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Gene polymorphism of ET-1 and its plasma concentration in men with hypertension and congestive heart failure

Hanna Palahniuk[1],Maryna Babii[2], Olena Sakovych[1], Iuliia Pashkova[1], Olena Maiko[1], Svitlana Franchuk[1], Vadym Zhebel[1]

[1]Department of Internal Medicine of the Medical Faculty №2, Vinnytsia National Pirogov Memorial Medical University, Vinnytsia, Ukraine
[2]Rehabilitation department of adults and children from 3 years with a lesion of the nervous system Khmelnytsky Regional Physiotherapy Hospital, Khmelnyk, Ukraine

Abstract— Nowadays the influence of polymorphism of the ET-1 gene on the plasma levels of this peptide in patients with essential hypertension and congestive heart failure is poorly understood. 191 men were examined: 79 from the control group, 62 with uncomplicated essential hypertension and left ventricular hypertrophy, and 50 men with essential hypertension and congestive heart failure IInd-IIIrd classes. All patients were between the age of 40-60 years, and were residents of the Podillya region in Ukraine.
Lys198Lys genotype and the Lys allele of the ET-1 gene dominate among residents of Podillia region in Ukraine. In patients with essential hypertension the level of ET-1 was significantly higher than that of the control group, with the highest concentration of peptide in individuals with congestive heart failure IInd-IIIrd classes. In men carriers of Lys198Lys genotype, the plasma concentration of ET-1 was significantly lower than in carriers of the Asn allele. In carriers of genotype Lys198Lys the highest level of peptide in plasma was determined in patients with congestive heart failure IInd-IIIrd classes. Men with uncomplicated essential hypertension with left ventricular hypertrophy, carriers of Lys198Lys genotype were more likely to encounter 2nd degree hypertension and carriers of Asn allele – 3rd degree hypertension respectively. In patients with congestive heart failure IInd-IIIrd classes, carrier of the Asn allele was seen to be associated with 3rd degree hypertension.

Keywords— EH: Essential hypertension, CHF:congestive heart failure, genotype Lys198Asn, ET-1:Endothelin-1 gene

1.Introduction

Essentialhypertension (EH) or elevated blood pressure (BP) is among the most prevalent, treatable risk factors for coronary artery disease, congestiveheart failure(CHF), and stroke. Systolic (SBP) and diastolic blood pressure (DBP) traits are highly heritable, with the total heritability for European/African ancestry individuals estimated to be 20/27% and 39/50% for SBP and DBP, respectively. [1] Genetic variance on blood pressure was shown about 100 years ago; a Mendelian inheritance was initially presumed. Platt and Pickering conducted a lively debate, whether blood pressure was inherited in a Mendelian fashion or whether the condition was polygenic. Genetic-hypertension research has appropriately followed both pathways.Genome-wide association studies, Pickering model, have identified more than 500 blood-pressure loci, the targets of which are waiting to be evaluated. [12]
One of the factors that may influence the level of blood pressure is a Single Nucleotide Polymorphism (SNP) of the endothelin-1(ET-1)gene (Lys198Asn), which is considered as a possible genetic marker of EH. [5,9,10] Nowadays, studies of SNP are well known among other genes. However, the effect of polymorphism of the ET-1 gene on the plasma levels by this peptide in patients with EH and CHF is poorly understood and it has not been well studied in the world. [6,8,11,15,16] Circulating plasma levels of ET-1 and related peptides generated during the synthesis of ET-1 from its precursor molecule pre-proendothelin-1 have been widely studied as potential risk markers for cardiovascular events. The associations of ET-1 with aging, blood pressure, lung function, and chronic kidney disease have been described, as have relations between ET-1 levels and evidence of cardiac remodeling, including increased left atrial diameter and increased left ventricular mass. [7]

The aim of the study is to improve diagnosis with relation to the stages of EH and CHF on the background of male citizens of the Podillia region in Ukraine by determining the plasma levels of ET-1 in patients with different ET-1 gene variantions.

2. Materials and Methods

The study involved 191 male residents of Podillia region in Ukraine. Amongst them, 62 men were diagnosed with uncomplicated EH along with left ventricular hypertrophy (LVH) and saved systolic function whose average age was (49,19±0,66) years old, 50 men with EH and CHF IInd-IIIrd classes according to NYHA Classification (50,14±0,99) years old and 79 men without signs of cardiovascular diseases whose average age was (49,01±0,73) years old, and they did not differ from patients with EH and made the control group ($p>0,05$).

The diagnosis of EH was established on the basis of the patients' complaints, anamnesis, physical examination, laboratory and instrumental methods of investigation according to the guidelines of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) 2018. [19] Systolic function of left ventricle was assessed in terms of ejection fraction (EF). Systolic function was considered saved when EF was over 40 %. All patients during the examination were treated at Vinnytsia regional specialized dispensary of radiation protection of the Ministry of Health of Ukraine, Military Medical Center of the Central Region of Ukraine and were observed from December 2013 to June 2014.

Exclusion criteria of the study was: secondary hypertension, renal and liver dysfunction, coronary heart disease the onset of which was before EH, endocrinological, hematological, neoplastic and autoimmune disorders, patients with EH complications: myocardial infarction, acute cerebrovascular accident. Genotyping of the ET-1 gene was conducted using polymerase chain reaction (PCR). The ET-1 concentration in plasma was determined by using ELISA method on enzyme-linked immunosorbent analyzer "Humareader single» (Germany). To determine the ET-1 plasma concentration a standard set of the firm «DRG» (USA) was used. The mathematical processing was performed on a personal computer using a standard statistical package STATISTICA 6,0. The frequency distribution of gene polymorphisms in the population had undergone a checking according to Hardy-Weinberg equilibrium law using a calculator genexpert to calculate the number of statistical parameters in the study "case-control" using SNP (State Scientific Center of the Russian Federation "HosNYY genetics", gen-exp.ru).

3. Results and Discussion

It was established that in the control group, frequency of Lys198Lys genotype of the ET-1 gene was 65,82% (n=52), Lys198Asn genotype– 27,85% (n=22) and Asn198Asn genotype - 6,33% (n=5) ($p_{Lys/Asn\text{-}Lys/Lys}<0,0001$; $p_{Asn/Asn\text{-}Lys/Lys}<0,0001$; $p_{Asn/Asn\text{-}Lys/Asn}<0,001$).The frequency of the Lys allele in individuals from the control group was 79,75%, the Asn allele - 20,25% ($p_{Lys\text{-}Asn}<0,0001$). Because of the low frequency of carriers of Asn198Asn genotype, men with Lys198Asn and Asn198Asn genotypes from the control group were put together in a group as combined carriers of the Asn allele. The frequency of combined carriers of the Asn allele in patients from the control group was 34,18% (n=27) ($p_{Asn\ allele\text{-}Lys/Lys}<0,00001$) (Table 1).

It was investigated that Lys198Lys genotype of ET-1 dominated in patients with uncomplicated EH and LVH - 56,45% (n=35), Lys198Asngenotype was in 33,87% (n=21) patients and Asn198Asn genotype - 9,68% (n=6) ($p_{Lys/Asn\text{-}Lys/Lys}<0,01$; $p_{Asn/Asn\text{-}Lys/Lys}<0,0001$; $p_{Asn/Asn\text{-}Lys/Asn}<0,001$).The frequency of the Lys allele in patients with uncomplicated EH and LVH was 73,39%, the Asn allele - 26,61% ($p_{Lys\text{-}Asn}<0,00001$). Because of the low frequency of carriers of Asn198Asn genotype, men with Lys198Asn genotypes and Asn198Asn with uncomplicated EH and LVH were put together as combined carriers of the Asn allele. The frequency of combined carriers of the Asn allele in males with uncomplicated EH and LVH was 43,55% (n=27) ($p_{Asn\ allele\ -Lys/Lys}>0,05$) (Table 1).

In patients with EH and CHF IInd-IIIrd classes the genotype Lys198Lys of the ET-1 gene was determined in 66% (n=33) men, the genotype Lys198Asn was in 28% (n=14), the Asn198Asn genotype was 6% (n=3) ($p_{Lys/Asn\text{-}Lys/Lys}<0,0001$; $p_{Asn/Asn\text{-}Lys/Lys}<0,0001$; $p_{Asn/Asn\text{-}Lys/Asn}<0,01$). The frequency of the Lys allele was 80%, the Asn allele was 20% ($p_{Lys\text{-}Asn}<0,001$). Because of the low frequency of the Asn198Asn genotype men with genotypes Lys198Asn and Asn198Asn were further united as combined carriers of the Asn allele

(Table 1).

Table 1 The distribution of the ET-1 gene genotypes in male citizens of Podillia region in Ukraine in the control group and the patients with EH, (%)

Groups	Genotype Lys/Lys	Combined carriers of the Asn allele	p
Control group (n=79)	65,82% (n=52)(1)	34,18% (n=27)(4)	$p_{4-1}<0,0001$
Men with uncomplicated EH and LVH (n=62)	56,45% (n=35)(2)	43,55% (n=27)(5)	$p_{5-2}>0,05$
Men with EH and CHF IInd-IIIrd classes (n=50)	66,00% (n=33)(3)	34,00% (n=17)(6)	$p_{6-3}<0,001$
p	$p_{2-1}>0,05$; $p_{3-1}>0,05$; $p_{3-2}>0,05$	$p_{5-4}>0,05$; $p_{6-4}>0,05$; $p_{6-5}>0,05$	

Comparing the frequencies of genotypes and alleles of the ET-1 gene it was found that no significant differences were defined between these groups ($p>0,05$).

The odds ratio was calculated to assess the risk of development of EH and CHF in carriers of different ET-1 genotypes in males of the Podillia region in Ukraine aged 40-60 yrs. It was established that the inherited ET-1 gene variant was not associated with the risk of development of EH and CHF after calculating the number of statistical parameters using the calculator genexpert (http://gen-exp.ru/calculator_or.php).

According to the results of the control group, the Lys198Lys genotype dominated and also the Lys allele of the ET-1gene corresponded to the results of researchers from Kazakhstan, Hungary and Sumy (Ukraine). [4,9,14] In patients with EH, the Lys198Lys genotype dominated and the Lys allele of the ET-1 gene as in number of other studies. L.O. Minushkin showed that amongst patients of both sexes with both complicated and uncomplicated EH, residents of Yakutia, had a higher frequency of Lys198Lys genotype of the ET-1 gene carriers compared to that of the group of patients with EH, Moscow residents. [13] However, the analysis of frequency distribution of genotypes polymorphism of the ET-1 gene in male residents of Kazakhstan have shown that Lys198Lys genotype in patients with EH occurs 1.3 times lesser compared to healthy individuals. Heterozygous Lys198Asn variant is equally common in patients with EH and in the control group. Asn198Asn genotype was identified only in patients with EH. [9] In Sumy in the general population, both in the control group and in patients with ischemic stroke the genotype Lys198Lys of the gene ET-1 dominated. In the control group of women the genotype Lys198Lys dominated, while in the group of patients with ischemic stroke, carriers of the Asn allele dominated(the genotypes Lys198Asn and Asn198Asn). However, in men, this difference between study groups was not found, but in the control group and in patients with ischemic stroke the genotype Lys198Lys prevailed. [14] J. J. Jin et al. (Japan) showed the frequency of the genotypes of the ET-1 gene in men and women with EH: the genotype Lys198Lys - 53%, Lys198Asn - 38%, Asn198Asn - 8%, the Lys allele - 72%, the Asn allele - 28 %. [10] In Hungary in adolescents of both sexes with EH, the genotypes Lys198Lys, Lys198Asn and the Lys allele were more likely to occur than the genotype Asn198Asn and the Asn allele, but there was no difference between the genotypes Lys198Lys and Lys198Asn. It should be noted that the genotype Lys198Lys and Asn198Asn were more common in the control group and the genotype Lys198Asn - in patients with EH. [4] In Prague the study of the polymorphism of the ET-1 gene did not reveal difference in the distribution of genotypes between the control group and the male and female distribution with respect to EH. However, both in patients with EH and the control group dominated the genotype Lys198Lys. [2]

The plasma ET-1 level in the control group was (1,79±0,08) fmol/ml. In patients with uncomplicated EH and LVH the average concentration of plasma ET-1 was (12,59±0,22) fmol/ml and in men with EH and

CHF it was (13,30±0,11) fmol/ml therefore the average difference in concentration was significantly higher than in the control group (p<0,0001). The highest ET-1 plasma concentration was in patients with EH and CHF (p<0,01) (Figure 1).

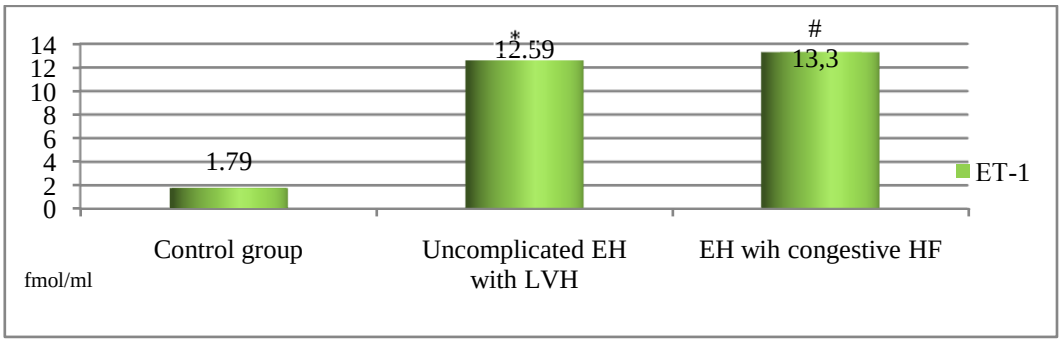

Figure 1 :Plasma ET-1 levels in the control group and in patients with EH, (M±m, fmol/ml)

Note: The difference is significant when compared to: * - control group (p<0,0001), # - men with EH and LVH (p<0,01).

It was found the difference in plasma concentration of ET-1 in carriers of various genotypes of the ET-1 gene (Figure 2).

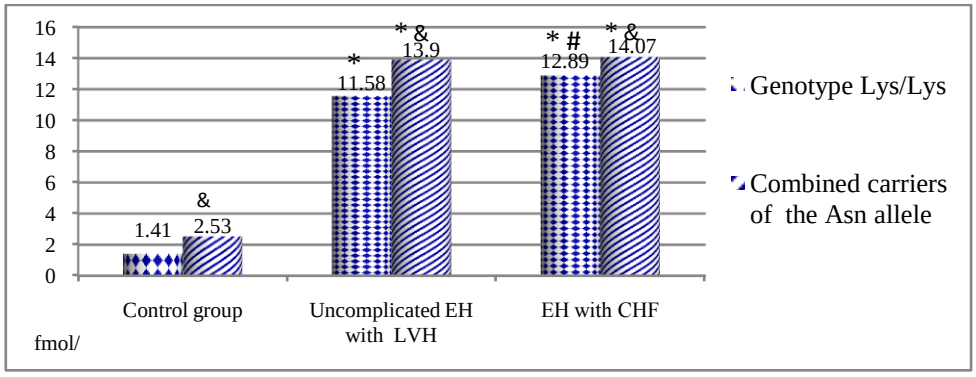

Figure 2: Plasma ET-1 levels in the control group and patients with EH carriers of various genotypes of the ET-1 gene (M±m, fmol/ml)

Note: The difference is significant when compared to: * - control group (p<0,0001), # - men with EH and LVH (p<0,0001), & - carriers of the genotype Lys198Lys

In the control group and in patients with EH the highest concentration of ET-1 was found in combined carriers of the Asn allele of the ET-1 gene and the lowest level was found in homozygote carriers of Lys198Lys genotype (p<0,0001) and that means that the presence of the Asn allele in the genotype of the ET-1 gene is associated with a higher plasma concentration of the peptide. Patients with EH have significantly higher concentration of ET-1 compared to the patients in the control group, carriers of all genotypes of the ET-1gene (p<0,0001).

The role of the genotype of the ET-1 gene as a possible regulator of the plasma concentration of peptide in patients with EH has not been well studied in the world. However, E. Berezikova showed similar studies compared to our findings: men and women living in Russia who are carriers of the Asn198Asn genotype had elevated plasma levels of ET-1 compared with carriers of the genotype Lys198Lys. In carriers of the Lys198Asn genotype the plasma level of ET-1 was intermediate but no differences from the carriers of the Asn198Asn and Lys198Lys genotypes were detected. [5] In Australian pregnant women the Asn198Asn genotype showed the highest plasma concentration of ET-1 (5,8 pg/ml) compared to the Lys198Asn

genotype (3,1 pg/ml) and the genotype Lys198Lys - 3,6 pg/ml. [3] At the same time, S. Tanaka et al. showed no difference in cell cultures in the levels of ET-1 in the Lys198Asn polymorphism. [16]
It table illustrates the frequency of different degrees of arterial hypertension in men with EH - and the carriers of various genotypes of the ET-1 gene (Figure 3,4).

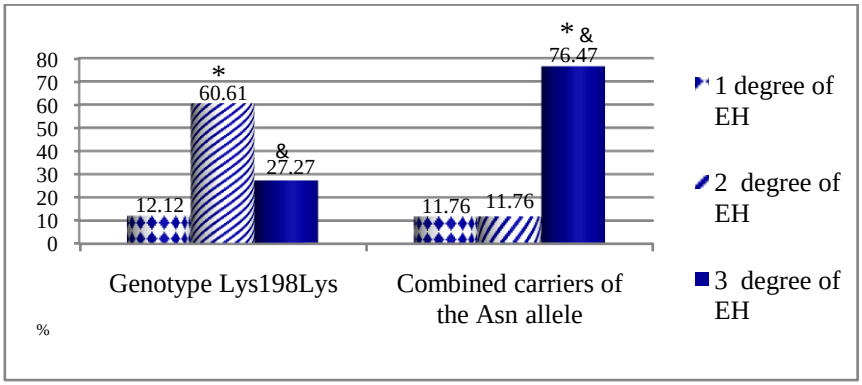

Figure 3 :Frequency of different degrees of hypertension in the presence of polymorphic genotypes of the gene ET-1 in men with uncomplicated EH and LVH, (%)

Note: The difference is significant when compared to: * - 1st degree of hypertension (p<0,01), & - 2d degree of hypertension (p<0,01).

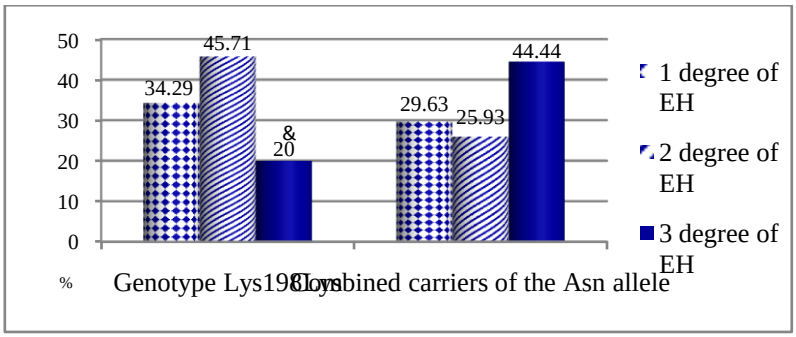

Figure 4 : Frequency of different degrees of hypertension in the presence of polymorphic genotypes of the gene ET-1 in men with EH and congestive HF, (%)

Note: The difference is significant when compared to: * - 1st degree of hypertension (p<0,01), & - 2d degree of hypertension (p<0,01).

It was established that men with uncomplicated EH and LVH carriers of genotype Lys198Lys are more likely to have 2nd degree hypertension and with combined carriers of the Asn allele – 3rd degree hypertension respectively. It was investigated that in patients with EH and CHF IInd-IIIrd classes combined carriers of the Asn allele is associated with 3rd degree hypertension. Similar data was obtained from the work of Russian scientists (Moscow), where it was investigated that 3d degree of hypertension was associated with the Asn allele of the ET-1 gene and the Lys198Lys genotype was protective. [12]

4. Conclusion

1. Lys198Lys genotype and the Lys allele of the ET-1 gene dominate among residents of Podillia region in Ukraine, between the age group of 40-60 years . The genotype of the ET-1 gene is not associated with an increased risk for the development of hypertension and CHF on its background.

2. The carriers of the Asn allele of the ET-1 gene have significantly higher plasma levels of the aforementioned peptide among the control group and in patients with EH who are required to take into

account during scientific and clinical studies using plasma concentrations of ET-1.
3. In men with uncomplicated EH with LVH carriers of Lys198Lys genotype are more likely to encounter 2nd degree hypertension and in carriers of Asn allele – 3rd degree hypertension. In patients with EH and CHF IInd-IIIrd carriers of the Asn allele are associated with 3rddegree hypertension.

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