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Proceeding Paper

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

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Abstract: *Background and Objectives*: Cardiovascular diseases (CVDs) are among the leading causes of death worldwide, although CVD 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. *Materials and Methods*: The PubMed and Scopus databases were searched for studies reporting onthe 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. *Results:* Five independent studies, including case-control, cross-sectional, and cohort research methods, were analyzed with 3123 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, $p < 0.001$). *Conclusions*: Our meta-analysis based on available studies using a fixed model shows that T3111C polymorphism in the CLOCK gene is associated with CVD susceptibility.

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 CVD 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 a feedback loop of the circadian rhythm gene. These oscillations of activation and inhibition

of circadian rhythm gene expression occur within 24 h. 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]. However, 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 a 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 20 February 2021 (Figure 1).

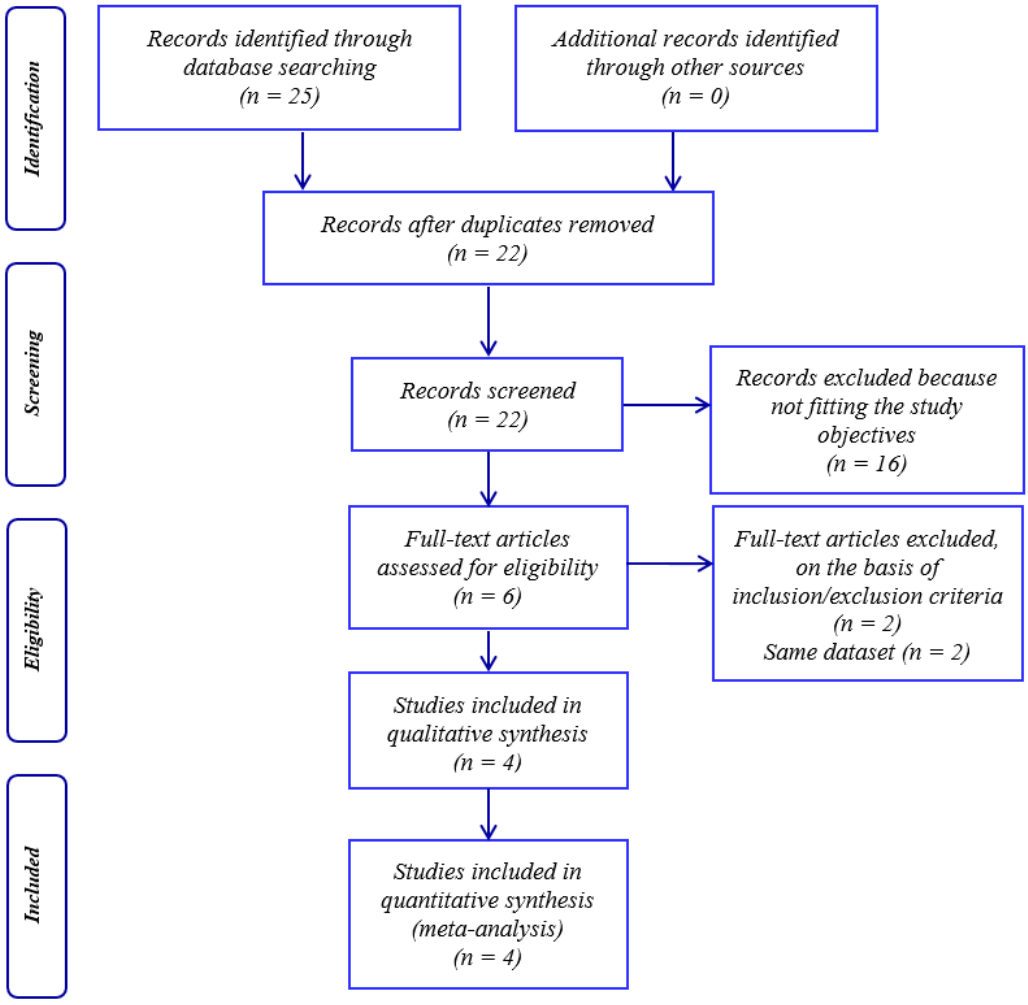

Figure 1. PRISMA flow diagram showing the exclusion and inclusion criteria and the number of studies eliminated and incorporated at each step of the database search.

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, cohort 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, and no departure from the 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 following 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
Study type	Case-control	Cross-sectional	Cross-sectional	Cross-sectional	Cross-sectional
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]. These studies included case-control, cross-sectional, and cohort research studies, analyzing 3123 subjects in total.

The evaluation of the individual studies' reliability and validity was assessed with the Newcastle–Ottawa Scale (NOS). The NOS assigns up to nine points for the risk of bias in three domains: selection (four points), comparability (two points), and exposure (three points) for case-control and the adapted Newcastle–Ottawa scale for cross-sectional studies assigns up to a maximum of ten points [16]. Additionally, there are three domains: selection (five points), comparability (two points), and outcome (three points). Therefore, studies with or more than nine points were considered very good, while studies with seven

to eight points were good. The main factors influencing the quality of the studies were the comparability of the study regarding whether the influences of confounding factors were taken into account. Overall, the qualities of the studies were optimal, with seven or eight NOS points.

The funnel plot of odds ratio (OR) versus standard error was symmetrical, suggesting no publication bias (Figure 2). In addition, Egger's and Begg's tests identified no significant publication bias (p = 0.17 and 0.14, respectively). Although the publication bias was not observed, it is possible due to the small number of included studies associated with T3111C polymorphism and the presence of statistical heterogeneity in meta-analysis, which might reflect population differences. Nevertheless, according to the meta-analysis results, the C allele or CC genotype carriers are at a higher risk of developing CVD.

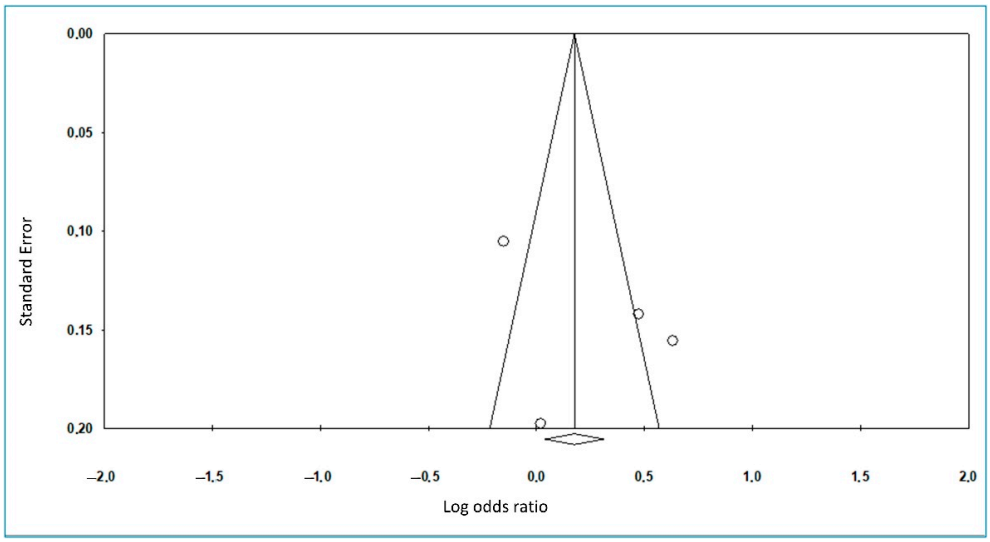

Figure 2. Funnel plot showing the log odds ratio versus standard error. There is no indication of publication bias (p = 0.17).

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* (%)	
	TT	TC	CC	T	C	TT	TC	CC	T	C
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 toward 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 by Monteleone et al. [12] was an exception, probably due to the 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 risk of CVDs.

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

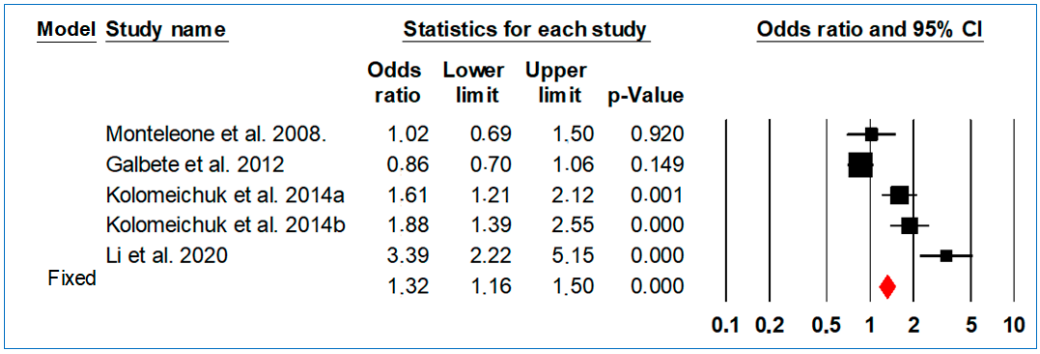

Figure 3. Forest plot for the association between T3111C polymorphism and cardiovascular diseases.

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 T allele or TT genotype carriers were less likely to develop 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. [17]. The patients with C allele have a leaning toward 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. However, they confirmed a significant association of the *CLOCK* genotype between obese (BMI > 40 kg/m^2) and overweight participants (BMI $\leq$ 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.

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 an association of T3111C with obesity as a risk factor for CVDs (Figure 4B).

Our study had some limitations. First, only a limited number of articles for most of the T3111C polymorphism was available. Second, the database search on manuscripts written in the English language, and accordingly, some publications might have been omitted. Third, the original studies utilized different diagnostic criteria, affecting the meta-analysis results and leading to relatively high heterogeneity. Fourth, subgroup analyses in different populations were not performed, although, in most included studies, populations were mainly Caucasian. Fifth, this meta-analysis was based on estimates without adjusting the population characteristics such as age and ethnicity. Despite these limitations, the present study has some advantages. First, the statistical power was increased because a large number of respondents were derived from different studies (a total of 3123 participants). Second, the association of T3111C with different risk factors for CVD was investigated in this meta-analysis.

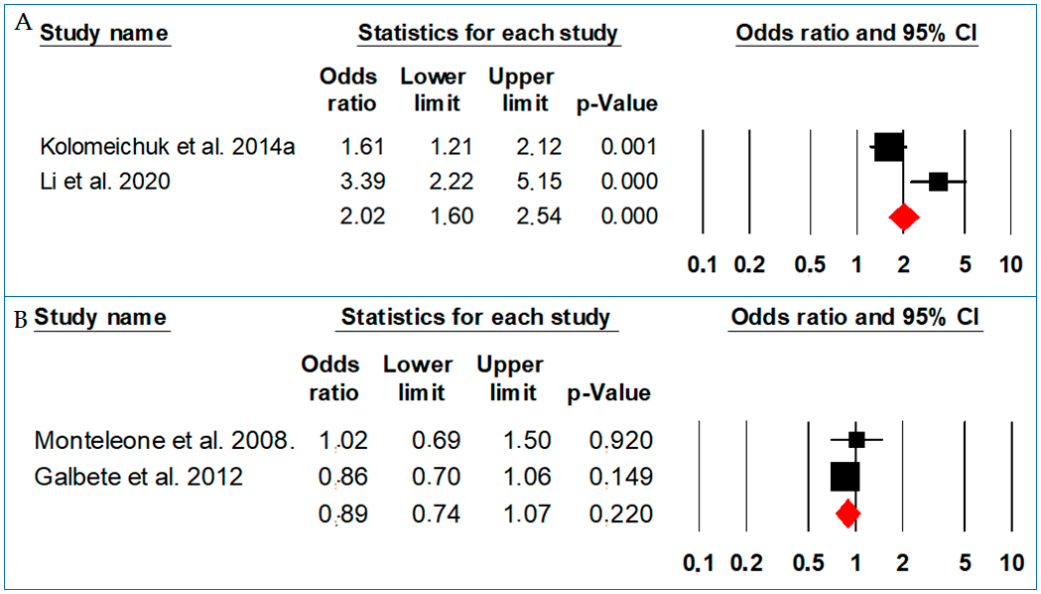

Figure 4. Forest plots for the association between T3111C polymorphism and (**A**) hypertension risk and (**B**) obesity risk.

4. Conclusions

The present meta-analysis based on available studies showed that T3111C polymorphism in the *CLOCK* gene is associated with CVD susceptibility. Furthermore, carriers of the C allele had a higher risk of developing CVD. Further studies are warranted to elucidate the mechanistic link between T3111C polymorphism and cardiovascular diseases.

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References

1. Škrlec, I.; Milić, J.; Heffer, M.; Peterlin, B.; Wagner, J. Genetic variations in circadian rhythm genes and susceptibility for myocardial infarction. *Genet. Mol. Biol.* **2018**, *41*, 403–409. [CrossRef] [PubMed]
2. Thosar, S.S.; Butler, M.P.; Shea, S.A. Role of the circadian system in cardiovascular disease. *J. Clin. Investig.* **2018**, *128*, 2157–2167. [CrossRef] [PubMed]
3. Škrlec, I. Circadian rhythm and myocardial infarction. *Med. Flum.* **2019**, *55*, 32–42. [CrossRef]
4. Crnko, S.; Du Pré, B.C.; Sluijter, J.P.G.G.; Van Laake, L.W. Circadian rhythms and the molecular clock in cardiovascular biology and disease. *Nat. Rev. Cardiol.* **2019**, *16*, 1. [CrossRef] [PubMed]
5. Škrlec, I.; Milić, J.; Heffer, M.; Wagner, J.; Peterlin, B. Circadian clock genes and circadian phenotypes in patients with myocardial infarction. *Adv. Med. Sci.* **2019**, *64*, 224–229. [CrossRef]
6. Scott, E.M.; Carter, A.M.; Grant, P.J. Association between polymorphisms in the Clock gene, obesity and the metabolic syndrome in man. *Int. J. Obes.* **2008**, *32*, 658–662. [CrossRef]

7. Škrlec, I.; Milić, J.; Cilenšek, I.; Petrovič, D.; Wagner, J.; Peterlin, B. Circadian clock genes and myocardial infarction in patients with type 2 diabetes mellitus. *Gene* **2019**, *701*, 98–103. [CrossRef]
8. Gorokhova, S.G.; Generozov, E.V.; Yu, A.O.; Muraseeva, E.V.; Naumov, V.A.; Babikova, E.A.; Zaharczevskaya, N.B.; Prigorovskaya, T.S. Different association of CRY1 and CLOCK circadian genes with coronary atherosclerosis. *J. Clin. Exp. Cardiol.* **2014**, *5*. [CrossRef]
9. Corella, D.; Asensio, E.M.; Coltell, O.; Sorlí, J.V.; Estruch, R.; Martínez-González, M.Á.; Salas-Salvadó, J.; Castañer, O.; Arós, F.; Lapetra, J.; et al. CLOCK gene variation is associated with incidence of type-2 diabetes and cardiovascular diseases in type-2 diabetic subjects: Dietary modulation in the PREDIMED randomized trial. *Cardiovasc. Diabetol.* **2016**, *15*, 4. [CrossRef] [PubMed]
10. Sookoian, S.; Gemma, C.; Gianotti, T.F.; Burgueño, A.; Castaño, G.; Pirola, C.J. Genetic variants of Clock transcription factor are associated with individual susceptibility to obesity. *Am. J. Clin. Nutr.* **2008**, *87*, 1606–1615. [CrossRef]
11. Škrlec, I.; Milić, J.; Steiner, R. The Impact of the Circadian Genes CLOCK and ARNTL on Myocardial Infarction. *J. Clin. Med.* **2020**, *9*, 484. [CrossRef] [PubMed]
12. Monteleone, P.; Tortorella, A.; Docimo, L.; Maldonato, M.N.; Canestrelli, B.; De Luca, L.; Maj, M. Investigation of 3111T/C polymorphism of the CLOCK gene in obese individuals with or without binge eating disorder: Association with higher body mass index. *Neurosci. Lett.* **2008**, *435*, 30–33. [CrossRef] [PubMed]
13. Galbete, C.; Contreras, R.; Martínez, J.A.; Martínez-González, M.Á.; Guillén-Grima, F.; Marti, A. Physical activity and sex modulate obesity risk linked to 3111T/C gene variant of the clock gene in an elderly population: The sun project. *Chronobiol. Int.* **2012**, *29*, 1397–1404. [CrossRef] [PubMed]
14. Kolomeichuk, S.N.; Kurbatova, I.V.; Topchieva, L.V.; Korneva, V.A.; Poltorak, A.N.; Chambers, T.C.; Nemova, N.N. Association between CLOCK genetic variants and individual susceptibility to essential hypertension and coronary artery disease in Russian population. *Exp. Clin. Cardiol.* **2014**, *20*, 1798–1813.
15. Li, G.Y.; Wang, H.; Chen, H. Association of insulin resistance with polymorphic variants of Clock and Bmal1 genes: A case–control study. *Clin. Exp. Hypertens.* **2020**, *42*, 371–375. [CrossRef] [PubMed]
16. Herzog, R.; Álvarez-Pasquin, M.J.; Díaz, C.; Del Barrio, J.L.; Estrada, J.M.; Gil, Á. Are healthcare workers intentions to vaccinate related to their knowledge, beliefs and attitudes? A systematic review. *BMC Public Health* **2013**, *13*, 1–17. [CrossRef] [PubMed]
17. Kolomeichuk, S.N.; Makeeva, I.V.V.; Topchieva, L.V.V.; Korneva, V.A.A.; Nemova, N.N.N.; Kolomeĭchuk, S.N.; Makeeva, I.V.V.; Topchieva, L.V.V.; Korneva, V.A.A.; Nemova, N.N.N. Association of T3111C polymorphism in 3′-untranslated region of the Clock gene with the risk of essential arterial hypertension and coronary artery disease in the Russian population (Residents of Karelia). *Russ. J. Genet.* **2011**, *47*, 1256–1259. [CrossRef]