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The Association of Cardiovascular Disease with the T3111C Polymorphism in the *CLOCK* Gene†

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Abstract: Cardiovascular diseases (CVDs) are among the leading causes of death worldwide, although CVDs mortality has decreased in developed countries. Numerous pathophysiological processes lead to the development of CVDs. The circadian rhythm coordinates many physiological processes, and its disruption can lead to many pathophysiological changes. One of the significant circadian rhythm genes is the CLOCK gene, whose polymorphisms are associated with CVD risk factors. Research findings of the association between CLOCK gene polymorphism and CVDs and its comorbidities are not consistent. This meta-analysis was conducted to quantify the associations between T3111C polymorphism and the risk of CVDs. The PubMed and Scopus databases were searched for studies reporting on the association between T3111C (rs1801260) in the circadian CLOCK gene and cardiovascular disease and its comorbidities such as obesity, hypertension, insulin resistance, and coronary artery disease. A fixed-effect model was used to calculate the pooled odds ratio and 95% confidence interval by comprehensive meta-analysis software. Five independent studies, including case-control, cross-sectional, and cohort research methods, were analyzed with 3,123 subjects in total. The meta-analysis revealed a significant association between T3111C polymorphism and cardiovascular disease (OR = 1.32, 95% CI: 1.16–1.50, $p < 0.001$) with significant heterogeneity ($I^2 = 91.1\%$, $p < 0.001$) and no publication bias. The subgroup analysis on comorbidity related to CVDs revealed that hypertension was associated with T3111C polymorphism (OR = 2.02, 95% CI: 1.60–2.54, $p < 0.001$). Our meta-analysis based on available studies using a fixed model shows that T3111C polymorphism in the CLOCK gene is associated with CVDs susceptibility. This research was funded by a grant from the Croatian Ministry of Science and Education and dedicated to multi-year institutional financing of scientific activity at the Josip Juraj Strossmayer University of Osijek, Osijek, Croatia grant number IP8-FDMZ-2020.

Keywords: *CLOCK* gene; cardiovascular disease; hypertension; obesity; T3111C polymorphism

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1. Introduction

Cardiovascular diseases (CVDs) are amongst the main reasons for death globally, although CVDs mortality has decreased in developed countries [1]. Numerous pathophysiological processes lead to the development of CVDs. The circadian clock coordinates numerous physiological processes, and its interruption can lead to numerous pathophysiological changes [2].

One of the significant circadian rhythm genes is the *CLOCK* gene. CLOCK protein (Circadian Locomotor Output Cycles Kaput) and ARNTL protein (Aryl hydrocarbon Receptor Nuclear Translocator–Like) stimulate the transcription of other circadian rhythm genes. CRY (Cryptochrome) and PER (Period) proteins inhibit the transcription of the *CLOCK* and *ARNTL* genes [3,4]. The alternation of light and darkness stimulates such

feedback loop of the circadian rhythm gene, and these oscillations of activation and inhibition of circadian rhythm gene expression occur within 24 hours. The circadian feedback loop affects the expression of circadian clock genes. Many physiological changes are influenced by these genes and under the control of the central clock located in the suprachiasmatic nucleus (SCN) [2,5]. Many *CLOCK* gene polymorphisms are associated with CVD risk factors [6,7]. Research findings of the association between *CLOCK* gene polymorphism and CVDs and its comorbidities are not consistent [8–11]. The rs1801260 or T3111C polymorphism is an SNP in the 3′ untranslated region of the *CLOCK* gene.

This meta-analysis was conducted to quantify the associations between T3111C polymorphism and the risk of CVDs.

2. Methods

The Scopus and PubMed databases were searched for studies reporting on the association between T3111C (rs1801260) polymorphism in the circadian *CLOCK* gene and cardiovascular disease and its comorbidities such as obesity, hypertension, insulin resistance, and coronary artery disease until 20th February 2021 (Figure 1).

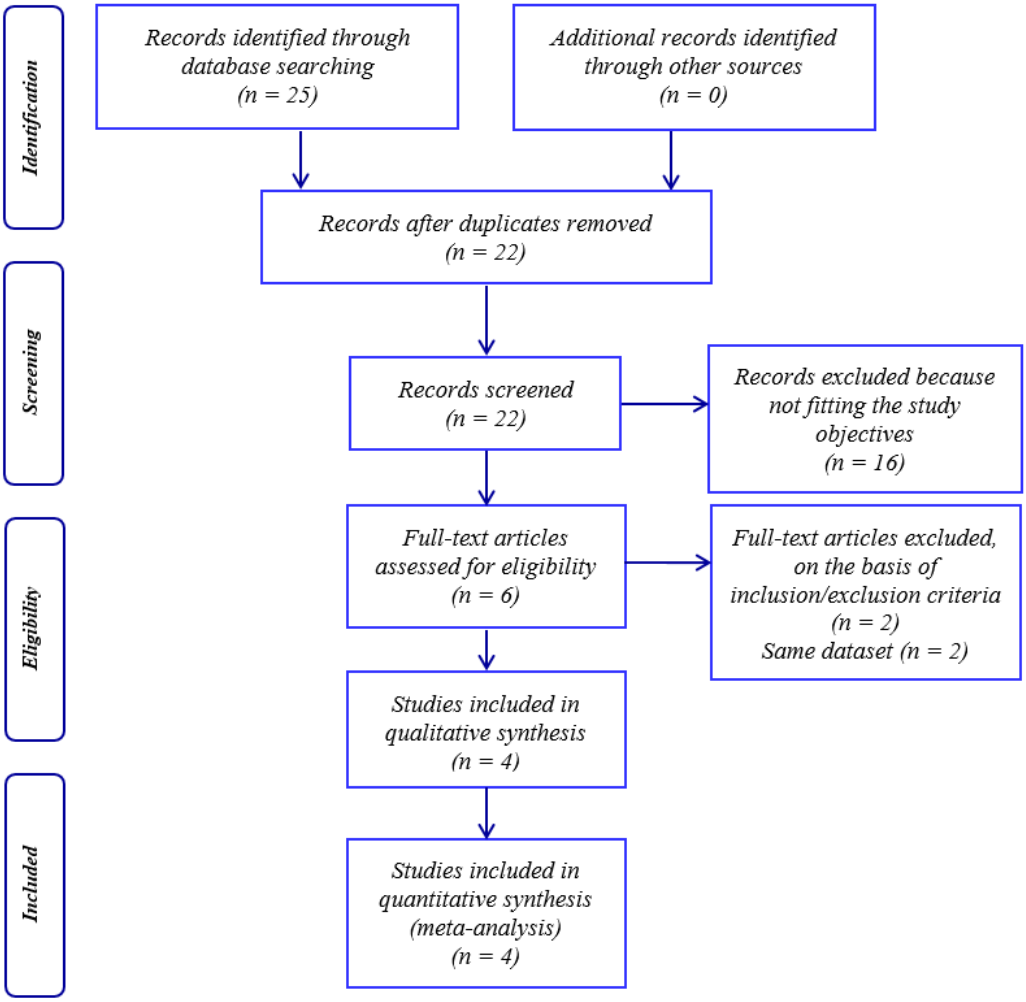

Figure 1. PRISMA flow diagram showing the exclusion and inclusion criteria and the number of studies eliminated and incorporated at each step of the database search.

2.1. Study Selection

The aim was to identify studies on the association between *CLOCK* gene polymorphism C3111T and CVDs using the inclusion criteria of cross-sectional, case-control, co-

hort studies, and only articles in the English language were taken into consideration. Additional inclusion criteria were allele and genotype frequencies, absence of the specific disorder in the control group, no departure from Hardy-Weinberg equilibrium in the control group. Studies were excluded if there was no control study group, lacking data on allele frequencies, a case report, a review, a meta-analysis, and studies by the same authors whose data overlap.

2.2. Data Extraction

For each study incorporated in the meta-analysis, the later data were extracted: authors, year of publication, studied population, study design, number of patients and control participants (female, male), selection criteria for patients and controls, the average age of the participants, prevalent ethnicity, type of CVD or CVD risk factor, and genotyping method (Table 1). In addition, allelic frequency in cases and controls, including odds ratios (OR) and *p* values.

Table 1. Characteristics of studies included in the meta-analysis.

First author	**Monteleone et al.**	**Galbete et al.**	**Kolomeichuk et al.**	**Kolomeichuk et al.**	**Li et al.**
Year	2008 [12]	2012 [13]	2014a [14]	2014b [14]	2020 [15]
Country	Italy	Spain	Russia	Russia	China
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Asian
Disorder	obesity	obesity	hypertension	coronary artery disease	hypertension / insulin resistance
Population type	general	general	hospital	hospital	hospital
Case	192	532	434	299	103
Control	92	371	435	434	231
Male (%)	14.79%	72.76%	48.33%	49.11%	57.78%
Age (year ± SD) cases	38.4 ± 10.9	69 ± 5	51.9 ± 6.9	52.3 ± 7.1	54 ± 13.81
Age (year ± SD) control	26.1 ± 4.6	69 ± 5	50.8 ± 8.1	50.8 ± 8.1	53.1 ± 11.27
Genotyping method	RFLP-PCR	RT-PCR	RFLP-PCR	RFLP-PCR	PCR sequencing
NOS score *	8	7	8	8	8

* NOS score – Newcastle-Ottava quality assessment scale.

2.3. Statistical Analyses

The Comprehensive Meta-analysis software version 3.3.070 (Biostat) was applied for the performed meta-analyses. If the p values were less than 0.05, associations were confirmed. Cochran's Q test assessed the statistical heterogeneity among the studies. The fixed-effects model was applied to compute the pooled odds ratio and 95% confidence interval. Publication bias was evaluated utilizing the funnel plot and Egger test for associations with p values less than 0.05.

3. Results and Discussion

The PRISMA flow diagram of the study collection is presented in Figure 1. According to our exclusion and inclusion criteria, five independent studies (one study included two different patient data sets) were incorporated in the present meta-analysis [12–15]. Those studies included case-control, cross-sectional, and cohort research studies, analyzing 3,123 subjects in total.

The funnel plot of odds ratio (OR) versus standard error was symmetrical, suggesting no publication bias (Figure 2). Egger's test identifies no significant publication bias ($p = 0.17$).

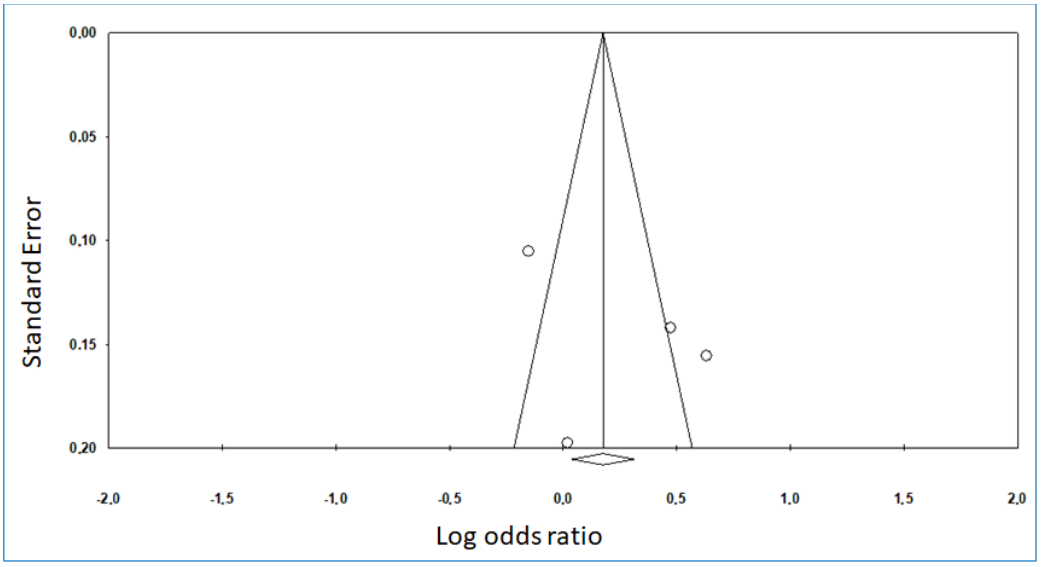

Figure 2. Funnel plot showing log odds ratio versus standard error. There is no indication of publication bias ($p = 0.17$).

Table 1 presents the properties of the qualified studies. The genotype and allele frequencies are presented in Table 2.

Table 2. The genotypes and allele distribution of the *CLOCK* T3111C polymorphism in each incorporated study in the present meta-analysis.

First author	Cases					Controls				
	Genotype frequencies, N (%)			Allele frequencies, N (%)		Genotype frequencies, N (%)			Allele frequencies, N (%)	
Monteleone et al.	103 (53.6)	68 (35.4)	21 (10.9)	272 (70.8)	112 (29.1)	46 (50)	39 (42.4)	7 (7.6)	131 (71.2)	53 (28.8)
Galbete et al.	278 (52.2)	217 (40.8)	37 (7)	789 (72.7)	297 (27.3)	181 (48.8)	151 (41.5)	36 (9.7)	516 (69.5)	226 (30.5)
Kolomeichuk et al. a	143 (33)	213 (49)	78 (18)	252 (58.1)	182 (41.9)	209 (48)	187 (43)	39 (9)	300 (69)	135 (31)
Kolomeichuk et al. b	81 (27)	161 (54)	57 (19)	162 (53.8)	137 (46.2)	209 (48)	187 (43)	39 (9)	300 (69)	135 (31)
Li et al.	51 (49.5)	44 (42.7)	8 (7.8)	146 (70.9)	60 (29.1)	186 (80.5)	40 (17.3)	5 (2.2)	412 (89.2)	50 (10.8)

Table 3 reviews the principal outcomes of the present meta-analysis. A statistically significant association between *CLOCK* T3111C polymorphism and CVD risk was found in the overall population (T versus C: OR = 0.73, 95% CI = 0.62–0.84, $p < 0.001$). The same trend towards CVD risk was seen in three other studies with OR = 1.32, 95% CI: 1.16–1.50, $p < 0.001$ (Figure 3). The study Monteleone et al. [12] was an exception, probably due to a low number of participants involved in the study. An exception was the study of Galbete et al. [13], apparently because of the higher average age of the involved participants.

Table 3. Meta-analysis results of the *CLOCK* T3111C polymorphism and CVDs risk.

Comparison		Test of association		Test of heterogeneity	
		OR (95% CI)	*p*	I^2	*p*
Allelic model	T vs C	0.73 (0.62–0.84)	<0.001	97.93	<0.001
Dominant model	TT+CT vs CC	0.59 (0.46–0.71)	<0.001	79.59	0.001
Recessive model	TT vs CT+CC	0.63 (0.54–0.73)	<0.001	91.64	<0.001

Although many studies showed inconsistency in the association of T3111C polymorphism and CVDs, the present meta-analysis revealed a notable association. An association between CVD and CLOCK T3111C was observed in the overall population under recessive and dominant genetic models and the allelic model. The meta-analysis results showed that the T allele or TT genotype carriers are at a higher risk of developing CVD.

No departure from the Hardy-Weinberg equilibrium was seen in any of the incorporated studies. A significant association between CVD risk factor, hypertension, and the *CLOCK* T3111C polymorphism was initially published in the research by Kolomeichul et al. [16]. The patients with C allele have a leaning towards insulin resistance and CVD with significant association ($p < 0.001$), according to the study by Li et al. [15]. In contrast, Monteleone et al. [12] showed that the *CLOCK* genotypes were not associated with obesity in humans. Although, they confirmed a significant association of the *CLOCK* genotype between obese (BMI > 40 kg/m^2) and overweight participants (BMI ≤ 40 kg/m^2). A possible explanation for these contradictory findings may be different inclusion criteria such as inadequate sample size (particularly in the study of Monteleone et al. [12]) and the appearance of confounding risk factors, such as eating disorders or the elderly population.

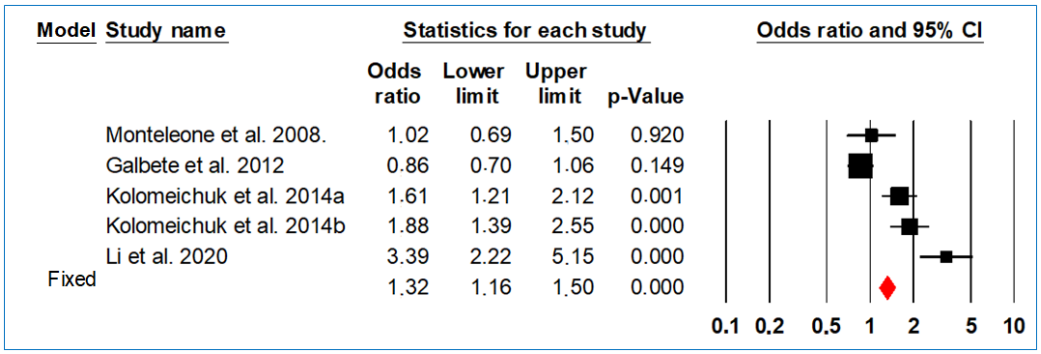

Figure 3. Forest plot for the association between T3111C polymorphism and cardiovascular diseases.

The subgroup analysis on comorbidity related to CVDs revealed that hypertension was associated with T3111C polymorphism (OR = 2.02, 95% CI: 1.60–2.54, $p < 0.001$, Figure 4A), while there was any association of T3111C with obesity as a risk factor for CVDs (Figure 4B).

Our study has some limitations. We limited the database search on manuscripts written in the English language, and accordingly, we might have omitted some publications. The number of the included study and subjects was relatively small.

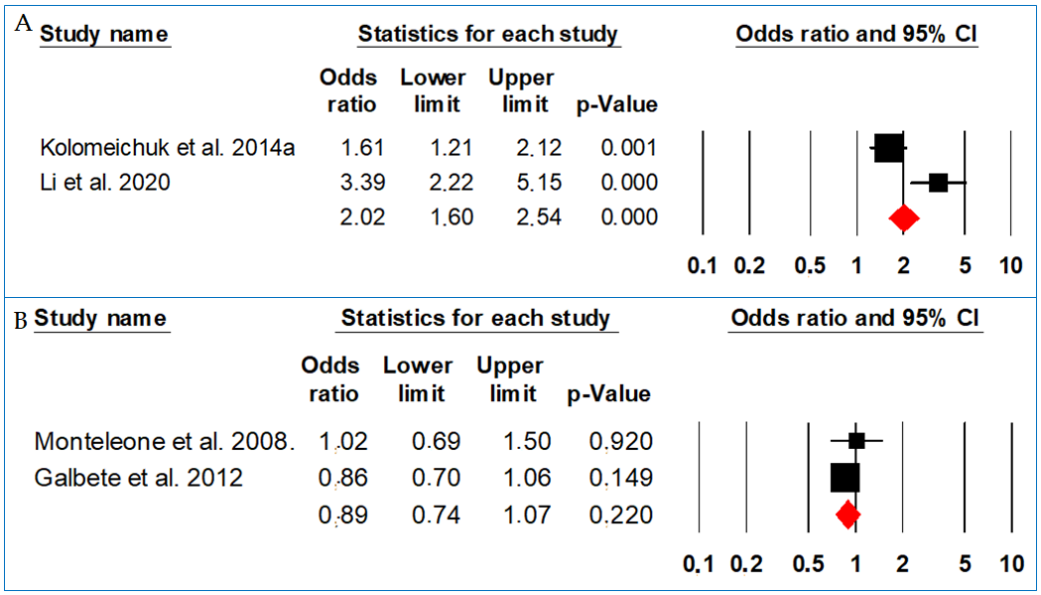

Figure 4. Forest plots for the association between T3111C polymorphism and (**A**) hypertension risk and (**B**) obesity risk.

3. Conclusion

Our meta-analysis based on available studies using a fixed model shows that T3111C polymorphism in the *CLOCK* gene is associated with CVDs susceptibility. Further studies are warranted to elucidate the mechanistic link between T3111C polymorphism and cardiovascular diseases.

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