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Left Ventricular Hypertrophy: the Impact of C825T Polymorphism of β3-Subunit G-protein Gene in Patients with Essential Hypertension

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Abstract

The C825T polymorphism of β3-subunit G-protein gene (GNB3) is associated with activation and cell growth that lead to left ventricular hypertrophy (LVH). The aim this research was to analyze the association between the C825T polymorphism GNB3 gene and some central hemodynamic parameters, left ventricular mass (LVM) and heart geometric in patients with essential hypertension (EH) and LVH. We used two-dimensional-controlled M-mode echocardiograms in 155 patients whith EH for evaluation of LVH, the C825T polymorphism GNB3 was determinated by polymerase chain reaction and restriction digestion. 49 (0.32) were homozygous for the C allele (CC), 86 (0.55) were heterozygous (CT), and 20 (0.13) were homozygous for the T allele (TT). C- and T alleles carries were 32% and 68% respestively, T allele frequency was 0.4. The differences between left ventricular internal end-diastolic diameter (LVEDD), intraventricular septum thickness (IST), posterior wall thickness (PWT), left ventricular mass (LVM) and left ventricular mass index (LVMI) in the patients with EH according to genotypes and alleles of C825T polymorphism GNB3 gene were statistical significance ($p=0.001$, $p<0.001$, $p=0.025$, $p<0.001$ and $p=0.001$ respectively). Normal geometry and LV remodeling (0.48 and 0.44 respectively) prevails among patients with EH versus eccentric and concentric LVH (0.03 and 0.05 respectively). The frequency of the T allele was significantly higher in patients with LV remodeling compare the patients with normal geometry (0.43 versus 0.365 respectively) and didn't find significant difference of frequency genotypes and alleles for eccentric and concentric types of LVH.

Thus, the T allele of C825T polymorphism of β3-subunit G-protein gene is associated with left ventricular hypertrophy, higher some echocardiographic parameters left ventricular, left ventricular mass, left ventricular mass index but not associated with types of left ventricular geometry in patients with essential hypertension in Ukrainian population.

Keywords: left ventricular hypertrophy, polymorphism of β3-subunit G-protein gene (GNB3), essential hypertension.

1. Introduction

Left ventricular hypertrophy (LVH) is a major independent risk factor for morbidity and mortality from cardiovascular disease. Obesity, diabetes and especially hypertension are important determinants of LVH. Epidemiological studies suggest that LVH may be influenced by genetic factors, such as: genes that encode components of hormonal pathways, genes of key sympathetic and parasympathetic receptors, genes that modify intracellular ion homeostasis, energy metabolism and motor unit composition and regulation [1; 2; 3]. Genes and their polymorphisms that associated with LVH are angiotensinogen gene, angiotensin-converting enzyme gene, angiotensin receptor type 1 gene, aldosterone synthase gene, nitric oxide synthase gene, type A natriuretic peptide receptor gene, beta(2)-adrenergic receptor gene, β3-subunit G-protein gene (GNB3) [3; 4; 5]. These research show significant impact of some gene polymarphysms on the intensity of protein synthesis in myocardiocytes and the development of hypertension leads to mechanical overload. Recent studies have demonstrated an interest in C825T polymorphism GNB3 gene due to participation in the initiation of cell growth and proliferation [6]. C825T polymorphism was detected in exon 10 of the gene *GNB3*, which encodes the β3-subunit of heterotrimeric G proteins that are key components of intracellular signal cell transduction [7].

Series of studies prove the association between the 825T allele and LVH in patients with essential hypertension (EH) [4; 8; 9; 10; 11]. By contrast, Sedlacek K. et al. 2002 showed any association between T allele GNB3 and cardiac structure [12]. Shlyakhto et al. 2002 also found no association between left ventricular structure or function and the GNB3 gene variant in a St. Petersburg population [13]. Wang X. et al. 2004 did not confer a significantly increased risk for the development of LVH [14]. So, it is not clear understand the role of C825T polymorphism in patient with LVH and its association with heart remodelling and hemodynamic parameters.

The aim this research was to analyze the association between the C825T polymorphism of the β3-subunit G-protein gene and some central hemodynamic parameters, left ventricular mass and heart geometric in patients with EH and LVH.

2. Subjects and methods

2.1 Study Subjects

155 patients whith EH involved in this study (87 men (56.1%), mean age 58 ± 1.0 years, 68 women (43.9%), mean age 64 ± 1.2 years), who had been under medical surveillance in the Sumy regional clinical hospital. A final diagnosis of EH was established on the basis of clinical, instrumental and laboratory investigations according to ESH/ESC 2013 [15]. Hypertension is defined as values >140mmHg systolic blood pressure (BP) and/or >90mmHg diastolic BP in 3 repeated measurements. Patients with any severe concomitant pathological condition, secondary hypertension, pregnant women and those taking contraceptive pills were excluded from the study.

The study had been previously approved by the Ethic Committee on Medical Research of Medical Institute of Sumy State University. An appropriate informed consent was obtained from all patients.

2.2 Evaluation of LVH

2.2.1. Two-dimensional-controlled M-mode echocardiograms were recorded with each patient in the partial left decubitus position after a rest of at least 10 minutes by "Sonoline Ultrasound Imaging System" (Siemens, Germany). Left ventricular internal end-diastolic diameter (LVEDD), intraventricular septum thickness (IST), and posterior wall thickness (PWT) were measured according to the Penn convention just below the tip of the mitral valve as recommended by the American Society of Echocardiography.

2.2.2. Left ventricular mass (LVM) was calculated according to the formula of Devereux et al.: LVM (in grams)=(1.04[EDD+IST+PWT]^3−EDD^3)−13.6g. The LVM indexed to body size (LVMI) was obtained according the allometric signal height$^{2.7}$). LVH was diagnosed if LWMI exceeded : >50 g/m$^{2.7}$ in men, >47 g/m$^{2.7}$ in women [16].

2.2.3. Patterns of left ventricular geometry were defined as proposed by Ganau et al.: normal geometry when LVMI was normal and relative wall thickness (RWT = 2 PWT/EDD) <0.45; LV remodeling when normal LVMI was combined with RWT >0.45; concentric LVH when LVH occurred with a RWT >0.45; and eccentric LVH when LVH and a RWT <0.45 were combined [17].

2.3. Genotype determination for GNB3 C825T polymorphism

Venous blood sampling for genotyping was performed under sterill conditions into 2,7 ml S–Monovette ("Sarstedt", Germany) containing EDTA potassium salt as an anticoagulant, the samples were frozen and stored at –20 ◦C. DNA was extracted from whole blood by polymerase chain reaction (PCR) using commercially available kits ("Isogene Lab Ltd", Russia) according to the manufacturer's protocol. To identify the GNB3 promotor C825T polymorphism (rs 5443) PCR with subsequent restriction fragment length polymorphism (RFLP) analysis were performed. The primer pair used for PCR amplification was 5′-TGACCCACTTGCCACCCGTGC-3′ (sense) and 5′-GCAGCAGCCAGGGCTGGC-3′ (antisense). The PCR product was digested with the restriction enzyme BseDI (Fermentas), electrophoretically resolved on 2.5% agarose, and visualized under UV illumination. For statistical analysis used nonparametric Mann-Whitney test, Kruskel-Wallis test and χ^2 test.

2.4. Statistical Analysis

The normal distribution and homogeneity of variances were tested before further statistical analyses. The association between genotype distribution and LVH was examined by χ2 test, in which odds ratios and 95% CIs were calculated. Values are expressed median (range) in variables due to abnormal distribution. Differences among genotypes of C825T polymorphisms of GNB3 gene were compared by nonparametric Mann-Whitney test and Kruskel-Wallis test. A value of $p<0.05$ was considered significant. All statistical analyses were performed by using the statistical software package SPSS version 21 (SPSS for Windows, version 21, SPSS Inc., Chicago, IL).

3. Results

3.1. Among the 155 patients with EH, 49 (0.32) were homozygous for the C allele (CC), 86 (0.55) were heterozygous (CT), and 20 (0.13) were homozygous for the T allele (TT). Thus 32% were C allele carriers, 68% were T allele carriers. The frequency of T allele was 0.4. The distribution of genotypes was in Hardy-Weinberg equilibrium.

Table 1 summarizes echocardiographic parameters of the patients with EH according to C825T polymorphism of GNB3 gene.

Table 1: Echocardiographic characteristics according to C825T polymorphism of GNB3 gene in patients with EH

	CC	CT	TT	p	C allele	T allele	p
LVED D, sm	5.3 (4.9-5.5)	5.5 (5.1-5.9)	5.8 (5.6-6.0)	0,001	5.3 (4.9-5.5)	5.6 (5.1-6.0)	0.001
IST, sm	1.2 (1.1-1.3)	1.3 (1.2-1.4)	1.3 (1.2-1.4)	< 0.001	1.2 (1.1-1.3)	1.3 (1.2-1.4)	< 0.001
PWT, sm	1.2 (1.1-1.2)	1.2 (1.07-1.3)	1.25 (1.2-1.3)	0.006	1.2 (1.1-1.2)	1.2 (1.1-1.3)	0.026
RWT	0.44 (0.42-0.48)	0.45 (0.4-0.51)	0.45 (0.42-0.48)	0.812	0.44 (0.42-0.48)	0.15 (0.41-0.50)	0.525
LVM, g	296.7 (259.5-331.1)	343.1 (293.8-417.4)	399.9 (338.8-496.8)	< 0.001	296.7 (259.5-331.1)	356.6 (295.9-423.2)	< 0.001
LVMI, g/m²	70.8 (61.4-80.8)	81.9 (66.2-99.8)	92.9 (82.2-114.3)	< 0.001	70.8 (61.4-80.8)	83.3 (71.1-103.9)	0.001

Values are median (range).

p – value of significance

LVEDD – left ventricular end-diastolic diameter; IST – interventricular septum thickness; PWT – posterior wall thickness; RWT – relative wall thickness ratio; LVM – left ventricular mass; LVMI – left ventricular mass index.

The differences between echocardiographic parameters in the patients with EH according to genotypes and alleles of C825T polymorphism of GNB3 gene are statistical significance, exept RWT (p=0.812). Patients with CT and TT genotypes had greater LV dimensions and thickness compare the patients with the CC genotype. Furthermore, LV dimensions and thickness in patients with CC genotype were less compare the patients with CT genotype. But these parameters were significaly higher in patients with TT genotype.

We observed increase LVM and LVMI in patients with CT and TT genotypes versus CC genotype. Moreover, these parameters were significantly higher in homozygous for the T allele compare heterozygous.

Generally, T allele carriers had significantly higher echocardiographic parameters compared with C allele carriers.

3.2. As shown in Table 2, the distribution of patients with EH according to types of heart geometry was statistical significance (p<0.001).

Table 2: Distribution of patients with EH according to types of heart geometry

Frequencies	heart geometric type			
	Normal geometry	LV remodeling	Eccentric LVH	Concentric LVH
N	74	68	5	8
Frequencies	0.48	0.44	0.03	0.05
χ^2	107.9			
p	<0.001			

- χ^2 tests were performed for analyse distributions frequencies types of heart geometric
- p – value of significance

Normal geometry and LV remodeling (0.48 and 0.44 respectively) prevails among patients with EH versus eccentric and concentric LVH (0.03 and 0.05 respectively).

3.3. It is advisable to investigate the distribution of genotypes and alleles of C825T polymorphism of GNB3 gene in patients with EH according to types of heart geometry (Table 3).

Table 3: Distribution of genotypes and alleles of C825T polymorphism of GNB3 gene in patients with EH according to types of heart geometry

	Frequencies						
	Genotypes				Alleles		
	CC	CT	TT	p	C	T	p
Normal geometry	28 (0,38)	38 (0,51)	8 (0,11)	<0,001	28 (0,38)	46 (0,62)	0,036
LV remodeling	18 (0,27)	41 (0,6)	9 (0,13)	<0,001	18 (0,27)	50 (0,73)	<0,001
Eccentric LVH	1 (0,2)	3 (0,6)	1 (0,2)	0,449	1 (0,2)	4 (0,8)	0,18
Concentric LVH	2 (0,25)	4 (0.5)	2 (0,25)	0,607	2 (0,25)	6 (0,75)	0,157

- p – value of significance

The frequency of CC genotype was in 3.5 times higher compare TT genotype in patients with normal geometry and in 2.5 times higher in patients with LV remodeling.

The frequency of the T allele was significantly higher in patients with LV remodeling compare the patients with normal geometry (0.43 versus 0.365 respectively). We didn't find significant difference of frequency genotypes and alleles for eccentric and concentric types of LVH.

4. Discussion

Potential candidate genes of LVH include ones encoding proteins regulating cardiac structure, hemodynamic load, calcium homeostasis, hormones, substrate metabolism, growth factors, energy metabolism and cell signaling [11]. One of them is C825T polymorphism of GNB3 gene. Furthemore, C825T polymorphisms of GNB3 gene associated with LV mass and LVH in hypertensive patients [18]. Hypertensive patients with echocardiographic criteria of LVH have higher risk cardiovascular complications and mortality compare without these criteria [19].

In the present study we showed the association of C825T polymorphism of the GNB3 with LVH in patients with EH and the impact of these polymorphisms on parameters of some echocardiographic characteristics and LV geometry.

The results showed that frequency genotypes and alleles among Ukrainian hypertensive patients (0.4) was similar to that reported in Russian and European populations [8; 20; 21]. The T allele frequency in hypertensive Asians, Caucasians and Africans was 0.46, 0.33 and 0.79 respectively [22]. Siffert et al.2003 founded that T allele frequency was the lowest in Germans (0,319), intermediate in Chinese (0.477) and the highest in Africans (0.814 to 0.841) [7].

Our results indicate that hypertensive patients with CT, TT genotypes and T allele carriers had greater LV dimensions and thickness compare patients with the CC genotypes and C allele carriers. Poch et al. 2000 had similar results from European population [8]. Semplicini A. et al. 2001, Sartori et al. 2004 showed that patients carrying the T allele of GNB3 gene had an increased LVM and LVMI in comparison to those with CC genotype and C allele that conformable with our results [18; 23].

In contrast our results, Schlyaktho E.V. et al. 2002 showed lack of association of C825T polymorphism of GNB3 gene in hypertensive patients. Russian researches did not get differences frequency of the T allele in patients with LVH compared to those without LVH and the LVMI, echocardiographic parameters were similar hypertensive patients with the CC, CT and TT genotypes [13]. The results from study of Taiwan and Emirati populations showed that C825T polymorphism of GNB3 gene not associated with LVH in hypertensive patients [24; 25].

In our study we found that in patients with EH prevails normal geometry and LV remodeling that are more favorable variants of heart geometric. But in the study of Illyash M.G. et al. 2014 among Ukrainian miners with EH prevailed eccentric type of LV geometry [26]. Results of Cuspidi C. et al. 2012 showed that eccentric LVH was more frequent than concentric LVH in contrast our results [27]. The distribution of genotypes and alleles of C825T polymorphism GNB3 in patients with EH according type of LV geometry didn't demonstrate association C825T polymorphism GNB3 and types of LV geometry. Bella J. et al. 2012 observed marginal impact of genetic variations on forms of LVH and explained it other predictors such as: obesity, high salt diets, glucose metabolism, age, level of blood pressure, BMI, duration arterial hypertension [11].

In summary, we showed that the T allele of C825T polymorphism GNB3 gene is associated with LVH, higher some echocardiographic parameters LV, LVM, LVMI but not associated with types of LV geometry in patients with EH in Ukrainian population.

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