Genetic Determinants of the Efficiency of Climatotherapy in Patients with Chronic Heart Failure

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We studied the dependence of climatotherapy effectiveness in patients with chronic heart failure (functional classes 0-II) on Ca^{2+}-ATPase, phospholamban, β_1-adrenoceptor, and insulin-like growth factor 1 gene polymorphisms and possible interaction of these genes during the realization of the effect of climatotherapy. The effectiveness of climatotherapy depended on polymorphism of the studied genes; the maximum effect was attained in patients with the GG polymorphism of the Ca^{2+}-ATPase gene, GT polymorphism of the phospholamban gene, ArgGly polymorphism of the β_1-adrenoceptor gene, and 19/19 polymorphism of the insulin-like growth factor 1 gene. We demonstrated additive interaction of Ca^{2+}-ATPase and β_1-adrenoceptor genes during the realization of the cardiotonic effect of climatotherapy.

Key Words: *climatotherapy; chronic heart failure; genetic polymorphism*

Chronic heart failure (CHF) is a complex clinical state accompanied by hemodynamic, neurohumoral, and metabolic disturbances and resulting from any disease leading to impairment of the contractile function of the myocardium [2,11]. At the present time, CHF is not considered as a syndrome or complication of cardiovascular diseases, but as a component of the cardiovascular continuum developing by the intrinsic mechanisms not depending on the primary etiologic factor, and characterized by multifactor inheritance [1,3]. The therapeutic effect of artificial physical factors depends on genetic polymorphism [6-8]. However, we found no published data on the genetic analysis of the action of natural therapeutic physical factors.

Here we studied genetic predisposition to a therapeutic effect of climatotherapy (CT) in patients with CHF of the initial functional classes was studied depending on polymorphisms of genes encoding sarcoplasmic reticulum Ca^{2+}-ATPase (ATP2A), phospholamban (PLN), β_1-adrenoceptor (ADRB1), and insulin-like growth factor 1 (IGF1), *i.e.* proteins playing a role in the pathogenesis of CHF [4,9,10].

MATERIALS AND METHODS

We examined 80 sanatorium and health resort patients with initial stages of CHF (functional classes I-II) in during the summer climatotherapeutic season (Sochi—Matsesta region). The sample included 37 men and 43 women aging 50-71 years. CHF was accompanied by coronary heart disease (CHD) in 20 patients) and by hypertension and CHD in 60 patients.

The diagnosis of CHF was made from complaints, anamnesis, and results of physical examination (6-min walking test and ultrasound cardiac assessment).

The patients were subjected to bicycle ergometry (Ergofitt 777 bicycle ergometer and Bioset

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8000 system), 24-h electrocardiographic monitoring (Holter monitoring, JNDHEM-AD-24 system), computer oscillography (APKO-8-RITs system), echocardiography (ALOKA 1400 ultrasound scanner), and heart rate monitoring (VNS-Spektr device). The functional class of CHF and tolerance to substress physical exercise were evaluated by the standard test of 6-min walking. Blood pressure reactivity was estimated in the test of continuous 3-min exercise (50 W) on an Ergofitt 777 bicycle ergometer [2,9,11]. The course of CT in patients of all groups included sea bathing, air baths (medium-degree cold load), and sun baths (100 biological doses). The effectiveness of CT was determined by the dynamics of the test parameters (distance and functional class of CHF) in the 6-min walking test and clinical signs of CHF [5].

All patients signed the informed consent form. For evaluation of gene polymorphism, the blood (5 ml) was taken from the cubital vein. DNA was isolated from blood lymphocytes. Genetic polymorphism was assayed using polymerase chain reaction (PCR) [8]. To this end, 250 ng genomic DNA was added to the reaction mixture (25 μl) containing 0.67 mM Tris-HCl (pH 8.8, 25°C), 16.6 mM $(NH_4)_2SO_4$, 6.7 mM $MgCl_2$, 6.7 μM EDTA, 10 mM 2-mercaptoethanol, 170 μg bovine serum albumin, 0.8 mM deoxynucleotide triphosphates mixture, thermostable DNA polymerase (0.2 U/μl, Sibenzim), and 0.1 optical units of each oligoprimer (Table 1). PCR was performed in 50 μl reaction mixture containing 66 ng of each primer on a PHC-2 amplifier (Teche) and programmed thermocycler (DNA-Technology).

The PCR product was hydrolyzed by restriction endonuclease in 10 μl reaction mixture containing 5 μl amplification product, 3 μl water, 1 μl 10× buffer (according to manufacturer's recommendations for each restrictase), and 10 U (0.5 μl) restriction endonuclease at 37°C for 16 h. The degree of hydrolysis was estimated by vertical electrophoresis in 6% polyacrylamide gel (20 cm) in Tris-borate buffer. Amplification yields products of different length corresponding to certain alleles of the gene. For visualization and identification of DNA fragments, the gels were stained with ethidium bromide (0.5 μg/ml), examined in UV light on a Macrovue transilluminator (Pharmacia LKB) and photographed using a Vilber Lourmat system of gel documentation.

The results were analyzed by methods of variational statistics.

RESULTS

The frequencies of ATP2A2 genotypes were: GG, 52 patients (65%); GA, 23 patients (29%); and AA, 5 patients (6%). The patients were divided into 3 groups according to polymorphism of the ATP2A2 gene.

In patients with the GG polymorphism, the 6-min walking distance and left-ventricular ejection fraction significantly increased after CT. These changes confirm the cardiotonic effect of CT and correction of circulatory insufficiency in patients of this group (Table 2). We revealed a decrease in systolic and diastolic pressure; physical exercise tolerance and double product at the maximum load

TABLE 1. Oligonucleotide Primers for PCR

Gene product	Gene and its locus	Polymorphism	Structure of oligoprimers
Sarcoplasmic reticulum Ca^{2+}-ATPase, type 2, A isoform	ATP2A2	G/A substitution in position 54 of intron 18	5'-TTTGAAGGCGTGGATTGTGC-3'
	12q23-q24.1		5'-TTGGGAAGGGAAGAACTGTC-3'
β_1-adrenoceptor	ADRB1	C/G substitution in the 1165-position (substitution of Arg for Gly in position 389 of the protein)	5'-TGGGCTACGCCAACTCGG
	10q24-q26		3'-GGCCCCGACGACATCGTC
Insulin-like growth factor 1	IGF1	CA repeats in the promoter region	5'-AGACTCCCTCTGTCATACAC-3'
	12q22-q23		5'-TACCCTTCTCCCAGAGTGGT-3'
Phospholamban	PLN	T/G substitution in position 11,680	5'-GAGGTGAATATAATTTATATTACAATC-3'
	6q22.1		5'-TTACTTTTCCAACTTGATTCTCA-3'

TABLE 2. Tolerance to Physical Exercise, Myocardial Contractility, and Central Hemodynamics in CHF Patients after CT in Dependence on Polymorphisms of ATP2A2 and PLN Genes (*M*±*m*)

Parameter	ATP2A2			PLN		
	GG (*1*)	GA (*2*)	AA (*3*)	GG (*1*)	GT (*2*)	TT (*3*)
Mean hemodynamic pressure, mm Hg	3.17±0.19*	0.09±1.90	2.40±3.01	2.83±2.40	1.33±1.16	-2.86±1.99
Reactivity of mean hemodynamic pressure, min	-0.34±0.10	-0.57±0.14*	-0.30±0.25	-0.17±0.17	-0.52±0.10*$^{+x}$	-0.29±0.20^{x}
Distance in the 6-min walking test, m	64.25±9.10**	58.52±9.50*	15.00±17.18^{xo}	48.22±11.23*	68.02±9.66*$^{+x}$	45.21±11.72*^{x}
Left-ventricular ejection fraction, %	2.52±1.04*	0.22±1.12^{+}	-1.00±3.35	1.39±1.80	1.13±0.90	3.71±2.29
Threshold load power, W	6.63±2.15*	1.09±2.47^{+}	3.1±2.1	0.4±2.02	7.71±2.45^{+}	3.57±2.57
Tolerance to physical exercise, scare	-0.2±0.04**	0.04±0.02^{+}	-0.08±0.04^{x}	0.03±0.02	-0.08±0.04^{+}	-0.07±0.02

Note. Here and in Table 3: *p<0.05, **p<0.01, and ***p<0.001 compared to the initial value; $^{+}$$p$<0.05 compared to (*1*); ^{x}p<0.05 compared to (*2*); ^{o}p<0.05 compared to (*3*).

on a bicycle ergometer tended to increase. These parameters illustrate coronary reserve of the myocardium. The 6-min walking distance significantly increased, while the time to recovery of systolic pressure after standard physical exercise decreased in patients with the AG polymorphism. The test parameters in patients with the AA polymorphism underwent little changes.

In patients with the GG polymorphism of the ATP2A2 gene, the factor loading for parameters characterizing myocardial contractility, coronary reserve, and tolerance to physical exercise decreased after CT; blood pressure also decreased in these patients, which attested to potent cardiotonic, antiischemic, and hypotensive effects of CT. In patients with the AG polymorphism, the factor load for parameters of myocardial contractility decreased less significantly, while in patients with AA polymorphism these changes were not found.

We compared the factor structure of signs that characterized myocardial function in CHF patients, as well as hemodynamic parameters in each group of CHF patients. CT had the most pronounced cardiotonic effect in patients with the GG polymorphism of the ATP2A2 gene. The factor loading for 6-min walking distance decreased from 89 to 54%. The left-ventricular ejection fraction decreased from 77 to 45% (p<0.05).

The PLN gene frequency appeared as follows: GG, 18 patients (23%); TG, 48 patients (60%); and TT, 14 patients (17%).

CT had a potent effect on the results of the 6-min walking test in patients of all groups. Blood pressure decreased most significantly in patients with the GT polymorphism (Table 2). The most significant changes in myocardial contractility and tolerance to physical exercise were found in patients with the GT polymorphism of the PLN gene. The number of altered parameters and degree of changes were minimum in patients with the GG polymorphism. The left ventricular end-systolic volume decreased significantly in patients with the TT polymorphism of the PLN gene, which reflected a cardiotonic effect of CT in this group of patients.

The factor loading for parameters of myocardial contractility and tolerance to physical exercise decreased in patients of all groups. The most significant changes in the factor loading were detected in CHF patients with the GT polymorphism of the PLN gene. We revealed a decrease in the factor loading for the threshold load power (from 69 to 42%) and mean hemodynamic pressure (from 79 to 52%, p<0.05).

The frequency of ADRB1 genotypes was the following: ArgArg (CC), 43 patients (53%); ArgGly

(CG), 34 patients (43%); and GlyGly (GG), 3 patients (4%).

CT increased the 6-min walking distance in patients with the ArgGly and ArgArg polymorphism, which reflected a cardiotonic effect of this treatment (Table 3). Systolic and diastolic pressure decreased in these patients. The tolerance to physical exercise tended to increase. The most significant changes were revealed in patients with the ArgGly polymorphism. Our results show that CT is most effective in heterozygotes, but has little effect in GlyGly homozygotes.

In patients with the AgrGly polymorphism of the ADRB1 gene, CT decreased the factor loading for 6-min walking distance (from 69 to 38%, $p<0.05$) and left-ventricular ejection fraction (from 81 to 47%, $p<0.05$). We found a decrease in parameters of blood pressure. The factor loading for mean hemodynamic pressure decreased from 72 to 55% ($p<0.05$). The factor loading for autonomic parameters reflecting the balance between the sympathetic and parasympathetic influences on cardiac function decreased in patients with the ArgArg polymorphism.

The frequency of IGF1 genotypes in CHF patients was the following: 17/19, 6 patients (8%); 19/19, 30 patients (37%); 19/21, 13 patients (16%); 18/19, 11 patients (14%); and 19/20, 20 patients (25%).

The results of the 6-min walking test significantly improved in patients of all groups (except for patients with the 17/19 polymorphism of the IGF1 gene, Table 3). The distance increased most significantly in patients with the 19/21 polymorphism (92.5±26.5 m). CT was followed by a significant decrease in systolic and diastolic pressure in patients with the 19/19, 19/21, and 18/19 polymorphism. The strongest hypotensive effect was observed in patients with the 19/19 polymorphism. The cardiotonic effect was most pronounced in patients with the 19/21 polymorphism and minimum in patients with the 17/19 polymorphism.

CT had a strong effect on myocardial contractility and blood pressure in patients with the 19/19, 19/21, and 19/20 polymorphism. It was mainly related to a decrease in the factor loading for 6-min walking distance (from 68 to 44%) and mean hemodynamic pressure (from 71 to 54%, $p<0.05$). The factor loading for test parameters remained unchanged in patients with the 17/19 polymorphism.

These data show that CT had a strong cardiotonic and hypotensive effect on CHF patients with the 19/19 polymorphism of the IGF1 gene. The patients of this group are characterized by normal secretion of IGF1 [10]. The genotype, which is accompanied by low secretion of IGF1, contributes

TABLE 3. Tolerance to Physical Exercise, Myocardial Contractility, and Central Hemodynamics in CHF Patients after CT in Dependence on Polymorphism of ADRB1 and IGF1 Genes

Parameter	ADRB1			IGF1		
	GlyGly (*1*)	GlyArg (*2*)	ArgArg (*3*)	19/19 (*1*)	19/20 (*2*)	19/21 (*3*)
Mean hemodynamic pressure, mm Hg	0.81±1.42	4.32±0.36^{++}	-1.67±1.60^{x}	1.54±1.33	-0.28±2.24	3.75±2.99
Reactivity of mean hemodynamic pressure, min	-0.53±0.11**	-0.25±0.11^{+}	-0.17±0.17	-0.39±0.13*	-0.33±0.18	-0.54±0.13**
Distance in the 6-min walking test, m	56.26±8.84*	68.00±10.76***	11.67±2.67^{xo}	54.4±10.1*	57.6±11.2**	92.5±26.5^{***xo}
Left-ventricular ejection fraction, %	0.30±0.99	3.35±1.30^{++}	1.33±0.33^{x}	1.46±1.64	2.28±1.50	-1.42±2.11
Threshold load power, W	3.95±2.13	5.88±1.60*	5.67±3.67	7.50±1.24**	0.02±0.01^{+}	5.4±1.50^{*xo}
Tolerance to physical exercise, scare	-0.05±0.03	-0.06±0.01*	-0.33±0.33	-0.04±0.04	0.04±0.01	-0.17±0.11

to less significant improvement of myocardial contractility and central hemodynamics.

The multifactor analysis of variance with pairs of factors was performed to evaluate the contribution of interaction between genes to the cardiotonic effect of CT in CHF patients. The increase in distance was measured in the 6-min walking test (Δ distance). The complete factor model in which the genes and gene interactions explain the major part of Δ distance dispersion was statistically reliable only in the combination of genetic polymorphism for ATP2A2+PLN, ATP2A2+ADRB1, and PLN+ADRB1.

The ATP2A2 gene polymorphism had the strongest effect on Δ distance dispersion. Polymorphism of ATP2A2+ADRB1 and PLN+ADRB1 contributed to Δ distance dispersion by 5 and 1%, respectively. Our results suggest the interaction between alleles of these genes. The degree of dispersion was higher for ATP2A2+PLN (19%). Hence, genetic polymorphism of ATP2A2+PLN had the strongest effect on Δ distance. This influence significantly exceeded the effect of interaction in pairs of ATP2A2+ADRB1 and PLN+ADRB1. The analysis of variance showed that ATP2A2 and PLN genes have an additive action on the cardiotonic effect of CT in CHF patients. LSD test was applied to estimate the difference between the mean values of Δ distance at three levels (*i.e.*, 3 variants of polymorphism for each gene), as well as in combination of these levels.

Network study allowed us to divide CHF patients into groups with the highest and lowest prognostic effectiveness of CT. We calculated the increase in Δ distance upon combination of the GG polymorphism of ATP2A2 and TG polymorphism of PLN (75 m), TG polymorphism of PLN and GlyGly polymorphism of ADRB1 (70 m), and GG polymorphism of ATP2A2 and GlyGly polymorphism of ADRB1 (66 m). CT was most effective in CHF patients with these combinations of genetic polymorphism. The effectiveness of CT was lowest in CHF patients with the following combinations: AA polymorphism of ATPA2A and ArgArg polymorphism of ADRB1 (Δ distance — 10 m), ArgArg polymorphism of ADRB1 and TT polymorphism of PLN (Δ distance — 14 m), and TT polymorphism of PLN and AA polymorphism of ATP2A2 (decrease in the walked distance by 50 m).

CT had the strongest cardiotonic, antiischemic, and hypotensive effect in CHF patients with the GG polymorphism of the ATP2A2 gene, GT polymorphism of the PLN gene, and ArgGly polymorphism of the ADRB1 gene and 19/19 polymorphism of the IGF1 gene, respectively. The effectiveness of CT in CHF patients strongly depended on the Ca^{2+}-ATPase gene polymorphism (alone or in combination with polymorphism of the phospholamban gene). Probably, the regulation of transmembrane Ca^{2+} fluxes play a key role in the development and progression of heart failure. Due to a considerable number of allelic variants of the IGF1 gene polymorphism, it was impossible to perform complete factor analysis of interaction between alleles of this gene and polymorphism of other genes.

Our results show that polymorphism of ATP2A2, PLN, and IGF1 genes contributes to the difference in a cardiotonic therapeutic effect of CT. Polymorphism of the ADRB1 gene mainly determines a hypotensive effect of CT in patients. Gene products of test genes form the unique metabolic axis that is responsible for contractile function of cardiomyocytes, including the response to endogenous sympathetic and paracrine influences.

The cardiotonic effect of CT was minimum in patients with the AA polymorphism of the ATP2A2 gene. It was probably associated with changes in spatial configuration of the Ca^{2+}-ATPase molecule in patients with the AA polymorphism, which results in a decrease in its activity. The interaction between pair of genes necessitates a significant increase in the sample of patients due to variations in the frequency of genetic polymorphism. This specific feature requires further investigations. The results of our pilot study indicate that the distance dispersion in the 6-min walking test and increase in this parameter after the course of CT mainly depend on polymorphism of ATP2A2 and ADRB1 genes.

Our results indicate that intracellular Ca^{2+} exchange and neurohormonal imbalance are the key pathogenetic stages of CHF. Therefore, these stages are most sensitive to the influence of therapeutic physical factors.

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