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ENGLISH VERSION: ON THE GENETIC REGULATION OF BRAIN NATRIURETIC PEPTIDE LEVEL IN PLASMA OF MEN WITH ESSENTIAL HYPERTENSION*

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We investigated the organization of the brain natriuretic peptide gene (BNP T-381C) and plasma levels of BNP, CNP in male citizens of Podillia region in Ukraine with essential hypertension stage II and with its complications of chronic heart failure stage IIA. Genotyping of the BNP gene was conducted using polymerase chain reaction. The levels of BNP and CNP plasma concentrations were determined by ELISA. It was established that among practically healthy men and patients with essential hypertension of varying severity, the genotype T381C and C allele of the BNP gene dominanted. The carriers of the C allele of the BNP gene have significantly higher plasma levels of brain and endothelial natriuretic peptides as against the control group and patients with essential hypertension. In addition, the highest levels of natriuretic peptides were determined in patients with symptoms of chronic heart failure stage IIA.

Keywords: essential hypertension, chronic heart failure, gene polymorphism of the brain natriuretic peptide, plasma concentration of brain natriuretic peptide, plasma concentration of endothelial natriuretic peptide..

Introduction

Natriuretic peptide system (NP) is a group of circulating hormones, which are physiological antagonists of the renin-angiotensin-aldosterone system, counteracting the increase of vascular tone and vascular wall hypertrophy [17]. It was determined that brain natriuretic peptide (BNP) possesses the greatest specificity and information capacity to myocardial function. It is significant pathophysiological importance in the diagnosis of heart failure, risk stratification and monitoring the effectiveness of treatment of chronic heart failure (CHF) [2,7]. The scientists of the Department of Internal Medicine of Medical Faculty №2, National Pirogov Memorial Medical University, revealed that in patients with essential hypertension (EH), the level of plasma BNP concentration is directly proportional to tensile and pressure overload of the left ventricle (LV) and correlates with left ventricle end-diastolic pressure and the presence of left ventricular hypertrophy [4]. Similar studies by T. Ohtani (2012), Q. Lin (2012), N. Nair (2013) and colleagues found that the level

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of plasma BNP concentration increases in the early stages of LV dysfunction in patients with EH, correlates with the level of left ventricular end-diastolic pressure, increases in patients with left ventricular hypertrophy, and is associated with the severity of heart failure [14,11,13].

C-type natriuretic peptide (CNP) belongs to the class of vasodilators, participates in the regulation of blood pressure, vascular tone, and heart work. According to modern concepts, CNP is an antagonist of the most powerful vasoconstrictor endothelin-1, also plays a role in preventing the occurrence of endothelial dysfunction, which is the base of EH and CHF on its background. [3]. It is still an open question as to the role of genetic influence on the plasma concentration of both BNP and CNP natriuretic peptides.

It is proved that BNP gene is situated on the first chromosome and consists of three exons and two introns. The most physiologically significant polymorphism of the gene BNP - replacing thymine for cytosine at position 381 (T-381C) have been defined and studied [1,8,12,15]. This polymorphism has not been studied in the Ukrainian population. Considering the modern achievements in the study of the pathogenesis of CHF, we can assume the influence polymorphism of the gene BNP in the development and progression of EH and CHF on its background. *The aim of this study was* to improve the prognosis and diagnosis of chronic heart failure in male citizens of Podillia region in Ukraine with essential hypertension by determining the plasma concentration levels of brain and endothelial natriuretic peptides in carriers of different BNP gene variants.

Methods

During the study we examined 191 men aged 40–60 living in Podillia region in Ukraine. Among them, 62 men from the first main group were diagnosed with EH stage II with LV hypertrophy and CHF, 0-I classes according to NYHA Classification, whose average age was 49.19±0.66, and 50 men from the second main group with EH complicated by CHF stage IIA, II-III classes according to NYHA Classification, whose average age was 50.14±0.99. 79 healthy men whose age (49.01±0.73) did not differ from patients with EH and constituted the control group (p>0.05). The diagnoses of EH and CHF were established on the basis of patients' complaints, anamnesis, physical examination, laboratory and instrumental methods of investigation according to the guideline of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) in 2013 and 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. All patients were observed from December 2013 to July 2014.

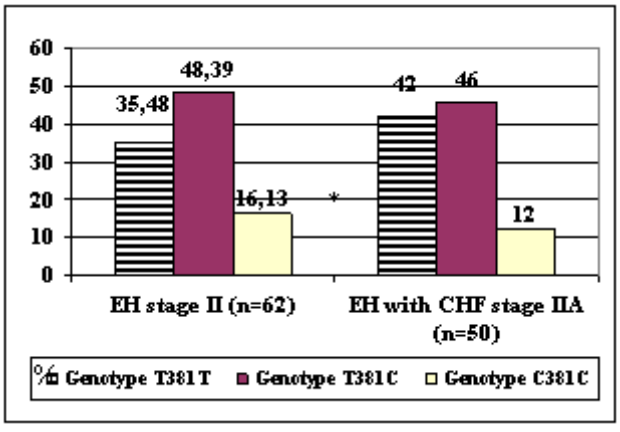
Exclusion criteria of the study were: secondary hypertension, renal and liver dysfunction, coronary heart disease the onset of which preceded EH, endocrine, hematological, neoplastic and autoimmune disorders, patients with EH complications: myocardial infarction, acute cerebrovascular accident.

Genotyping of the BNP gene was conducted using polymerase chain reaction after isolation of genomic DNA from white blood cells of venous blood. This study was carried out jointly with Research Institute for Genetic and Immunological Grounds of Pathology and Pharmacogenetics "Ukrainian Medical Stomatological Academy" (Poltava, headed by prof. I.P. Kaidashev). The plasma levels of BNP and CNP were determined by using ELISA method on enzyme-linked immunosorbent analyzer "Numareader single" (Germany) at 450 nm and differential filter 630 nm. To determine the BNP plasma concentration, a standard set of «Peninsula laboratories Inc.» (USA) was used. To determine the CNP plasma concentration, a standard set of «BIOMEDICA» (Germany) was used. The mathematical processing was performed on a personal computer using a standard statistical package STATISTICA 6.1. Checking the distribution of genes polymorphisms frequencies in the population according to Hardy-Weinberg equilibrium law was conducted using a calculator gene expert for estimation of the number of statistical parameters in the "case-control" studies which use SNP (*gen-exp.ru*). Boundary BNP level determined by the method proposed by M.U. Antomonov in collaboration with V.M. Zhebel et al. [5,6].

Results and discussion

It was established that in the male control group the frequency of the genotype T381T of the BNP gene was 31.65% (n=25), the genotype T381C – 49.37% (n=39) and the genotype C381C – 18.90% (n=15) (p_{CC-TT}>0.05; p_{TC-CC}> 0.05; p_{TC-TT}≤0.05). The frequency of the T allele in male from the control group was 43.67%, the C allele – 56.33% (p<0.05).

It was determined that in men with EH stage II and patients with symptoms of CHF stage IIA the genotype T381C and C allele of the BNP gene dominanted, but there were no significant differences in frequency carriage of the genotype variants of the BNP gene as compared with those in the control group (p>0.05) (Figure 1).



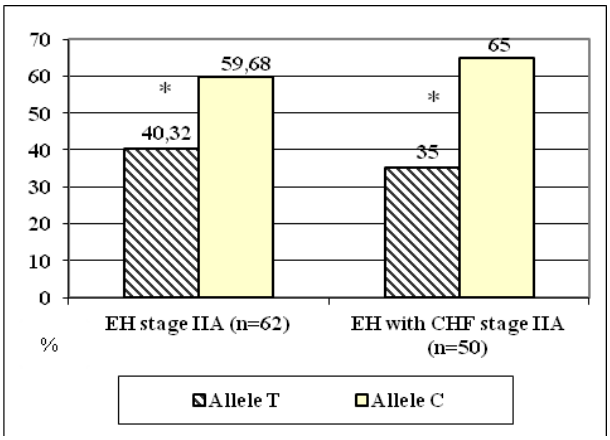


Fig. 1. The distribution of the BNP gene genotypes frequencies and alleles in male citizens of Podillia region with EH stage II and patients with EH complicated by CHF stage IIA, (%)

*Note: The difference is significant (p<0.05) when compared to: * - the T381C genotype/the C allele within each group.*

The study results are broadly consistent with the data conducted by researches among people from other populations. It was found that carriers of the genotype T381C and C allele of the BNP gene dominate among the residents of the US with EH [8], the inhabitants of the Novosibirsk region of Russian Federation [1] and Germany [9]. The frequencies of polymorphic genotypes of the BNP gene did not differ significantly in patients from different populations.

During the statistical analysis due to the small number of homozygotes C381C of the BNP gene we combined heterozygotes T381C and homozygotes C381C in a joint group – carriers of C allele. In groups of carriers of different genotypes the plasma levels of BNP, CNP were determined.

It has been proved that in men who live in Podillia region in Ukraine with EH stage II and its complications of CHF stage IIA, plasma concentration of BNP is significantly higher in carriers of C allele (93.49±0.94 pg/ml and 207.50±5.70 pg/mL respectively) than in carriers of the genotype T381T (48.16±0.63 pg/ml and 156.00±6.99 pg/ml respectively) (p<0.0001). It has been established that the plasma BNP level in men with EH complicated by CHF stage IIA was significantly higher than in patients with EH stage II and healthy men as in the carriers of the genotype T381T of the BNP gene in carriers of C allele. The plasma CNP level in men with EH stage II and patients with symptoms of chronic heart failure stage IIA, which are carriers of C allele, was significantly higher than in the homozygotes T381T of the BNP gene (p<0.05). When compared within each genotype, it was established that in men with EH stage II and patients with symptoms of chronic heart failure stage IIA the plasma levels of CNP were significantly higher than in the control group of the study (p<0.05), but they not differ in patients with varying severity of EH, carriers of polymorphic genotypes of the BNP gene (Table 1).

Table 1

Plasma levels of BNP and CNP in representatives of the control group and patients with varying severity of EH, carriers of polymorphic genotypes of the BNP gene, (pg/ml; pmol/ml)

Groups	Plasma concentration of BNP, pg/ml	Plasma concentration of CNP, pmol/ml
1	2	3
Control group (n=79)		
Homozygotes T381T	15.95±0.69 (n=25) **(1)**	2.26±0.56 (n=25) **(1)**
Carriers of C allele	24.41±0.48 (n=54) **(4)**	2.67±0.54 (n=54) **(4)**
Patients with EH stage II (n=62)		
Homozygotes T381T	48.16±0.63 (n=22) **(2)**	4.53±0.07 (n=22) **(2)**
Carriers of C allele	93.49±0.94 (n=40) **(5)**	5.74±0.78 (n=40) **(5)**
Patients with EH complicated by CHF stage IIA (n=50)		
Homozygotes T381T	156.00±6.99 (n=21) **(3)**	4.81±0.94 (n=21) **(3)**
Carriers of C allele	207.50±5.70 (n=29) **(6)**	6.02±0.67 (n=29) **(6)**
p	p_{4-1}<0.05; p_{5-2}<0.0001; p_{6-3}<0.0001; p_{2-1}<0.0001; p_{3-1}<0.0001; p_{3-2}<0.0001; p_{5-4}<0.0001; p_{6-4}<0.0001; p_{6-5}<0.0001	p_{4-1}<0.05; p_{5-2}<0.0001; p_{6-3}<0.0001; p_{2-1}<0.0001; p_{3-1}<0.0001; p_{3-2}>0.05; p_{5-4}<0.0001; p_{6-4}<0.0001; p_{6-5}>0.05

The data obtained are consonant with the results of studies by foreign authors. Among patients of the US population, in male and female persons the relationship between polymorphism of the BNP and presence and severity of EH and coronary arterial spasm was established. In addition, carriers of the genotypes C381C have significantly higher plasma levels of BNP than carriers of the genotypes T381T [10]. Y. Takeishi et al. (2007) conducted a study among Japanese population and found that both male and female CHF carriers of the genotypes C381C revealed significantly higher plasma levels of BNP in comparison with carriers of the genotypes T381T [16]. L.C. Costello-Boerrigter (2011) determined that the inheritance of genotypes with the presence of C allele of the BNP gene – T381C, C381C was associated with the high plasma concentration of brain NP in persons of different sex with EH [8]. However, the studies to determine plasma concentrations of CNP in carriers of polymorphic genotypes of the BNP gene have not been found in the scientific literature.

The research results indicate the need for determining the range of plasma concentrations of brain NP in carriers of certain variant genotype of the BNP gene. BNP levels for screening diagnosis of EH with CHF stage IIA were established in male citizens of Podillia region in Ukraine, aged 40-60 years that can be applied during examining of large groups of people to identify persons who need to undergo full examination including ultrasound of the heart and determine the causes of resistant high blood pressure:

- The BNP level ≥ 98.62 pg/ml (sensitivity – 86.00%, specificity – 85.40% correctness – 86.10%, false

negative answer – 12.00%, false positive answer – 17.00%) enables to diagnose EH with chronic heart failure stage IIA in males. We consider that this boundary level can be used in screening examination of large groups of people.

However, the BNP plasma concentration can be influenced by genetics. The results indicated that the presence of the C allele in the genotype of the BNP gene is associated with higher plasma concentrations of peptide so it was decided to calculate the BNP boundary levels for the C allele carriers (heterozygote genotype T381C and homozygote genotype C381C) and carriers of the homozygote genotype T381T:

- The BNP level ≥ 110.74 pg/ml (sensitivity – 96.32%, specificity – 7924% correctness – 82.00%, false negative answer – 6.40%, false positive answer – 15.21%) enables to diagnose EH with chronic heart failure stage IIA in males the C allele carriers of the BNP gene;

- The BNP level ≥ 79.68 pg/ml (sensitivity – 86.56%, specificity – 75.20% correctness – 88.00%, false negative answer – 6.71%, false positive answer – 4.53%) enables to diagnose EH with chronic heart failure stage IIA in males with the homozygote genotype T381T of the BNP gene.

That is to say, the individual genetic features of BNP plasma concentration in the aforementioned patients should be considered during the medical-expert and scientific researches using BNP as a biomarker.

Conclusions

1. The carriers of C allele of the BNP gene have significantly higher plasma levels of brain and endothelial natriuretic peptides as against the control group and patients with varying severity of essential hypertension in comparison with carriers of the homozygote genotype T381T. In addition, the highest levels of natriuretic peptides were determined in patients with symptoms of chronic heart failure stage IIA.

2. Boundary levels of MNP can be used in screening examination of large groups of people for early diagnosis of EH complicated by chronic heart failure stage IIA, carriers of different BNP gene variants.

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