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Mitochondrial mutations associated with cardiac angina

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Abstract

Aim: Cardiac angina is a disease in which discomfort or retrosternal pain may occur. Atherosclerosis of coronary arteries is one of the main risk factors for cardiac angina. The aim of the investigation was to analyze the association of 11 mitochondrial genome mutations with cardiac angina. In our preliminary studies an association of these mutations with atherosclerosis, a risk factor for cardiac angina, was found.

Methods: We used samples of white blood cells collected from 192 patients with cardiac angina and 201 conventionally healthy study participants. DNA from blood leukocyte samples was isolated using a phenol-chloroform method. DNA amplicons containing the investigated regions of 11 mitochondrial genome mutations (m.12315G>A, m.652delG, m.5178C>A, m.14459G>A, m.3336T>C, 652insG, m.3256C>T, m.1555A>G, m.15059G>A, m.13513G>A, m.14846G>A) were pyrosequenced. The heteroplasmy level of mitochondrial DNA (mtDNA) mutations was analyzed using a method developed by our laboratory on the basis of pyrosequencing technology.

Results: According to the obtained data, three mitochondrial mutations of human genome correlated with cardiac angina. A positive correlation was observed for mutation m.14459G>A ($P \leq 0.05$). One single nucleotide substitution m.5178C>A ($P \leq 0.1$) had a trend for positive correlation. A negative correlation for mutation m.15059G>A with cardiac angina ($P \leq 0.05$) was found.

Conclusion: MtDNA mutations m.14459G>A and m.5178C>A can be used for evaluation the predisposition of individuals to atherosclerotic lesions. At the same time, mitochondrial genome mutation m.15059G>A may be used for gene therapy of atherosclerosis.

Keywords: Cardiac angina, gene, mutation, heteroplasmy level, mitochondrial genome, molecular cellular models

INTRODUCTION

Cardiac angina is a disease in which discomfort or retrosternal pain may occur. Discomfort is felt by patients as pressure or retrosternal burning. Pain often occurs during physical exertion, excessive food ingestion, stress, being in cold air or with a sharp increase in blood pressure[1-5]. Cardiac angina is supposed to be caused by narrowing of the arterial lumen up to 50%-75%. As a result, there is a discrepancy between the blood flow to the heart and its need for blood. In this case, acute insufficiency of blood supply to the heart happens. The redox processes in the heart muscle become disrupted[2-6]. An excessive accumulation of insufficiently oxidized metabolic products (lactic, pyruvic, carbonic and phosphoric acids) and other metabolites occurs. Cardiac angina occurs most often in men over 40, and in women over 50 years. The prevalence of cardiac angina increases with age. For example, in patients who were older than 65 years, the frequency of occurrence of cardiac angina reached 10%-20%. One of the main risk factors for cardiac angina is a atherosclerosis of coronary arteries[2-6]. Other risk factors for cardiac angina include hypertension, diabetes mellitus, obesity, smoking; stress, hypodynamia, infectious diseases, allergic lesions and genetic mutations[7-12].

Molecular genetic markers for cardiac angina could help identification of predisposition to the disease much earlier than clinical methods for examining patients. At the present time, such studies are mainly devoted to polymorphisms of the genes in nuclear genome.

Our research group found a number of mitochondrial genome mutations associated with cardiac angina. It should be noted that in the study we investigated those mitochondrial mutations for which, in our preliminary studies, we detected an association with atherosclerosis[13-17]. Since atherosclerosis is a risk factor for cardiac angina, we decided to investigate whether these mutations are linked with cardiac angina.

It should be noted that during the investigation of the mitochondrial genome mutations, the level of heteroplasmy is determined. The ratio of the number of mutant mitochondrial DNA (mtDNA) copies in a sample to the total number of mtDNA copies is estimated[13-17]. This is the difference between quantitative analysis of mutations in the mitochondrial genome and the analysis of nuclear mutations. In the quantitative analysis of nuclear genome mutations, the number of homozygotes in which both alleles are either mutant or normal. The number of heterozygotes is detected too. Afterwards the mutation frequency in the investigated sample is estimated[18,19].

The level of heteroplasmy in mitochondrial genome mutations was measured using a quantitative method developed in our laboratory[14,17,20]. This method is based on the pyrosequencing technology[21,22]. Short DNA fragments (6-10 bp), containing the area of mutation were investigated. Such a small length of the studied DNA fragments significantly reduces the number of errors during sequencing.

METHODS

We used samples of white blood cells collected from 192 patients with cardiac angina and 201 conventionally healthy study participants. These individuals were examined in Moscow State University clinic. In order to

Table 1. The size of DNA amplicons and primers for PCR

Mutation	Primers	Size of DNA amplicons
m.12315G>A	F: bio-CTCATGCCCCCATGTCTAA(12230-12249) R: TTACTTTTATTTGGAGTTGCAC(12337-12317)	108 bp
m.652delG	F: TAGACGGGCTCACATCAC(621-638) R: bio-GGGGTATCTAATCCCAGTTTGGGT(1087-1064)	467 bp
m.3336T>C	F: bio-AGGACAAGAGAAATAAGGCC(3129-3149) R: ACGTTGGGGCCTTTGCGTAG(3422-3403)	294 bp
m.14459G>A	F: CAGCTTCCTACACTATTAAAGT(14303-14334) R: bio-GTTTTTTTAATTTATTTAGGGGG(14511-14489)	209 bp
m.5178C>A	F: bio-GCAGTTGAGGTGGATTAAAC(4963-4982) R: GGAGTAGATTAGGCGTAGGTAG(5366-5345)	383 bp
m.13513G>A	F: CCTCACAGGTTTCTACTCCAAA(13491-13512) R: bio-AAGTCCTAGGAAAGTGACAGCGAGG(13825-13806)	335 bp
m.652insG	F: TAGACGGGCTCACATCAC(621-638) R: bio-GGGGTATCTAATCCCAGTTTGGGT(1087-1064)	467 bp
m.3256C>T	F: bio-AGGACAAGAGAAATAAGGCC(3129-3149) R: ACGTTGGGGCCTTTGCGTAG(3422-3403)	294 bp
m.15059G>A	F: bio-CATTATTCTCGCACGGACT(14671-14689) R: GCTATAGTTGCAAGCAGGAG(15120-15100)	450 bp
m.1555A>G	F: TAGGTCAAGGTGTAGCCCATGAGGTGGCAA(1326-1355) R: bio-GTAAGGTGGAGTGGGTTTGGG(1704-1684)	379 bp
m.14846G>A	F: bio-CATTATTCTCGCACGGACT(14671-14689) R: GCTATAGTTGCAAGCAGGAG(15120-15100)	450 bp

bp: base pairs

compare the samples of patients with cardiac angina and conventionally healthy study participants more correctly, the samples were composed so that they did not have significant differences in age and sex.

The work was conducted in complance with the Declaration of Helsinki. The study protocol has been accepted by Ethics Community of National Medical Research Center of Cardiology, and all subjects signed an informed consent for inclusion in the research.

DNA from blood leukocyte samples was isolated using a phenol-chloroform method[13,14,23-25]. DNA amplicons containing the investigated regions of 11 mitochondrial genome mutations (m.12315G>A, m.652delG, m.5178C>A, m.14459G>A, m.3336T>C, 652insG, m.3256C>T, m.1555A>G, m.15059G>A, m.13513G>A, m.14846G>A) were pyrosequenced. The heteroplasmy level of mtDNA mutations was analyzed using a method developed by our laboratory.

The size of DNA amplicons and primers for PCR are listed in Table 1[13-16,20].

In order to be able to perform pyrosequencing of DNA amplicons, one of the primers for PCR was biotinylated.

The total volume of PCR reaction mixtures for each sample was 30 mL. The composition of the reaction mixture for PCR[13-16,20]: 0.4-0.6 mg mitochondrial DNA, 0.3 pmol/L of each primer, 200 mmol/L of each deoxyribonucleotriphosphate, 16.6 mmol/L $(NH_4)_2SO_4$, $MgCl_2$ (1.5 mmol/L for mutations m.14846G>A, m.15059G>A and m.14459G>A; 2.5 mmol/L for the rest of investigated mutations), 67 mmol/L tris-HCl (pH 8.8), and 3 units of Taq-polymerase.

In PCR, the following annealing temperature was used for the primers[13-16,20]:
1. For mutations m.3336T>C, m.14846G>A, m.13513G>A, m.15059G>A and m.3256C>T - 55 °C;
2. For mutations m.5178C>A, m.652delG and m.652insG - 60 °C;
3. For mutations m.12315G>A, m.14459G>A and m.1555A>G - 50 °C.

Table 2. Primers for pyrosequencing

Mutation	Primer
m.12315G>A	TTTGGAGTTGCAC(12328-12316)
m.652delG	CCCATAAACAAATA(639-651)
m.3336T>C	TGCGATTAGAATGGGTAC(3354-3337)
m.14459G>A	GATACTCCTCAATAGCCA(14439-14456)
m.5178C>A	ATTAAGGGTGTTAGTCATGT(5200-5181)
m.13513G>A	AGGTTTCTACTCCAA(13497-13511)
m.652insG	CCCATAAACAAATA(639-651)
m.3256C>T	AAGAAGAGGAATTGA(3300-3286)
m.15059G>A	TTTCTGAGTAGAGAAATGAT(15080-15061)
m.1555A>G	ACGCATTTATATAGAGGA(1537-1554)
m.14846G>A	GCGCCAAGGAGTGA(14861-14848)

PCR was conducted using "PTC DNA Engine 200"[13-16,20].

The DNA amplicons were analyzed on automated pyrosequencing device PSQTMHS96MA (Biotage, Sweden)[10,11]. Primers for pyrosequencing are listed in Table 2[13-16,20].

For statistical analysis of the obtained results software package SPSS 22.0 was used[26]. Bootstrap analysis was also conducted. Correlation was considered statistically significant at the level of $P \leq 0.05$. The results at the significance level of $P \leq 0.1$ were considered to show a tendency to statistical significance.

RESULTS

The age characteristics for study participants are presented in Table 3. The age of conventionally healthy participants ranged from 51 to 73 years. In the meantime, the age of patients with cardiac angina ranged from 52 to 76 years. The average age of conventionally healthy study participants was 2 years less than the age of patients with cardiac angina. This age difference between samples of patients with cardiac angina and conventionally healthy participants was not statistically significant.

Demographic characteristics for study participants are presented in Table 4. The data in Table 4 is presented as an average value with indicating the standard deviation (in parentheses).

According to Table 4, statistically significant differences by clinical and anthropometric characteristics between samples of patients with cardiac angina and conventionally healthy study participants were not found.

The aim of the investigation was to analyze the association of 11 mitochondrial genome mutations with cardiac angina: m.12315G>A, m.652delG, m.5178C>A, m.14459G>A, m.3336T>C, 652insG, m.3256C>T, m.1555A>G, m.15059G>A, m.13513G>A, m.14846G>A. In our preliminary studies, an association of these mutations with atherosclerosis, a risk factor for cardiac angina, was identified. Therefore, we decided to investigate whether these mutations have a link with cardiac angina.

Statistical analysis of the link of these mitochondrial genome mutations with cardiac angina is presented in Table 5.

As illustrated in Table 5, three mitochondrial mutations of human genome correlated with cardiac angina. A positive correlation was observed for mutation m.14459G>A ($P \leq 0.05$). One single nucleotide substitution m.5178C>A ($P \leq 0.1$) had a trend for positive correlation with this disease. We suppose that in case of

Table 3. Age characteristics of the study participants

Investigated individuals	Age			Standard deviation
	Minimum, (years)	Mean, (years)	Maximum, (years)	
Conventionally healthy study participants	51	62	73	8.3
Patients with cardiac angina	52	64	76	8.1

Table 4. Demographic characteristics of the study participants

Parameter	Conventionally healthy study participants	Patients with cardiac angina	Significance of differences
Sex, M/F	91:101	103:98	0.146
Age, years	62 (8.3)	64 (8.1)	0.111
Body mass index, kg/m^2	24.8 (5.9)	26.5 (6.3)	0.152
Systolic blood pressure, mmHg	123 (16)	147 (26)	0.214
Diastolic blood pressure, mmHg	82 (18)	91 (23)	0.319
Smoking, %	29	38	0.167

expansion of the sample, positive correlation m.5178C>A with cardiac angina will become significant. For mutation m.15059G>A a significant negative correlation with this disease was found ($P \leq 0.05$).

DISCUSSION

From the data obtained in this study, it can be concluded that mitochondrial genome mutations m.14459G>A and m.5178C>A are risk factors for the occurrence and development of cardiac angina. Meanwhile, the mutation m.15059G>A had a protective effect in this disease.

The detected mutations were localised in the coding region of mtDNA. Single nucleotide replacements m.14459G>A and m.5178C>A were localised in the genes of the second and sixth subunits of NADH dehydrogenase. We assume that the defects of this mitochondrial respiratory chain enzyme is a trigger of pathological mechanisms in the human body, as a result of which ATP deficiency occurs. Energy deficit, in turn, leads to the emergence and development of cardiac angina.

At the same time, mtDNA mutation m.15059G>A is localised in the cytochrome B gene. Perhaps this mutation is involved in molecular cell processes which protect a person from the occurrence of cardiac angina.

Mitochondrial genome mutations m.14459G>A and m.5178C>A may be candidates for the creation of molecular cell models in the development of drug therapy for patients with cardiac angina. Mutation m.15059G>A can be used for creating gene therapy approaches to this disease.

Molecular genetic markers for cardiac angina could help the identification of predisposition to the disease much earlier than clinical methods for examining patients. At the present time, such studies are mainly devoted to polymorphisms of nuclear genome genes. Studies of mitochondrial genome mutations in cardiac angina are practically absent. Therefore, analysis of the association of mtDNA mutations with cardiac angina, conducted by our research group, is very relevant.

In conclusion, according to the obtained data, three mitochondrial mutations of human genome correlated with cardiac angina. A positive correlation was observed for mutation m.14459G>A ($P \leq 0.05$). One single nucleotide substitution m.5178C>A ($P \leq 0.1$) had a trend for positive correlation. A negative correlation for mutation m.15059G>A with cardiac angina ($P \leq 0.05$) was found.

Table 5. Spearman correlation analysis of 11 mitochondrial genome mutations with cardiac angina

Mutation	Correlation coefficient	Significance
m.12315G>A	0.079	0.121
m.652delG	0.061	0.224
m.3336T>C	0.047	0.342
m.14459G>A	0.116	0.034**
m.5178C>A	0.092	0.068*
m.13513G>A	-0.075	0.129
m.652insG	-0.078	0.124
m.3256C>T	0.069	0.218
m.15059G>A	-0.122	0.036**
m.1555A>G	-0.065	0.217
m.14846G>A	-0.068	0.222

$^{**}P \leq 0.05$; $^{*}P \leq 0.1$

MtDNA mutations m.14459G>A and m.5178C>A can be used for evaluation the predisposition of individuals to atherosclerotic lesions. At the same time, mitochondrial genome mutation m.15059G>A may be used for gene therapy of atherosclerosis.

DECLARATIONS

Authors' contributions

Conception, design and statistical analysis: Sazonova MA
Pyrosequencing of PCR fragments: Sazonova MA, Sinyov VV
PCR: Ryzhkova AI, Shkurat TP
DNA extraction: Sazonova MD, Nikitina NN
Administrative and material support: Sobenin IA, Orekhov AN

Availability of data and materials

The data were strictly obtained from medical records according to the privacy policy and ethics code of our institute.

Financial support and sponsorship

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The study was carried out in accordance with the Declaration of Helsinki. The study protocol was inspected and approved by the Ethics Committee of the Institute of General Pathology and Pathophysiology. Each study participant has signed a written informed consent to participate in this investigation.

Consent for publication

Not applicable.

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