838524|            |                   |                    |         |
| CRY2   | C         | A         |            | 0.25              | 0.24               | 0.616   |
| CRY2   | C         | G         |            | 0.54              | 0.50               | 0.232   |
| CRY2   | G         | A         |            | 0.19              | 0.26               | 0.052   |
| PER2   | rs35333999| rs934945  |            |                   |                    |         |
| PER2   | C         | C         |            | 0.78              | 0.73               | 0.101   |
| PER2   | C         | T         |            | 0.17              | 0.19               | 0.646   |
| PER2   | T         | C         |            | 0.05              | 0.09               | 0.033   |
Human studies have identified genetic variants and expression patterns of circadian clock genes, such as ARNTL, CLOCK, CRY2, NPAS2 or PER2, that are associated with metabolic syndrome, hypertension or diabetes mellitus type 2 (Ohkura et al., 2006; Scott, 2015). Circadian clock genes play a major role in hemostatic balance by regulating the fibrinolytic systems, and CLOCK and CRY genes are directly involved in this activity (Ohkura et al., 2006), and therefore increase the risk for CVD. A role of the circadian rhythm in cardiovascular function is firmly supported in all those studies, but our study found the connection of myocardial infarction and some of the circadian rhythm genes SNPs.

Although some of the obtained results are significant, they are hardly suitable for diagnosis and prognosis purposes. A limitation of our study is its sample size. The sample size was relatively small and could yield false positive results. Furthermore, the high ORs and broad 95% CIs, as well as the low frequency of some genotypes does not allow adjusting for clinical and demographic confounders (i.e., multiple regression analysis), and low frequency of some genotypes may have resulted in insufficient statistical power to detect a positive association. For participants in control groups, there is a risk of developing some of the CVD.

In conclusion, we provide data indicating that genetic variability in the CRY2 and PER2 genes may be associated with MI. This suggests a role for the circadian rhythm in the development of myocardial infarction, but genetic variations in ARNTL and CLOCK genes are not directly associated with MI. Further verification and mechanistic analysis of the circadian system in MI are possible.

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