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Genetic and Functional Features of Peripheral Blood Leukocyte Mitochondria in Patients with Coronary Heart Disease and High Risk of Sudden Cardiac Death

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ABSTRACT

Aim. To assess the relationship between the respiration of mitochondria of peripheral blood leukocytes and mitochondrial DNA (mtDNA) polymorphism in patients with coronary heart disease (CHD) depending on the risk of developing sudden cardiac death (SCD).

Materials and methods. We formed two groups of patients: the main group – patients with CHD and the high risk of SCD (n = 107); the comparison group – patients with stable course of CHD without the risk of SCD (n = 50). Using methods of high-throughput sequencing, we determined patients' haplogroup and carriage of mtDNA polymorphisms A2706G, G3010A, and G9055A. The respiratory activity of isolated mitochondria from peripheral blood leukocytes was assessed by amperometric method using NAD- and FAD-dependent oxidation substrates.

Results. In both studied groups, H, U, and J haplogroups were predominant (74.5% and 92.5%, respectively, for the main group and the comparison group). There were more minor haplogroups in the main group than in the comparison group. The frequencies of occurrence of polymorphisms A2706G, G3010A, and G9055A did not significantly differ in intergroup comparison. In the main group, carriage of the A2706G polymorphism was associated with a decrease in the respiratory control ratio (RC) in FAD-dependent respiration (p = 0.05), and in the comparison group, it was associated with a decrease in oxygen consumption rate (OCR) in the V4 metabolic state in both NAD- and FAD-dependent respiration (p = 0.002 and p = 0.008, respectively) without changes in RC. In the main group, carriage of the G9055A polymorphism was associated with a decrease in OCR in the V3 metabolic state (p = 0.037) in FAD-dependent respiration. For the G3010A polymorphism, no association with mitochondrial respiration was found in the studied groups.

Conclusion. In patients with CHD, regardless of the risk of SCD, the frequencies of haplogroups H, U, and J and mtDNA polymorphisms A2706G, G3010A, and G9055A do not differ significantly. In patients with high risk of SCD, carriage of the A2706G polymorphism is associated with a decrease in RC in FAD-dependent respiration, and the G9055A polymorphism is associated with a decrease in OCR in V3 during FAD-dependent respiration.

Keywords: mitochondria, peripheral blood mononuclear cells, coronary heart disease, sudden cardiac death, mtDNA polymorphism

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Biomedical Ethics Committee of Research Institute of Medical Genetics, Tomsk National Research Medical Center of the Russian Academy of Sciences (Minutes No. 20 dated February 14, 2024).

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Генетические и функциональные особенности митохондрий лейкоцитов периферической крови пациентов при ишемической болезни сердца с высоким риском внезапной сердечной смерти

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РЕЗЮМЕ

Цель. Оценить взаимосвязь дыхательной активности митохондрий лейкоцитов периферической крови с полиморфизмом митохондриальной ДНК (мтДНК) у пациентов с ишемической болезнью сердца (ИБС) в зависимости от наличия риска развития внезапной сердечной смерти (ВСС).

Материалы и методы. Были сформированы две группы пациентов: основная группа – пациенты с ИБС и высоким риском ВСС (n = 107), группа сравнения – пациенты со стабильным течением ИБС без риска ВСС (n = 50). Пациентам определяли гаплогруппу, носительство полиморфизмов A2706G, G3010A и G9055A мтДНК методами высокопроизводительного секвенирования. Оценивали дыхательную активность изолированных митохондрий из лейкоцитов периферической крови амперометрическим методом при использовании NAD- и FAD-зависимых субстратов окисления.

Результаты. В обеих исследованных группах гаплогруппы H, U, J являлись превалирующими (74,5 и 92,5% для основной группы и группы сравнения соответственно). В основной группе минорных гаплогрупп было больше, чем в группе сравнения. Частоты встречаемости полиморфизмов A2706G, G3010A, G9055A не имели значимых межгрупповых различий. В основной группе носительство замены A2706G ассоциируется со снижением коэффициента дыхательного контроля (ДК) при FAD-зависимом дыхании (p = 0,05), а в группе сравнения – со снижением скорости потребления кислорода (СПК) в метаболическом состоянии V4 при NAD- и FAD-зависимом типах дыхания (p = 0,002 и p = 0,008 соответственно) без изменения ДК. Носительство замены G9055A в основной группе ассоциировано со снижением СПК в метаболическом состоянии V3 (p = 0,037) при FAD-зависимом дыхании. Для полиморфизма G3010A мтДНК не выявлено связи с респираторной активностью митохондрий в исследованных группах.

Заключение. У пациентов с ИБС вне зависимости от риска развития ВСС частоты гаплогрупп H, U, J и полиморфизмов A2706G, G3010A, G9055A мтДНК не имеют значимых различий. У пациентов высокого риска ВСС носительство полиморфизма A2706G связано с падением ДК при FAD-зависимом дыхании, а полиморфизма G9055A – со снижением СПК в V3 при FAD-зависимом дыхании.

Ключевые слова: митохондрии, лейкоциты периферической крови, ишемическая болезнь сердца, внезапная сердечная смерть, полиморфизм мтДНК

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Chronic coronary heart disease (CHD) is recognized as a primary pathogenetic factor in the development of not only heart failure (HF) [1], but also an increased risk of life-threatening ventricular arrhythmias, such as ventricular tachycardia [2]. The latter could be the cause of sudden cardiac death among above-mentioned patient population [3]. The search for the markers that can improve the stratification of patients with a high risk of sudden cardiac death (SCD) is actively ongoing, since the markers available to cardiologists are not always sufficient [4].

According to some data, mitochondrial DNA (mtDNA) polymorphism may contribute to the development of pacemaker cell dysfunction resulting in cardiac arrhythmias including life-threatening ones [5]. It is not possible to directly assess the function and genome of cardiac myocytes in routine clinical practice. However, if somatic mutations of mtDNA are not taken into account, DNA isolated from peripheral blood leukocytes can be used for genotyping.

The aim was to assess the relationship between the respiration of peripheral blood leukocyte mitochondria and mitochondrial DNA polymorphism in patients with CHD depending on the risk of SCD.

MATERIALS AND METHODS

As part of the study, we divided patients into two groups. The main group encompassed 107 patients diagnosed with CHD, who underwent implantation of cardiac resynchronization therapy devices with a defibrillator function as primary and secondary prevention of life-threatening ventricular tachyarrhythmias according to clinical guidelines [6]. The comparison group encompassed 50 patients with stable CHD (without a history of cardiovascular events, such as myocardial infarction, stroke, thromboembolism, and sudden circulatory arrest) and without indications for implantation of resynchronization therapy devices. Clinical and laboratory characteristics of the patients are summarized in Table 1. The study protocol was approved by the local Ethics Committee of the Research Institute of Medical Genetics, Tomsk National Research Medical Center (Minutes No. 20 dated February 14, 2024). A signed informed consent was obtained from each patient included in the study.

Table 1

Clinical and Laboratory Characteristics of the Patients at the Time of Enrollment

Parameters	Main group (n = 107)	Comparison group (n = 50)	p-value
Age, *Me* (Q_1; Q_3), years	64.0 (59.0; 71.0)	67.0 (63.0; 72.0)	0.147
Men, *n* (%)	83 (77.6%)	22 (44.0%)	<0.001
Angina pectoris, *n* (%)	83 (77.6%)	36 (72.0%)	0.320
Myocardial infarction, *n* (%)	72 (67.3%)	0 (0%)	
Coronary atherosclerosis, *n* (%)	47 (43.9%)	35 (70.0%)	0.003
LVEF, *Me* (Q_1; Q_3), %	42 (33; 58)	65 (64; 67)	<0.001
NYHA HF FC I, *n* (%)	11 (10.3%)	20 (40.0%)	<0.001
NYHA HF FC II, *n* (%)	58 (54.2%)	21 (42.0%)	0.121
NYHA HF FC III, *n* (%)	38 (35.5%)	9 (18.0%)	0.036
Hypertension, *n* (%)	99 (92.5%)	49 (98.0%)	0.552
Body mass index, *Me* (Q_1; Q_3), kg/m^2	29.1 (26.2; 33.1)	31.2 (26.5; 34.5)	0.326
Obesity, *n* (%)	47 (43.9%)	25 (50.0%)	0.699
Dyslipidemia, *n* (%)	78 (72.9%)	28 (56.0%)	0.020
Diabetes mellitus, *n* (%)	23 (21.5%)	7 (14.0 %)	0.244
Carotid artery atherosclerosis, *n* (%)	53 (49.5%)	40 (80.0%)	<0.001
Femoral artery atherosclerosis, *n* (%)	35 (32.7%)	25 (50.0%)	0.076
Thyroid gland diseases, *n* (%)	10 (9.3%)	11 (22.0%)	0.068
ACEi/ARA, *n* (%)	85 (79.4%)	38 (76.0%)	0.310
BAA, *n* (%)	88 (82.2%)	29 (58.0%)	<0.001
Anticoagulants, *n* (%)	44 (41.1%)	12 (24.0%)	0.029
SGLT2i, *n* (%)	24 (22.4%)	5 (10.0%)	0.055
Statins, *n* (%)	93 (86.9%)	43 (86.0%)	0.403
Diuretics, *n* (%)	49 (43.9%)	18 (36.0%)	0.208
Antiarrhythmics, *n* (%)	37 (34.6%)	10 (20.0%)	0.053
CCBA, *n* (%)	15 (14.0%)	21 (42.0%)	<0.001
Antiplatelets, *n* (%)	68 (63.6%)	33 (66.0%)	0.693
Glucose, *Me* (Q_1; Q_3), mmol/l	5.69 (5.22; 6.60)	5.57 (5.05; 6.19)	0.204
Total cholesterol, *Me* (Q_1; Q_3), mmol/l	4.14 (3.62; 5.00)	4.35 (3.60; 5.50)	0.466

End of Table 1

Parameters	Main group (n = 107)	Comparison group (n = 50)	p-value
Triglycerides, *Me* (Q_1; Q_3), mmol/l	1.27 (0.92; 1.86)	1.47 (1.13; 1.87)	0.307
HDL, *Me* (Q_1; Q_3), mmol/l	1.21 (0.93; 1.46)	1.15 (1.03; 1.45)	0.879
LDP, *Me* (Q_1; Q_3), mmol/l	2.25 (1.55; 3.21)	2.40 (1.70; 3.10)	0.855

Note. LVEF – left ventricular ejection fraction; FC – functional class; CHF – chronic heart failure; NYHA – New York Heart Association; ACEi – angiotensin-converting enzyme inhibitor; ARA – angiotensin II receptor antagonist; BAA – beta-adrenoreceptor antagonist; SGLT2i – sodium-glucose transporter type 2 inhibitors; CCBA – calcium channel-blocking agent; HDP – high-density lipoproteins; LDP – low-density lipoproteins.

All patients had blood collected in vacutainers with EDTA. Isolation of peripheral blood leukocytes was carried out using Histopaque-1077 density gradient (Sigma, USA). The resulting "ring" containing leukocytes was washed in phosphate buffered saline (pH = 7.40) (Sigma). Isolated mitochondria were obtained using the commercial Mitochondria Isolation Kit for Cultured Cells (ThermoScientific, USA) according to the manufacturer's instructions. The resulting pellet was resuspended in a minimum volume of 0.25 M sucrose for further work. The methodology of studying respiration during NAD-dependent (pyruvate + malate) and FAD-dependent (succinate) substrate oxidation was described previously [7]. The assessed parameters of mitochondrial respiration were V3 – phosphorylating metabolic state (in the presence of oxidation substrates, inorganic phosphate and ADP), V4 – non-phosphorylating metabolic state (after ADP depletion), and respiratory control coefficient (RC) – V3/V4.

To study mtDNA, total DNA was isolated from peripheral blood leukocytes. The complete mtDNA sequence was determined using high-throughput sequencing as described previously [8]. The mtDNA haplogroup of each patient was determined using the mtDNA-Server 2 program [9]. The search for associations with cellular respiration was carried out for three mtDNA polymorphisms: A2706G (marker of haplogroup H), G3010A (marker of haplogroup H1), and G9055A (marker of haplogroup K).

The choice of the above-noted polymorphisms for analysis was determined by the results of our own research and literature data. For haplogroup H, associations with myocardial infarction, ischemic cardiomyopathy, and postoperative atrial fibrillation were previously found [10–12]; for haplogroup H1, associations with increased risk of cardiovascular accidents, including sudden cardiac death, were found [13; 14]; haplogroup K, according to the results of several studies, showed a protective effect, but only in neurodegenerative disease [15; 16], whereas we previously revealed a higher frequency of the G9055A polymorphism in individuals who survived cardiac arrest [17].

Statistical data processing was carried out using STATISTICA 10.0 software package. The hypothesis of normal distribution of quantitative data was tested using the Shapiro – Wilk test. The differences between quantitative variables were assessed using the non-parametric Mann – Whitney test. The results were presented as median, upper and lower quartiles (*Me* (Q_1; Q_3)). Differences in frequencies were studied using the Pearson's chi-square test. The results were presented as absolute frequencies (n) and percentages (%). Differences were considered statistically significant at $p < 0.05$.

RESULTS

According to the data presented in Table 1, men predominated in the main group (80.6% vs. 44.0% compared to the comparison group, $p < 0.001$). Patients of this group more often had NYHA functional class II HF ($p = 0.036$), dyslipidemia ($p = 0.020$), and their LVEF was on average classified as mildly reduced, whereas in the comparison group, all patients had preserved LVEF. Also, patients in the main group more often took β-blockers ($p < 0.001$) and anticoagulants ($p = 0.029$), and less often took calcium antagonists ($p < 0.001$).

Genotyping and determination of mtDNA haplogroups were carried out in samples of 102 patients of the main group and 40 patients of the comparison group (Table 2). The frequencies of predominant haplogroups among patients in both studied groups corresponded to the population distribution [8]. The highest frequencies of occurrence were registered for haplogroups H, U, and J, and the total contribution of the listed mtDNA variants in the comparison and main groups was 92.5% and 74.5%, respectively. Also, haplogroup T was one of the most common mtDNA haplogroups in the main group of patients (10.8%). In the comparison group, the minor haplogroups were the following: T, D, and M-G. Carriage of these haplogroups was established in only 3 patients (1 case for each haplogroup). In the main group, there were more minor mtDNA haplogroups. In this group, these

included the following haplogroups: M-G, V, W, A, F, N, I, X, HV, C, and R. The obtained results are consistent with previous data obtained on a sample of patients with ischemic heart failure [8].

Table 2

Frequencies of Occurrence of mtDNA Haplogroups Among the Studied Patients, *n* (%)

mtDNA haplogroup	Comparison group ($n = 40$)	Main group ($n = 102$)	*p*-value
H, *n* (%)	19 (47.5%)	42 (41.2%)	0.86
U, *n* (%)	13 (32.5%)	25 (24.5%)	0.87
J, *n* (%)	5 (12.5%)	9 (8.8%)	0.45
T, *n* (%)	1 (2.5%)	11 (10.8%)	0.22
D, *n* (%)	1 (2.5%)	0 (0%)	0.14
A/M-G, *n* (%)	1 (2.5%)	1 (1.0%)	0.58
V, *n* (%)	0 (0%)	2 (2.0%)	0.32
W, *n* (%)	0 (0%)	1 (1.0%)	0.49
A, *n* (%)	0 (0%)	2 (2.0%)	0.32
F, *n* (%)	0 (0%)	1 (1.0%)	0.49
N, *n* (%)	0 (0%)	2 (2.0%)	0.32
I, *n* (%)	0 (0%)	1 (1.0%)	0.49
X, *n* (%)	0 (0%)	2 (2.0%)	0.32
HV, *n* (%)	0 (0%)	1 (1.0%)	0.49
C, *n* (%)	0 (0%)	1 (1.0%)	0.49
R, *n* (%)	0 (0%)	1 (1.0%)	0.49

Note. Here and in Table 3: mtDNA – mitochondrial DNA.

Analysis of the carriage of mtDNA A2706G, G3010A, and G9055A polymorphisms was carried out in 97 samples from the main group and 40 samples from the comparison group. Results are presented in Table 3. There were no significant differences in the occurrence frequencies of these mtDNA variants between the studied groups.

Table 3

Frequencies of mtDNA A2706G, G3010A, and G9055A Polymorphisms in the Studied Groups, *n* (%)

mtDNA polymorphism	Comparison group ($n = 40$)	Main group ($n = 94$)	*p*-value
A2706G, *n* (%)	23 (60.0%)	62 (66.0%)	0.91
G3010A, *n* (%)	12 (30.0%)	20 (21.3%)	0.36
G9055A, *n* (%)	4 (10.0%)	10 (10.6%)	0.92

Table 4 demonstrates the results of the assessment of the respiration of peripheral blood leukocyte mitochondria in carriers of the A2706G polymorphism in both studied groups. The presented data show that in carriers of this polymorphism in the comparison group, oxygen consumption rate in V4 metabolic state significantly decreased in both NAD- and FAD-dependent substrate oxidation ($p = 0.002$ and $p = 0.008$, respectively). In both cases, there were no significant changes in RC. In the main group, carriage of this polymorphism was associated with a decrease in RC in FAD-dependent substrate oxidation ($p = 0.05$).

The presence of guanine at position 2706 of mtDNA was associated with a decrease in the efficiency of phosphorylation only in succinate oxidation in patients of the main group, whereas in the comparison group, it exerted a protective effect reducing oxygen consumption rate in non-phosphorylating state. This is consistent with the data indicating increased production of reactive oxygen species as a result of electron leakage from the respiratory chain in carriers of haplogroup H (haplotype 2706A [18]), despite high levels of oxygen consumption (VO2max) during physical activity [19].

It is likely that carriage of this haplogroup is very common among patients with cardiovascular diseases, since long-term exposure to reactive oxygen species is damaging, including in relation to cardiomyocytes and their mitochondria. Together with risk factors and comorbidity, this results in a progressive decrease in myocardial contractile activity and the development of arrhythmias, including life-threatening ones.

In carriers of the G9055A polymorphism, a simultaneous decrease in mitochondrial respiration in V3 ($p = 0.037$) and V4 ($p = 0.037$) metabolic states with a fall in RC ($p = 0.13$), which did not reach statistical significance, was shown among patients with high risk of sudden cardiac death in FAD-dependent substrate oxidation (Table 5).

Carriage of the G3010A mtDNA polymorphism was not associated with a significant change in mitochondrial respiration in any of the studied groups in either NAD- or FAD-dependent substrate oxidation (Table 6).

Table 4

Peripheral Blood Leukocyte Mitochondria Respiration in the Presence and the Absence of mtDNA A2706G Polymorphism, (*Me* (Q_1; Q_3))

Parameter	Comparison group			Main group		
	NAD-dependent substrates					
	A2706	G2706	*p*-value	A2706	G2706	*p*-value
V3, nmol O_2/min/mg mitochondrial protein	196.44 (125.42; 245.15)	125.19 (93.69; 137.09)	0.07	123.18 (82.57; 194.71)	104.93 (61.81; 161.98)	0.52

End of table 4

Parameter	Comparison group			Main group		
	NAD-dependent substrates					
	A2706	G2706	*p*-value	A2706	G2706	*p*-value
V4, nmol O_2/min/mg mitochondrial protein	67.19 (52.08; 123.06)	41.47 (34.90; 47.78)	0.002	42.64 (30.38; 54.69)	41.87 (27.50; 55.15)	0.87
RC, RU	2.25 (1.89; 3.07)	2.47 (1.92; 3.57)	0.54	2.57 (2.47; 2.70)	2.35 (2.23; 2.88)	0.22
	FAD-dependent substrate					
V3, nmol O_2/min/mg mitochondrial protein	235.75 (156.25; 312.50)	175.93 (108.42; 193.73)	0.19	82.57 (71.88; 150.24)	140.31 (74.22; 169.96)	0.74
V4, nmol O_2/min/mg mitochondrial protein	87.50 (52.82; 106.21)	36.46 (28.91; 61.34)	0.008	29.20 (20.90; 54.69)	49.46 (32.50; 66.96)	0.19
RC, RU	2.96 (2.58; 3.38)	2.91 (2.45; 3.80)	1.00	3.23 (2.30; 4.18)	2.57 (2.16; 2.76)	0.05

Note. Here and in Tables 5 and 6. V3 – phosphorylating metabolic state; V4 – non-phosphorylating metabolic state; RC – respiratory control ratio (V3/V4)

Table 5

Peripheral Blood Leukocyte Mitochondria Respiration in the Presence and the Absence of mtDNA G9055A Polymorphism, (*Me* (Q_1; Q_3))

Parameter	Comparison group			Main group		
	NAD-dependent substrates					
	G9055	A9055	*p*-value	G9055	A9055	*p*-value
V3, nmol O_2/min/mg mitochondrial protein	125.32 (85.33; 199.42)	131.32 (127.81; 140.54)	0.79	107.95 (76.39; 161.98)	53.35 (50.65; 57.40)	0.09
V4, nmol O_2/min/mg mitochondrial protein	44.95 (34.09; 56.25)	51.53 (47.67; 55.13)	0.62	45.96 (30.27; 54.69)	23.95 (23.95; 25.95)	0.09
RC, RU	2.32 (1.88; 3.10)	2.55 (2.34; 2.87)	0.62	2.53 (2.30; 2.88)	2.23 (2.15; 2.61)	0.13
	FAD-dependent substrate					
V3, nmol O_2/min/mg mitochondrial protein	165.18 (72.62; 225.00)	254.32 (154.18; 260.33)	0.15	128.68 (75.76; 162.55)	32.67 (28.51; 45.29)	0.037
V4, nmol O_2/min/mg mitochondrial protein	56.25 (29.71; 88.14)	84.77 (51.46; 92.52)	0.52	43.66 (26.69; 64.41)	15.24 (14.40; 29.87)	0.037
RC, RU	2.67 (2.33; 3.53)	3.00 (2.41; 3.37)	0.46	2.64 (2.28; 3.23)	2.14 (2.01; 2.56)	0.13

Table 6

Peripheral Blood leukocyte Mitochondria Respiration in the Presence and the Absence of mtDNA G3010A Polymorphism, (*Me* (Q_1; Q_3))

Parameter	Comparison group			Main group		
	NAD-dependent substrates					
	G3010	A3010	*p*-value	G3010	A3010	*p*-value
V3, nmol O_2/min/mg mitochondrial protein	131.32 (120.31; 199.42)	164.30 (93.16; 235.86)	0.96	120.86 (64.95; 203.61)	106.06 (79.78; 114.48)	0.56
V4, nmol O_2/min/mg mitochondrial protein	51.86 (44.94; 78.13)	41.59 (36.32; 71.35)	0.26	48.65 (27.50; 91.02)	33.44 (30.13; 50.89)	0.10
RC, RU	2.29 (1.88; 3.57)	2.50 (2.29; 3.07)	0.35	2.52 (2.16; 2.70)	2.49 (2.30; 3.32)	0.52
	FAD-dependent substrate					
V3, nmol O_2/min/mg mitochondrial protein	170.55 (69.90; 254.32)	132.33 (106.25; 172.28)	0.57	128.68 (65.00; 178.31)	133.93 (81.21; 163.10)	0.69
V4, nmol O_2/min/mg mitochondrial protein	58.80 (29.71; 95.24)	43.03 (28.91; 87.50)	0.66	43.66 (29.20; 66.96)	38.07 (23.36; 65.10)	0.65
RC, RU	2.94 (2.57; 3.81)	2.95 (2.34; 3.16)	0.48	2.61 (2.17; 2.87)	3.17 (2.43; 4.07)	0.10

DISCUSSION

The search for associations between the carriage of mtDNA polymorphisms, the belonging of mtDNA to a certain haplogroup, and cardiovascular disease progression type is an actively developing field today. The aim of the study was to reveal the relationship between the carriage of single polymorphisms, A2706G, G3010A, and G9055A, as well as the belonging of mtDNA of individuals of the studied groups to certain haplogroups and the risk of developing sudden cardiac death in patients diagnosed

with ischemic heart disease. When comparing the group of patients without risk of sudden cardiac death and the group of patients with high risk of sudden cardiac death, no differences in the frequencies of mtDNA haplogroups were found. The predominant mtDNA haplogroup in both groups was haplogroup H (47.5% in the comparison group and 41.2% in the main group), which is consistent with literature data.

In point of fact, this haplogroup is the main one for our population; its frequency of occurrence in our country reaches an average of 40% [20]. Less frequently, patients were identified as carriers of haplogroups U (32.5% and 24.5% for the comparison group and the main group, respectively) and J (12.5% and 8.8%, respectively), which also correlates with previous literature data. The main group was characterized by a greater variety of minor mtDNA haplogroups, such as A/M-G, C, D, F, HV, I, N, R, V, W, and X.

The analysis of available literature data did not reveal any studies aimed to identify the role of the relationship between the carriage of the A2706G polymorphism (encodes the 12S rRNA subunit; marker polymorphism of haplogroup H) and mitochondrial respiration. In our study, this polymorphism was associated with a decrease in oxygen consumption rate in V4 metabolic state in oxidation of various substrates ($p = 0.002$ and $p = 0.008$ in NAD- and FAD-dependent respiration, respectively) in patients diagnosed with CHD without the risk of sudden cardiac death. It could be assumed that in this group of patients, the carriage of this polymorphism partially exerts a protective effect aimed to reduce respiratory activity in V4 metabolic state to reduce oxygen consumption for processes not associated with the synthesis of ATP by mitochondria.

In patients with CHD and high risk of sudden cardiac death, the carriage of the A2706G polymorphism was associated with lower RC ratio in FAD-dependent substrate oxidation. With respect to other respiration parameters, carriage of this polymorphism did not make a significant contribution to changes in mitochondrial respiratory parameters in patients of this group. It may be concluded that the carriage of this polymorphism has a probable protective effect in patients with CHD without the risk of developing life-threatening tachyarrhythmias. This effect is the opposite in patients with high risk of developing life-threatening arrhythmias.

There is no valid evidence in the available sources on the influence of the G3010A polymorphism carriage (replacement in the 12S rRNA; marker polymorphism of haplogroup H1) on mitochondrial function in cardiovascular diseases. A number of studies have only revealed associations between an unfavorable course of cardiovascular diseases, including the development of sudden cardiac death [13, 14]. In this study, carriage of mentioned mtDNA polymorphism was not associated with any changes in mitochondrial respiration or oxidation-phosphorylation coupling, neither in NAD-dependent nor in FAD-dependent substrates oxidation, both in the group of patients without the risk of sudden cardiac death and in the group of patients with high risk of sudden cardiac death.

The G9055A mtDNA polymorphism determines an amino acid substitution in the ATPase-6 subunit of ATP synthase. Only a small number of studies aimed to evaluate the effect of this polymorphism on energy metabolism in various diseases not related to cardiovascular pathology (Parkinson's disease, autism, and breast cancer) have been published [21, 22]. The results of the study showed that the contribution of this mtDNA polymorphism to the mitochondrial respiration resulted in a significant decrease in oxygen consumption rate in succinate oxidation in patients of the main group who carry this polymorphism, i.e., it had a negative effect on the functioning of the electron transport chain. Otherwise, in patients with complicated CHD, this polymorphism was not associated with any significant changes in mitochondrial respiration. In patients of the comparison group, carriage of this polymorphism was not accompanied by any significant changes in mitochondrial respiration. Most likely, for these patients, the G9055A polymorphism is neutral and does not make a significant contribution to the course of the underlying disease.

CONCLUSION

In this study, a comprehensive study of peripheral blood leukocyte mitochondria was carried out in cardiac patients diagnosed with CHD and high risk of developing life-threatening arrhythmias, namely their functional activity, haplogroups, and certain mtDNA polymorphism (A2706G, G3010A, and G9055A). The frequency distribution of haplogroups in CHD patients with and without high risk of developing life-threatening tachyarrhythmias corresponds to the population distribution. As in the population, haplogroups H, U, and J were predominant for CHD patients regardless of the risk of developing sudden cardiac death. The presence of guanine at position 2706 of mtDNA was associated with a decrease in the

activity of non-phosphorylating state of mitochondrial respiration only in case of CHD without the risk of developing sudden cardiac death. In case of CHD with high risk of sudden cardiac death, carriage of the G9055A polymorphism was associated with a decrease in the intensity of mitochondrial respiration in both phosphorylating and non-phosphorylating states in FAD-dependent substrate oxidation. For the G3010A mtDNA polymorphism, no association with changes in respiration of peripheral blood leukocyte mitochondria was revealed.

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