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Research paper

Circadian clock genes and myocardial infarction in patients with type 2 diabetes mellitus

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ABSTRACT

Disruption of circadian clock may trigger the onset of diabetes mellitus and myocardial infarction. Type 2 diabetes mellitus (T2DM) is well-known risk factors for cardiovascular diseases and myocardial infarction.

We performed a case-control study, where we explored the possible association between single nucleotide polymorphisms in three circadian rhythm genes (ARNTL, CLOCK, and PER2) and myocardial infarction in 657 patients with T2DM. The study group consisted of 231 patients with myocardial infarction and T2DM and a control group of 426 T2DM patients. We hypothesized that variations in the circadian rhythm genes in patients with T2DM could be an additional risk factor for myocardial infarction. The statistically significant difference was found in allelic ($p = 1.1 \times 10–5$) and genotype distribution ($p = 1.42 \times 10–4$) between two groups of the rs12363415 at the ARNTL gene locus.

We provide evidence that genetic variability in the ARNTL gene might be associated with myocardial infarction in patients with T2DM.

1. Introduction

Circadian rhythm refers to physiological processes that repeat approximately every 24 h and ensure that physiological processes are synchronized with the environment (Ferrell and Chiang, 2015). Several physiological factors can stimulate the emergence of type 2 diabetes mellitus (T2DM) and myocardial infarction (MI), and some of these factors are known to oscillate with circadian rhythms (Kanth et al., 2013). Some of those are blood pressure (Dashti et al., 2014; Englund et al., 2009; Woon et al., 2007), glucose homeostasis (Dashti et al., 2014; Lipkova et al., 2014), vascular endothelial function, myocardial contractions and metabolism (Bonney et al., 2013a; Ebisawa et al., 2001; Martino and Sole, 2009).

Glucose maintenance is essential for the physiological function of almost all cell types, and brain and red blood cells practically exclusively use glucose as an energy source (Ferrell and Chiang, 2015). There are 24-h rhythmic changes in blood glucose levels caused by changes in insulin sensitivity and insulin secretion (Kurose et al., 2014; Polonsky et al., 1988). In the pancreas, the beta cells have their circadian clock which dependent on oscillations of ARNTL and CLOCK proteins. Those proteins regulate insulin secretion depending on the stage of alertness. Disruption of that clock may trigger the onset of diabetes (Dierickx et al., 2018; Gómez-Abellán et al., 2012; Huang et al., 2011; Klerman, 2005; Takahashi et al., 2008). Type 2 diabetes mellitus belongs to a heterogeneous group of metabolic disorders caused by reduced insulin activity and decreased insulin production (Tibaut and Petrovič, 2016), leading to the disturbed use of peripheral glucose. The inability to prevent gluconeogenesis and hepatic glucose production contribute to the disease development (Moussa and Li, 2012).

T2DM is a well-known risk factor for cardiovascular diseases (CVDs), including myocardial infarction, and circadian rhythm might represent the risk for T2DM and myocardial infarction. Excessive weight is one of the primary causes because the fatty tissue is an active

Abbreviations: ARNTL, Aryl Hydrocarbon Receptor Nuclear Translocator Like; BMAL1, Brain and Muscle ARNTL-like protein 1; CLOCK, Circadian Locomotor Output Cycles Kaput; CRY, cryptochrome; CVD, cardiovascular diseases; HbA1c, glycated hemoglobin; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; MI, myocardial infarction; PCR, polymerase chain reaction; PER2, period 2; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus; WHO, World Health Organisation

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endocrine organ that secretes low-level inflammatory mediators that promote the development of metabolic syndrome and CVDs (Gómez-Abellán et al., 2012; Marcheva et al., 2010; Prasai et al., 2008). CVDs are the world's leading cause of death (WHO, 2015), and CVDs in people with diabetes are two to four times more frequent than in non-diabetics and are more difficult and more progressive. The presence of other risk factors, such as hyperlipidemia, hypertension, smoking, and obesity, further complicates the severity of CVDs. T2DM and CVDs are heterogeneous disorders, with environmental and genetic factors being involved in their pathogenesis (Tibaut and Petrovič, 2016). Studies have shown that polymorphisms of the *CLOCK* gene are associated with body weight and the risk for metabolic syndrome (Dierickx et al., 2018; Gómez-Abellán et al., 2012; Huang et al., 2011; Klerman, 2005; Takahashi et al., 2008). One of the most common CVDs is the myocardial infarction (MI). The incidence of MI in developed countries has decreased in recent years. The MI is defined as myocardial cells death due to prolonged ischemia (Tibaut et al., 2016).

Circadian clock network consists of molecular components where *ARNTL* (also referred to as *BMAL1*), *CLOCK, CRYs* and *PERs* 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 *CRYs, PERs*, and other clock-related genes. Heterodimers of the CRY/PER proteins assemble the negative feedback loop and inhibit the transcriptional activity of the *ARNTL* and *CLOCK* genes (Takeda and Maemura, 2010, 2016).

This study aimed to explore a possible association of the genetic variability of the *ARNTL*, *CLOCK,* and *PER2* genes with myocardial infarction in patients with type 2 diabetes mellitus. We implemented a case-control study on a population of patients with myocardial infarction and type 2 diabetes mellitus in comparison with a control population solely with type 2 diabetes mellitus.

2. Materials and methods

2.1. Participants

Participants with type 2 diabetes mellitus, patients from the University Medical Centre Ljubljana, Slovenia and the Institute of Histology and Embryology, Faculty of Medicine, University Ljubljana were included in the study.

They were divided into two groups. The first group consisted of patients with a history of myocardial infarction and type 2 diabetes mellitus (cases, N = 231). The second group consisted of patients solely with type 2 diabetes mellitus (controls, N = 426) and no history of angina pectoris. The Slovenian National Medical Ethics Committee approved the research. All subjects were Slovenian and were not related. Patients were classified as having type 2 diabetes according to the current report of the American Diabetes Association (American Diabetes Association, 2012). The study was conducted according to the Declaration of Helsinki and its amendments. Written informed consent was obtained from all participants in the study.

2.2. Biochemical analyses

Fasting glucose, glycated hemoglobin (HbA1c) were determined by standard biochemical methods. Total cholesterol, HDL cholesterol, and triglycerides were measured by an enzymatic colorimetric method on an AU680 Chemistry System analyzer (Beckman Coulter, Brea, USA) using reagents from the same manufacturer. The plasma level of the LDL cholesterol was calculated from measured values of total cholesterol, triglycerides, and HDL cholesterol.

2.3. Genotyping

In this study, we genotyped eight genetic variants in three circadian rhythm regulating genes, *ARNTL*, *CLOCK*, and *PER2*. SNPs were chosen on the known genetic linkage according to HapMap Phase 3 (http://www.hapmap.org). The set of most representative SNPs for *ARNTL*, *CLOCK*, and *PER2* gene regions was obtained using the Tagger algorithm available through the Haploview software (Haploview, version 4.2) (de Bakker et al., 2005).

Genomic DNA was extracted from the peripheral blood lymphocyte using standard procedures (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany). Genotyping 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 allelic discrimination analysis was performed using SDS 7500 Software Version 2.3 (Applied Biosystems, Foster City, CA, USA).

2.4. Statistical analysis

Chi-square tests (χ^2) on contingency tables were used to compare allelic and genotype frequencies in both groups. Analyses were performed using SNPStats web tool (Solé et al., 2006). An additional level of genotyping quality control was achieved using Chi-Square goodness-of-fit test, by comparing our genotype distribution with those predicted by Hardy-Weinberg equilibrium. Logistic regression, performed with Statistical Package for Social Sciences (SPSS) Version 20, was used to estimate the effect of *ARNTL* (rs3789327, rs4757144, rs12363415), *CLOCK* (rs11932595, rs6811520, rs13124436) and *PER2* (rs35333999, rs934945) genotypes on the odds of a patient having the phenotype for myocardial infarction. Age, history of hypertension, diastolic blood pressure, HbA1c, patients on statin therapy, total, LDL and HDL cholesterol were used as a covariate. The association between the genotypes and risk factors was tested using Kruskal-Wallis test. Associations were considered significant when they reached the p value of equal to or < 0.05. Appropriate corrections of significance values were also applied using the Benjamini-Hochberg correction method (false-discovery rate – FDR values) because of multiple SNPs were investigated. The q values of < 0.05 were considered to be significant.

3. Results

In the study population of 657 participants, clinical and laboratory characteristics of cases and controls are presented in Table 1.

Table 1
Clinical and laboratory characteristics of cases and controls.

Characteristics	Cases	Controls	p value
Number	231	426	
Age (years)	66.5 ± 9.0	62.0 ± 8.5	< **0.0001**
Male sex (%)	129 (55.8)	217 (50.9)	0.26
Duration of diabetes (years)	15.02 ± 8.5	14.16 ± 7.63	0.27
Patients on insulin therapy (%)	137 (59.3)	234 (54.9)	0.71
Systolic blood pressure (mm Hg)	150 ± 21	149 ± 20	0.60
Diastolic blood pressure (mm Hg)	81 ± 12	85 ± 12	**0.001**
BMI (kg/m^2)	30.67 ± 4.0	30.39 ± 4.35	0.48
History of hypertension (%)	208 (90.0)	349 (81.9)	**0.005**
Smokers (%)	20 (8.6)	42 (9.8)	1.00
Total cholesterol (mmol/l)	4.55 ± 1.15	4.8 ± 1.12	**0.011**
LDL cholesterol (mmol/l)	2.53 ± 0.9	2.77 ± 0.92	**0.002**
HDL cholesterol (mmol/l)	1.15 ± 0.29	1.28 ± 0.36	< **0.0001**
Triglycerides (mmol/l)	2.16 ± 1.66	1.89 ± 1.42	0.057
S-fasting glucose [mmol/l]	8.77 ± 2.96	8.53 ± 2.63	0.30
HbA1c [%] [a]	**7.98 ± 1.38**	**7.75 ± 1.17**	**0.01**
Patients on statin therapy (%)	**157 (68.0)**	**210 (49.3)**	< **0.0001**
Patients on insulin therapy, n (%)	140 (60.6)	236 (55.4)	0.42

Significant p values obtained by Mann-Whitney U test are bolded.
[a] HbA1c – glycated hemoglobin.

Table 2
Allele and genotype distribution and frequencies of the *ARNTL*, *CLOCK*, and *PER2* polymorphisms (N = 657).

Gene	SNP	Minor allele	MAF[a] cases	MAF[a] controls	p value	q value	Genotype	Genotype frequency, N (%)				
								Cases	Controls	p Value	X^2	q Value
ARNTL	rs3789327[b]	C	0.43	0.56	2.25×10^{-5}	4.49×10^{-5}	CC	62 (0.27)	145 (0.34)	6.58×10^{-6}	23.86	1.75×10^{-5}
							CT	74 (0.32)	183 (0.43)			
							TT	95 (0.41)	98 (0.23)			
	rs4757144	G	0.46	0.46	0.975	0.974	AA	74 (0.32)	128 (0.30)	0.599	1.02	0.702
							AG	104 (0.45)	196 (0.46)			
							GG	53 (0.23)	98 (0.23)			
	rs12363415	G	0.04	0.11	$\mathbf{1.1 \times 10^{-5}}$	$\mathbf{3.29 \times 10^{-5}}$	AA	215 (0.93)	346 (0.81)	$\mathbf{1.00 \times 10^{-4}}$	17.72	$\mathbf{2.84 \times 10^{-4}}$
							AG	16 (0.07)	72 (0.17)			
							GG	0	8 (0.02)			
CLOCK	rs11932595	G	0.46	0.46	0.883	0.883	AA	53 (0.23)	85 (0.20)	0.614	0.97	0.702
							AG	109 (0.47)	217 (0.51)			
							GG	69 (0.30)	124 (0.29)			
	rs6811520[b]	C	0.31	0.67	2.35×10^{-35}	7.06×10^{-35}	CC	136 (0.59)	47 (0.11)	5.41×10^{-38}	171.6	4.33×10^{-37}
							CT	42 (0.18)	187 (0.44)			
							TT	53 (0.23)	192 (0.45)			
	rs13124436[b]	A	0.10	0.30	8.49×10^{-16}	1.7×10^{-15}	AA	9 (0.04)	51 (0.12)	1.47×10^{-9}	40.68	5.88×10^{-9}
							AG	18 (0.08)	107 (0.25)			
							GG	204 (0.88)	268 (0.63)			
PER2	rs35333999	T	0.03	0.04	0.328	0.656	CC	217 (0.94)	392 (0.92)	0.485	1.44	0.702
							CT	14 (0.06)	30 (0.07)			
							TT	0	4 (0.01)			
	rs934945	T	0.17	0.17	0.927	0.927	CC	157 (0.68)	294 (0.69)	0.98	0.04	0.98
							CT	67 (0.29)	124 (0.29)			
							TT	7 (0.03)	8 (0.02)			

q value – corrected significant p value by Benjamini-Hochberg method.
Significant p values obtained by Chi-square test are bolded.
[a] MAF – minor allele frequency.
[b] Departure from the Hardy-Weinberg equilibrium in the group with myocardial infarction plus type 2 diabetes mellitus.

Allele frequencies and the genotype distribution of the *ARNTL*, *CLOCK*, and *PER2* polymorphisms are presented in Table 2. *ARNTL* rs3789327 and rs12363415, and *CLOCK* rs6811520 and rs13124436 SNPs were associated with MI. SNPs rs3789327 of the *ARNTL* gene and rs6811520 and rs13124436 of the *CLOCK* gene departed from the Hardy-Weinberg equilibrium in the group with myocardial infarction and type 2 diabetes mellitus ($p > .05$).

A logistic regression model was fitted to estimate the independent effect of the selected polymorphism after adjustment for cardiovascular risk factors. *ARNTL* SNP rs12363415 was associated with an increased risk of MI in our study. In the dominant model GG + AG versus AA, the p-value was $p < .01$ (OR = 7.37; 95% CI: 4.15–13.08). A model showed a borderline significant interaction between the risk of myocardial infarction and age ($p = .05$) (Table 3). Significant difference was found under the recessive genotype model for the rs3789327 SNP in the *ARNTL* gene (GG versus AG + AA, $p = .0003$, OR = 0.53 with 95% CI 0.38–0.75). Under dominant genotype model significant difference was for the rs6811520 and rs13124436 SNPs in the *CLOCK* gene (CT + TT versus CC, $p < .0001$, OR = 0.44 with 95% CI 0.32–0.61, and AG + AA versus GG, $p < .0001$, OR = 3.02 with 95% CI 2.10–4.33, respectively).

Table 3
Odds ratios for myocardial infarction adjusted for cardiovascular risk factors included in the logistic regression model.

Risk factor	OR (95% CI)	p Value
age	1.06 (0.99–1.11)	0.050
Diastolic blood pressure	0.98 (0.94–1.02)	0.400
History of hypertension	1.56 (0.62–2.62)	0.100
Total cholesterol	1.40 (0.15–1.85)	0.300
LDL cholesterol	0.53 (0.06–1.53)	0.300
HDL cholesterol	0.30 (0.58–1.32)	0.200
HbA1c	0.88 (0.38–3.05)	0.500
rs12363415 (GG + AG)	2.0 (1.1–3.4)	< **0.001**

Significant p values obtained by the logistic regression analyses are bolded.

Table 4 shows the association between cardiovascular risk factors and tested circadian clock gene SNPs in cases and controls.

4. Discussion

Based on the results of the polymorphism analysis at the *ARNTL*, *CLOCK* and *PER2* genes loci in this study, we showed that variations in the circadian rhythm genes might be an additional risk factor for MI in type 2 diabetics' patients. These data are consistent with data from the literature where it has been shown that metabolic syndrome is a significant risk factor for CVDs and contributes to the emergence of CVDs (Kelly et al., 2012; Prasai et al., 2008; Scott et al., 2008). Combining genetic association studies of circadian clock gene polymorphisms and T2DM and myocardial infarction, as relevant diseases, might help to identify susceptibility markers of MI and physiological pathways from gene to clinical outcomes. In this study, we found evidence of an association between myocardial infarction in patients with type 2 diabetes mellitus and gene variants rs12363415 and rs3789327 of the *ARNTL* gene, and rs6811520 and rs13124436 of the *CLOCK* gene in a sample of 657 participants.

The common pathophysiological processes contribute to the development of diabetes and CVDs (Prasai et al., 2008). Atherosclerotic changes in blood vessels in people with diabetes are more difficult than those in non-diabetics, they are diffuse and present in small blood vessels (Boras and Ljubičić, 2009; Paneni et al., 2013). In addition to microvascular changes, such as nephropathy, retinopathy, and neuropathy, diabetes also leads to macrovascular changes or atherosclerosis. Pathophysiological mechanisms responsible for cardiovascular changes in diabetic patients are multiple and complex. They include insulin resistance, hyperinsulinemia, hyperglycemia, elevated levels of free fatty acids, dyslipidemia and hypertension. Metabolic disorders lead to endothelial dysfunction, vasoconstriction, inflammatory reaction and prothrombotic state, oxidative stress, and eventually atherosclerosis (Dokken, 2008; King and Grant, 2016). Circadian clock genes are involved in regulating vascular endothelial function (Shimizu et al.,

Table 4
The association between cardiovascular risk factors and circadian clock gene SNPs in cases and controls.

	ARNTL						*CLOCK*						*PER2*			
	rs3789327		rs4757144		rs12363415		rs11932595		rs6811520		rs13124436		rs35333999		rs934945	
	Cases	Ctrl	Cases	Ctrl	Cases	Ctrl	Cases	Ctrl	Cases	Ctrl	Cases	Ctrl	Cases	Ctrl	Cases	Ctrl
Age	0.748	0.664	0.437	0.656	0.311	0.761	0.952	0.292	0.186	0.584	0.579	0.218	0.072	0.909	0.717	0.605
Hypertension	0.323	0.483	**0.044**	0.383	**< 0.001**	**< 0.001**	0.244	0.800	**< 0.001**	0.123	**0.013**	**0.011**	0.714	0.418	**0.008**	0.718
Systolic blood pressure	0.309	0.819	0.592	0.191	0.274	0.224	0.210	0.875	0.959	0.999	0.243	0.142	0.865	0.816	0.398	0.474
Diastolic blood pressure	0.340	0.104	0.911	0.061	0.624	0.714	0.810	**0.031**	0.233	0.558	0.910	0.693	0.922	**0.027**	0.589	0.920
BMI	0.192	0.675	0.500	0.169	0.156	0.578	0.952	0.596	0.528	0.290	0.824	0.950	0.709	0.249	0.551	0.142
Total cholesterol	0.204	0.853	0.266	**0.038**	0.052	0.593	0.672	0.478	0.236	0.808	0.112	0.058	0.459	0.184	0.293	0.936
LDL cholesterol	0.740	0.885	0.508	0.273	0.249	0.771	0.427	**0.035**	0.385	0.216	0.760	0.371	0.307	0.101	0.478	0.768
HDL cholesterol	0.115	0.733	0.135	0.086	**0.037**	0.386	0.414	0.347	0.925	0.630	0.978	**0.026**	0.183	0.802	0.381	0.490
Triglycerides	0.306	**0.029**	0.731	0.761	0.154	0.385	0.533	0.827	0.573	0.378	0.055	0.616	0.402	**0.008**	0.365	0.303
Fasting glucose	0.325	0.270	0.320	0.838	0.964	0.258	0.796	0.303	0.101	0.300	0.837	0.853	0.674	0.277	0.382	0.142
HbA1c	0.871	0.313	0.446	0.282	0.773	**0.003**	0.148	0.467	0.864	0.971	0.764	0.117	0.244	0.558	0.459	0.838

In table are presented Kruskal-Wallis test p-value.
Significant p values are bolded.

2016).

In this study, T2DM patients with MI had higher HbA1c levels, whereas diastolic blood pressure, total cholesterol, LDL cholesterol, and HDL cholesterol levels were significantly lower compared to the control group. The levels of HbA1c are an independent factor for microvascular and macrovascular complication in diabetic patients (Poljičanin and Metelko, 2009). Elevated HbA1c levels are associated with increased incidence of cardiovascular disease and mortality (Gosavi et al., 2016; King and Grant, 2016; Lazzeri et al., 2014), what is the case here. The patients with diabetes and MI had a higher fasting glucose level than the controls. Fasting glucose is a simple tool for predicting mortality in nondiabetic patients with MI but is not a valuable prognostic marker in patients with diabetes (Aronson et al., 2007). Elevated blood glucose levels in MI patients without T2DM are considered to be associated with a less favorable prognosis than in diabetic patients (Karetnikova et al., 2016). In our study, the majority of patients in the cases group had been on statin therapy, which was resulting in significantly lower LDL cholesterol levels. Statins are an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase and have anti-inflammatory and antithrombotic properties and antioxidant effects. From the literature, it is seen that statins have a little protective effect for MI, with a 27% lower risk in MI. Studies suggested that a reduction of MI injury after statin treatment is associated with an attenuated inflammatory response (Han et al., 2018).

Expression of *BMAL1*, *CLOCK*, *CRY1* and *PER2* genes in the fatty tissue is associated with metabolic syndrome in humans (Gómez-Abellán et al., 2008; Green et al., 2008; Scott, 2015). *BMAL1* plays an essential role in adipose tissue development and contributes to the lipogenesis and adipocyte differentiation (Eckel-Mahan and Sassone-Corsi, 2013). Genetic variations of the *BMAL1* 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 (Woon et al., 2007). In the present study *ARNTL* rs4757144 was associated with hypertension in cases. Polymorphisms of *CLOCK* gene are associated with metabolic syndrome in humans, and the inheritance of metabolic syndrome is estimated to be around 40% in the European population (Scott et al., 2008). Polymorphisms of the *CLOCK* gene are associated with the type 2 diabetes and cardiovascular disease in humans (Corella et al., 2016; Scott, 2015; Scott et al., 2008). Corella et al. showed that *CLOCK* gene SNP (rs4580704) associated with the development of the CVDs in type 2 diabetic patients, and the same variant associated with stroke. Although this SNP is in the intron region, it is associated with the other SNP in the 3'UTR region that is modulated through microRNA molecules (Corella et al., 2016). We found the association of rs6811520 and rs13124436 of the *CLOCK* gene with a history of hypertension in patients with MI and T2DM.

Genetic variations in *PER2* might be associated with MI (Škrlec et al., 2018). *PER2* polymorphism rs934945 is associated with hypertension in MI patients (Škrlec et al., 2019) just as at patients with MI and T2DM in this research. The cardiac *PER2* has a vital role in fatty acid metabolism, inflammation during myocardial ischemia and reperfusion. It has been shown that PER2 protein has a cardioprotective role during myocardial ischemia in mice (Bonney et al., 2013a, 2013b).

Disturbances in the circadian rhythm gene affect the rhythm itself and are the possible cause of the increased incidence of diabetes worldwide (Kurose et al., 2011). Genetic variation in *CLOCK* and *BAML1* genes might play a role in the development of obesity and type 2 diabetes mellitus in humans (Kumar Jha et al., 2015; Scott et al., 2008; Sookoian et al., 2008; Woon et al., 2007). The allele frequency of the circadian rhythm genes varies among different populations (Allebrandt and Roenneberg, 2008), and it is essential to be aware of the ethnic composition of the studied population. Biological and epidemiological studies suggest a direct link between lifestyle and metabolic disorders (Dibner and Schibler, 2015), but the genetic and biochemical linkage of human circadian rhythm with metabolic disorders has not been fully explored. The importance of circadian rhythms in maintaining "energy" homeostasis and metabolism are apparent.

Studies have shown that the risk of developing CVDs in diabetic patients is two-three times higher than in healthy subjects (Martín-Timón et al., 2014). People with diabetes usually have a higher heart rate in sleep and lower heart rate variability over the day than non-diabetics, which causes unnecessary oxygen consumption in the myocardium with reduced blood supply (Boras and Ljubičić, 2009; Paneni et al., 2013). It was noted that hypertensive patients with type 2 diabetes have a two-fold increase in blood pressure than hypertensive patients without diabetes (Gamaldo et al., 2014).

Within the regulation of diabetes in humans, it is crucial to control circadian glucose metabolism, and changes in the standard cyclic pattern of glucose tolerance are characteristic for type 2 diabetes (Green et al., 2008). Circadian rhythm disorder is an environmental trigger linked to the development of T2DM. Apart from environmental changes in circadian rhythm, genetic mutations in the central clock gene, such as *CLOCK* and *BMAL1*, can also promote the development of diabetes (Gale et al., 2011). Study on mice showed that loss of *BMAL1* in the whole body increases insulin sensitivity (Bass, 2012). Genetic variability of *ARNTL* gene (Leea et al., 2018) and two *BMAL1* haplotypes are linked with T2DM in humans (Gómez-Abellán et al., 2008; Maury et al., 2010; Woon et al., 2007). Circadian rhythm disorders are associated with cardiovascular and metabolic changes in the human population (Kurose et al., 2011).

This study is a novel investigation of the association between different SNPs of the circadian rhythm genes in patients with T2DM and

myocardial infarction. The study compared the frequency of polymorphisms and genotype models between patients with MI and T2DM, and T2DM patients. A large number of genes with modest influence contribute to a manifestation because T2DM and myocardial infarction are complex traits.

The limitation of this study is the sample size. With a higher number of participants, a better connection between genotypes and phenotypes could be achieved. Estimated power of 80%, with an odds ratio of 1.5, at a significance level of 0.05 for HapMap-predicted minor allele frequency of 0.06 (rs35333999) to 0.41 (rs11932595) of a potential susceptibility marker and a prevalence of the type 2 diabetes mellitus of 8% (Cokolic et al., 2017) yield the sample size of 600 for cases and 1100 for controls under the dominant model. Another limitation is the unavailability of the insulin levels in participants because circadian clock genes could affect insulin secretion. The age difference between patients and controls could be considered as a limitation of the study, although there was not any significant association of age and genotypes in cases and controls. Also, it is possible that the polymorphisms of the *ARNTL*, *CLOCK*, and *PER2* genes are not functionally related to myocardial infarction or T2DM, and in this case, functional SNPs or a group of functionally significant polymorphisms that might be more strongly associated with T2DM or MI should be found. This approach to the problem of MI creates an opportunity to continue the research in multicenter studies with a large number of respondents.

The results of this research enhance our understanding of the pathophysiological processes involved in the development of the MI and T2DM and require further investigation. Circadian rhythm gene polymorphisms may be useful in predicting the risk, prognosis, or prediction of the response of patients with CVD and T2DM. In today's personalized medicine, knowledge of the circadian rhythm of an individual can be significant in managing patients with increased CVD and T2DM risk and can be included as an important constituent of the diagnostic process.

Conflicts of interest

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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