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The association of genotype and alleles 174G/C (rs1800795) frequency of Il6 gene with clinical data in patients with atrial fibrillation, arterial hypertension in combination with extra-cardiac pathology

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Objective. *To study the genotype and allele frequency of 174G/C (rs1800795) polymorphism of Il6 gene in patients with atrial fibrillation, arterial hypertension in combination with extra-cardiac pathology and to establish its association with clinical data.*

Materials and methods. *161 patients with persistent and paroxysmal AF and second stage AH aged 53.3±7.1 years were included in the study. We estimated clinical, anthropometrical and laboratory parameters, the results of instrumental tests: 24-Hour Holter ECG monitoring, 24-Hour arterial pressure monitoring, transthoracic echo-*

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cardiography. The test of -174G / C (rs1800795) polymorphism of the IL6 gene was performed by polymerase chain reaction followed by restriction fragment length polymorphism analysis.

Results. *We had 7.19 % cases of emergency admission. 4.19 % of them were due to cardiac embolism, 14.37 % — due to CHF decompensation and 15.57 % due to recurrent AF. We proved the association of rs1800795 polymorphism of IL6 gene and DM (p=0.024). The factors connected with AF were: left atrial volume (p = 0.027), end-diastolic volume (p = 0.021) and the presence of C allele of polymorphic marker G (–174) C of IL-6 gene (p = 0.003). When analyzing the frequencies of the genotypes with the rs1800795 polymorphism of the IL6 gene in patients with various comorbidities with recurrent AF, we found that the frequency of CC genotype is higher in patients with recurrent AF in the group with subclinical hypothyroidism. The frequency of heterozygous CG genotype of rs1800795 was higher in patients with cardiac embolism (OR 2.25; 95 % CI 1.01–5.04 p=0.05) compared with patients without cardiac embolism. Patients with CC genotype had higher level of galectin-3 (p<0.022) compared with patients with other genotypes.*

Conclusion. *Screening of exposure genes and studying their polymorphisms become an important research area, since gene polymorphism can influence the progression and development of atrial fibrillation complications.*

Key words: *atrial fibrillation, arterial hypertension, polymorphism, rs1800795 polymorphism of IL6 gene*

Conflicts of interest: nothing to declare.

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Introduction

Atrial fibrillation (AF) is one of the most common stable arrythmias [1]. The frequency of AF has doubled over the last years. Possible reasons of such increase may include: increasing number of elderly patients, improvement of AF diagnosis at outpatient level, increasing number of acute myocardial infarction survivors (AMI), etc. [2].

It is remarkable that the frequency of AF is increasing and progressing. The term «AF progression» means the development of chronic AF from paroxysmal form [3]. 2.2 million of US citizens had paroxysmal or persistent AF that in 67 % of cases progressed to chronic AF during the 5-year follow-up. 8.2 million people of 512 million European population have AF with 1:4 risk of progression in men and women aged 40 years. It is predicted that the number of people with this arrhythmia will increase from 2.5 million in the early 2000s up to 15 million in 2050 in the United States [4]. Nowadays many clinical studies are devoted to risk factors (RF) of AF, including the main factor — arterial hypertension (AH), which contributes to ventricular hypertrophy and atrial dystrophy. However, the progression of AF needs more attention [5, 6].

As genetics is developing, idiopathic AF becomes less frequent. Familiar AF is autosomal dominant disease due to impaired function of various potassium channels during phase 3. Less frequent, AF may be autosomal recessive or sex linked disease – due to impaired function of sodium channels [7].

Familiar AF may be an independent nosological unit, or may be accompanied by channelopathies, for example short or long QT syndrome, Brugada syndrome and catecholaminergic polymorphic ventricular tachycardia. AF may also be associated with structural genetic cardiomyopathies, such as familial dilated cardiomyopathy, hypertrophic cardiomyopathy, idiopathic restrictive cardiomyopathy, arrhythmogenic right ventricular dysplasia, and with unclassified diseases (non-compaction cardiomyopathy, endocardial fibroelastosis) [8].

During large meta-analysis involving over ten GWAS (genome wide association study) of different ethnicity, some phenotypic traits were associated with polymorphisms: high density lipoproteins (HDL), triglycerides, blood pressure (BP) [9]. Some of identified loci are common for all traits, some lie in extragenic spaces, some locate in genes of proteins that were unknown to be involved in biological pathway of this trait. A number of authors showed the association of rs2200733 and rs1800795 polymorphisms with postoperative AF [10]. The IL-6 protein is produced by endothelial cells, vascular smooth muscle cells, and myocytes during ischemia. Its level is associated with AF in patients with

coronary artery disease, after cardiac surgery, cardioversion and catheter ablation [11, 12].

Objective

To study the genotype and allele frequency of 174G/C (rs1800795) polymorphism of IL6 gene in patients with atrial fibrillation, arterial hypertension in combination with extra-cardiac pathology and to establish its association with clinical data.

Materials and methods

161 patients took part in prospective cohort study. The criteria of inclusion were: age of 45–65 years, stage 3 of AH (ESH/ESC, 2018), paroxysmal of persistent AF (RSC, ASSA, ACSR, Moscow, 2017) and one of the following diseases: type 2 diabetes mellitus (DM (EASD/ESC, 2017), subclinical hypothyroidism (SCH), central obesity (CO (AACE/ACE, 2014), chronic obstructive pulmonary disease (COPD (ERS, 2017). We estimated clinical, anthropometrical and laboratory parameters, the results of instrumental tests: ECG, 24-Hour Holter ECG monitoring, 24-Hour arterial pressure monitoring using SCHILLER monitoring system (Schiller, Switzerland), echocardiography (Echo CG) in M and 2D modes using Vivid 7 ultrasound machine (General Electric, USA). The level of galectin-3 was determined with enzyme immunoassay using «Human Galectin-3 ELISA kit; eBioscience» (Bender MedSystems GmbH, Austria), minimum concentration of determination — 0.12 ng/ml. The level of NT-proBNP was determined using the «NTproBNP-ELISA-Best kit». CRP (C-reactive protein) was determined using ELISA test system (Biomerica), USA.

The test of -174G / C (rs1800795) polymorphism of the IL6 gene was performed by polymerase chain reaction followed by restriction fragment length polymorphism analysis.

DNA isolation from blood leukocytes was performed using phenol-chloroform extraction method [Smith K., 1990]. The test of -174G / C (rs1800795) polymorphism of the IL6 gene was performed by polymerase chain reaction followed by restriction fragment length polymorphism analysis (PCR with RFLP). The study design is presented in table 1.

According to the definition of an expert consensus document (HRS / EHRA / ECAS, 2012), the term «progression of AF» means the development of chronic AF from paroxysmal form.

All patients signed an informed consent to participate in research. The study was approved by the local ethical committee of Novosibirsk State Medical University.

Statistical analysis. Statistically significant predictors of complications were determined by multifactor logistic regression analysis. Optimal models of multifactor regression analysis were developed using the direct and inverse step models. In multifactor model we estimated: the stage and FC of CHF (NYHA), Echo CG data, left ventricular mass index (LVMI) and biochemical markers of myocardium remodeling: galectin-3 and NT-proBNP. We also estimated hemodynamic parameters: left atrium diameter (LA), end-diastolic diameter (EDD), heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP); biochemical parameters: C-reactive protein (CRP) as a marker of inflammation, uric acid, glomerular filtration rate (GFR) and fibrinogen. The level of significance (p) was taken as 0.05. The lower threshold of statistical power was 80 %. Statistical analysis of obtained data was done using Rstudio software (version 0.99.879 — © 2009–2016 RStudio, Inc., USA, 250 Northern Ave, Boston, MA 02210 844–448-121, info@rstudio.com).

Results and discussion

Average age of participants was 53.3±7.1 year. During the period of follow-up of 167 patients with AF and AH, 15.57 % had AF progression, 14.37 % — CHF decompensation, 4.19 % — cardiac embolism and 7.19 % were admitted due to emergency (Figure 1).

Table 1. **The study design**

Observational cohort study of patients with AF and AH aged 45–60 years (n=161)				
AF/AH — comparison group n=37	AF/AH/COPD n=30	AF/AH/SCH n=25	AF/AH/DM n=36	AF/AH/CO n=33

Estimation of clinical parameters	ECG, 24-Hour Holter ECG monitoring, 24-Hour arterial pressure monitoring «Schiller»	Echo CG «General Electric, USA»,	NTproBNP Galectin-3 «Human Galectin-3 ELISA kit; eBioscience», «NTproBNP-ELISA-Best kit»	Biochemical spectrum «Vector Best», ELISA using ELISA test system, Biomerica, США	The test of -174G / C (rs1800795) polymorphism. PCR with RFLP. SibEnzyme, Russia.

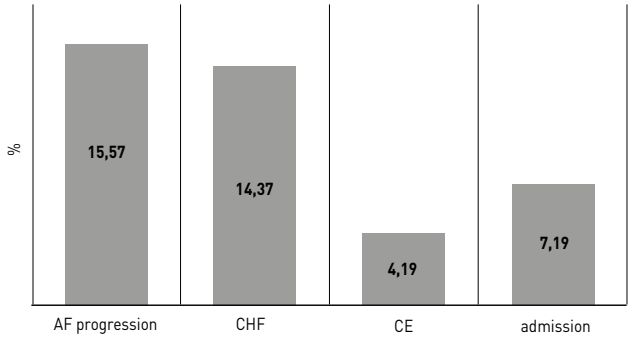

Figure 1. Development and progression of AF complications over the year

When analyzing the frequencies of the genotypes and alleles rs1800795 of the IL6 gene in patients with various comorbidities, we found statistically significant differences in DM group (p=0.047).

The analysis of the frequencies of the genotypes and alleles rs1800795 polymorphism of the IL6 gene in patients with various comorbidities is presented in table 2.

The odds ratio of SS genotype in DM group is significantly lower compared with comparison group (5.6% vs 27.0; p = 0.024). The frequency CC genotype of rs1800795 is different between DM and AH groups (p=0.025), and DM and CO groups (p=0.020). The frequency of allele C did not increase significantly in COPD group. Although, there are data on association between the development of AF in patients with COPD, inflammation and rs1800795. The factors associated with AF were: the volume of the LA (p = 0.027), EDD (p = 0.021) and carriage of allele C of the polymorphic marker G (–174) C of the IL-6 gene (p = 0.003) [13].

When comparing the frequencies of the genotypes and alleles rs1800795 of the IL6 gene in patients with various comorbidities with and without recurrent AF, no statistically significant differences were found. This may be associated with relatively large investigated group. Earlier, a number of authors showed an association of the rs1800795 polymorphism with postoperative AF. The IL-6 protein is produced by endothelial cells, vascular smooth muscle cells, and myocytes during ischemia [15–17].

When analyzing the frequencies of the genotypes with the rs1800795 polymorphism of the IL6 gene in patients with various comorbidities with and without recurrent AF, we found that the frequency of CC genotype is higher in patients with recurrent AF in the group with subclinical hypothyroidism and in comparison group. The frequency of CC genotype was lower in COPD group and it was the same in patients with and without recurrent AF in DM group. The frequency of recurrent AF was lower in SCH group in patients with CG genotype, p=0.030 (Table 3).

Some patients had CE over the year after treatment. When comparing the frequencies of the genotypes with the rs1800795 polymorphism of the IL6 gene in patients with and without CE, the frequency of heterozygous CG genotype of rs1800795 was higher in

Table 2. **The frequencies of the genotypes and alleles rs1800795 polymorphism of the IL6 gene in patients with various comorbidities**

Genotypes	Comparison group		COPD		SCH		DM		CO	
	n	%	n	%	n	%	N	%	n	%
CC	10	27.0	7	23.3	7	28.0	2	5.6	9	27.3
CG	17	46.0	14	46.7	13	52.0	21	58.3	15	45.4
GG	10	27.0	9	30.0	5	20.0	13	36.1	9	27.3
Alleles	**%**		**%**		**%**		**%**		**%**	
C	50.0		46.7		54.0		34.7		50.0	
G	50.0		53.3		46.0		65.3		50.0	

Table 3. **The frequencies of the genotypes with the rs1800795 polymorphism of the IL6 gene in patients with various comorbidities with and without recurrent AF**

	Comparison group				DM			
	Recurrent AF				Recurrent AF			
	No		Yes		No		Yes	
Genotypes	n	%	n	%	n	%	N	%
CC	3	17.6	7	35.0	1	5.9	1	5.3
CG	10	58.8	7	35.0	9	52.9	12	63.2
GG	4	23.5	6	30.0	7	41.2	6	31.6
Genotypes	SCH				COPD			
CC	1	12.5	6	35.3	4	36.4	3	15.8
CG	7	87.5	6	35.3	3	27.3	11	57.9
GG	0	0	5	29.4	4	36.4	5	26.3

patients with CE (OR 2.25; 95% CI 1.01–5.04 p=0.05) (Table 4). The association between stroke and allele G rs1800795 carriage was shown in population [18].

Table 4. **The frequencies of the genotypes with the rs1800795 polymorphism of the IL6 gene in patients with and without CE**

Genotypes	CE			
	No		Yes	
	n	%	N	%
CC	33	24.8	4	12.5
CG	61	45.9	21	65.6
GG	39	29.3	7	21.9
Significance of differences, p	0.117			
	n	%	N	%
CG+GG	100	75.2	28	87.5
CC	33	24.8	4	12.5
Significance of differences, p	0.161			
	n	%	N	%
CC+GG	72	54.1	11	34.4
CG	61	45.9	21	65.6
Significance of differences, p	0.050			
Relative risk	2.25			
95% CI RR	1.01–5.04			

When analyzing the frequencies of the genotypes with the rs1800795 polymorphism of the IL6 gene in patients with various comorbidities, CHF development and admission, no statistically significant differences were found.

When comparing average values of various parameters in patients with different genotypes of rs1800795 polymorphism of IL6 gene using the Kruskal-Wallis test, we found significant differences by the level of HDL, creatinine and galectin-3.

When comparing the values of the studied parameters in patients of the CC genotype with a group of patients with CG and GG genotypes, the significance of differences remains the same (table 5). Patients with CC genotype had higher level of galectin-3 (p<0.022) compared with patients with other genotypes, p=0.022.

Conclusion

When analyzing the frequencies of the genotypes with the rs1800795 polymorphism of the IL6 gene in patients with various comorbidities with recurrent AF, we found that the frequency of CC genotype is higher in patients with recurrent AF in the group with SCH. When comparing the frequencies of CG genotype of rs1800795 of the IL6 gene in patients with and without CE, the frequencies of heterozygous CG genotype was higher in patients with CE. According to logistic regression analysis, the factors associated with AF include: volume of the LA, EDD and carriage of allele C of the polymorphic marker G (–174) C of the IL-6 gene. Patients with CC genotype had higher level of galectin-3 compared with patients with other genotypes.

Conflict of interest: None declared.

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