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INDIVIDUAL DIFFERENCES IN THE NUMBER OF MITOCHONDRIAL DNA COPIES: THE EFFECT OF SOCIO-DEMOGRAPHIC FACTORS

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According to the published data, the number of copies of mitochondrial DNA (mtDNA copy-number) reflects the efficiency of mitochondria functioning and, hence, the amount of cell-produced energy (in the form of ATP), which is an important indicator of the normal functioning of an organism. Differences in mtDNA copy-number are caused by a complex effect of various environmental factors including chemical, biological or physiological stress, age, and embryonic development. However, to date it remains unknown, which factors and at what age have the most significant impact on mtDNA copy-number and cause greater allostatic load. Therefore, the present study aimed to assess the involvement of various socio-demographic parameters in manifesting mtDNA copy-number in healthy individuals aged 18-25 years (N=1065). The analysis of the relative mtDNA copy-number was carried out by quantitative real-time PCR. As a result of multiple linear regression analysis including socio-demographic factors as predictors of the relative mtDNA copy-number, the optimal model was obtained (r2 = 0.03; F = 10.83; P <0.05), where age (β = -0.02; P <0.01) and childhood maltreatment (β = 0.05; P = 0.05) were the most statistically significant predictors. The data obtained confirm the role of mitochondria as key components in the physiological response to stress in humans and also indicate that the processes of gradual reduction in the energy efficiency of mitochondria begin even at the age of 18-25 years.

Keywords: mitochondrial DNA, mitochondria, environmental factors, mitochondrial theory of aging, allostatic load.

Introduction. Mitochondria represent intracellular organelles present in almost all eukaryotic cells, whose main function is to produce biochemical energy in the form of adenosine triphosphate (ATP) [1]. Moreover, mitochondria play an important role in the regulation of iron and calcium homeostasis, hormone synthesis, apoptosis, and n maintain the redox balance [18]. Mitochondria differ from all other animal organelles by the presence of their own DNA (mitochondrial DNA, mtDNA), which encodes 37 genes in humans: 2 ribosomal RNA genes, 22 transport RNA genes, 13 protein-encoding genes [4]. According to the published data, mtDNA copy-number reflects the efficiency of mitochondria functioning and, hence, the amount of energy produced by cells (ATP), which is an important indicator of the normal functioning of an organism [9]. Molecular epidemiological studies have demonstrated that mtDNA copy-number in leukocytes might increase the risk of developing cancer, diabetes, cardiovascular diseases, aging-associated diseases, and mental disorders such as depression [11]. The mtDNA copy-number in the human body is strictly regulated during cell differentiation; moreover, the cells requirement for ATP positively correlates with mtDNA copy-number [8]. In addition, a number of studies demonstrating that environmental factors can influence mtDNA copy-number exist. Together with an environmental stress related to the environmental pollution, mtDNA copy-number can also be affected by psychoemotional stress associated with psychological environment, which affected individual especially in early childhood [13]. Moreover, health state of an organism and individual lifestyle represent the important factors that directly affect the optimal functioning of mitochondria, including mtDNA copy-number [19]. However, to date the certain factors and the age of the most significant effect on the changes in mtDNA copy-number, which cause a greater allostatic load remain unknown. In turn, the identification of such environmental predictors of the mtDNA copy-number will allow us to better understand the nature of several socially significant diseases. Therefore, the present study aimed to assess the involvement of various socio-demographic parameters in individual variance in mtDNA copy-number in healthy individuals even in early adulthood.

Materials and methods. The study included 1065 individuals (79.25% women, aged 18–25 years), who were students at the Universities of the Republic of Bashkortostan and the Udmurt Republic. The examined sample consisted of Russians – 357, Tatars – 340, Udmurts – 234, individuals of mixed ethnicity – 134. Participation in the study was voluntary, informed consent was obtained from all the participants. The design of the study was approved by the Ethics Committee of the IBG UFRC RAS. Information on the studied socio-demographic parameters was obtained via questionnaires, including 16 different indicators.

A quantitative analysis of the mtDNA copy number was carried out using real-time PCR on the CFX96 DNA Analyzer (BioRad, USA) according to the Wang et al. (2018) [16]. Each experimental sample was analyzed in triplicate. For further analysis, the average value of the Ct threshold cycle for the ND1 and HGB genes was used. A relative mtDNA copy number was estimated by the formula 2- ΔΔCt, where ΔΔCt = (Ct mtDNA (sample) – Ct ntDNA (calibrator)) - (Ct HGB

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(sample) – Ct HGB (calibrator)). Pooled DNA of several healthy individuals (calibrator) was used as a control sample and was identical in each run of the analyzer. Statistical analysis was carried out using a series of multiple linear regressions with an inclusion of examined socio-demographic parameters as the independent variables, followed by a backward stepwise exclusion of the least significant predictor until the most significant model was obtained. During the backward stepwise selection of variables, the Akaike information criterion, the coefficient of determination (r2) and the statistical significance level (p-value) were used to obtain the optimal model (according to the number of variables). Statistical analysis and data visualization was carried out under R v.4.1.2. The level of statistical significance was assumed to be 0.05.

Results and discussion. The mean values and a list of the studied socio-demographic indicators are shown in Table.

As a result of multiple regression analysis including 16 various socio-demographic factors as predictors of relative copy-number of mtDNA we obtained the optimal model ($R2 = 0.03$; $F = 10.83$; $P < 0.05$) including age ($\beta = -0.02$; $P < 0.01$) and maltreatment in childhood ($\beta = 0.05$; $P = 0.05$) (Table 2). According to the constructed model, relative copy-number of mtDNA was increased in individuals, who experienced maltreatment in childhood, whereas a negative association was reported with age (Fig. 1).

According to the published data, early adverse events may cause irreversible changes in a number of biochemical and molecular-genetic parameters [21], including changes in the number of mtDNA copy-number [14]. One of the potential systems, which can modulate these modifications, is the endocrine system, which functioning is mediated by an activation of the hypothalamic-pituitary-adrenal axis in individuals experienced childhood abuse [7]. Stress is known to induce the synthesis of glucocorticoids, which trigger molecular mechanisms [12] resulting in the changed mitochondrial density and functioning via modified expression of mitochondrial and nuclear genes [17]. Moreover, glucocorticoids can regulate the functioning of mitochondria by activating glucocorticoid receptors on mitochondrial membranes, thus regulating their membrane potential and triggering intracellular signaling pathways that significantly affect biogenesis and functional activity of mitochondria [12, 17].]. In addition, stressful life events occurring at an early age, which also include childhood maltreatment, are known to increase oxidative stress, which, in turn, causes mitochondrial damage [15]. To date, specific mechanisms responsible for regulating the mtDNA copy-number remain unknown, however, a hypothesis on the relation between the initiation of mtDNA replication and mitochondrial dysfunction caused by the effect of hypothalamic-pituitary system hormones was suggested [5]. The experiments with model animals demonstrated that mtDNA copy-number was increased by 210% in the group of stress- or corticosterone-exposed mice compared to the control group [3]. Therefore, the mtDNA copy-number seems to reflect the functional changes occurred in mitochondria, which affect the energy production by cells. Assuming the abovementioned data, stress significantly impacts the organism's state, primarily, homeostasis of cells, organs and tissues, resulting in enhanced demand for energy, which synthesis depends on mitochondria.

Table1

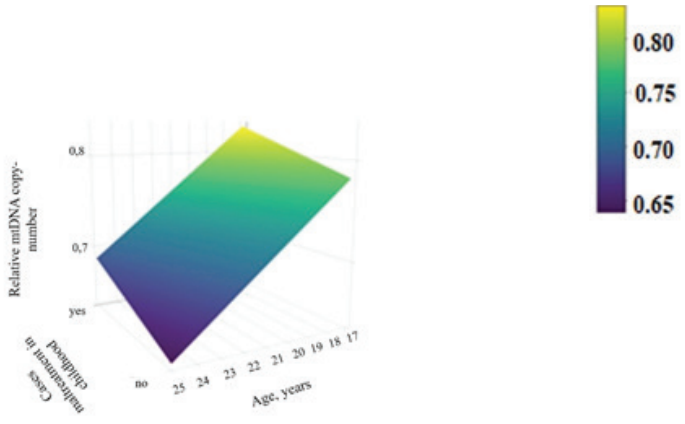
Studied socio-demographic parameters

Parameter	N (%)	Parameter	N (%)
Sex		Childhood maltreatment	
Men	221 (20.75)	yes	104 (9.78)
Women	844 (79.25)	no	961 (90.22)
Ethnicity		Bilingual rearing	
Russians	357 (33.52)	yes	591 (55.49)
Tatars	340 (31.92)	no	474 (44.51)
Udmurts	234 (21.97)		
Mixed	134 (12.59)		
Place of residence		Chronic disorders	
Urban	609 (57.14)	yes	344 (32.26)
Rural	456 (42.86)	no	721 (67.74)
Birth order		Smoking	
1	646 (60.65)	yes	95 (8.88)
2	327 (30.69)	previously	91 (8.57)
>3	92 (8.66)	non-smoking	879 (82.55)
Sibship size		Maternal care	
1	216 (20.29)	high	772 (72.49)
2	564 (53.00)	low	293 (27.51)
>3	285 (26.71)		
Premature birth		Maternal protection	
no	964 (90.53)	high	590 (55.44)
yes	101 (9.47)	low	475 (44.56)
Income level		Paternal care	
Under intermediate	113 (10.57)	high	564 (52.95)
Intermediate	878 (82.45)	low	501 (47.05)
Above intermediate	74 (6.98)		
Rearing in a full family		Paternal protection	
yes	892 (83.72)	high	500 (46.91)
no	173 (16.28)	low	565 (53.09)

Table2

The predictors of mtDNA copy-number variance included in the model

Predictor	β	Standard error	t-criterion	p
Intercept	1.09	0.10	11.46	<0.01
Childhood maltreatment	0.05	0.02	1.96	0.05
Age	-0.02	< 0.01	-3.92	<0.01



Relation between a relative mtDNA copy-number and childhood maltreatment

The second important result obtained in the present study is a negative association between individual age mtDNA copy-number, which is consistent with the mitochondrial theory of aging. According to this theory, the accumulation of damages within mtDNA replication (mtDNA has a lower replication accuracy and repair efficiency compared to nuclear DNA) results in impaired processes of oxidative phosphorylation and ATP synthesis [10]. In turn, these disturbances are accompanied by enhanced levels of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radical. ROS can cause oxidative damage to proteins, lipids, nucleic acids, redox enzymes, as well as cell membranes and organelles, including mitochondria, also promoting diminished ATP synthesis [20]. Moreover, ROS can cause new impairments in mtDNA, resulting to greater increase in ROS level in the organism. The mitochondrial theory of aging is confirmed by literature data indicating an increased heteroplasmy and a decreased mtDNA copy-number with age [6, 18], as well as by the results of the present.

Conclusion. Therefore, within the present study, the effect of a number of socio-demographic parameters on inter-individual variance in mtDNA copy-number was examined in healthy individuals aged 18-25 years. Among 16 parameters, statistically significant findings were shown for such variables as childhood maltreatment and age, confirming the role of mitochondria as key components in the physiological response to stress in humans. Previously, a decreased mtDNA copy-number was considered to start at ~50 years [18]; however, within the present study it was primarily shown that a negative relation between age and mtDNA copy-number was statistically significant even at the student age, hence, the processes of gradual decrease in the energy efficiency of mitochondria begin even at this age. Several studies describing the changes in the mtDNA copy-number accompanying the clinical symptoms of depression [11] and post-traumatic stress disorder [2] have been published, which indicates the role of mitochondrial dysfunction in the pathophysiological mechanisms of mental illness. A relation of the mtDNA copy-number and the psychoemotional state of a person has been also confirmed by the present study, thus, individual mtDNA copy-number varied depending on the presence of negative childhood experiences; however, the causal relationships have to be studied. Therefore, the results obtained by our group open up new opportunities for further research in gerontology and psychiatry.

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THE USE OF REAL-TIME PCR FOR THE DIAGNOSIS OF Z GENE MUTATION PI IN PATIENTS WITH ALPHA-1 ANTITRYPSIN DEFICIENCY

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The aim of this study is to develop a method for diagnosing PiZ mutation associated with alpha-1 antitrypsin deficiency using real-time PCR technology.

The number of patients and the control group was 503 and 81 individuals, respectively.

A simple method is proposed for detecting one of the most frequent mutations of the Pi gene PiZ associated with alpha-1 antitrypsin deficiency. Priority exclusion of the PiZ mutation as the most significant one may allow to speed up diagnosis and make it more accessible to practical healthcare.

Keywords: SERPINA1; alpha-1-antitrypsin deficiency, real-time PCR.

Introduction. The aim of this study is to develop a method for diagnosing frequent mutations associated with alpha-1 antitrypsin deficiency using real-time PCR technology.

Alpha-1 antitrypsin deficiency is a hereditary disease associated with a number of mutations in the Pi protease inhibitor gene. The gene controlling the structure of AAT - SERPINA1 is located on the long arm of chromosome 14 (14q31-32.2), contains 7 exons (four coding (2-4 and 5) and three non-coding (1a, 1b, 1c), for which more than 200 [5] allelic variants are known, inherited by autosomal codominant type (OMIM 107400).

The designation of alleles of the Pi gene is carried out by letters of the Latin alphabet from A to Z, depending on the position of the product in the gel during isoelectric focusing. The most common variants of alleles are PiM alleles, in which the concentration of AAT in the blood serum is within normal values (90-200 mg/dl or 16.5-36.8 μmol/L according to the method we used). Alleles associated with insufficient protein levels are registered much less frequently and in some cases are characterized, in addition to deficiency, by a decrease in the functional activity of AAT, such as the Z allele, which is the most significant in clinical practice [4]. It is known that 95% of patients with AATD have the PiZZ genotype [2].

Alpha-1 antitrypsin deficiency is a widespread, but to this day rarely diagnosed disease [6]. Diagnosis is often delayed for several years and there may be many undiagnosed individuals with AATD in the population [9]. Since PiZ is the most common pathological allele in most populations, depending on the spectrum of mutations in a particular population, it makes sense to start molecular diagnostics with it. The proposed method of genotyping can help speed up and make the diagnosis of AATD more accessible.

One of the most popular and widespread methods of molecular diagnostics is Real-Time PCR, which has a number of advantages over the classical method with the detection of PCR products by gel electrophoresis and in some cases, for example, over Luminex technology used for multiplex genotyping [8]:

- Reducing the risk of contamination and, accordingly, obtaining unreliable results. The method does not require working directly with the PCR product;
- Acceleration of the experiment as a result of the exclusion of gel electrophoresis, the method of restriction fragment length polymorphism (RFLP) and additional steps associated with the use of separate detection systems;
- The possibility of full automation of the test from DNA extraction to interpretation of the result;
- More accessible reagents and equipment for real-time PCR. The actions against the ongoing SARS-CoV-19 coronavirus pandemic and the use of RT-PCR as the main method of early diagnosis has contributed to a significant expansion of the global fleet of real-time PCR equipment. Laboratories working mainly on this technology are widespread in practical healthcare.

We propose a method for verifying the PiZ mutation using real-time PCR technology. The priority exclusion of the PiZ mutation as the most significant one can speed up the diagnosis of alpha-1 antitrypsin deficiency and make it more accessible for practical healthcare.

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