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# MtDNA mutations linked with left ventricular hypertrophy

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## Abstract

**Aim:** In left ventricular hypertrophy (LVH), the heart muscle thickens. One third of individuals with LVH never complain of heart problems. However, such patients have a high risk of sudden death. LVH can be caused by arterial atherosclerotic lesions. The linkage of mtDNA mutations 652insG, m.5178C>A, m.3336T>C, m.14459G>A, 652delG, m.14846G>A, m.1555A>G, m.15059G>A, m.3256C>T, m.12315G>A and m.13513G>A with atherosclerosis was described earlier by our laboratory. The aim of the study was to analyze the linkage of these mtDNA mutations with LVH.

**Methods:** DNA from white blood cells was isolated using a phenol-chloroform method. PCR-fragments of DNA contained the region of the investigated mutations. The heteroplasmy level of mtDNA mutations was analyzed using a pyrosequencing-based method developed by our laboratory.

**Results:** We investigated two groups of individuals. One hundred and ninety-four patients with LVH. Two hundred and ten were conventionally healthy. It was found that mtDNA mutation m.5178C>A was significantly associated with LVH. Single nucleotide replacement m.1555A>G was associated with LVH at the level of significance  $P \leq 0.1$ . At the same time m.12315G>A and m.3336T>C were significantly associated with the absence of this pathology. Single nucleotide replacement m.14459G>A was associated with the absence of LVH at the significance level  $P \leq 0.1$ .

**Conclusion:** MtDNA mutations m.5178C>A and m.1555A>G can be used for molecular genetic assessment of the predisposition of individuals to the occurrence of left ventricular hypertrophy. They can also be used for the family analysis of this pathology. Mutations m.12315G>A, m.3336T>C and m.14459G>A can be used in the development of LVH gene therapy methods.



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**Keywords:** Left ventricular hypertrophy, heteroplasmy level, mutation, mitochondrial genome, mtDNA

## INTRODUCTION

In case of left ventricular hypertrophy (LVH), the heart muscle thickens. Often septum between the left and right ventricles mutates in this disease<sup>[1]</sup>. In LVH muscle fibers in the myocardium are arranged randomly. The main criterion for LVH is considered to be an increase in myocardial thickness larger than or equal to 1.5 cm in the presence of left ventricular diastolic dysfunction<sup>[1-3]</sup>. The third part of individuals with LVH never complain of heart problems. However, such patients have a high risk of sudden death, which reaches 4% per year<sup>[2,3]</sup>. Echocardiography helps to identify such patients. It can be used to identify a left ventricle and left atrium enlargements. It can also detect heart rhythm disorders. This helps to assess the risk of sudden death. About 50% of deaths from left ventricular hypertrophy per year happen precisely because of ventricular arrhythmias. The second cause of death of patients with LVH is congestive heart failure. It is most common in patients older than 40 years<sup>[4,5]</sup>.

The onset and development of left ventricular hypertrophy can be caused by atherosclerotic lesions of the arteries, in particular, atherosclerotic plaques and thickening of the intima-medial layer of these vessels<sup>[6-10]</sup>. Risk factors for LVH include diabetes mellitus, stress, smoking, hyperlipoproteinemia, hypodynamia, arterial hypertension, hyperfibrinogenemia, homocysteinemia, obesity, hypothyroidism and metabolic syndrome<sup>[11-15]</sup>.

In addition, left ventricular hypertrophy may occur due to hereditary and somatic mutations of the human genome. At present, many scientists are studying, basically, the single-nucleotide polymorphism (SNP) of the nuclear genome associated with this pathology<sup>[16-20]</sup>. However, nuclear polymorphisms are associated only with a small number of LVH cases. Meanwhile mitochondrial genome mutations with left ventricular hypertrophy were analyzed by a very small number of research groups around the world<sup>[21-23]</sup>.

It should be noted that in human cells there are plenty of mitochondria. Each mitochondria contains several copies of the mitochondrial genome. Therefore, during the analysis of DNA samples from the study participants it is necessary to determine the heteroplasmy level of each investigated mitochondrial genome mutation (ratio of mtDNA molecules containing the mutation to the total number of mtDNA molecules)<sup>[24-28]</sup>. It differs significantly from SNP analysis of the nuclear genome, where it is necessary to identify homozygous and heterozygous individuals according to this SNP.

The linkage of mtDNA mutations 652insG, m.5178C>A, m.3336T>C, m.14459G>A, 652delG, m.14846G>A, m.1555A>G, m.15059G>A, m.3256C>T, m.12315G>A and m.13513G>A with atherosclerosis was described earlier by our laboratory researchers<sup>[24,28-31]</sup>. Since LVH has common risk factors with atherosclerosis, it was decided to analyze the relationship of these mutations to mtDNA with left ventricular hypertrophy.

## METHODS

In this study two groups of study participants were examined. One hundred and ninety-four patients had left ventricular hypertrophy. Two hundred and ten study participants were conventionally healthy. For identifying patients with LVH among the study participants, the method of echocardiography was used. The main criterion of LVH was considered to be an increase in myocardial thickness of more than or equal to 1.5 cm in the presence of left ventricular diastolic dysfunction. Individuals with diabetes mellitus, hypercholesterolemia and patients, who used drugs, were excluded from the study. In order to compare the samples of patients with LVH and conventionally healthy study participants more correctly, the composition of the samples was changed so that they did not contain significant differences in age, sex, diastolic and systolic blood pressure.

Clinical, anthropometric and age characteristics were determined for patients with left ventricular hypertrophy and conventionally healthy individuals [Tables 1 and 2]

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 National Medical Research Center of Cardiology. Each study participant has signed a written informed consent to participate in this investigation.

DNA from white blood cells was isolated using the phenol-chloroform method<sup>[32-34]</sup>. PCR-fragments of DNA were obtained. They contained the region of studied mutations (652insG, m.5178C>A, m.3336T>C, m.14459G>A, 652delG, m.14846G>A, m.1555A>G, m.15059G>A, m.3256C>T, m.12315G>A and m.13513G>A). It should be noted that biotin was attached to one of the DNA chains of the PCR fragment using the primer “bio-” (biotinylated). This was necessary for the analysis of the biotinylated DNA chain of the investigated amplificate using the pyrosequencing<sup>[35-37]</sup>.

PCR primer sequences, taken for the present research<sup>[24,26,28-30]</sup>:

1. For m.652insG  
F: TAGACGGGCTCACATCAC (621-638)  
R: bio-GGGGTATCTAATCCCAGTTTGGGT (1087-1064)
2. For m.5178C>A  
F: bio-GCAGTTGAGGTGGATTAAAC (4963-4982)  
R: GGAGTAGATTAGGCGTAGGTAG (5366-5345)
3. For m.3336T>C  
F: bio-AGGACAAGAGAAATAAGGCC (3129-3149)  
R: ACGTTGGGGCCTTTGCGTAG (3422-3403)
4. For m.14459G>A  
F: CAGCTTCCTACACTATTAAAGT (14303-14334)  
R: bio-GTTTTTTTAATTTATTTAGGGGG (14511-14489)
5. For m.652delG  
F: TAGACGGGCTCACATCAC (621-638)  
R: bio-GGGGTATCTAATCCCAGTTTGGGT (1087-1064)
6. For m.14846G>A  
F: bio-CATTATTCTCGCACGGACT (14671-14689)  
R: GCTATAGTTGCAAGCAGGAG (15120-15100)
7. For m.1555A>G  
F: TAGGTCAAGGTGTAGCCCATGAGGTGGCAA (1326-1355)  
R: bio-GTAAGGTGGAGTGGGTTTGGG (1704-1684)
8. For m.15059G>A  
F: bio-CATTATTCTCGCACGGACT (14671-14689)  
R: GCTATAGTTGCAAGCAGGAG (15120-15100)
9. For m.3256C>T  
F: bio-AGGACAAGAGAAATAAGGCC (3129-3149)  
R: ACGTTGGGGCCTTTGCGTAG (3422-3403)
10. For m.12315G>A  
F: bio-CTCATGCCCCCATGTCTAA (12230-12249)  
R: TTACTTTTATTTGGAGTTGCAC (12337-12317)
11. For m.13513G>A  
F: CCTCACAGGTTTCTACTCCAAA (13491-13512)  
R: bio-AAGTCCTAGGAAAGTGACAGCGAGG (13825-13806)

**Table 1. Clinical and anthropometric characteristics of the groups of individuals**

Characteristic	Patients with left ventricular hypertrophy/standard deviation	Conventionally healthy individuals/standard deviation	Significance of differences
Diastolic blood pressure, mmHg	86/23	83/17	0.395
Systolic blood pressure, mmHg	128/23	122/21	0.228
Age, years	64/8.6	58/8.2	0.119
Smoking, %	35	28	0.111
Sex (man/women)	112/82	94/116	0.118
Body mass index, kg/m <sup>2</sup>	33.8/4.5	28.9/4.1	0.149
Total cholesterol, mol/L	6.51/1.12	6.45/1.05	0.135
Triglycerides, mol/L	1.65/0.64	1.48/0.61	0.115
High-density lipoprotein, mol/L	1.55 (0.53)	1.68 (0.44)	0.104
Low-density lipoprotein, mol/L	4.36 (1.23)	4.04 (1.21)	0.142

**Table 2. Age of participants in the study groups**

Groups of individuals	Age minimum	Mean age	Age maximum	Standard deviation
Patients with left ventricular hypertrophy	53 years old	64 years old	75 years old	8.3
Conventionally healthy individuals	54 years old	58 years old	62 years old	7.9

PCR fragments of the following size were obtained<sup>[24,26,28-30]</sup>:

- (1) m.652insG - 467 bp;
- (2) m.5178C>A - 383 bp;
- (3) m.3336T>C - 294 bp;
- (4) m.14459G>A - 209 bp;
- (5) m.652delG - 467 bp;
- (6) m.14846G>A - 450 bp;
- (7) m.1555A>G - 379 bp;
- (8) m.15059G>A - 450 bp;
- (9) m.3256C>T - 294 bp;
- (10) m.12315G>A - 108 bp;
- (11) m.13513G>A - 335 bp.

The reaction mixture for PCR was 30 µL. It contained:

- (1) 0.3 pM of each primer;
- (2) 67 mM tris-HCl (pH 8.8);
- (3) MgCl<sub>2</sub>: 2.5 mM for m.652insG, m.5178C>A, m.3336T>C, m.652delG, m.1555A>G, m.3256C>T, m.12315G>A and m.13513G>A; 1.5 mM for G14846A, G15059A and G14459A;
- (4) 16.6 µM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>;
- (5) 3 units of Taq-polymerase;
- (6) 200 µM of each deoxyribonucleotriphosphate;
- (7) 0.4-0.6 µg<sup>[24,26,28-30]</sup>.

Annealing temperature for the PCR is shown in [Table 3](#).

To carry out the polymerase chain reaction, we used thermocycler “PTC DNA Engine 200”.

Pyrosequencing of PCR fragments was performed on an automated pyrosequencing device PSQTMHS96MA (Biotage, Sweden).

For pyrosequencing the following primer sequences were used<sup>[24,26,28-30]</sup>:

1. For m.652insG

**Table 3. Annealing temperature for the PCR**<sup>[24,26,28-30]</sup>

Mutations	Annealing temperature for primers
m.15059G>A	
m.3336T>C	
m.13513G>A	55 °C
m.3256C>T	
m.14846G>A	
m.652insG	
m.5178C>A	60 °C
m.652delG	
m.14459G>A	
m.1555A>G	50 °C
m.12315G>A	

CCCATAAACAAATA (639-651);

2. For m.5178C>A

ATTAAGGGTGTTAGTCATGT (5200-5181);

3. For m.3336T>C

TGCGATTAGAATGGGTAC (3354-3337);

4. For m.14459G>A

GATACTCCTCAATAGCCA (14439-14456);

5. For m.652delG

CCCATAAACAAATA (639-651);

6. For m.14846G>A

GCGCCAAGGAGTGA (14861-14848);

7. For m.1555A>G

ACGCATTTATATAGAGGA (1537-1554);

8. For m.15059G>A

TTTCTGAGTAGAGAAATGAT (15080-15061);

9. For m.3256C>T

AAGAAGAGGAATTGA (3300-3286);

4. For m.12315G>A

TTTGGAGTTGCAC (12328-12316);

8. For m.13513G>A

AGGTTTCTACTCAA (13497-13511).

The heteroplasmy level of mtDNA mutations was analyzed using a quantitative method developed on the basis of pyrosequencing technology by our laboratory<sup>[24-26,38,39]</sup>. The statistical analysis was performed using SPSS 22.0 software package<sup>[40]</sup>. The bootstrap analysis and the Spearman correlation coefficient were used. The results were considered statistically significant at  $P \leq 0.05$ . In addition, the results were taken into account, the significance level of which was  $P \leq 0.1$ . It was supposed that such results had a tendency to have statistical significance. They may be significant if the sample is expanded.

## RESULTS

According to [Table 1](#), statistically significant differences by clinical and anthropometric characteristics between samples of patients with left ventricular hypertrophy and conventionally healthy study participants were not found.

It should be noted that the age of patients with left ventricular hypertrophy ranged from 53 to 75 years. At the same time, the age of conventionally healthy participants ranged from 54 to 62 years [[Table 2](#)]. The mean age of patients with left ventricular hypertrophy was 6 years higher than the age of conventionally healthy study participants. This age difference between samples of patients with left ventricular hypertrophy

**Table 4. Spearman correlation analysis of 11 mtDNA mutations with left ventricular hypertrophy**

Mutations	Spearman correlation coefficient	Significance
m.652insG	-0.036	0.357
m.5178C>A	0.114	0.038 <sup>a</sup>
m.3336T>C	-0.126	0.014 <sup>a</sup>
m.14459G>A	-0.082	0.082 <sup>b</sup>
m.652delG	0.065	0.214
m.14846G>A	-0.028	0.427
m.1555A>G	0.096	0.064 <sup>b</sup>
m.15059G>A	0.064	0.236
m.3256C>T	0.057	0.258
m.12315G>A	-0.122	0.017 <sup>a</sup>
m.13513G>A	-0.034	0.369

<sup>a</sup> $P \leq 0.05$ ; <sup>b</sup> $P \leq 0.1$

and conventionally healthy participants was not statistically significant. The linkage of mtDNA mutations 652insG, m.5178C>A, m.3336T>C, m.14459G>A, 652delG, m.14846G>A, m.1555A>G, m.15059G>A, m.3256C>T, m.12315G>A and m.13513G>A with atherosclerosis was described earlier by our laboratory researchers<sup>[24,28-31]</sup>. Since LVH has common risk factors with atherosclerosis, it was decided to analyze the relationship of these mutations to mtDNA with left ventricular hypertrophy.

The results of this analysis are presented in [Table 4](#).

The direction of the linkage of mtDNA mutations with left ventricular hypertrophy was detected using the coefficient of correlation. If the Spearman correlation coefficient was positive, the investigated mutation was associated with left ventricular hypertrophy. If the Spearman correlation coefficient was negative, the mutation was associated with the absence of left ventricular hypertrophy.

According to the obtained results, mtDNA mutation m.5178C>A was significantly associated with LVH. Single nucleotide replacement m.1555A>G was associated with left ventricular hypertrophy at the level of significance  $P \leq 0.1$ . It showed a tendency to a positive correlation with LVH. Meanwhile m.12315G>A and m.3336T>C were significantly associated with the absence of this pathology. Single nucleotide replacement m.14459G>A was associated with the absence of left ventricular hypertrophy at the significance level  $P \leq 0.1$ . Mutation m.14459G>A showed a tendency to negative correlation with LVH.

## DISCUSSION

Due to the fact that several mitochondria can be found in a human cell, and several copies of the mitochondrial genome can be found in the mitochondria, in particular the level of mtDNA mutations in the mitochondrial genome was analyzed. The presence of heteroplasmy threshold level of a mitochondrial genome mutation may be associated with the occurrence of the disease. Our previous article was devoted to the detection of threshold heteroplasmy level of mitochondrial genome mutations 652insG, m.5178C>A, m.3336T>C, m.14459G>A, 652delG, m.14846G>A, m.1555A>G, m.15059G>A, m.3256C>T, m.12315G>A and m.13513G>A, which are associated with atherosclerosis and its risk factors<sup>[25]</sup>.

Mutations m.5178C>A and m.1555A>G can be used to assess the molecular genetic predisposition of individuals to occurrence of left ventricular hypertrophy. They can also be used for family analysis of this pathology. Mutations m.12315G>A, m.3336T>C and m.14459G>A could be used in the development of LVH gene therapy methods.

It is noteworthy that one of the mutations of the mitochondrial genome (m.5178C>A) can lead to a defect in the respiratory chain enzyme (NADH dehydrogenase), leading to a decrease in ATP synthesis and an energy

deficit in the mitochondria and cells of humans. At the same time, two other mutations (m.3336T>C and m.14459G>A) of this enzyme seem to have a protective, stabilizing effect and positively affect mitochondria and cardiac muscle cells. In our preliminary studies, it was found that mutation m.12315G>A, localized in the transfer RNA-Leucine gene (recognition codon CUN), was associated with atherosclerosis<sup>[29]</sup>. However, in the present study it was found that this mutation has a protective effect on left ventricular hypertrophy. A possible reason for this may be a difference in the mechanisms of the occurrence and development of these pathologies.

It should be noted that single nucleotide substitutions which have a protective (antipathological) effect on diseases are called “protective mutations”, but not polymorphisms. Since polymorphisms are neutral, they exist in populations without influencing the occurrence and development of diseases. In addition, polymorphisms do not have a protective effect in various pathologies. Therefore, the name “protective (antipathological) mutations” seems to us more correct.

It is necessary to say that in literary sources there are very few studies that have investigated the linkage of mitochondrial genome mutations with LVH. In particular, in the article of Zhu *et al.*<sup>[21]</sup> the association of mutation m.4401A>G with left ventricular hypertrophy was found. In a research work by Govindaraj *et al.*<sup>[22]</sup> heteroplasmic mutations m.4797C>M and m.8728T>Y MT-tRNA, were found to be associated with hypertrophic cardiomyopathy. In the article by Bates *et al.*<sup>[23]</sup> the association of mtDNA mutation m.3243A>G with concentric hypertrophic remodelling and subendocardial dysfunction was studied. In none of such studies the association of the heteroplasmy level of the detected by us mtDNA mutations with left ventricular hypertrophy was analyzed.

In conclusion, five mutations of the mitochondrial genome associated with left ventricular hypertrophy were found in the present study. They can be used for molecular genetic assessment of the predisposition of individuals to the occurrence of LVH, family analysis and gene therapy of this pathology.

## DECLARATIONS

### Authors' contributions

Conception, design and statistical analysis: Sazonova MA  
Pyrosequencing of PCR fragments: Sazonova MA, Sinyov VV  
PCR: Ryzhkova AI, Khasanova ZB  
DNA extraction: Sazonova MD  
Administrative and material support: Sobenin IA

### Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

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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 National Medical Research Center of Cardiology. Each study

participant has signed a written informed consent to participate in this investigation.

### Consent for publication

Not applicable.

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