

The analysis of endothelial nitric oxide synthase gene polymorphism in intron 4 with hypertension disease

Leila Zarmakhi, Mehrdad Hashemi*

Department of Genetics, Islamic azad university, Tehran medical branch, Tehran, Iran

*Corresponding Author: email address: mhashemi@iautmu.ac.ir (M. Hashemi)

ABSTRACT

Nitric oxide is an important vasorelaxant factor that it inhibits platelet adhesion and proliferation of smooth muscle cells. NO is synthesized from L-arginine by means of endothelial nitric oxide synthase (eNOS) which is an isoform of Nitric Oxide Synthase. In the present study, we examined possible association between the 27 base pair (bp) repeat polymorphism in intron 4 of the eNOS3 gene and hypertension in an Iranian population. 40 patients with hypertension and 40 healthy controls were included in the research. Genotyping was performed by polymorphism chain reaction (PCR). The frequency of 4a4a, 4a4b, 4b4b genotypes were 52.6%, 26.3%, 15.7% respectively in hypertension groups. The frequency of 4a4a, 4a4b, 4b4b genotypes were 73.8%, 15.7%, 10.5% respectively in control groups. The hypertensive patient group showed a significant higher frequency of the 4a allele compared to the controls ($p < 0.01$). The present study showed a significant association between the eNOS3 gene polymorphism and hypertension in the Iranian population.

Keywords: Hypertension; Nitric oxide synthase gene; Polymorphism

INTRODUCTION

Hypertension is a multifactorial disease involving both environmental and genetic components that their pathogenic mechanisms haven't been clear yet. Clinical and Experimental studies suggest that an alteration in nitric oxide (NO) metabolism is a feasible contributing factor in the pathogenesis of hypertension [1-3]. Nitric oxide (NO) is a gaseous free radical and an important molecular mediator of many physiologic processes in virtually every organ [4]. Nitric oxide in the cardiovascular system is an important vasorelaxant factor that it also inhibits platelet adhesion and proliferation of smooth muscle cells [5]. Endothelial nitric oxide synthase (eNOS) is one of three isoforms of nitric oxide synthase which exhibits homology of sequence and function [6]. NO is synthesized from L-arginine by means of endothelial nitric oxide synthase (NOS), which is an isoform of Nitric Oxide Synthase (NOS), as well as it is dominant in the blood vessel walls [4,7].

The gene encoding eNOS is located on chromosome 7(7q35-q36) and it contains 26 exons with an entire length of 21 kb [4,8]. Some studies considered the role of endothelial NOS in hypertension was provided by the observation that disruption of the NOS3 gene in knockout mice resulted in increase blood pressure [3]. Association studies of intron 4 of eNOS gene 27 bp polymorphism and hypertension disease have reported variable results [9-14]. This study is carried out in order to show the association of intron 4 polymorphism of the NOS3 gene with the incidence of hypertension in Iran population.

MATERIALS AND METHODS

80 subjects were enrolled in this study. They are included 40 hypertension patients (14 men and 26 women) with age average 61/78 and 40 normotensive subjects (26 men and 13 women) with age average 44/15. Arterial hypertension was defined according to the criteria of the World Health Organization that they are included a systolic blood pressure and diastolic blood

pressure of $\geq 140/90$ mmhg [9]. Family history for early cardiovascular events were analyzed as previously reported [10]. Normal control subjects weren't exposed to antihypertensive treatment and their SBP and DBP were less than 140 and 90 mmhg, respectively.

DNA analysis

Genomic DNA was isolated from peripheral white blood cells by phenol-chloroform extraction and for genotyping was taken into EDTA-containing receptacles. The 27 base pair (bp) repeat polymorphism in intron 4 of the eNOS3 gene was analyzed with polymerase chain reaction followed by the method of Wang et al(15). Oligonucleotide primers of PCR were designed as follows: sense 5'-AGG CCC TAT GGT AGT GCA TTT-3' and antisense 5'-TCT CTT AGT GCT GTG CTC ACC-3. The amplified fragments were separated on 1 % agarose gel with ethidium bromide staining.

Statistical analysis

Statistical analysis was performed by using SPSS (Statistical Package for Social Science, Version 16.0). The Student's t test was used for continuous variables, and the X^2 test was used for categorical variables to test for statistical significance.

RESULTS

From the 40 hypertensive subjects, 100 % of them remained on anti hypertensive therapy. Table .1 summarizes the demographic and clinical characteristic of all subjects. There were

Table 1. Demographic and clinical characteristics of the study population.

	Normotensive subjects=40	Hypertensive patients=40
Age %	44.15	61.78
Sex(male) %	65	35
BMI(kg/m ²) %	24.78	28.01
Diabetes %	2.5	22.5
Smokers %	30	12.5
Family history %	32.5	57.5
27 bp polymorphism %	22	47.5

DISCUSSION

In the present study, it is found that there is a significant correlation between eNOS gene polymorphism and the rate of hypertension in

significant differences between two groups with respect to age and gender.

Apart from blood pressure, BMI, diabetes, family history and 27 bp polymorphism were significantly higher in cases than in controls ($P=0.009$). A 420 bp band indicated five repeats of the 27 bp (eNOS4b allele), and a 393 bp band four repeats (eNOS4a allele) (Fig1).

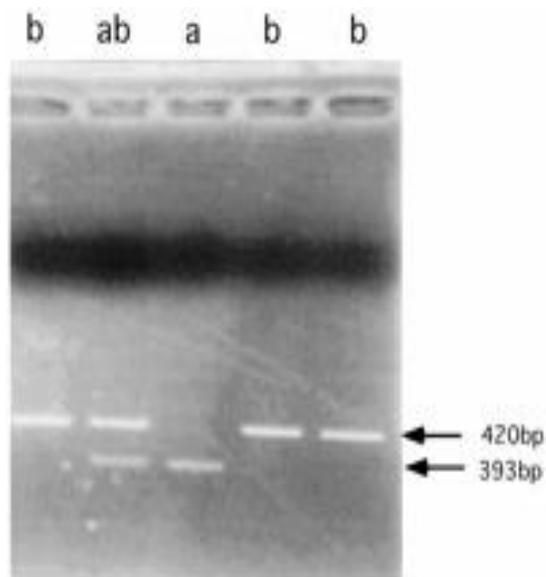


Figure1. Genotype of intron 4 of eNOS gene 27 bp polymorphism

The hypertensive patient group showed a significant higher frequency of the 4a allele compared to the controls (Fig 2).

Iran_tehran population. Our results showed that 4a allele frequency of the eNOS gene intron 4a4b VNTR polymorphism were significantly higher in patients with hypertension. Some researchers

have investigated the association between the eNOS gene intron 4a4b VNTR polymorphism and

hypertension, with conflicting results; in 1998, Miyamoto et al [16] reported the 894T

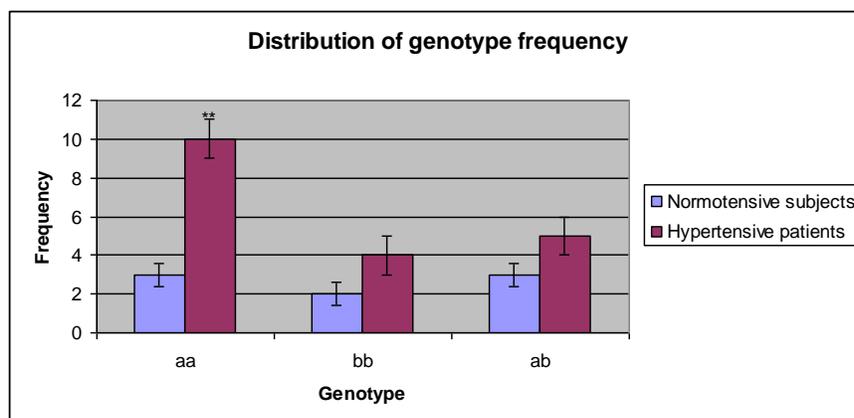


Figure2. Distribution of genotype frequency

allele was associated with increased risk of hypertension in Japanese, whereas Lacolley et al [17] claimed that the 894G allele was risk associated allele in Caucasians. However, two following Japanese studies with relatively large sizes did not replicate these positive results, they didn't reveal any association between the G894T polymorphism and hypertension [18,19]. Uwabo et al [20] reported that the 4a4b polymorphism was correlated with hypertension, which is concordant to our study

The mechanism by which the NOS34a4b polymorphism confers susceptibility to hypertension hasn't understood yet. The 4a4b polymorphism is associated with altered plasma NO levels, influencing both NO and enzyme production [21]. According to several studies concerning human and experimental hypertension, it is exhibited that production of NO is decreased the NO-dependent dilatation is blunted [22,23]. The main factor for the impairment of the NO-cyclic GMP pathway in hypertension seems to be located at the level of the NOS3. Additional evidences implicated dysfunction of NO system in hypertension [23,24] with fort et al [1]. Wang et al [24] observed that the intron 4b/a coordinated with the T-786C and regulated the transcription efficiency in a

haplotype-specific fashion in vitro experiment. In addition, it is also observed that the haplotype-specific effects of T-786C and intron 4a/b on the eNOS gene promoter efficiency were modified by cigarette smoking in vitro experiment. In this study, we also examined whether gender, age, BMI, family history and smoking (Table 1) status exert influence on the relationship of the three eNOS gene polymorphism and all possible haplotypes with hypertension by adding interaction terms. Although we did not find any significant results, it could not excluded the possibility that the eNOS gene variants are involved in hypertension within other environmental or genetic context. Tanus-santos et al [25] observed that the distribution of these three eNOS polymorphism frequencies were markedly different among Caucasians, African-Americans and Asians.

CONCLUSION

The present study showed a significant association between the eNOS3 gene polymorphism and hypertension in the Iran-Tehran population. For the future study it is necessary to select the other genes which involved in hypertension condition.

REFERENCES

1. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* 1989; 2:997-1000.
2. Forte P, Copland M, Smith M, Milne E, Sutherland J, Benjamin N, Basal nitric oxide synthase in essential hypertension. *Lancet* 1997;349;837-42.
3. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature* 1995;377:239-42.
4. Derebecka N, Holysz M, Trzeciak W.H. Genes of nitric oxide synthases: Structure, regulation of expression, protein products. *Postępy Biochemii* 2000; 2: 130–139
5. An P, Freedman BI, Hanis CL, Chen YD, Weder AB, Schork NJ, Boerwinkle E, Province MA, Hsiung CA, Wu X, Quertermous T, Rao DC. Genome-wide linkage scans for fasting glucose, insulin, and insulin resistance in the national heart, lung, and blood institute family blood pressure program: evidence of linkages to chromosome 7q36 and 19q13 from meta-analysis. *Diabetes* 2005; 54 (3): 909-14
6. Stuehr DJ. Structure-function aspects in the nitric oxide synthases. *Annu Rev Pharmacol Toxicol* 1997;37:339–5.
7. Chen, PF, Tsai AL, Berka V, Wu KK. Endothelial nitric oxide synthase. *J. Biol. Chem.* 1996; 271: 14631–14635
- 8 Marsden PA, Heng HHQ, Scherer SW, Stewart RJ, Hall AV, Shi XM, Tsui LC. Schappert KT. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. *J. Biol. Chem.* 1993;268: 17478–1748
9. World health organization. International society of hypertension guideline for the management of hypertension. *J hypertens* 1999;17:151-183.
10. Licata G, Scaglione R, Corrao S, Ganguzza A, Mazzola G, Arnone S, Dichiaro MA, Licata A, Merlino G, Di Chiara T. Heredity and obesity associated hypertension. Impact of hormonal characteristics and left ventricular mass. *J Hypertens* 1995; 13:611-618.
11. Aras O., Hanson NQ, Bakanay SM, Tsai My, Gulec S. Endothelial nitric oxide gene polymorphism (Glu298Asp) is not associated with coronary artery disease in Turkish population. *Thromb Haemost.* 2002;87(2):347-349.
12. Cakir DU, Mete N. The role of endothelial nitric oxide synthase gene polymorphism in clinically classified patients with coronary artery disease. *Biotechnology and Biotechnological Equipment.* 2004;4:2117-2121
13. Ichihara S, Yamada Y, Fujimura T, Nakashima N, Yokota M. Association of a polymorphism of the endothelial constitutive nitric oxide synthase gene with myocardial infarction in the Japanese population. *Am J Cardiol* 1998; 18 : 83-6.
14. Park JE, Lee WH, Hwang TH, Chu JA, Kim S, Choi YH, Kim JS, Kim DK, Lee SH, Hong KP, Seo JD, Lee WR. Aging affects the association between endothelial nitric oxide synthase gene polymorphism and acute myocardial infarction in the Korean male population. *Korean J. Intern.* 2000;15: 65-70.
15. Wang XL, Sim AS, Bandenhop RF, McGredie RM, Wilken DE. A smoking dependent risk of coronary artery disease associated with polymorphism of the endothelial nitric oxide synthase gene. *Nat Med* 1996;2: 41-45.
16. Miyamoto Y, Saito Y, Kaiyama N, Yoshimura M, Shimasaki Y, Nakayama M. Endothelial nitric oxide synthase gene is positively associated with essential hypertension. *hypertension* 1998; 32:3-8.
17. Lacolley P, Gautier S, Poirier O, Pannier B, Cambien F, Benetons A. Nitric oxide synthase gene polymorphism, blood pressure and aortic stiffness in normotensive and hypertensive subjects. *J Hypertens* 1998;16;31-35.
18. Kato N, Sugiyama T, Morita H, Nabika T, Kurihara H, Yamori Y. Lack of evidence for association between the endothelial nitric oxide synthase gene and hypertension. *hypertension* 1999;33;933-936.
19. Tsujita y, Baba S, Yamauchi R, Mannami T, Kinoshita M, Yamamoto R. Association analyses between genetic polymorphism of endothelial nitric oxide synthase gene and hypertension in Japanese: The Suita study. *J Hypertens* 2001;19:1941-1948.

20.Uwabo J, Soma M, Nakayama T, Kanmatsuse K. Association of a variable number of tandem repeats in the endothelial constitutive nitric oxide synthase gene with essential hypertension in Japanese. *Am J hypertens* 1998;11:125-8.

21.Tsukada T, Yokoyama K, Arai T, et al. Evidence of association of the eNOS gene polymorphism with plasma NO metabolite concentration in humans. *Biochem Biophys Res Commun* 1998;245: 190-3.

22.Linder L, Kiowski W, Buhler FR, Lucher T. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo:blunted response in essential hypertension. *Circulation* 1990;81:1762-7.

23.Lucher TF, Raji L, Vanhoutte P. Endothelium-dependent vascular responses in normotensive and hypertensive Dahl rats. *Hypertension* 1987;9: 157-63.

24.Wang J, Dudley D, Wang XL. Haplotype-specific effects on endothelial NO synthase promoter efficiency:modifiable by cigarette smoking. *Arterioscler thrombvasc Bio* 2002;22:el-4.

25.Tanus-Santos JE, Desai M, Flockhart DA. Effects of ethnicity on the distribution of clinically relevant endothelial nitric oxide variants. *Pharmacogenetics* 2001;11:719-725.