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Polymorphism of ABCA1, APOC3, and PON1 genes and indicators of the central nervous system in patients of European race with chronic heart failure of ischaemic origin

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Abstract

Aim

To examine the association of the polymorphic variants of the -455 T>C, -482 C>T; 3238 C>G in the APOC3 gene; R219K G>A in the ABCA1 gene; L55M A>T and Q192R A>G in the PON1 gene, and indicators of the central nervous system (CNS) in patients of European race with chronic heart failure (CHF) of ischaemic origin.

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Materials and methods

54 patients with CHF of ischaemic origin, who were no older than 65 years and had no other related diseases and conditions that could be a cause of pathology of the brain, had numerous tests and examinations. These included a physical examination; magnetic resonance imaging (MRI) of the brain; an assessment of cognitive functions by means of Wechsler's 5 and 7 subtests; proofreading Bourdon's test; mini mental state examination (MMSE); genetic polymorphism analysis of the -455 T>C, -482 C>T, 3238 C>G in the APOC3 gene; R219K G>A in the ABCA1 gene; and, L55M A>T and Q192R A>G in the PON1 gene.

Results

There were no significant differences in the results of the cognitive assessment and the state of the brain determined by MRI, depending on the R219K G>A polymorphism in the ABCE1 gene and 3238C>G polymorphism in the APOC3 gene. The AA genotype of the L55M A>T polymorphism in the PON1 gene, AA genotype of the Q192R A>G polymorphism in the PON1 gene, the presence of C allele of the -455 T>C polymorphism in the APOC3 gene, and T allele of the -482 C>T polymorphism in the APOC3 gene is associated with better cognitive functions in patients with CHF of ischaemic origin. Atrophic changes in the brain in patients with CHF, within the context of coronary artery disease (CAD), are associated with the CC genotype of the -482 C>T polymorphism in the APOC3 gene and G allele of the Q192R A>G polymorphism in the PON1 gene.

Conclusion

Determining the polymorphic variants of the -455 T>C in the APOC3 gene, -482 C>T in the APOC3 gene, Q192R A>G and L55M A>T in the PON1 gene can be effective for predicting the development of atrophic changes in the brain and cognitive dysfunction in patients with CHF of ischaemic origin.

Keywords

Chronic heart failure, coronary artery disease, cognitive functions, genetic polymorphism

Introduction

Chronic heart failure (CHF) is a complex syndrome accompanied by many systemic disorders, including changes in the CNS which are important [1-4].

Along with hypertension and atherosclerotic vascular disease, CHF is one of the extracerebral causes leading to the onset and deterioration of existing cognitive disorders, which can progress to some degree of dementia [1-5].

Prevention of diseases is often more important than their treatment. Identification of genetic factors associated with the high risk of cardiovascular disease and cognitive dysfunction among the population would make it possible to carry out preventive measures well before the onset of clinical symptoms. This preventive diagnosis might delay the onset of the cognitive disorders due to CHF, and perhaps in some cases even prevent their development. Each genetic locus characterizes a certain level of variation that is expressed by the presence of different variants of a gene (alleles) in different individuals. Changes in the sequence of deoxyribonucleic acid (DNA) (mutations) can result in the development of alternative variants of genes. If the mutation occurs at a frequency of 1.5-3% or more and does not lead to obvious phenotypic manifestations of the disease, it is considered a polymorphism. Genetic polymorphism in the human genome in 95% of cases is associated with single nucleotide substitutions – a single nucleotide polymorphism (SNP) [6,7]. Establishing links of certain polymorphisms of some genes, associated with lipid metabolism, with such parameters as the severity of CHF, the severity of cognitive dysfunction, and the presence of any morphological changes in the brain in patients with CHF of ischaemic origin might be useful, of course, for the development of a preventive approach at the population level.

Materials and methods

General inclusion criteria for the participants of the study were: the presence of CHF occurred on the background of proven CAD; not older than 65 years; the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, and beta-blockers in a stable dose for four weeks before entering the study. The study excluded patients with acute or subacute CAD; diabetes; acute cerebrovascular accident, including in medical history; atherosclerotic plaques of the arteries of the head and neck leading to the development of hemodynamically significant stenoses – narrowing >50% of the arterial lumen, according to the duplex ultrasound of the vessels; signs of

dementia according to MMSE; alcohol abuse; intake during 90 days before inclusion in the study of neurometabolic, neurotrophic drugs, as well as any other substances that can directly or indirectly affect the cognitive functions of patients; myocarditis; thyroid disorders; manifest valvular lesions; laboratory signs of manifest disorders of the liver and kidneys; other somatic diseases which, in the opinion of a physician-scientist, could be an independent cause of cognitive impairment; contraindications for MRI.

The main clinical characteristics of patients are shown in Table 1.

Table 1. **The main clinical characteristics from the groups of patients studied (median and quartiles)**

Indicator	Patients with CHF (n=54)
Age, years	57.23 (54;62)
Male sex, n (%)	65 (58)
Higher education, n (%)	34 (63)
Height, cm	171 (160.5;174.5)
Body weight, kg	84.8 (74.5;95.5)
Myocardial infarction, n (%)	28 (52)
Hypertension, n (%)	49 (91)
Duration of CAD, months	60.1 (35.7;86.1)
Duration of CHF, months	46.2 (20.6;68.7)

All respondents were residents of Saratov (Russian city). They were of the European race, Slavs.

Data of past medical history and physical examination were recorded in a formalized medical history. If the presence of decompensated heart failure was noted in a patient he/she was included in the study 1 month after stabilization. All studies were performed in the morning after the procedure of signing the informed consent form. The study protocol was approved by the local ethics committee at the "Saratov State Medical University named after V. I. Razumovsky" of the Ministry of Healthcare of the Russian Federation.

To assess the morphological state of the CNS of patients, in addition to physical examination, MRI of the brain was performed on the Philips Achieva 1.5 T. The thickness of gray matter (GM) in the occipital, frontal, parietal, and temporal lobes of the brain was determined. To investigate the status of the white matter (WM) of the brain, an average width of the middle cerebellar peduncles was measured. In addition to the standard method of diffusion weighted imaging of the brain, diffusion coefficients (DC) of water molecules in the GM and WM of the occipital, frontal, parietal, and temporal lobes, and in the basal ganglia were calculated. Cognitive functions were assessed by verbal and nonverbal Wechsler's subtests (5 and 7) and proofreading Bourdon's test. MMSE was used in order to exclude dementia.

For genetic analysis venous blood sampling was carried out on an empty stomach. To study polymorphism of the genes involved in lipid metabolism, pyrosequencing, using AxyPrep Blood Genomic DNA Miniprep Kit for DNA isolation, was performed. Polymerase chain reaction was carried out in the MaxyGene Therm-1000 followed by obtaining a single-stranded DNA and sequencing, using a genetic analysis PyroMark Q24 system. Characteristics of the studied polymorphic variants are presented in Table 2.

Statistical analysis was performed by the program Statistica 6.0. Univariate analysis of variance, nonparametric correlations (Kendall's coefficient), and frequency analysis (cross-tabulation) with application of χ^2 and Fisher's criteria were used.

Based on the fact that some of the studied mutations were inherited in an autosomal dominant pattern and given that in some cases the frequency of homozygotes for the mutant allele was extremely small (Table 3), in the further comparative statistical analysis the division of patients into two groups was used, namely in accordance with the presence or absence of the mutant allele in a genotype.

Results

It is obvious that statistical analysis of the relationships between genetic polymorphisms in a relatively small group of patients is possible at a sufficiently high frequency of each of the studied polymorphism variants and moreover, compliance of selective frequency distribution, and prevalence of the studied variants in the population. Genotype frequencies of the studied polymorphisms of the ABCA1, APOC3

Table 2. **Characteristics of the polymorphisms studied**

Locus	Product	Polymorphism	rs	Types of genotype
ABCA1	ABCA1	R219K G>A	2230806	GG, GA, AA
APOC3	Apolipoprotein C3	-455 T>C	2854116	TT, CT, CC
APOC3	Apolipoprotein C3	-482 C>T	2854117	CC, CT, TT
APOC3	Apolipoprotein C3	3238C>G	5128	CC,CG, GG
PON1	Paraoxonase 1	L55M A>T	854560	AA, AT, TT
PON1	Paraoxonase 1	Q192R A>G	662	AA, AG, GG

and PON1 genes mostly correspond to this condition. Established distribution of the genotypes was in line with the expected, based on the Hardy-Weinberg equilibrium (Table 3).

When analyzing the results of the cognitive assessment in patients with CHF of ischaemic origin, there were established significant differences in cognitive indicators, depending on the polymorphism variants of the -455 T>C and -482 C>T in the APOC3 gene, and L55M A>T and Q192R A>G in the PON1 gene. The presence of the mutant C allele of the -455 T>C polymorphism in the APOC3 gene is associated with significantly better memory and attention defined by Wechsler's 7-subtest (28.6±9.3 points in patients with the TT genotype and 37.2±10.3 in patients with the CC and CT genotypes) and proofreading Bourdon's test, namely the speed of doing this test by patients with CC and CT genotypes was 115.98 ± 22.31 signs/min, and in patients with TT genotype – 99.77±20.12 signs/min; attention switching, also determined by the Bourdon's test, in patients with CT and TT genotypss was 46.75±4.25 conv. u, and in patients with CC genotype – 33.89±5.83 conv.u. The -482 C>T polymorphism in the APOC3 gene was associated with the speed of proofreading Bourdon's test, namely patients with CT and TT genotypes completed the test faster: 117.02±24.04 signs/min compared with patients with CC genotype – 104.17±18.60 signs/min. Thus, atherogenic mutations of the APOC3 gene (-455C and -482T) are associated with better memory and attention in patients, mutation carriers, compared with patients – homozygous for the normal allele.

A number of significant differences in the results of the cognitive assessment were established depending on a polymorphism of the PON1 gene. The average 7 minute attention span, determined by the proofreading Bourdon's test, in patients with the AA genotype of the L55M A>T polymorphism in the PON1 gene was 0.91±0.06 conv. u, and in patients with AT and TT genotypes – 0.83±0.11 conv. u. Significant differences in the average 7 minute attention span, determined by the proofreading Bourdon's test, were established depending on the Q192R A>G polymorphism in the PON1 gene. In patients with the AA genotype, it was 0.93±0.04 conv. u, and in patients with AG and GG genotypes – 0.85±0.10 conv. u.

Significant differences in the results of the cognitive assessment depending on the R219K G>A polymorphism in the ABCE1 gene have not been established.

Significant changes in the thickness of the GM and DC of water molecules in the brain were established depending on the -482 C>T polymorphism in the APOC3 gene and Q192R A>G polymorphismin the PON1 gene, whereas the polymorphism in the ABCA1 gene, just as in the analysis of its effects on cognitive functions, was not significant.

Table 3. **Distribution of genotype frequencies of some polymorphisms of genes ABCA1, APOC3 and PON1 in patients with CHF and its comparison with the population**

Gene	Polymorphism	Distribution of genotypes		x^2
ABCA 1	R219K G>A	GG	64.7%	22.3
		GA	20.2%	
		AA	15.1%	
		GA+AA	35.3%	
APOC3	-455 T>C	TT	22.5%	24.5
		CT	62.5%	
		CC	15%	
		CT+CC	77.5%	
APOC3	-482 C>T	CC	48.1%	24.4
		CT	44.5%	
		TT	7.4%	
		CT+TT	51.7%	
APOC3	3238 C>G	CC	1.8%	25.5
		GC	20.8%	
		GG	77.4%	
		GC+CC	98.2%	
PON1	L55M A>T	AA	50.0%	25.3
		AT	35.1%	
		TT	14.9%	
		AT+TT	50.0%	
PON1	Q192R A>G	AA	41.9%	25.6
		AG	40.6%	
		GG	17.5%	
		AG+GG	58.1%	

It should be noted that the -482 C>T polymorphism in the APOC3 gene had no significant effect on the width of the cortex or middle cerebellar peduncles, and established significant differences were related to the diffusion of water molecules in the GM and WM of the brain (Table 4).

Thus, the DC of water molecules in the GM of the frontal and parietal lobes and in the WM of the parietal and occipital lobes is significantly lower if a genotype has the mutant T allele of the -482 C>T polymorphism in the APOC3 gene. It is known that the DC increases, in particular in reducing the number of neurons in the brain which indirectly reflects the severity of the micromorphological changes in the brain [3,8-10]. Decrease in the diffusion of water molecules in the WM can be observed in violation of the myelination of the nerve fibers [3,8].

Regarding the Q192R A>G polymorphism in the PON1 gene, it was found that the average width of the GM of the parietal lobes was significantly lower in the presence of the G allele in a genotype (3.18±0.71 compared to 3.80±0.54, P<0.05).

Discussion

Apolipoprotein C3 is a transport protein which is predominantly a part of of the very low density lipoproteins (VLDL) and chylomicrons [6,7,11-14]. When there is an increased expression of the APOC3 gene, an excessive inhibition of lipoprotein lipase occurs which, in turn, is accompanied by increased levels of triglycerides, low density lipoprotein (LDL), and chylomicrons [11,15-17]. In the present study, the atherogenic APOC3 gene mutations (-455C and -482T) are associated with better memory and attention in patients, carriers of mutations, compared with patients – homozygous for the wild gene. Expressed hemodynamically significant atherosclerosis of the head and neck, as well as the presence of diabetes or acute cerebrovascular accident were the exclusion criteria in the study, and it is unknown what kind of relationship of the investigated polymorphic variants in the APOC3 gene with cognitive functions of patients with these diseases and conditions it could have been. As for the patients included in the present study, the revealed impairments of cognitive functions were caused mainly by the presence of CHF [4]. It can be assumed that in this case the atherogenic T-455C and C-482T polymorphisms in the APOC3 gene perform some protective role in respect of cognitive functions. This may be partly explained by the fact that cholesterol and other lipids are structural components of the brain, involved in the formation of cell membranes, myelin sheaths, and are necessary for normal functioning of the CNS [18-20].

The presence in a genotype of the minor allele of the L55M A>T or Q192R A>G polymorphisms in the PON1 gene is associated with the worst performance of the cognitive assessment and the signs of brain atrophy. These results are not surprising. The presence of these alleles in a genotype reduces the stability of the paraoxonase 1 which is one of the most important antioxidant enzymes in the body, which contributes to oxidation processes, including lipid peroxidation, and decrease in cellular resistance to oxidative stress [21-23]. Probably, these processes are important in the development of cognitive dysfunction.

Conclusion

Significant differences in the results of the cognitive assessment and the state of the brain determined by MRI, depending on the R219K G>A polymorphism in the ABCE1 gene and 3238C>G polymorphism in the APOC3 gene, have not been established.

Better indicators of cognitive function in patients with CHF of ischaemic origin were associated with the homozygous AA genotype of the L55M A>T polymorphism in the PON1gene, homozygous AA genotype of the Q192R A>G polymorphism in the PON1 gene, the presence of the C allele of the -455 T>C polymorphism in the APOC3 gene and T allele of the -482 C>T polymorphism in the APOC3 gene. Atrophic changes of the brain in patients with CHF on the background of CAD are associated with the homozygous CC genotype of the -482 C>T polymorphism in the APOC3

Table 4. **Diffusion coefficients (DC) of water molecules in different parts of the brain in patients with CHF of ischaemic origin, depending on the -482 C>T polymorphism in the APOC3 gene, (M±SD)***

Parameter	-482 C>T polymorphism in the APOC3 gene		Significant difference, *P*
	Patients with CC genotype (n=26)	Patients with CT and TT genotypes (n=28)	
DC of the GM of frontal lobes, m^2/c	0.43±0.04	0.38±0.05	0.046
DC of the GM of parietal lobes, m^2/c	0.44±0.03	0.39±0.05	0.04
DC of the WM of parietal lobes, m^2/c	0.44±0.05	0.37±0.08	0.03
DC of the WM of occipital lobes, m^2/c	0.43±0.02	0.37±0.07	0.048

Note: * – there are only statistically significant differences (*P*<0.05)

gene and the presence of the G allele of the Q192R A>G polymorphism in the PON1 gene.

To predict the development of atrophic changes in the brain and cognitive dysfunction in patients with CHF of ischaemic origin, determination of the polymorphic variants of the -455 T>C and -482 C>T in the APOC3 gene, Q192R A>G and L55M A>T in the PON1 gene are recommended.

Conflict of interest: None declared

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