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GENETICS

MTNR1A and *MTNR1B* Gene Variants of the Melatonin Receptor and Arterial Stiffness in Persons without Arterial Hypertension

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A comparative analysis of vascular stiffness indices and the results of blood test was carried out in 85 healthy donors aged 19-64 years, carriers of polymorphic variants of type 1 and type 2 melatonin receptor genes. The associations of polymorphic markers of type 1 *MTNR1A* (*rs34532313*) and type 2 *MTNR1B* (*rs10830963*) melatonin receptor genes with parameters of vascular stiffness and blood parameters in healthy patients were studied. Genotyping was performed using allele-specific PCR. In all patients, 24-h BP monitoring with assessment of arterial stiffness was performed. Allele *C* homozygotes of *MTNR1A* differed significantly from carriers of the major *T* allele by elevated triglyceride, LDL, and fibrinogen levels. The major allele *C* of the *rs10830963* polymorphic variant of the *MTNR1B* gene is associated with elevated LDL and triglycerides, as well as with individual differences in the elastic properties of the vascular wall in the examined subjects.

Key Words: *arterial stiffness; melatonin; polymorphism; melatonin receptors; 24-h BP monitoring*

Pineal gland hormone melatonin plays a pivotal role in biological systems as a mediator of fluctuations in the intensity of light flux on the physiology and behavior of animals and humans [1]. There are ample data on the effect of melatonin on the cardiovascular system. Melatonin affects the heart through both type 1 (MTNR1A) and type 2 (MTNR1B) receptors, as well as through receptor-independent mechanisms [2,3]. Associations between melatonin patterns and body temperature were experimentally found [4]. A decrease in melatonin levels has been reported in some pathological conditions such as arterial hypertension [5], heart failure [6], coronary heart disease [7], including the condition after acute myocardial infarction [8]. It is known that melatonin plays an important role in various pathological processes in the cardiovascular system due to its anti-inflammatory, antioxidant, and antihypertensive effects [9]. At the same time, the mechanisms of regulation of arterial wall stiffness are insufficiently studied. For effective prevention of cardiovascular and associated diseases of other body systems, it seems relevant to assess the relationship of melatonin receptor genes and vascular stiffness

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indicators in a sample of healthy population living in the North of Russia.

Our aim was to study the association of polymorphic markers of *MTNR1A* (*rs34532313*) and *MTNR1B* (*rs10830963*) melatonin receptor genes with parameters of vascular stiffness and blood parameters in healthy patients.

MATERIALS AND METHODS

The study included 85 subjects (58 men, 27 women) with normal BP aged 19-67 years (mean age 36.4±11.3 years). The study was approved by the Ethics Committee of the Ministry of Health of the Republic of Karelia (Protocol No. 41, September 6, 2018) and was performed in accordance with the ethical principles of the WMA Declaration of Helsinki. Medical histories were analyzed in all participants, load tests, laboratory tests (lipid spectrum, glucose, and fibrinogen) and echocardiography were performed according to indications. The exclusion criteria were abnormalities in the blood test, body mass index $\geqslant$27.5 kg/m^2, impaired glucose tolerance, hypotension or hypertension. The inclusion criteria are the absence of medical contraindications and patient's consent.

The blood samples were collected between 08.00 and 09.00 and 24-h BP monitoring was performed using a BPLab monitor MnSDP-3 with the Vasotens function (Peter Telegin Company) according to the recommendations [7]. BP curves over 24 h were analyzed using BPLabVasotens technology with a special automatic mathematical algorithm. This algorithm allows calculating the parameters of the central pulse wave from the peripheral pulse wave. The details of the module and phase characteristics of the Vasotens transfer function were reported earlier [7]. Quality control included visual evaluation of curves on the Vasotens clinical report screen. On average, 90 (standard deviation=8.1) successful measurements were performed during the monitoring period. DNA was isolated from 100 μl of peripheral blood using the DiaGene kit for genomic DNA isolation (Dia-M) according to the manufacturer's instructions. Genotyping was carried out using polymorphic markers of melatonin receptor genes *MTNR1A* (*rs34532313*) and *MTNR1B* (*rs10830963*) by PCR with allele-specific primers (Syntol). The Q5 programmable thermal cycler (Bio-Rad) was used for amplification.

Statistical data processing was carried out using Statgraphics 2.1 software (Statgraphics Technologies, Inc.); the nonparametric Kruskal–Wallis test and ANOVA were applied. The normality of the data distribution was checked using the Shapiro–Wilk test. The level of statistical significance was set at 5%.

RESULTS

The main biochemical parameters of the examined patients were within the normal range: total cholesterol 5.67±1.10 mmol/liter, HDL 1.37±0.25 mmol/liter, LDL 3.8±1.0 mmol/liter, triglycerides 1.55±0.78 mmol/liter, glucose 4.37±0.60 mmol/liter, and fibrinogen 3.77±0.91 mmol/liter. The mean body mass index was 25.2±1.4 kg/m^2.

Genotyping showed that the frequencies of the *C* and *G* alleles of the polymorphic marker *rs10830963* of the *MTNR1B* gene in the studied sample fit the Hardy–Weinberg law χ^2=1.28 (p=0.82). The frequency of the minor allele *G* was 0.24. The distribution of allele and genotype frequencies of the *rs34532313* polymorphic marker of the *MTNR1A* gene corresponded to the data on the population of the Volga region of Russia [10]. Similar results on the genotype frequencies of the *rs10830963* polymorphic marker were obtained in a study of the *MTNRB1* marker for the Russian population of St. Petersburg [11]. In our sample, the allele frequency of the *rs34532313* marker was distributed as follows: 0.65 for the *C* allele and 0.35 the *T* allele. The distribution of the *rs10830963* marker genotypes also fit the Hardy–Weinberg equilibrium (p=0.85).

Table 1 presents data on laboratory blood parameters depending on the polymorphic markers *MTNR1A* and *MTNR1B*. It was found that homozygotes for the *C* allele of the *rs34532313* marker of the *MTNR1A* gene had significantly (p=0.023) increased levels of fibrinogen in healthy male donors.

Analysis of the dependence of vascular stiffness indices and polymorphic markers *MTNR1A* and *MTNR1B* did not reveal significant differences for alleles of the *rs10830963* marker (Hbc/1A). Allele *C* of the polymorphic marker *rs34532313* of the *MTNR1A* gene was significantly associated with the minimum augmentation index in healthy patients (Fig. 1). No significant differences in the parameters of arterial stiffness (pulse wave propagation velocity, and morning BP rise) were found.

Homozygotes for the *C* allele of the *MTNR1A* gene significantly differed from the carriers of the major *T* allele by increased levels of triglycerides, LDL, and fibrinogen (Table 1).

Melatonin is a multifunctional indoleamine that shows significant positive effects on cellular toxicity caused by oxidative stress. The effect of melatonin on the cardiovascular system has been proven, which is associated with its powerful antioxidant and anti-inflammatory properties [3]. The antioxidant effect of melatonin on LDL oxidation has been studied. Melatonin has also been shown to reduce plasma levels of total and VLDL-cholesterol, as well as LDL-cholesterol

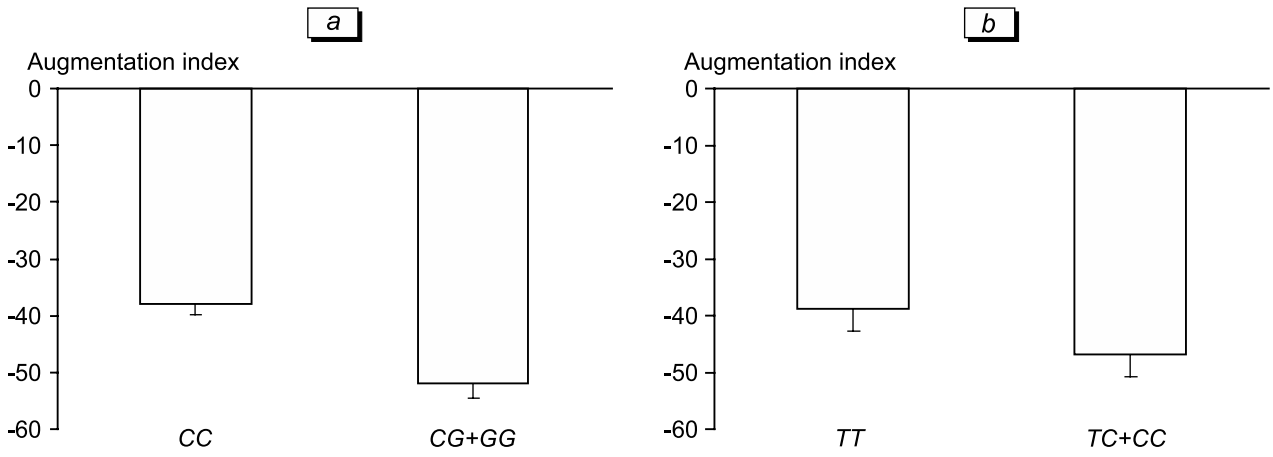

Fig. 1. Augmentation index for polymorphic markers *rs10830963* (*a*) and *rs34532313* (*b*).

TABLE 1. Clinical Parameters for Carriers of Genetic Markers *MTNR1A* and *MTNR1B*

Parameter	*MTNR1A*		*MTNR1B*	
	T/T+T/C	*C/C*	*C/C*	*C/G+G/G*
Total cholesterol, mmol/liter	5.57±0.31	5.55±0.55	6.40±1.43	5.79±1.94
HDL, mmol/liter	3.62±0.23*	3.34±0.51	4.67±1.34	3.92±1.74
LDL, mmol/liter	1.37±0.07	1.35±0.28	2.71±0.62	1.37±0.31^{+}
TG, mmol/liter	1.43±0.1	1.44±0.35	2.81±0.87	1.48±0.99^{+}
Glucose, mmol/liter	4.36±0.25	4.37±0.17	4.48±0.83	4.45±0.11
Fibrinogen, mmol/liter	3.48±0.27*	3.93±0.97	5.45±1.92	4.3±1.34^{+}

Note. $p \leq 0.05$ in comparison with *genotype *C/C* (*MTNR1A*), $^{+}$genotype *C/C* (*MTNR1B*).

subfraction in hypercholesterolemic rats. Melatonin can exhibit these effects by increasing the clearance of endogenous cholesterol [11]. According to published reports, the activity of blood coagulation system undergoes circadian fluctuations [12]. A dose-dependent correlation was also found between the level of melatonin in blood plasma and coagulation activity [13].

Carriers of the *CC* genotype have significantly higher levels of systolic BP. Previously, when studying glaucoma, we found that the *MTNR1A* gene polymorphism *rs34532313* of the can play a role in modulating the circadian pattern without affecting the mean level of intraocular pressure. Carriers of the minor *T* allele have more pronounced nocturnal rise in intraocular pressure compared to carriers of the *C* allele [14].

The *rs10830963* polymorphic marker of the *MTNR1B* gene located in intron 1 is a single nucleotide *C/G* substitution that is associated with increased receptor expression and mRNA levels in pancreatic β-cells. This mutation also leads to a violation of insulin secretion and an increase in the plasma glucose level, irrespective of age and body mass index. Overexpression of *MTNR1B* leads to disruption of the melatonin release mechanism, which can affect the vascular tone in mammals.

A relationship has been shown between an increased level of oxidized LDL in the blood serum at night and a decrease in the level of melatonin in the blood in patients with acute myocardial infarction [15]. These results support the hypothesis that melatonin, through its receptors, can reduce the total cholesterol levels and stimulate HDL synthesis.

Thus, the data presented in this work allow us to conclude that the major allele *C* of the *rs10830963* polymorphic variant of the *MTNR1B* gene is associated with increased levels of LDL and triglycerides, as well as with individual differences in the elastic properties of the vascular wall in the examined patients.

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