Advances in Medical Sciences 64 (2019) 224–229

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original research article

Circadian clock genes and circadian phenotypes in patients with myocardial infarction

Ivana Škrlec[a,b,*], Jakov Milić[b], Marija Heffer[b], Jasenka Wagner[a,b], Borut Peterlin[c]

[a] Department of Biology and Chemistry, Faculty of Dental Medicine and Health, J. J. Strossmayer University of Osijek, Osijek, Croatia
[b] Department of Medical Biology and Genetics, Faculty of Medicine, J. J. Strossmayer University of Osijek, Osijek, Croatia
[c] Clinical Institute of Medical Genetics, University Medical Center Ljubljana, Ljubljana, Slovenia

ARTICLE INFO

Keywords:
Cardiovascular diseases
Chronotype
Circadian rhythm
Clock genes
Myocardial infarction

ABSTRACT

Purpose: Human physiological activities and diseases are under the control of the circadian rhythm. There are strong epidemiological associations between disrupted circadian rhythms, sleep duration and diseases. Sleep disorders are associated with vascular outcomes, such as myocardial infarction (MI).
Methods: We conducted an association study of genotype-phenotype interaction, to determine which circadian clock gene variants might be associated with the circadian phenotypes in patients with MI. In the present study, we analyzed the allele frequencies of 10 single nucleotide polymorphisms in four circadian clock genes in two independent samples: MI patients and controls. Chronotype was assessed using the Morningness - Eveningness Questionnaire (MEQ) and daytime sleepiness using the Epworth Sleepiness Scale (ESS).
Results: Chronotype was associated with the *ARNTL* genetic variant rs12363415 in MI patients. The polymorphisms rs11932595 of the *CLOCK* gene and rs934945 of the *PER2* gene were associated with daytime sleepiness in the patient group.
Conclusion: Our data suggest that genetic variations in some circadian clock genes might be related to circadian phenotype (i.e., chronotype and daytime sleepiness) in patients with myocardial infarction.

1. Introduction

Human physiological activities and diseases are under the control of the circadian rhythm. Several physiological factors may trigger myocardial infarction (MI), and several of these factors are known to fluctuate with the circadian rhythm [1]. Circadian rhythm is under the influence of lifestyle, and it may, consequently, lead to MI. MI is the third leading cause of death in Croatia [2]. Despite numerous research studies conducted on MI, its etiology is still largely unknown [3].

There is increasing data that circadian rhythms have a crucial role in controlling homeostasis and proper body function, including cardiac metabolism [4,5]. Circadian rhythm is associated with many cardiovascular parameters [6]. Many intrinsic vasoactive and cardioactive substances such as angiotensin II, melatonin, plasminogen activator inhibitor 1 (PAI-1), glucocorticoids, epinephrine, norepinephrine, and nitrogen oxide show a specific circadian pattern. The circadian clock regulates the endothelial response to vascular injury [7]. Several physiological factors can stimulate the emergence of MI, and some of these factors are known to oscillate with circadian rhythms [8]. Some of those are blood pressure [7,9], glucose homeostasis [10], vascular endothelial function, myocardial contractions and metabolism [11,12]. Metabolic syndrome is a significant risk factor for cardiovascular diseases (CVDs) and contributes to the emergence of MI [12]. CVDs and diabetes mellitus share common pathophysiological mechanisms underlying insulin resistance and risk factors for CVDs. Excessive weight is one of the primary causes because the fatty tissue is an active endocrine organ that secretes low-level inflammatory mediators that promote the development of metabolic syndrome and CVDs, among which is MI [10]. Acute myocardial infarction as a consequence of circadian rhythm is an issue of significant scientific interest [13]. Humans may be categorized into different circadian phenotypes (morningness, i.e. chronotype) depending on their preferred time of sleep and wakefulness [14]. Sleep is one of the primary requirements for the regular psychophysical functioning of every person [15], and sleep disorders are associated with vascular outcomes, such as MI [16]. Clark et al. in their study showed that daytime sleepiness is an independent risk factor for cardiovascular events [17] but, to the authors' knowledge, there are no studies regarding the association of chronotype and daytime sleepiness with circadian clock gene variations in patients with MI. Some cardiovascular risk factors, such as obesity, body mass index, type 2 diabetes

* Corresponding author at: Department of Biology and Chemistry, Faculty of Dental Medicine and Health, Crkvena 21, Osijek HR-31000, Croatia.
E-mail address: iskrlec@fdmz.hr (I. Škrlec).

https://doi.org/10.1016/j.advms.2018.12.003
Received 13 March 2018; Accepted 7 December 2018
Available online 26 February 2019

mellitus and hypertension, are known to be associated with chronotype and sleep [18,19]. Desynchronization of the circadian rhythm of the organism from its environment, either through shift-work schedules or genetic alteration, augments the risk of developing cardiovascular disease [20].

The circadian clock network consists of molecular components, where *ARNTL*, *CLOCK, CRY2* and *PER2* genes represent the central nodes in the system [21]. These clock genes constitute the internal clock and are involved in negative and positive transcriptional and translational feedback loops. Heterodimers of the ARNTL/CLOCK proteins initiate the transcription of the *CRY2, PER2*, and other clock genes. Heterodimers of the CRY2/PER2 proteins act as a negative feedback loop, inhibiting the *CLOCK* and *ARNTL* transcriptional activity [22,23].

We hypothesized that genetic variations of the *ARNTL*, *CLOCK, CRY2* and *PER2* genes might be associated with circadian phenotypes (i.e., chronotype and daytime sleepiness) in patients with myocardial infarction. We undertook a case-control study of a population of patients with myocardial infarction, in comparison with a control population.

2. Material and methods

2.1. Participants

Patients with myocardial infarction that were hospitalized at the Clinical Department of Cardiovascular Diseases and Intensive Care of the University Hospital of Osijek from August 2012 to December 2013, and who survived, were included in the study. MI was defined as the presence of at least two of the following: an average increase in the biochemical marker of myocardial necrosis – cardiac troponin T (above the 99th percentile), ischemic chest pain symptoms lasting more than 30 min, and electrocardiographic changes (ECG) indicative of ischemia [24]. Patients were excluded from the study if they did not meet these criteria. Some patients were excluded from the study because they underwent percutaneous coronary intervention (20 patients) or coronary artery bypass grafting (18 patients). Additionally, 53 patients refused to participate in the study, and 9 patients withdrew from the study (Fig. 1).

The control group consisted of 200 healthy sex- and age-matched participants, whose medical documentation did not show any history of cardiovascular diseases. They were chosen by their primary care physician in the doctor's surgery after examination. Patients with cardiovascular disease, MI, type 2 diabetes mellitus or sleep problems were excluded. We excluded any patient's relatives from the control group because of the complex heritability of cardiovascular risk factors showed in monozygotic twins [25], and because of the heritability of chronotype features [20,26].

2.2. Questionnaires

Systematic information on their medical history was collected from all participants. All data given were checked in the patients' medical records.

The Horne-Östberg Morningness-Eveningness Questionnaire (MEQ) was used to evaluate the individual chronotype after the patient's medical condition had been stabilized [27,28]. We used a previously published Croatian version of the MEQ [29]. The MEQ consists of 19 questions, and it classifies participants as morning, intermediate, or evening types.

Daytime sleepiness was assessed using the validated Croatian version of the Epworth Sleepiness Scale (ESS, contact information and permission to use: Mapi Research Trust, Lyon, France) [30–32], one of the most commonly used scales in sleep medicine, also after the patient's medical condition had been stabilized, in up to 48 h from patients' admission. The ESS score for each participant is the sum of the results of their eight responses (range 0–24), and the sum can be converted into four types of daytime sleepiness.

2.3. SNP selection and genotyping

In the present study, genetic variants in the four circadian rhythm regulating genes, *ARNTL*, *CLOCK*, *CRY2,* and *PER2* were genotyped. Genes were selected for their involvement in the molecular mechanisms of the regulation of circadian rhythms, and the SNPs chosen had been associated with cardiovascular risk factors in previous studies (Table 1).

Genomic DNA was extracted from the peripheral blood lymphocytes using the standard procedures (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany). Genotyping was carried out by the real-time PCR method performed on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using TaqMan SNP genotyping assays. The allelic discrimination analysis was performed using SDS 7500 Software Version 2.3 (Applied Biosystems, Foster City, CA, USA).

2.4. Statistical analysis

The Chi-square test (χ^2) was used to obtain the significance of the associations between allelic frequencies and chronotype and daytime sleepiness. The association between the genotypes and chronotype and daytime sleepiness was tested using one-way analysis of variance (ANOVA), *t*-test and the Mann-Whitney U test. An additional level of genotyping quality control was performed using the $\chi 2$ goodness-of-fit test with the SHEsis web tool [33], by comparing our genotype distribution with those predicted by the Hardy-Weinberg equilibrium. The

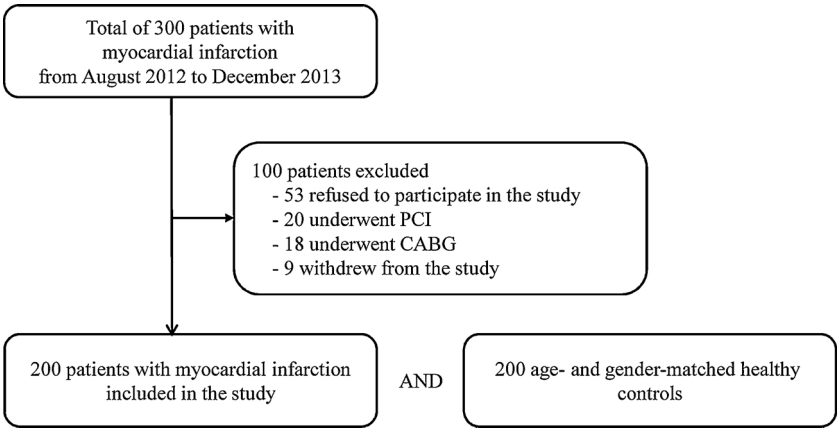

Fig. 1. Patient selection flow chart.
Abbreviations: CABG – coronary artery bypass grafting, PCI – percutaneous coronary intervention.

Table 1
Circadian rhythm gene SNPs and references linked to cardiovascular risk factors.

Gene	SNP	Gene region	Related phenotype	Citation
ARNTL	rs3789327	intronic region	Hypertension and T2D	Garaulet and Madrid [7]
ARNTL	rs4757144	intronic region	Hypertension and T2D	Woon et al. [5]
ARNTL	rs12363415	intronic region	Hypertension and T2D	Woon et al. [5]
CLOCK	rs11932595	intronic region	Obesity	Sookoian et al. [10]
CLOCK	rs6811520	intronic region	Hypertension	Leu et al. [9]
CLOCK	rs13124436	intronic region	Sleep disturbance	Allebrandt et al. [46]
CRY2	rs2292912	intronic region	T2D	Kelly et al. [11]
CRY2	rs10838524	intronic region	Depression	Lavebratt et al. [58]
PER2	rs35333999	Coding (Iso/Val)	Chronotype	Lane et al. [55]
PER2	rs934945	Coding (Glu/Gly)	Metabolic syndrome	Englund et al. [12]

T2D – type 2 diabetes mellitus.

significance threshold was set at $p < 0.05$. All analyses were adjusted by age and performed using Statistica 12 (StatSoft, Inc., version 12, Tulsa, OK, USA) system for Windows.

2.5. Ethical issues

This study was approved by the Ethics Committee of the Faculty of Medicine, Josip Juraj Strossmayer University of Osijek (No. 2158-61-07-12-21) and by the Ethics Committee of the

University Hospital Osijek (No. 25-1:3160-3/2012). The study was conducted according to the Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants of the study.

3. Results

The mean age of the study population was 64 ± 13 years. Our sample consisted mostly of males (114 males, 86 females in the patient group, and 104 males, 96 females in the control group). Table 2 presents the relationship of cardiovascular risk factors with chronotype and daytime sleepiness in MI patients. The MEQ score in the patient group varied from 41 to 74, with a mean of 58.88 ± 6.52, while the observed MEQ score in the control group ranged from 32 to 72, with a mean score of 58.46 ± 7. The ESS score in the patient group ranged from 0 to 13, and the mean score was 5.66 ± 3.18, whereas the ESS score in the control group was from 0 to 17, with a mean score of 5.85 ± 3.65. There were no significant differences in MEQ ($p = 0.601$) or ESS ($p = 0.912$) scores between the patient and control groups.

The genotype frequencies of the investigated polymorphisms were as predicted by the Hardy-Weinberg equilibrium in the patient and control groups ($p > 0.05$), except for rs6811520, which was excluded from further analyses.

The association between *ARNTL, CLOCK, CRY2,* and *PER2* allele frequencies and self-reported chronotype and daytime sleepiness are presented in Table 3. For the ESS score in the patient group, significant associations were found for AA-GG genotypes of the rs378927 of the *ARNTL* gene ($t = 1.99$, $df = 98$, $p = 0.017$, *t*-test) and for the CT-TT and CC-TT genotypes of the rs934945 SNP of the*PER2* gene ($t = -0.89$, $df = 60$, $p = 0.001$ and $t = -0.82$, $df = 143$, $p = 0.007$, respectively; *t*-test). In the control group, an association was found only for the MEQ scores and the CT-TT genotypes of *PER2* gene, rs35333999 ($t = 1.01$, $df = 30$, $p = 0.016$; *t*-test). ANOVA showed an association of the MEQ scores and the following SNPs: rs13124436 of the *CLOCK* gene, and rs12363415 of the *ARNTL* gene ($p = 0.025$ and $p = 0.043$, respectively) in the control group. Table 4 shows the association between cardiovascular risk factors and circadian clock gene SNPs in MI patients.

Table 2
The relationship of cardiovascular risk factors with chronotype and daytime sleepiness in MI patients.

	Chronotype[a]	Daytime sleepiness[a]
Smoking	0.229	**0.028**
Hypertension	0.845	0.355
Dyslipidemia	0.429	**0.005**
Respiratory diseases	0.806	0.466
Thyroid gland diseases	0.428	0.331
Kidney diseases	0.565	0.158
Liver diseases	0.705	0.273
Positive family history of CVD	0.252	**0.004**

The family history of CVD was defined as evidence of coronary artery disease in first-degree relatives before 60 years of age.
CVD – cardiovascular disease.
[a] Mann-Whitney U test p-value.

Table 3
The association between self-reported chronotype and daytime sleepiness scores at allele level.

	Chronotype*		Daytime sleepiness*	
	Patients	Controls	Patients	Controls
ARNTL				
rs3789327	0.239	0.766	0.439	0.642
rs4757144	0.853	0.992	0.743	0.263
rs12363415	**0.004**	0.873	0.521	0.789
CLOCK				
rs11932595	0.974	0.769	**0.047**	0.875
rs6811520[a]	0.520	0.311	0.480	0.318
rs13124436	0.259	0.509	0.720	0.349
CRY2				
rs2292912	0.672	**0.028**	0.096	0.336
rs10838524	0.590	0.799	0.652	0.927
PER2				
rs35333999	0.907	**0.027**	0.616	0.391
rs934945	0.451	0.811	**0.044**	0.865

* p-value Pearson Chi-square test.
[a] *CLOCK* SNP rs6811520 showed a departure from the Hardy-Weinberg equilibrium and was excluded.

4. Discussion

We conducted an association study of a genotype-phenotype interaction to determine which circadian rhythm gene variants might be associated with the circadian phenotypes (i.e., chronotype and daytime sleepiness) in MI patients. We analyzed the allele frequencies of 10 single nucleotide polymorphisms in four circadian genes. We found an association between gene variants of the *ARNTL, CLOCK* and *PER2* gene and chronotype or daytime sleepiness in MI patients, while *CRY2* polymorphisms were not associated with circadian phenotypes in the patient group.

Circadian rhythms are involved in a broad diversity of physiological and metabolic functions, and any interruption of these rhythms may

Table 4
The association between cardiovascular risk factors and circadian clock gene SNPs in MI patients.

	ARNTL			*CLOCK*		*CRY2*		*PER2*	
	rs3789327	rs4757144	rs12363415	rs11932595	rs13124436	rs2292912	rs10838524	rs35333999	rs934945
Smoking	0.421	0.072	0.595	0.170	0.390	0.518	0.940	0.723	0.870
Hypertension	0.741	0.983	0.326	0.086	0.073	0.747	0.585	0.652	**0.009**
Dyslipidemia	0.661	0.546	0.287	**0.010**	0.361	0.230	0.470	0.359	0.484
Respiratory diseases	0.505	0.512	0.528	0.086	0.797	0.755	0.221	0.666	0.345
Thyroid gland diseases	0.434	0.570	0.291	0.921	0.880	0.933	**0.046**	0.380	**0.022**
Kidney diseases	0.919	0.988	0.735	0.974	0.254	0.651	0.510	0.784	0.117
Liver diseases	0.747	0.231	0.508	0.851	0.255	0.221	0.929	0.871	0.550
Positive family history of CVD	0.882	0.104	0.561	0.623	0.774	0.800	0.470	0.314	0.138

Mann-Whitney U test p-value.
The family history of CVD was defined as evidence of coronary artery disease in first-degree relatives before 60 years of age.
CVD - cardiovascular disease.

influence human health [4]. There is a body of literature exploring the association of chronotype, or diurnal preference, with human behavior, performance, and disease [34,35]. Correlations between genotypes and the elements of the circadian phenotype (such as chronotypes and daytime sleepiness) have not been adequately investigated in order to clarify the association between genetic susceptibility and related clinical outcomes, in this case – myocardial infarction. Combining genetic association studies of circadian clock gene polymorphisms and chronotypes and daytime sleepiness, as relevant phenotypes, might help to identify susceptibility markers of MI and physiological pathways from gene to phenotypic outputs. Disruption of the circadian rhythm has been involved in the pathogenesis of cardiovascular disease, for which hypertension is a significant factor [36]. In animal models, mutations in circadian clock genes can lead to signs of metabolic syndrome [37], which can lead to cardiovascular disease.

The *ARNTL* gene polymorphism rs12363415 was only associated with chronotype in the patient group, which means that this SNP might be a potential biomarker for MI. Genome-wide association studies (GWAS) show that both circadian clock genes and non-circadian genes are associated with individual differences in chronotype. Chronotype is a phenotypic aspect of circadian activity in humans [38]. Genetic variations of the *BMAL1* gene, the mouse analog of the human *ARNTL* gene, are associated with type 2 diabetes mellitus and hypertension, providing evidence for the role of *ARNTL* variants in the pathology of cardiovascular disease in humans [5]. Cardiovascular disease and type 2 diabetes mellitus share common pathophysiological mechanisms, underlying insulin resistance and cardiovascular risk factors, such as metabolic syndrome [39,40]. In people with diabetes, atherosclerotic changes in blood vessels are more severe than in healthy people [41,42].

Studies on *CLOCK* mutant mice indicate an essential role of the myocardial *CLOCK* gene in energy metabolism, myocardial contractility, and in diurnal heart rate control [43]. CLOCK is a transcription factor and a master regulator of the circadian rhythm gene expression of numerous transcription factors involved in the regulation of a large number of circadian physiological and behavioral functions [44,45]. GWAS studies show that circadian clock genes and non-circadian genes are associated with differences in sleep parameters [38]. Genetic variant rs11932595 of the *CLOCK* gene was associated with sleep duration in two independent European populations [46]. In the present study, rs11932595 was associated with daytime sleepiness in MI patients. Other research by Vanderlind et al. found that rs11932595 is associated with sleep quality [47]. Short sleep time harms health and is associated with increased mortality, coronary artery disease, type 2 diabetes mellitus, obesity, and hypertension. Loss of regular sleep leads to changes in the immune system, leading to a proinflammatory state that can eventually lead to obesity and increased risk for cardiovascular disease. Apart from known cardiovascular risk factors, such as hypertension, obesity, hyperlipidemia, and smoking, sleep problems are also associated with coronary heart disease and myocardial infarction [48].

In the present study, we found that rs11932595 SNPs of the *CLOCK* gene are associated with dyslipidemia, and daytime sleepiness is associated with dyslipidemia in MI patients. *PER2* polymorphism rs934945 is associated with hypertension in MI patients. It is known that sleep disorders are associated with a higher risk of developing hypertension and dyslipidemia, and thereby increase the risk for cardiovascular disease [48]. According to *Laugsand* et al., it is considered that problems with falling asleep and irregular sleep are associated with the risk of having the first myocardial infarction [49]. Sleep difficulties and sleep maintenance are associated with an increased risk of cardiovascular mortality, as demonstrated in the meta-analysis by Meng et al. [50].

We found that the *PER2* gene, polymorphism rs934945, is associated with daytime sleepiness in MI patients. This may be due to *PER2* being an essential regulator of circadian rhythms and one of the most crucial clock genes. A variant of *PER2* is associated with contractions of the iris furrow, and the same association was found in a GWAS study [19]. This suggests a link between the iris function and chronotype and daytime sleepiness. The cardiac *PER2* has a vital role in fatty acid metabolism, inflammation during myocardial ischemia and reperfusion [51,52]. It has been shown that PER2 protein has a cardioprotective role during myocardial ischemia in mice [4], and mutation of the *PER2* gene is associated with the shorter circadian period during constant darkness [53]. The *PER2* polymorphism rs35333999 was related to self-reported chronotype in three genome-wide association studies [19,54,55]. In the present study, rs35333999 associated with chronotype in the controls, but not in the MI patients.

Genetic variability of the circadian genes might incline individuals to be less capable of adjusting their circadian rhythms in harmony with their environment, and to be prone to sleep disorders [56]. Our results are consistent with new etiological assumptions, in which circadian rhythm abnormalities are core vulnerability dimensions of MI. The present study showed that there is a difference in the genetic background of chronotype and daytime sleepiness in MI patients. Most studies investigated the association of chronotype and daytime sleepiness with the genetic background of circadian rhythm in healthy volunteers.

A potential interpretation of why no associations were found for all SNPs and circadian phenotypes, could be that polymorphisms in those clock genes have a smaller contribution to determining chronotype or daytime sleepiness independently, but only when they are taken together, where a specific combination of polymorphisms gives a significant association. This is relevant, since circadian clock genes are tightly linked together in the clock mechanism. A large number of genes with modest influence contribute to a phenotypic manifestation because chronotype and sleep are complex traits [57].

4.1. Limitations of the study

Some of the limitations of our study need to be considered. The set of circadian genes that we selected based on previous publications might be found to be limited since recent GWAS studies have demonstrated the relevance of numerous other circadian and non-circadian genes involved in the circadian clock and clock-controlled genes [19,54,55]. Chronotype and sleep duration were self-reported, and because we did not have objective measures of sleep or circadian parameters, they are subject to reporting bias. Our sample had the advantage of being relatively homogenous for demographic variables such as age, ethnicity, and social environment, but the sample size was relatively small and could yield a false positive result. For participants in the control group, there is still a risk of developing some of the cardiovascular diseases.

5. Conclusions

In conclusion, this genotype-phenotype interaction study suggests that SNPs in *ARNTL, CLOCK, CRY2,* and *PER2* might act on some essential components of circadian rhythms observed in MI patients. These findings might expand our understanding of the pathophysiological processes involved in MI and require reproduction in separate samples of MI patients tested for chronotypes and other relevant circadian phenotypes. Further verification and mechanistic analysis of the circadian system in MI are possible.

Conflict of interest

The authors declare no conflict of interests

Financial disclosure

This research was supported by the Josip Juraj Strossmayer University of Osijek, Croatia – in the UNIOS project 'Variation in circadian rhythm genes in patients with myocardial infarction' under grant number IZIP-2014-150.

The author contribution

Study design: Marija Heffer, Borut Peterlin.
Data collection: Ivana Škrlec, Jakov Milić.
Statistical analysis: Ivana Škrlec, Jakov Milić.
Data interpretation: Ivana Škrlec, Jakov Milić, Borut Peterlin.
Manuscript preparation: Ivana Škrlec.
Literature search: Ivana Škrlec, Jakov Milić, Marija Heffer, Jasenka Wagner, Borut Peterlin.
Funds collection: Jasenka Wagner.

References

[1] Kanth R, Ittaman S, Rezkalla S. Circadian patterns of ST elevation myocardial infarction in the new millennium. Clin Med Res 2013;11:66–72. https://doi.org/10.3121/cmr.2013.1120.
[2] Hrabak Žerjavić V, Kralj V, Dika Ž, Jelaković B. Epidemiology of hypertension, stroke and myocardial infarction in Croatia. MEDIX 2010;16:102–7.
[3] Erdmann J, Linsel-Nitschke P, Schunkert H. Genetic causes of myocardial infarction: new insights from genome-wide association studies. Dtsch Arztebl Int 2010;107:694–9. https://doi.org/10.3238/arztebl.2010.0694.
[4] Bonney S, Hughes K, Harter PN, Mittelbronn M, Walker L, Eckle T. Cardiac period 2 in myocardial ischemia: clinical implications of a light dependent protein. Int J Biochem Cell Biol 2013;45:667–71. https://doi.org/10.1016/j.biocel.2012.12.022.
[5] Woon PY, Kaisaki PJ, Bragança J, Bihoreau M, Levy JC, Farrall M, et al. Aryl hydrocarbon receptor nuclear translocator-like (BMAL1) is associated with susceptibility to hypertension and type 2 diabetes. Proc Natl Acad Sci U S A 2007;104:14412–7. https://doi.org/10.1073/pnas.0703247104.
[6] Guo YF, Stein PK. Circadian rhythm in the cardiovascular system: chronocardiology. Am Hear J 2003;145:779–86. https://doi.org/10.1016/S0002-8703(02)94797-6.
[7] Garaulet M, Madrid JA. Chronobiology, genetics and metabolic syndrome. Curr Opin Lipidol 2009;20:127–34. https://doi.org/10.1097/MOL.0b013e3283292399.
[8] Virag JAI, Lust RM. Circadian influences on myocardial infarction. Front Physiol 2014;5:422. https://doi.org/10.3389/fphys.2014.00422.
[9] Leu H-B, Chung C-M, Lin S-J, Chiang K-M, Yang H-C, Ho H-Y, et al. Association of circadian genes with diurnal blood pressure changes and non-dipper essential hypertension: a genetic association with young-onset hypertension. Hypertens Res 2015;38:155–62. https://doi.org/10.1038/hr.2014.152.
[10] Sookoian S, Gemma C, Gianotti TF, Burgueño A, Castaño G, Pirola CJ. Genetic variants of Clock transcription factor are associated with individual susceptibility to obesity. Am J Clin Nutr 2008;87:1606–15.
[11] Kelly MA, Rees SD, Hydrie MZI, Shera AS, Bellary S, O'Hare JP, et al. Circadian gene variants and susceptibility to type 2 diabetes: a pilot study. PLoS One 2012;7:e32670. https://doi.org/10.1371/journal.pone.0032670.
[12] Englund A, Kovanen L, Saarikoski ST, Haukka J, Reunanen A, Aromaa A, et al. NPAS2 and PER2 are linked to risk factors of the metabolic syndrome. J Circadian Rhythms 2009;7:5. https://doi.org/10.1186/1740-3391-7-5.
[13] Holmes DR, Aguirre FV, Aplin R, Lennon RJ, Nestler DM, Bell MR, et al. Circadian rhythms in patients with ST-elevation myocardial infarction. Circ Cardiovasc Qual Outcomes 2010;3:382–9. https://doi.org/10.1161/CIRCOUTCOMES.109.913343.
[14] Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, et al. Epidemiology of the human circadian clock. Sleep Med Rev 2007;11:429–38. https://doi.org/10.1016/j.smrv.2007.07.005.
[15] Thomas M, Sing H, Belenky G, Holcomb H, Mayberg H, Dannals R, et al. Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. J Sleep Res 2000;4:335–52.
[16] Ohayon M, Partinen M. Insomnia and global sleep dissatisfaction in Finland. J Sleep Res 2002;4:339–46.
[17] Clark A, Lange T, Hallqvist J, Jennum P, Rod NH. Sleep impairment and prognosis of acute myocardial infarction: a prospective cohort study. Sleep 2014;37:851–8. https://doi.org/10.5665/sleep.3646.
[18] Merikanto I, Lahti T, Puolijoki H, Vanhala M, Peltonen M, Laatikainen T, et al. Associations of chronotype and sleep with cardiovascular diseases and type 2 diabetes. Chronobiol Int 2013;30:470–7. https://doi.org/10.3109/07420528.2012.741171.
[19] Jones SE, Tyrrell J, Wood AR, Beaumont RN, Ruth KS, Tuke MA, et al. Genome-wide association analyses in 128,266 individuals identifies new morningness and sleep duration loci. PLoS Genet 2016;12:e1006125. https://doi.org/10.1371/journal.pgen.1006125.
[20] Portaluppi F, Tiseo R, Smolensky MH, Hermida RC, Ayala DE, Fabbian F. Circadian rhythms and cardiovascular health. Sleep Med Rev 2012;16:151–66. https://doi.org/10.1016/j.smrv.2011.04.003.
[21] Reppert SM, Weaver DR. Coordination of circadian timing in mammals. Nature 2002;418:935–41. https://doi.org/10.1038/nature00965.
[22] Takeda N, Maemura K. Circadian clock and the onset of cardiovascular events. Hypertens Res 2016;39:383–90. https://doi.org/10.1038/hr.2016.9.
[23] Partch CL, Green CB, Takahashi JS. Molecular architecture of the mammalian circadian clock. Trends Cell Biol 2014;24:90–9. https://doi.org/10.1016/j.tcb.2013.07.002.
[24] Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD. Third universal definition of myocardial infarction. Nat Rev Cardiol 2012;9:620–33. https://doi.org/10.1038/nrcardio.2012.122.
[25] Elder SJ, Lichtenstein AH, Pittas AG, Roberts SB, Fuss PJ, Greenberg AS, et al. Genetic and environmental influences on factors associated with cardiovascular disease and the metabolic syndrome. J Lipid Res 2009;50:1917–26. https://doi.org/10.1194/jlr.P900033-JLR200.
[26] Carpen JD, Archer SN, Skene DJ, Smits M, von Schantz M. A single-nucleotide polymorphism in the 5'-untranslated region of the hPER2 gene is associated with diurnal preference. J Sleep Res 2005;14:293–7. https://doi.org/10.1111/j.1365-2869.2005.00471.x.
[27] Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol 1976;4:97–110.
[28] Horne J, Ostberg O. Individual differences in human circadian rhythms. Biol Psychol 1977;5:179–90.
[29] Milić J, Kvolik A, Ivković M, Babić A, Labak I, Benšić M, et al. Are there differences in students' school success, biorhythm, and daytime sleepiness depending on their school starting times? Coll Antropol 2014;38:889–94.
[30] Johns MW. A new method for measure daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540–5.
[31] Mapi Research Trust n.d. http://www.mapi-trust.org/ (Accessed 1 January 2012).
[32] Pecotic R, Dodig IP, Valic M, Ivkovic N, Dogas Z. The evaluation of the Croatian version of the Epworth sleepiness scale and STOP questionnaire as screening tools for obstructive sleep apnea syndrome. Sleep Breath 2012;16:793–802. https://doi.org/10.1007/s11325-011-0578-x.
[33] Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005;15:97–8. https://doi.org/10.1038/sj.cr.7290272.
[34] Ingram KK, Ay A, Bin Kwon S, Woods K, Escobar S, Gordon M, et al. Molecular insights into chronotype and time-of-day effects on decision-making. Sci Rep 2016;6:29392. https://doi.org/10.1038/srep29392.
[35] Fabbian F, Bhatia SS, De Giorgi A, Maietti E, Bhatia SS, Shanbhag A, et al. Circadian periodicity of ischemic heart disease: a systematic review of the literature. Heart Fail Clin 2017;13:673–80. https://doi.org/10.1016/J.HFC.2017.05.003.
[36] Kovanen L, Donner K, Kaunisto M, Partonen T. CRY1, CRY2 and PRKCDBP genetic variants in metabolic syndrome. Hypertens Res 2015;38:186–92. https://doi.org/10.1038/hr.2014.157.
[37] Yu JH, Yun C-H, Ahn JH, Suh S, Cho HJ, Lee SK, et al. Evening chronotype is

associated with metabolic disorders and body composition in middle-aged adults. J Clin Endocrinol Metab 2015;100:1494–502. https://doi.org/10.1210/jc.2014-3754.
[38] Goel N. Genetic markers of sleep and sleepiness. Sleep Med Clin 2017;12:289–99. https://doi.org/10.1016/J.JSMC.2017.03.005.
[39] Gómez-Abellán P, Madrid JA, Ordovás JM, Garaulet M. Chronobiological aspects of obesity and metabolic syndrome. Endocrinol Nutr 2012;59:50–61. https://doi.org/10.1016/j.endoen.2011.08.002.
[40] Marcheva B, Ramsey KM, Buhr ED, Kobayashi Y, Su H, Ko CH, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature 2010;466:627–31. https://doi.org/10.1038/nature09253.
[41] Boras J, Ljubičić A. Macrovascular complications of diabetes mellitus. Medix 2009;15:136–42.
[42] Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. Eur Heart J 2013;34:2436–43. https://doi.org/10.1093/eurheartj/eht149.
[43] Scott EM. Circadian clocks, obesity and cardiometabolic function. Diabetes Obes Metab 2015;17:84–9. https://doi.org/10.1111/dom.12518.
[44] Menet JS, Pescatore S, Rosbash M. CLOCK:BMAL1 is a pioneer-like transcription factor. Genes Dev 2014;28:8–13. https://doi.org/10.1101/gad.228536.113.
[45] Riestra P, Gebreab SY, Xu R, Khan RJ, Gaye A, Correa A, et al. Circadian CLOCK gene polymorphisms in relation to sleep patterns and obesity in African Americans: findings from the Jackson heart study. BMC Genet 2017;18:58. https://doi.org/10.1186/s12863-017-0522-6.
[46] Allebrandt KV, Teder-Laving M, Akyol M, Pichler I, Müller-Myhsok B, Pramstaller P, et al. CLOCK gene variants associate with sleep duration in two independent populations. Biol Psychiatry 2010;67:1040–7. https://doi.org/10.1016/j.biopsych.2009.12.026.
[47] Vanderlind W, Beevers C, Sherman S, Trujillo L, McGeary J, Matthews M, et al. Sleep and sadness: exploring the relation among sleep, cognitive control, and depressive symptoms in young adults. Sleep Med 2014;15:144–9. https://doi.org/10.1016/j.sleep.2013.10.006.
[48] Khan MS, Aouad R. The effects of insomnia and sleep loss on cardiovascular disease. Sleep Med Clin 2017;12:167–77. https://doi.org/10.1016/J.JSMC.2017.01.005.
[49] Laugsand L, Vatten L, Platou C, Janszky I. Insomnia and the risk of acute myocardial infarction: a population study. Circulation 2011;124:2073–81. https://doi.org/10.1161/CIRCULATIONAHA.111.025858.
[50] Meng L, Zheng Y, Hui R. The relationship of sleep duration and insomnia to risk of hypertension incidence: a meta-analysis of prospective cohort studies. Hypertens Res 2013;36:985. https://doi.org/10.1038/hr.2013.70. 3611.
[51] Bonney S, Kominsky D, Brodsky K, Eltzschig H, Walker L, Eckle T. Cardiac Per2 functions as novel link between fatty acid metabolism and myocardial inflammation during ischemia and reperfusion injury of the heart. PLoS One 2013;8:e71493. https://doi.org/10.1371/journal.pone.0071493.
[52] Khaper N, Bailey CDC, Ghugre NR, Reitz C, Awosanmi Z, Waines R, et al. Implications of disturbances in circadian rhythms for cardiovascular health: a new frontier in free radical biology. Free Radic Biol Med 2017. https://doi.org/10.1016/J.FREERADBIOMED.2017.11.006. pii: S0891.
[53] Vukolic A, Antic V, Van Vliet BN, Yang Z, Albrecht U, Montani J. Role of mutation of the circadian clock gene Per2 in cardiovascular circadian rhythms. Am J Physiol Regul Integr Comp Physiol 2010;298:627–34. https://doi.org/10.1152/ajpregu.00404.2009.
[54] Hu Y, Shmygelska A, Tran D, Eriksson N, Tung JY, Hinds DA. GWAS of 89,283 individuals identifies genetic variants associated with self-reporting of being a morning person. Nat Commun 2016;7:10448. https://doi.org/10.1038/ncomms10448.
[55] Lane JM, Vlasac I, Anderson SG, Kyle SD, Dixon WG, Bechtold DA, et al. Genome-wide association analysis identifies novel loci for chronotype in 100,420 individuals from the UK Biobank. Nat Commun 2016;7:10889. https://doi.org/10.1038/ncomms10889.
[56] Etain B, Jamain S, Milhiet V, Lajnef M, Boudebesse C, Dumaine A, et al. Association between circadian genes, bipolar disorders and chronotypes. Chronobiol Int 2014;31:807–14. https://doi.org/10.3109/07420528.2014.906445.
[57] Kalmbach DA, Schneider LD, Cheung J, Bertrand SJ, Kariharan T, Pack AI, et al. Genetic basis of chronotype in humans: Insights from three landmark GWAS. Sleep 2016;40:1–10. https://doi.org/10.1093/sleep/zsw048.
[58] Lavebratt C, Sjöholm LK, Soronen P, Paunio T, Vawter MP, Bunney WE, et al. CRY2 is associated with depression. PLoS One 2010;5:e9407. https://doi.org/10.1371/journal.pone.0009407.