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FUNCTIONAL ABILITY OF MITOCHONDRIA AND MITOCHONDRIAL GENOME POLYMORPHISM AS FACTORS AFFECTING ARRHYTHMOGENESIS IN CHRONIC CORONARY ARTERY DISEASE

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Aim. To investigate functional state of mitochondria and mitochondrial DNA (mtDNA) polymorphism in coronary artery disease (CAD) patients with life-threatening cardiac rhythm disorders (CRD).

Methods. We investigated venous blood samples of 45 patients with uncomplicated CAD and 120 CAD patients with CRD. Oxygen consumption rate of mitochondrias of leukocytes in V3 and V4 states were determined in pyruvate-malate and succinate buffers, as well as in the presence of palmitic acid (PA). In patients with complicated CAD, mtDNA haplogroup and substitutions in gene encoding proteins of the respiratory chain complexes and mitochondrial rRNA were determined. Statistical analysis was performed using Mann-Whitney, Wilcoxon tests and Chi-square test with Yates' correction.

Results. In CAD and CAD with CRD, oxygen consumption rate of intact mitochondria did not different in either pyruvate-malate or succinate buffers. In uncomplicated CAD, PA supplementation increases oxygen consumption rate by mitochondria in both succinate and pyruvate-malate buffers. The majority of patients (41%) with CAD and CRD were carriers of the haplogroup «H» and, in this indicator, the sample did not differ from patients with uncomplicated CAD. However, mtDNA of patients with complicated CAD was characterized by a more frequent combined carriage of two and more missense substitutions in genes of respiratory chain and rRNA.

Conclusion. Mitochondria of patients with coronary artery disease and life-threatening cardiac rhythm disorders have reduced functional reserve. The distribution of frequencies of main mtDNA haplogroups of patients with coronary artery disease with life threatening cardiac rhythm disorders corresponds to the population. The mtDNA of such patients is characterized by a high frequency of carriage of combined polymorphisms in gene of electron transport chain proteins and rRNA.

Key words: coronary artery disease; cardiac arrhythmia; mitochondria; mitochondrial DNA; polymorphisms; haplogroup

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Cardiac rhythm disorders (CRD) represent one of the formidable complications of coronary heart disease (CHD) [1]. They are the ones that often become the causes of sudden cardiac death (SCD) [2]. Cyclicity of the electromechanical coupling process in cardiomyocytes is provided due to the energy of adenosine triphosphate synthesized by the electron-transport chain of mitochondria. The coding part of the electron-transport chain proteins (13 subunits comprising four enzyme complexes) is determined by mitochondrial DNA (mtDNA). In addition, mtDNA encodes 2 rRNA genes and 22 tRNA genes that are required for the realization of translation in mitochondria [3]. In human populations, mtDNA is characterized by a pronounced polymorphism. Inherited combinations of mtDNA variants are grouped into separate haplogroups. Carriage of such genetic variants may determine the functional capabilities of the mitochondrial respiratory chain. This circumstance affects the energetics of cells and whole organs, contributing negatively to the development of cardiovascular pathology [4].

The aim of this study was to investigate the functional status of mitochondria and mtDNA polymorphism in patients with ischemic heart disease who have heart rhythm disorders.

METHODS

Forty-five patients diagnosed with CHD without CRD and a history of myocardial infarction and 120 patients with CHD with CRD in the form of ventricular tachyarrhythmias (ventricular fibrillation or ventricular tachycardia (VT)) and implanted cardioverter-defibrillators (ICDs) were included in the study. The inclusion criteria were: age over 40 years, presence of documented CHD, functional class of heart failure I-III according to NYHA, signed informed consent; for patients with CHD and CRD additionally - presence of implanted cardioverter-defibrillator. ICD implantation was performed for primary or secondary prevention of SCD. Patients who underwent ICD implantation for primary prevention of SCD were at high risk of developing SCD. Patients who underwent ICD implantation for secondary prevention of SCD had a history of episodes of sustained VT and/or ventricular fibrillation, symptoms of cardiac rhythm disturbances (syncope, ventricular tachyarrhythmias recorded on ECG), and a history of CSD. According to clinical guidelines, the persistence of VTs was assessed by their duration: VTs with a duration of 30 seconds or more were considered stable; unstable VTs with a duration of less than 30 seconds were considered unstable. Exclusion criteria were: myocardial infarction (for patients with CHD without CRD), canalopathies (prolonged QT syndrome, Brugada syndrome, etc.), cancer, pregnancy, neurological disorders or psychoemotional conditions that could potentially interfere with the study. Also, patients who were candidates for heart transplantation or who withdrew from the study at any stage were not included. Cardiac rhythm disorders (VT, ventricular fibrillation) in patients were verified by ECG recordings, 24-hour ECG Holter monitoring and ICD endogram recordings. All patients included in the study had stable CHD without indications for myocardial revascularization. Before ICD implantation, coronary angiography was performed in all patients with CHD and CRD to exclude indications for revascularization. ICD implantation was performed not earlier than 3 months after myocardial infarction and not earlier than 6 months after stenting or bypass surgery. A brief clinical and anamnestic picture of patients in both groups is summarized in Table 1. MtDNA genotyping was performed for 81 patients with a complicated course of CHD. Patients were observed in specialized departments of the Research Institute of Cardiology of Tomsk NIMC. The study was approved by the Biomedical Ethics Committee of the Cardiology Research Institute of Tomsk NIMC (protocol #219 of 26.10.2021).

Venous blood sampling in patients with CHD without CRD was performed at the follow-up visit, in patients with CHD and CRD - either before ICD implan-

Table 1.

Clinical characteristics of patients

Indicator	Patients with CHD without CRD (n=45)	Patients with CHD with CRD (n=120)	p
Age, years	67.0 (63.0; 70.8)	64.0 (59.0; 72.0)	0.13
Sex, male, % (n)	21 (46.7)	92 (76.7)	<0.001
Myocardial infarction, n (%)	0 (0)	72 (60.0)	
CHF FC I (NYHA), n (%)	19 (42.2)	16 (13.3)	<0.001
CHF FC II (NYHA), n (%)	19 (42.2)	65 (54.2)	0.17
CHF FC III (NYHA), n (%)	7 (15.6)	39 (32.5)	0.03
Unstable VTs, n (%)	0 (0)	93 (77.5)	
Persistent VTs, n (%)	0 (0)	17 (14.2)	
VF, n (%)	0 (0)	10 (8.3)	
AH, n (%)	44 (97.8)	112 (93.3)	0.25
Obesity, n (%)	25 (59.5)	48 (40.0)	0.07
BMI, kg/m^2	31.2 (27.0; 34.5)	28.4 (25.6; 32.5)	0.05
iACEs; ARBs, n (%)	34 (75.6)	70 (58.3)	0.04
BABs, n (%)	26 (57.8)	102 (85.0)	0.02
Anticoagulants, n (%)	10 (22.2)	50 (41.7)	0.07
Antiplatelet, n (%)	30 (66.6)	75 (62.5)	0.26
Statins, n (%)	39 (86.7)	91 (75.8)	0.16

Notes: CHD - coronary heart disease; CRD - cardiac rhythm disturbances; CHF FC (NYHA) - New York Heart Association (NYHA) functional class of congestive heart failure; VT - ventricular tachycardia; VF -ventricular fibrillation; AH - arterial hypertension; BMI - body mass index; iACEs - angiotensin-converting enzyme inhibitors; ARBs - angiotensin II receptor blockers; BABs - beta-adrenoblockers.

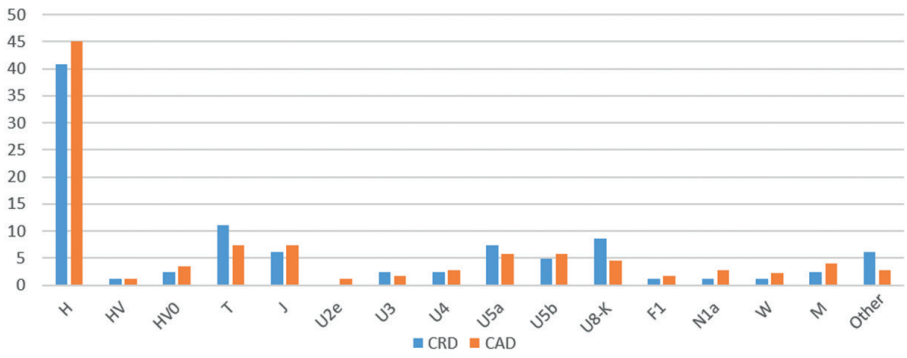

Fig. 1. Distribution of frequencies of major mtDNA haplogroups among patients, where on the ordinate axis - frequency of haplogroups (in percent); on the abscissa axis - major human mtDNA haplogroups; hereinafter CRD - patients with CHD with life-threatening cardiac rhythm disturbances; CHD - patients with uncomplicated CHD (according to previously published data) [9].

tation or at the follow-up visit of the patient for routine check of ICD parameters. Blood was withdrawn into vacutainers with EDTA anticoagulant. Peripheral blood leukocytes were isolated on a Histopaque-1077 density gradient (USA). A ring of isolated cells was washed in phosphate-salt buffer (pH=7.4) (Sigma, USA). The cell sediment was transferred to sucrose medium (0.25 M) with EDTA and lysis was performed by gentle pipetting. Leukocyte mitochondria were isolated by differential centrifugation in a sucrose gradient (0.25 M) [5]. The content of mitochondria in the studied samples was monitored by the concentration of total protein estimated by the Lowry method. For incubation of isolated mitochondria, we used medium of the following composition (in mM): sucrose, 250.0; KCI, 10.0; KH_2PO_4, 5.0; $MgCI_2$, 1.25; HEPES, 5.0; pyruvate, 6; malate, 8, or succinate, 5; pH = 7.35-7.40. Palmitic acid added to the incubation medium at a concentration of 20 μM/L was used as an additional metabolic substrate [6]. After oxygenation of the incubation medium, mitochondrial suspension (0.5-1 mg protein) was added. Oxygen content was estimated by polarographic method using a Clark electrode in a 1 mL thermostated chamber at 25 °C and constant stirring. Oxygen uptake rate was calculated in nM O_2/min/mg protein. The respiratory control ratio (RC) was calculated using the formula V3/V4, where V3 is the rate of oxygen uptake in the presence of an oxidation substrate (succinate or a mixture of pyruvate and malate) and a phosphorylation substrate (200 μM ADP), and V4 was calculated upon ADP depletion [7].

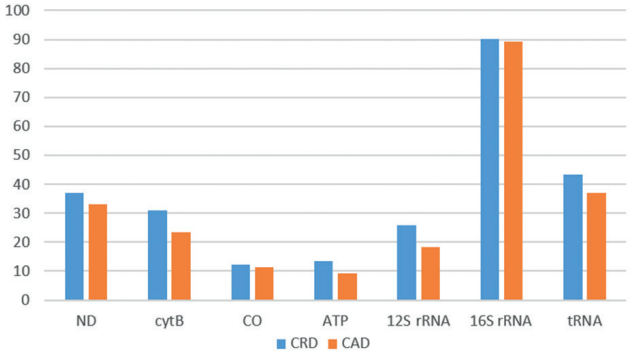

Fig. 2. Prevalence of missense polymorphisms in genes encoding proteins of mitochondrial electron-transport chain, as well as in RNA genes localized in mtDNA, where the ordinate axis is the number of patients in % having the corresponding polymorphism; on the abscissa axis - mtDNA genes encoding proteins of respiratory chain complexes, rRNA and tRNA; ND - NADH dehydrogenase genes; cytB - cytochrome B gene; CO - cytochrome C oxidase genes; ATP - ATP synthase genes; 12S rRNA, 16S rRNA, tRNA - RNA genes.

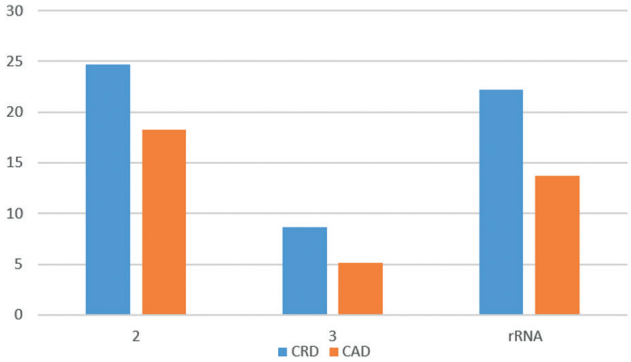

Fig. 3. Frequency of co-carriage of two or more missense polymorphisms in genes of electron-transport chain and rRNA localized in mtDNA, where on the ordinate axis - patients in %, on the abscissa axis - multiple missense polymorphisms in genes (2 - in two complexes of electron-transport chain, 3 - in three complexes of electron-transport chain; rRNA - in two rRNA genes).

Genomic DNA was isolated from peripheral blood mononuclear cells using the phenol-chloroform method [8]. The concentration of the obtained DNA was measured on a spectrophotometer «Nanodrop-2000C» (Thermo Fisher Scientific, USA). To determine whether the studied samples belonged to a specific mtDNA haplogroup, sequencing of the first hypervariable segment (HVS1) of the mtDNA D-loop and genotyping of individual polymorphic restriction sites were performed according to the previously described protocol [9]. For each patient, the haplotype was determined as a list of substitutions compared to the human mtDNA reference sequence [10]. Based on this information, patients' mtDNA was categorized into haplogroups and confirmed to belong to these haplogroups by restriction. The human mtDNA database and family tree mtDNA tree Build 17 [11] were used for classification and to search for missense substitutions in individual haplogroups.

Statistical analysis

Statistical processing of the obtained data was performed using the STATISTICA 13.0 statistical program package. The Shapiro-Wilk test was used to check whether the distribution of quantitative variables obeyed the normal law. The nonparametric Mann-Whitney test was used to compare independent groups of data, and the Wilcoxon test was used for dependent variables. Values are presented as median and interquartile range (Me (Q1; Q3). Qualitative data are presented as frequencies and percentages, and comparisons were made using the Chi-square criterion with Yates correction. The critical level of significance p is taken as $p<0.05$.

RESULTS

Examination of the rate of oxygen uptake by mitochondria of patients with CHD uncomplicated by CRD and CHD with CRD revealed the following: intact isolated mitochondria of patients of these groups, in pyruvate malate buffer in V3 state had similar (p=0.44) rates of oxygen consumption 127.7 (85.3; 196.4) and 119.1 (82.6; 174.0) nM O_2/min/mg protein, respectively. In the V4 condition, oxygen consumption rates were also not significantly different (p=0.29), being 46.6 (38.5; 56.3) and 43.7 (27.5; 66.7) nM O_2/min/mg protein, respectively. Using succinate buffer, in the V3 condition, oxygen consumption rates for the CHD and CHD with CRD groups were 125.5 (64.4; 172.3) and 112.0 (65.0; 165.0) nM O_2/min/mg protein, respectively (p=0.50), and in the V4 condition -44.2 (27.5; 71.9) and 40.2 (21.1; 68.7) nM O_2/min/mg protein, respectively (p=0.58). Degrees of conjugation of oxidation and phosphorylation processes (RC

coefficient) in uncomplicated and complicated CHD also had no significant differences, being, respectively, 2.5 (2.0; 3.3) and 2.4 (2.1; 2.7) for pyruvate malate buffer (p=0.50), and 2.7 (2.33; 3.22) and 2.6 (2.1; 2.8) for succinate buffer (p=0.34).

The addition of palmitic fatty acid to the incubation medium affected the oxygen consumption of mitochondria in the groups of patients under consideration to different degrees. Thus, in the group of patients without CRD, against a background of palmitic acid, the rate of oxygen consumption in pyruvate malate buffer for the V3 state was characterized by a statistically significant (p=0.04) increase to 198.3 (162.7; 234.9) nM O_2/min/mg protein. In the V4 condition, the rate of oxygen consumption was also statistically significantly (p=0.02) higher and was 64.4 (60.3; 87.7) nM O_2/min/mg protein. In succinate buffer, for this group of patients, the increase in the rate of oxygen consumption in states V3 and V4 did not reach statistically significant values (p=0.07), being 184.3 (47.3; 406.3) and 86.1 (36.9; 159.4) nM O_2/min/mg protein, respectively. In contrast, in the CHD group with CRD, the addition of palmitic acid had no effect on the rate of oxygen consumption in the V3 and V4 states and RC using both pyruvate malate and succinate buffers.

Genotyping of mtDNA of patients with CHD and CRD revealed that the predominant haplogroup in the studied samples was mtDNA of the H branch (41%) (Fig. 1). In addition to the haplogroups shown in the figure, single cases of mtDNA carriers of haplogroups A, X, U7, U8a1, N1b1, R1a, R0a, and N2a were registered in the sample we studied.

The results of assessment of carriage of missense polymorphisms of genes encoding mitochondrial electron-transport chain proteins (amino acid substitutions in proteins), as well as rRNA and tRNA genes, in IBS patients with life-threatening cardiac rhythm disturbances are presented in Fig. 2. These results were almost similar to those shown previously when mtDNA was analyzed in patients with CHD without life-threatening rhythm disturbances [9]. The situation is different when assessing the frequency of combined occurrence of two or more missense substitutions in genes of different electron-transport chain complexes, as well as simultaneously in 12S and 16S rRNA genes. Fig. 3 shows that in contrast to patients with uncomplicated CHD, in our study mtDNA of patients with life-threatening cardiac rhythm disorders were 1.5 times more likely to have multiple missense polymorphisms in genes of proteins belonging to two different electron-transport chain complexes. For genes with missense substitutions in three electron-transport chain complexes, as well as with simultaneous substitutions in 12S and 16S rRNA, these differences were even more pronounced and amounted to 1.6- and 1.7-fold, respectively.

DISCUSSION

On the basis of the obtained data we can say that in contrast to patients with uncomplicated CHD, patients of the group with complicated course of CHD are characterized by reduced functional capacity of mitochondria. Since these differences were obtained by examining mitochondria isolated from leukocytes and not from cardiomyocytes, they cannot be considered the result of specific pathologic factors characteristic of CHD. Rather, the hereditary nature of such differences can be hypothesized. In particular, given the possible role of mitochondria in arrhythmogenesis [12], the cause may be the influence of mtDNA polymorphism on the functional features of the electron-transport chain components encoded by it.

To test this assumption, we performed mtDNA genotyping of patients with CRD to determine the haplogroups to which individual mtDNAs belong. The results were compared with previously published data obtained for patients with CHD without life-threatening cardiac rhythm disturbances [9]. Fig. 1 shows that the predominant haplogroup in the studied samples is mtDNA of the H branch (41%). In addition to the haplogroups shown in the figure, single cases of mtDNA carriers of haplogroups A, X, U7, U8a1, N1b1, R1a, R0a, and N2a were registered in the sample we studied. Such results are in good agreement with the literature data. Thus, haplogroup H is known to be prevalent among Caucasoids [13]. Moreover, the association of mtDNA haplogroup H carriage with CHD or CHD-related phenotypes has been shown [9, 14, 15].

It is well known that the belonging of each individual's mtDNA to a certain haplogroup means that it is a carrier of all nucleotide substitutions that have consistently occurred in the history of this haplogroup and are reflected in the phylogenetic scheme of human mtDNA [11].

We evaluated the carriage and occurrence of missense polymorphisms of genes encoding mitochondrial electron-transport chain proteins (amino acid substitutions in proteins), as well as rRNA and tRNA genes in the sample under consideration. Figure 2 presents data reflecting the prevalence of such substitutions in CHD patients with life-threatening cardiac rhythm disturbances. These results were almost similar to those shown when mtDNA was analyzed in patients with CHD without life-threatening rhythm disturbances [9]. The situation is different when assessing the frequency of combined occurrence of two or more missense substitutions in genes of different electron-transport chain complexes, as well as simultaneously in 12S and 16S rRNA genes. As shown in Fig. 3, in contrast to patients with uncomplicated CHD, in our study mtDNA of patients with life-threatening cardiac rhythm disorders were 1.5 times more likely to have multiple missense polymorphisms in genes of proteins belonging to two different electron-transport chain complexes. For genes with missense substitutions in three electron-transport chain complexes, as well as with simultaneous substitutions in 12S and 16S rRNA, these differences were even more pronounced and amounted to 1.6- and 1.7-fold, respectively.

Our results are consistent with previously published data on the role of metabolic component in arrhythmogenesis in ischemic heart disease [16]. The higher frequency of carriage of combined amino acid substitutions in electron-transport chain proteins, as well as 12S and 16S rRNA, that we found requires further genetic studies. This will improve the accuracy of predicting the development of life-threatening cardiac rhythm disturbances in CHD.

CONCLUSION

1. Mitochondria of patients with coronary artery disease and life-threatening heart rhythm disorders have a reduced functional reserve.
2. The frequency distribution of the major mtDNA haplogroups of patients with coronary artery disease and life-threatening cardiac rhythm disorders is consistent with the population.
3. Patients with ischemic heart disease with life-threatening rhythm disturbances are characterized by a high frequency of carriage of combined polymorphisms in mtDNA genes encoding mitochondrial electron-transport chain proteins and mitochondrial rRNAs.

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