The Association Between Polymorphism of Lys198Asn of Endothelin-1 Gene and Arterial Hypertension Risk in Kazakh People

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ABSTRACT

Aim: *We researched frequency distribution of genotypes and alleles Lys198Asn of polymorphism of endothelin -1 gene associated with arterial hypertension development in Kazakh people*

Method: *The research included 120 patients suffering arterial hypertension of 2-3 degree, average, high and very high degree of risk, and control group included 80 actually healthy person of Kazakh nationality.*

Result: *The research discovered that development of arterial hypertension in the present ethnic group associated with polymorphism of endothelin-1 gene.*

Conclusion: *At that, allele 198Asn and AsnAsn genotype of endothelin-1 gene act as marker of increased risk of arterial hypertension development.*

Key words: *Genes, endothelin, hypertension, genetic, blood pressure*

Kazaklarda Endotelin-1 geninin Lys198Asn polimorfizmi ile arteriyel hipertansiyon gelişme riski arasındaki ilişki

Amaç: *Kazaklarda arteriyel hipertansiyon gelişimi ile ilişkili endotelin-1 geninin genotipleri ve allellerinin Lys198Asn polimorfizmi sıklığının dağılımını araştırdık.*
Metod: *Çalışma evre 2-3 hipertansiyonu olan ortalama, yüksek ve çok yüksek riskli 120 hastayı içeriyordu. Kontrol grubu olarak ise 80 sağlıklı Kazak denek alındı.*
Bulgular: *Araştırmanın sonuçları çalışılan etnik grupta arteriyel hipertansiyon gelişiminin endotelin-1 gen polimorfizmi ile ilişkili olduğunu göstermiştir.*
Sonuç: *Endotelin-1 geninin allel 198Asn ve AsnAsn genotipi arteriyel hipertansiyon gelişimi için bir risk belirteci olarak rol oynamaktadır.*
Anahtar kelimeler: *Genler, endotelin, hipertansiyon, genetik, kan basıncı*

INTRODUCTION

A vital part of essential hypertension development belongs to the genetic factors. The epidemiological studies show that 20-40% variations of blood pressure are defined by (1). Data about uptake of arterial hypertension in members of one family and concordance of blood pressure levels in relatives by blood is available (2). The inheritance of essential hypertension has some particular properties, which confirm its polygenic character. First, the prevalence of essential hypertension in population rises up to 15-20%, which is considerably higher than the prevalence of classical genetic diseases heritable by Mendelian type. Second, the beginning of the disease as usual relates to 3d-4th decade of life, this fact also the evidence of non-Mendelian type of inheritance (3). In this case the method of so called 'candidate genes' isolation is applied. One of the genetic markers of cardiovascular diseases is the polymorphism of Lys198Asn of endothlin-1 gene (END1) and its role in this pathology forming. In research of ECTIM and Glasgow Heart Scan Study a connection between structural mutation of Lys198Asn and arterial hypertension in overweight patients (4,5). Homozygous individuals by 198Asn allele with Body Mass Index more than 26kg/m2 had significantly higher level of blood pressure, more clearly defined hypertonic reaction on treadmill stress in comparison with normal weight patients. No association between polymorphism of Lys198Asn of endotheline-1 gene and arterial hypertension is found in Russian population of Moscow city (6), and population of Yakutia (7). Thus we can observe the contradiction of literature data in studies dedicated to the role of polymorphism of Lys198Asn of endotheline-1 gene in cardiovascular diseases development, which stipulates the necessity of further research work.

Aim of the research, to study the frequency distribution of genotypes and alleles of Lys198Asn polymorphism of endothelin-1 gene interrelated with arterial hypertension development in Kazakh.

MATERIALS AND METHODS

120 patients (men) of Kazakh nationality, hospitalized in cardiologic department of NSMC of Astana city with Arterial hypertension of 2-3 degree, risk 2-4, without concomitant diseases, non-relatives, were examined. Average age of patient was 47,0±0,7 years (Table 1).

The control group included 80 actually healthy men without any clinical manifestation of cardiovascular diseases, having no burdened familial heredity of abovementioned disease, with normal values of cardiac ultrasound and ECG examinations, stress testing, the average age was 42,5±0,9 years.

Patients of all the studied groups were subjected to ulnar vein blood sampling in quantity of 5-10 ml, samples were placed into sterile plastic vials which contained 100 microliters of 0.5M EDTA pH8.0 as coagulant. The collected blood has been freezed and kept at -20°C.

The DNA was allocated from blood leucocytes with standard phenol chloroform method (8,9). The identification of polymorphism of Lys198Asn in EDN1 gene was made by the method suggested before. For amplification of the necessary fragment of EDN1 gene two primers were used (6):

5'-ATGATCCCAAGCTGAAAGGCTA-3'

5'-CAGGGCTCTCCGTGGAGGCTAT-3'

The amplification was carried out in 20 microliters of reaction mixture, consisting 50 mM KCl, 10 mM Tris-HCl pH 9.0 at 25°C, 0.1% triton X-100, 2.5 mM MgCl2, 0.01mg/

***Table 1**. The general characteristics of surveyed patients.*

	Essential hypertension	*Healthy group*
Number of patients	*n:120*	*n:80*
Age, years	*47.0±0.7*	*42.5±0.9*
Duration of hypertension, years	*13.3±1.4*	*-*
SBP, mm.Hg.	*172.4±5.4*	*116.6±1.8*
DBP, mm.Hg.	*93.7±3.3*	*72.5±1.7*
BMI, kg/m2	*27.4±1.6*	*23.5±1.1*
eGFR, ml/min	*94.4±11.2*	*113±5.02*

SBP - systolic blood pressure, DBP - diastolic blood pressure, BMI - body mass index, eGFR - estimated glomerular filtration rate by Cockroft-Gault formula.

ml BSA, 0.25 mcM of each primer, 200 mcM dNTP, 1unit of activity (1 un.) Taq of DNA polymerase and 0.5-1 mcg of genomic DNA. To prevent the evaporation of the reaction mixture during Polimerase Chain Reaction, 25 microliters of liquid paraffin was stratified. After primary denaturation at 93˚C during 3min, 35 cycles of amplification were carried out in the next temperature-time conditions: melting +93˚C – 50 seconds, annealing: +52˚C – 50 seconds, synthesis: +72˚C – 50 seconds. After completion of 35 cycles of amplification , the final synthesis was made at +72˚C during 10 min. The product received during Polimerase Chain Reaction, sized 116 n., was subjected to restriction by Nhe I ferment (1unit) in solution with 3.3mM Tris-acetate pH 7.9, 1mM Mg acetate, 6,6 mM K acetate, 0.01mg/ml BSA during 12 hours at +37˚C. After restriction the fragments of DNA were subjected to electrophoresis division in 6% polyacrylamide gel. The normal allele consist site of restriction and 97 and 19 n. fragments are generated, in case of mutant allele restriction site is missed and the 116 n. fragment remains (Figure 1).

The results were processed using Statistica 6.0. program. To check if distribution of genotypes complies to Hardy-Weinberg balance and to compare the genotype distribution between groups the criterion was x2 used.

RESULTS

As a result of analysis of frequency distribution of genotypes of polymorphism of Lys198Asn of endoteline-1 gene (Table 2) it is determined that frequency of LysLys genotype in patients suffering essential hypertension is 1,3 times less than in control group (55%, 67,5%, respectively). Frequency of LysAsn heterozygous genotype is actually equal in the compared groups (30%; 32,5%, respectively). AsnAsn homozygous genotype was identified only in patients with essential hypertension, the control

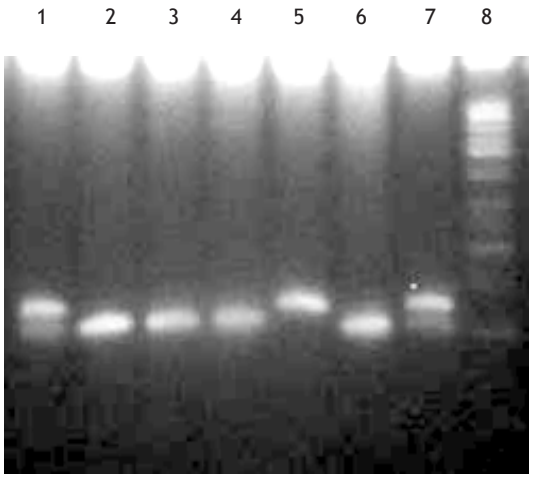

***Figure 1**. The identification of polymorphism of Lys198Asn of EDN1: 2,3,4,6 - genotype LysLys; 1,7 - genotype LysAsn, 5 - genotype AsnAsn, 8 - marker of molecular weight*

***Table 2**. Frequency distribution of genotypes and alleles of Lys198Asn END1gene*

	Essential hypertension		*Healthy group*	
Genitype	*n*	*%*	*n*	*%*
LysLys	*66*	*55*	*54*	*67,5*
LysAsn	*36*	*30*	*26*	*32,5*
AsnAsn	*18*	*15*	*0*	*0*
Total	*120*	*100*	*80*	*100*
Alleles				
Lys	*168*	*70*	*134*	*83,8*
Asn	*72*	*30*	*26*	*16,2*
Total	*240*	*100*	*160*	*100*

***Table 3**. Diffusion of alleles of END1 gene in different ethnic groups*

Alleles	Kazakh	Russian	Ethnic groups Yakuts	Japanese	American white	American black	Irish
Lys198	*75,5%*	*78,9%*	*85,5%*	*72,5%*	*78,1%*	*82%*	*78%*
198Asn	*24,5%*	*21,1%*	*14,5%*	*27,5%*	*21,9%*	*18%*	*22%*

group did not have this mutant genotype. This difference is statistically confident (x^2 =13,81, p=0,001).

The analysis of frequency distribution of alleles of this gene (Table 1) discovered that the most diffused allele in patients and healthy groups was Lys allele (70%, 83,8% respectively) and the mutant allele Asn is more seldom (30%, 16,2%, respectively). At the same time occurrence of mutant allele Asn in patients is 2 times more frequent and this difference is statistically confident (x^2 =7,27; p=0,007).

DISCUSSION

As a result of our research, the interconnection of polymorphism of Lys198Asn of END1 gene with the arterial hypertension development in Kazakh people is discovered. Carriage of mutant allele 198Asn and genotype AsnAsn of END1 gene causes arterial hypertension development in Kazakh people.

The analysis of association of polymorphous marker of endothelin-1 gene in (10) excess weight patients suffering arterial hypertension in Japan, showed confident association of 198Asn allele with arterial hypertension. The carriage of this allele is confidently accompanied with high indices of systolic and diastolic pressure in comparison with Lys198 allele of END1 gene (P=0,001).

Heterogeneity of distribution of alleles and genotypes of END1 gene researched by different investigators discovered next tendencies: diffusion of Lys198 allele depends on race (Table 3). The biggest diffusion was observed in Yakut (85,5%) (7) and Afro-american (82%) (11) and the smallest in Japanese (72,5%) (10).

The comparison of frequency distribution of alleles of END1 gene in Kazakh complies to the results of other research works carried out in Asian populations (10), where Lys198 allele occurs more frequently than 198Asn allele (in Japanese Lys198-72,5% and 198Asn-27,5%). Probably, such variety of alleles distribution of END1 gene reflects not only the region of habitation, but considerably determined by ethnic identity.

Thus, the results of our investigation lead us to next conclusions:

1. The research of polymorphism of Lys198Asn of END1 gene in patients suffering arterial hypertension of 2 and 3 degree showed that the frequency of Lys198 allele is revealed in 70%, frequency of 198Asn allele – in 30%, the distribution of genotypes is next: LysLys – 55%, LysAsn-30%, AsnAsn-15%.

2. The association between polymorphism of Lys198Asn of END1 gene and risk of arterial hypertension development in Kazakh people. The carriage of 198Asn allele, and 198AsnAsn genotype of endotheline-1 gene causes development of arterial hypertension in Kazakh people.

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