Advances in Bioresearch
Adv. Biores., Vol 7 (5) September 2016: 16-21
©2016 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV Value 8.21 [2014]

Advances in Bioresearch

ORIGINAL ARTICLE

Evaluation of NOS3 gene rs1800779 Polymorphism in Iranian Patients affected by Coronary- Heart disease Patients and Normal individuals

Masoud Zakerjafari[1] , Alireza Mousa Mayali[2*] , Pegah Tavangar[3]
1-Department of biology, Ardabil Branch,Islamic Azad university, Ardabil, Iran
2- Department of Biology, Islamic Azad University, North Tehran branch, Tehran, Iran
3- Department of Biology, Faculty of Pharmacy, Mazandaran University of medical Sciences, Sari,Iran
Correspondence author's email: mosamayali_ar@yahoo.com

ABSTRACT
Coronary- heart disease is one of several subtypes of heart disease. Coronary heart disease (CHD) is a disease in which a waxy substance called plaque builds up inside the coronary arteries. Several studies have shown that Nitric oxide (NO) has several important roles in Coronary- heart disease. Studies have demonstrated that the NO level of plasma, serum and postmortem from patients with Coronary- heart disease are increased and they have an abnormal NOS/NO pathway. NO is created by three nitric oxide synthase (NOS) isoenzymes, including endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS).Evidence suggests that eNOS might play a role in this mysterious disease, as a result we studied NOS3 gene rs1800779 polymorphism in Iranian patient affected by Coronary- heart disease. In the present case-control study, the polymorphism of NOS3 gene rs1800779 polymorphism has been investigated in 40 Coronary- heart disease patients and 60 healthy subjects by using ARMS-PCR method. Then, the data were analyzed by SPSS software. The results of this study showed considerable association between Coronary- heart disease and NOS3 gene rs1800779 polymorphism in Iranian population. It is important to define a possible role of polymorphisms in brain disorders as a consequence of a decreased risk of developing Coronary- heart disease in Iranian population.
Keywords: Endothelial nitric oxide synthase, Coronary- heart disease, NOS3, Polymorphism, rs1800779

Received 24/03/2016 Accepted 02/06/2016

How to cite this article:
M Zakerjafari , A M Mayali , P Tavangar. Evaluation of NOS3 gene rs1800779 Polymorphism in Iranian Patients affected by Coronary- Heart disease Patients and Normal individuals. Adv. Biores., Vol 7 [5] September 2016: 16-21.
DOI: 10.15515/abr.0976-4585.7.5.1621

INTRODUCTION

Coronary artery disease has a number of well determined risk factors. The most common risk factors include smoking, family history, hypertension, obesity, diabetes, lack of exercise, stress, and high blood lipids [1,2]. Smoking is associated with about 36% of cases and obesity 20%. Lack of exercise has been linked to 7–12% of cases. Job stress appears to play a minor role accounting for about 3% of cases [3]. In one study, women who were free of stress from work life saw an increase in the diameter of their blood vessels, leading to decreased progression of atherosclerosis [4,5]. In contrast, women who had high levels of work-related stress experienced a decrease in the diameter of their blood vessels and significantly increased disease progression [6,7]. Having a type A behavior pattern, a group of personality characteristics including time urgency, competitiveness, hostility, and impatience is linked to an increased risk of coronary disease [8, 9].

Typically, coronary artery disease occurs when part of the smooth, elastic lining inside a coronary artery (the arteries that supply blood to the heart muscle) develops atherosclerosis.(10) With atherosclerosis, the artery's lining becomes hardened, stiffened, and swollen with calcium deposits, fatty deposits, and abnormal inflammatory cells - to form a plaque. Deposits of calcium phosphates (hydroxyapatites) in the muscular layer of the blood vessels appear to play not only a significant role in stiffening arteries but also for the induction of an early phase of coronary arteriosclerosis. This can be

seen in a so-called metastatic mechanism of calciphylaxis as it occurs in chronic kidney disease and haemodialysis [11, 12]. Although these patients suffer from a kidney dysfunction, almost fifty percent of them die due to coronary artery disease [13, 14]. Plaques can be thought of as large "pimples" that protrude into the channel of an artery, causing a partial obstruction to blood flow [15]. Patients with coronary artery disease might have just one or two plaques, or might have dozens distributed throughout their coronary arteries. A more severe form is chronic total occlusion (CTO), when a coronary artery is completely obstructed for more than 3 months [16].

Different studies have represented that Nitric oxide and NO synthase genes are related with Coronary-heart disease. NO is one of the simplest of biologically active molecules with exclusive chemical properties that constructed by three nitric oxide synthase (NOS) isoenzymes, including endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS(iNOS).Studies have showed that the NO level of plasma, serum and postmortem brain from patients with Coronary- heart disease are amplified and they have an abnormal NOS/NO pathway. eNOS is responsible for most of the NO production in the vascular system, thus, it is more possible that eNOS owns a main role inCoronary- heart disease .In addition, several studies have suggested that eNOS may interfere with Coronary- heart disease (17-19).As a result, we studied NOS3 gene rs1800779 polymorphism in Iranian patient affected by Coronary- heart disease .

MATERIAL AND METHODS

Patients and controls

DNA was obtained from 40 patients with clinically Coronary- heart disease. A total of 60 healthy controls aged 42-65 years or younger were also analyzed to define a possible role for the NOS3 gene rs1800779 polymorphism in Iranian patients in late onset Coronary- heart disease. Genomic DNA was amplified by polymerase chain reaction (PCR) with proper primers and then, the data were analyzed by (SPSS) software.

DNA extraction and NOS3 gene rs1800779 polymorphism genotyping

Genomic DNA from venous blood samples were isolated using Quick Micro Prep Kit (Zymo Research, U.S.A.) according to manufacturer's instructions .The NOS3 gene rs1800779 polymorphism genotyping was performed base on ARMS-PCR by eNOS- READY GENE ZG Kit(Zima gene, Iran).The thermal cycling conditions for ARMS-PCR were as follows: 1 cycle at 95°C for 7 min followed by 35 cycles of 94°C for 75 s and 59°C for 55 sand 72ºC for 30 s and final extension step at 72ºC for 5 min. The electrophoresis was carried out using 2%GelRedstained agarose gel, at 110V for 45 min. After electrophoresis, the amplified PCR products were visualized under U. V. light.

This polymorphism was genotyped by ARMS-PCR, with the primer pairs:

Primer	Sequence (5'->3')	Length	Tm	GC%
F_C	AAGGCAGGAGACAGTGGATG	20	59.38	55.00
R_N	TGAAGGAAGAGTTCTGGTGGC	21	59.93	52.38
R_M	TGAAGGAAGAGTTCTGGTGGA	21	58.31	47.62

Specificationsprimers usedforinternal control:

Primer	Sequence (5'->3')	Length	Tm	GC%
F	GTGTACCCCACCTGCATTCT	20	59.67	55.00
R	CCCAGCAAGGATGTAGTGAC	20	57.97	55.00

PCR program used for ARMS-PCR:

cycle	temperature(Celsius)	Time
first	95	7Minutes
Two tothirty-five	94 59 72	1minute and15seconds 55Seconds 30seconds

RESULTS

We genotyped and analyzed 40 patients with Coronary- heart disease (average above65years), and 60 healthy controls younger than 65 years (range 46 to 65), for NOS3 gene rs1800779 polymorphism.

NOS3 gene rs1800779 polymorphism frequencies were in equilibrium in patients and controls. Patients showed an extensively increased frequency of the NOS3 gene rs1800779 polymorphism allele compared with controls. Thus the NOS3 gene rs1800779 polymorphism allele would confer a slightly increased risk of developing late onset Coronary- heart disease in Iranian population.

Carriers of the NOS3 gene rs1800779 polymorphism were at a slightly but significantly increased frequency in patients compared with controls. Both groups of healthy controls, older than 85 and younger than 65 years, did not had similar gene frequencies, suggesting that this polymorphism is related with Coronary- heart disease in Iranian population (table).

Genotype Table:

Table1: Genotype Table of NOS3 gene rs1800779 polymorphism:

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Genotype * Group	100	100.0%	0	.0%	100	100.0%

Table2: Genotype * Group Cross tabulation

Genotype * GROUP Cross tabulation

Count

		Group		
		CASE	CONTROL	Total
Genotype	GG	15	51	66
	GT	21	8	29
	TT	4	1	5
Total		40	60	100

Table3: Chi-Square Tests for Genotype of NOS3 gene rs1800779 polymorphism:

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	24.233[a]	2	.000
Likelihood Ratio	24.689	2	.000
Linear-by-Linear Association	21.920	1	.000
N of Valid Cases	100		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 2.00.

Table4: Symmetric Measures for Genotype of NOS3 gene rs1800779 polymorphism:

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	-.471	.087	-5.279	.000[c]
Ordinal by Ordinal	Spearman Correlation	-.491	.088	-5.574	.000[c]
N of Valid Cases		100			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

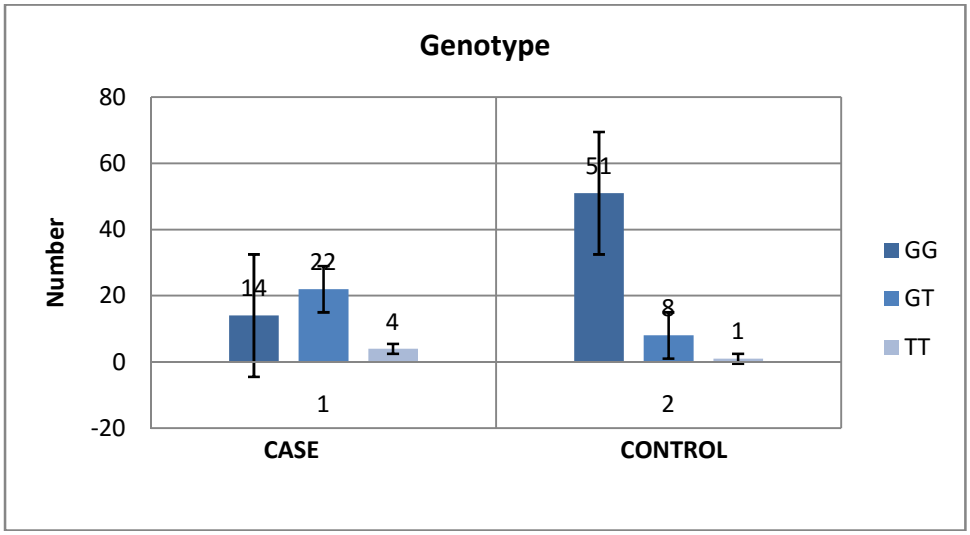

Chart1: Genotype chart of NOS3 gene rs1800779 polymorphism:

Table5: Risk Estimate for Genotype of NOS3 gene rs1800779 polymorphism:

Risk Estimate

	Value
Odds Ratio for Genotype (1 / 2)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

ALLELE Table:

Table6: ALLEL Table of NOS3 gene rs1800779 polymorphism:

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Genotype * Group	200	100.0%	0	.0%	200	100.0%

Table7: Group Cross tabulation and Chi-Square Tests

GENOTYP * GROUP Cross tabulation

Count

		Group		
		1	2	Total
Genotype	G	51	110	161
	T	29	10	39
Total		80	120	200

Table8: Chi-Square Tests for ALLEL of NOS3 gene rs1800779 polymorphism:

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	23.831[a]	1	.000		
Continuity Correction[b]	22.086	1	.000		
Likelihood Ratio	23.741	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	23.712	1	.000		
N of Valid Cases	200				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 15.60.
b. Computed only for a 2x2 table

Table9: Symmetric Measures for ALLEL of NOS3 gene rs1800779 polymorphism:

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	-.345	.066	-5.175	.000[c]
Ordinal by Ordinal	Spearman Correlation	-.345	.066	-5.175	.000[c]
N of Valid Cases		200			

a. Not assuming the null hypothesis.
b. Using the asymptotic standard error assuming the null hypothesis.
c. Based on normal approximation.

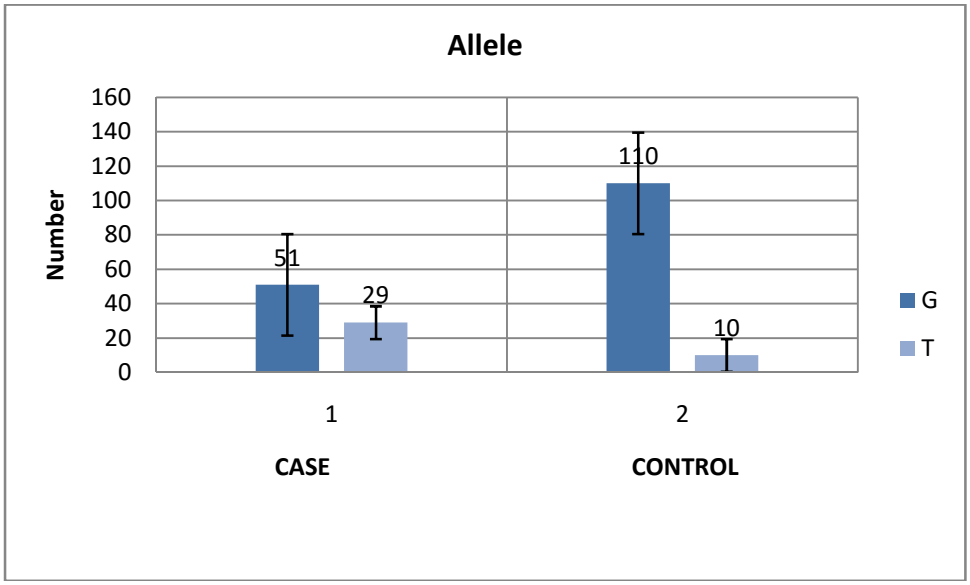

Chart 2: ALLEL Table of NOS3 gene rs1800779 polymorphism:

Table10: Risk Estimate for ALLEL of NOS3 gene rs1800779 polymorphism:

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Genotype (1 / 2)	.160	.072	.353
For cohort Group = 1	.426	.318	.571
For cohort Group= 2	2.665	1.545	4.594
N of Valid Cases	200		

DISCUSSION

There is growing evidence that the NOS3 gene rs1800779 polymorphism plays a part in the pathogenesis of Coronary- heart disease. Several lines of evidence suggest that NOS3 gene rs1800779 polymorphism may play a part in Coronary- heart disease by modulating inflammation. In accordance with this, an increased frequency of the allele among patients with Coronary- heart disease has been seen in this study. This study analyzed different populations, and found an increased frequency of the allele. We also found an association between the allele and Alzheimer's disease in our population. However, the difference between patients and controls was significant in Iranian population, and the fact that a similar result has

been found in different populations suggests that the NOS3 gene rs1800779 polymorphism is truly involved in the development of Coronary- heart disease.

The NOS3 gene rs1800779 polymorphism has been associated with heart disease. To define a possible role of this polymorphism in heart disease as a consequence of a decreased risk of developing Coronary-heart disease , we genotyped healthy controls. Gene frequencies were almost identical between controls, indicating that the NOS3 gene rs1800779 polymorphism is related with Coronary- heart disease in Iranian population.

REFERENCES

1. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. (1977). Diabetes, blood lipids, and the role of obesity in coronary heart disease risk for women.Ann Intern Med. ;87:393–397.
2. The Expert Panel. National Cholesterol Education Program Second Report. The expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel II). Circulation. 1994;89:1333–1445.
3. Anderson KM, Odell PM, Wilson PWF, Kannel WB. Cardiovascular disease risk profiles. Am Heart J. 1991;121:293–298.
4. Anderson KM, Wilson PWF, Odell PM, Kannel WB. An updated coronary risk profile: a statement for health professionals. Circulation. 1991;83:357–363.
5. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families: the Framingham Offspring Study. Am J Epidemiol. 1979;110:281–290.
6. Kannel WB, Wolf PA, Garrison RJ. Monograph Section 34: Some Risk Factors Related to the Annual Incidence of Cardiovascular Disease and Death Using Pooled Repeated Biennial Measurements: Framingham Heart Study, 30-Year Followup. Springfield, Mass: National Technical Information Service; 1987:1–459.
7. Wilson PWF, Castelli WP, Kannel WB. Coronary risk prediction in adults: the Framingham Heart Study. Am J Cardiol. 1987;59:91–94.
8. Corti MC, Guralnik JM, Salive ME, Harris T, Field TS, Wallace RB, Berkman LF, Seeman TE, Glynn RJ, Hennekens CH, Havlik RJ. HDL cholesterol predicts coronary heart disease mortality in older persons. JAMA. 1995;274:539–544.
9. Wilson PWF, Kannel WB. Hypercholesterolemia and coronary risk in the elderly: the Framingham Study. Am J GeriatCardiol. 1993;2:52–56.
10. McNamara JR, Cohn JS, Wilson PWF, Schaefer EJ. Calculated values for low-density lipoprotein cholesterol in the assessment of lipid abnormalities and coronary disease risk. Clin Chem. 1990;36:36–42.
11. Grover SA, Abrahamowicz M, Joseph L, Brewer C, Coupal L, Suissa S. The benefits of treating hyperlipidemia to prevent coronary heart disease: estimating changes in life expectancy and morbidity. JAMA. 1992;267:816–822.
12. Grover SA, Coupal L, Hu XP. Identifying adults at increased risk of coronary disease: how well do the current cholesterol guidelines work? JAMA.1995;274:801–806.
13. The 4S Group. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S).Lancet. 1994;344:1383–1389.
14. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ, West of Scotland Coronary Prevention Study Group. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med. 1995;333:1301–1307.
15. Myers RH, Kiely DK, Cupples LA, Kannel WB. Parental history is an independent risk factor for coronary artery disease: the Framingham Study.Am Heart J. 1990;120:963–969.
16. Ernst E, Resch KL. Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature. Ann Intern Med. 1993;118:956–963.
17. Akyol O, Zoroglu SS, Armutcu F, Sahin S, Gurel A. Nitric oxide as a physiopathological factor in neuropsychiatric disorders. In Vivo. 2004;18(3):377-90.
18. Bernstein HG, Bogerts B, Keilhoff G. The many faces of nitric oxide in Coronary- heart disease . A review. Coronary- heart disease research. 2005;78(1):69-86.
19. Bernstein HG, Keilhoff G, Steiner J, Dobrowolny H, Bogerts B. Nitric oxide and Coronary- heart disease : present knowledge and emerging concepts of therapy. CNS & neurological disorders drug targets. 2011;10(7):792-807.