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ORIGINAL ARTICLE

Evaluation of NOS3 T-C 786 Gene Polymorphism in Iranian Patients affected by Migraine and Normal individuals

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ABSTRACT
A migraine is a serious, annoying headache which may be preceded or followed closely by sensory warning symptoms for instance flash of light, blind spots, tingling in the RFLP and legs, nausea, vomiting, and increased sensitivity to light and sound. The precise reason behind migraine headaches is unidentified; it's regarded as a result of abnormal brain activity causing a provisional alteration in the nerve signals, chemicals and blood flow in the brain.Facts proposed that NOS3 might have a role in this disorder; as a result we studied NOS3 T-C 786 gene polymorphism in Iranian patients affected by Migraine and normal individuals to realize the association between them. The present research was conducted including number of 60 Iranian patients affected by Migraine and 60 normal individuals by employing RFLP-PCR process. To conclude, the information and statistics received from this work was analyzed by SPSS software. To sum up, the end outcome of present study explains substantial relation between NOS3 T-C 786 gene polymorphism in Iranian patients affected by Migraine and normal individuals. It could be an important genetic predisposition feature.
***Key words**: Migraine, T-C 786 gene polymorphism, NOS3, headache*

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INTRODUCTION

A migraine is generally a rigorous headache felt as a throbbing pain at the front or side of the head [1]. Usually the headache affects half of the head, is pulsating naturally, and may last from 2 hours to 3 days. Related symptoms could contain nausea, vomiting, and sensitivity to light, sound, or smell [2]. The pain is usually made worse by physical activity. Up to one-third of men and women with migraine headaches perceive an aura: a temporary visual, sensory, language, or motor trouble which signals that the headache will quickly happen. Infrequently an aura could occur with minimum headache following it [3].

Migraines are considered to be as a result of combination of environmental and genetic aspects. About two-thirds of instances run in family units [4, 5]. Changing hormone levels could also play an important role, as migraines affect slightly more boys than girls before puberty, but about two to three times more women than men. The chance of migraines frequently is reduced during pregnancy [4]. The precise mechanisms of migraine aren't recognized. It's however, thought to be a neurovascular disorder. The principal theory relates to increased excitability of the cerebral cortex and abnormal control of pain neurons in the trigeminal nucleus of the brainstem [6,7].

Endothelial NOS is just a nitric oxide syntheses generating NO in blood vessels and is a part of regulating vascular tone by inhibiting smooth muscle contraction and platelet aggregation. NOS3 (Nitric Oxide Synthase 3 (Endothelial Cell)) is just a Protein Coding gene [8]. Variations in this gene are connected with susceptibility to coronary spasm [9,10]. A relationship between a polymorphism in the gene and late-onset Migraine disease has been reported.

Consequently, we conducted a research to study NOS3 T-C 786 gene polymorphism in Iranian patients affected by Migraine and normal individuals.

MATERIAL AND METHODS

This research was performed on 60 patients with Migraine and 60 healthy controls. The patient's samples were casually extracted from Hazrat-e-Abolfazl Mental Rehabilitation Center, Hamadan, Iran. The control group was selected from random participants whose health was established by medical diagnostic.

DNA extraction and PCR Reaction

Genomic DNA from venous blood samples were isolated using DNA Extraction Kit PGS (Model: PGS0051) in accordance with manufacturer's instructions. DNA were quantified with the NanoDrop technology (Thermo Scientific / NANODROP 1000 Spectrophotometer).The NOS3 T-C 786 gene polymorphism genotyping was performed base on the amplification-refractory mutation sequencing RFLP. The Thermal cycling conditions for RFLP-PCR were the following. Figure1 Utilizing the BIOER TECHNOLLOGY CO .LTD. (Model: TC-24/H.b) For The PCR We Used 20 µL Sample: 1 µL Forward Primer, 1 µL Reverse Primer, 6 µL Diluents 'Water, 2 µL DNA 50 ng/ml, 10 µL Master Mix Sequence of Primers was 5'- GTT CCT TTC CCC AGC AGT G -3' ´as forward primer,5´-5'-AGA ATG CAT GTC ACG CTC T -3'as reverse normal primer and 5'-AGA ATG CAT GTC ACG CTC C -3'as reverse mutant primer.

PCR program used for NOS3 T-C 786 gene polymorphism:

cycle	temperature(Celsius)	Time
first	95	Minutes 7
Two to thirty-five	94 59 72	1 minute and15vseconds 55 Seconds 30 seconds
thirty-six	72	5Minutes

Gel Electrophoresis

The electrophoresis was carried out using 1% Gel Red stained agarose gel, at 80V for 35 min We Use Horizontal Electrophoresis Cell with TBE Buffer (PH=8.3) , Ladder Were Used 50bp DNA Ladder After electrophoresis, the amplified PCR products were Perceive under U. V. light.

Statistical analysis

Statistical analyses were conducted using with the SPSS software (Statistical Package for Social Sciences) version18. Chi- square test ($\chi 2$), was used to check the association between two categorical variables or even to detect difference between several proportions. Pearson chi-square was used to investigate the connection involving the NOS3 T-C 786 gene polymorphism and Migraine.

RESULTS

We analyzed 60 patients with Migraine, and 60 healthy controls, for the NOS3 T-C 786 gene polymorphism.

T-C 786 gene polymorphism frequencies were in equilibrium in patients and controls. Patients showed an extensively increased frequency of the T-C 786 gene polymorphism allele compared with controls. Thus the T-C 786 gene allele would confer a slightly increased risk of developing late onset Migraine.

Table1: Genotype Table of NOS3 T-C 786 gene polymorphism:

	GROUP		
Total	Control	Case	
73	51	22	1 = TT Genotype
40	8	32	2 = TC
7	1	6	3 = CC
120	60	60	Total

The results of genotyping are depicted in Table1: The following genotypes were identified for NOS3 T-C 786 gene polymorphism.

Table2: Chi- square test (χ2) for analyzing NOS3 T-C 786 gene polymorphism:

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	46.734[a]	1	.000		
Continuity Correction[b]	44.126	1	.000		
Likelihood Ratio	55.247	1	.000		
Fisher's Exact Test				.000	.000
N of Valid Cases	120				

DISCUSSION

Migraine is just a regular neurological disorder affecting between 10 and 20% of the populace [11]. The clinical appearance is heterogeneous and includes continuing headache attacks, associated apparent symptoms of vegetative disorder, and hypersensitivity of numerous functional systems of the nervous system. About one-third of migraine further occurrence transient neurological symptoms frequently concerning the visual system ahead of or within a migraine attack, which are identified as migraine aura [12].Heredity has been demonstrated to play an important role in migraine pathogenesis. About 50% of affected individuals have a first-degree relative also struggling with migraine [13,14]. Furthermore, family and twin studies support the notion of MO and MA being different phenotypes of the similar entity, with a heritability including 33 to 57% [15, 16]. Migraine has been reported as a chance factor for ischaemic stroke in men and premenopausal in women [17]. Two reports have exposed that classical migraine (with aura) poses an increased risk than simple migraine (without aura). Migraine seems to become a background risk factor for stroke [18, 19].

The evidence exposed in the piece of writing confirms that NOS3 T-C 786 gene polymorphism plays an important role in Iranian patients affected by Migraine. In accordance with this, an increased frequency of the allele among patients with Migraine has been seen.

By analyzing a group of Iranian patients, it is understood that the NOS3 T-C 786 gene has been connected with this disorder. As a result NOS3 T-C 786 gene polymorphism is actually a noteworthy genetic tendency factor for in Iranian Migraine patients. Therefore, NOS3 T-C 786 gene polymorphism may be a genetic predisposing factor for Migraine disorder treatment in Iranian population.

REFERENCES

1. Headache Classification Subcommittee of the International Headache Society (2004). "The International Classification of Headache Disorders: 2nd edition". *Cephalalgia* **24** (Suppl 1): 9–160.
2. Stovner LJ, Zwart JA, Hagen K, Terwindt GM, Pascual J (April 2006). "Epidemiology of headache in Europe". *European Journal of Neurology* **13** (4): 333–45.
3. Gilmore, B; Michael, M (2011-02-01). "Treatment of acute migraine headache".*American family physician* **83** (3): 271–80.
4. Buzzi, MG; Cologno, D; Formisano, R; Rossi, P (Oct–Dec 2005). "Prodromes and the early phase of the migraine attack: therapeutic relevance". *Functional neurology* **20** (4): 179–83.
5. Derry S, Rabbie R, Moore RA (2012). Moore, Maura, ed. "Diclofenac with or without an antiemetic for acute migraine headaches in adults". *Cochrane Database Syst Rev* **2**: CD008783.
6. Tepper Stewart J., S. J.; Tepper, Deborah E. (2010). "Breaking the cycle of medication overuse headache". *Cleveland Clinic Journal of Medicine* **77** (4): 236–42.
7. Posadzki, P; Ernst, E (2011). "Spinal manipulations for the treatment of migraine: a systematic review of randomized clinical trials". *Cephalalgia : an international journal of headache* **31** (8): 964–70.
8. Liu Q, Gross SS (1996). "Binding sites of nitric oxide synthases". *Meth. Enzymol.* **268**: 311–24.
9. Mungrue IN, Husain M, Stewart DJ (2002). "The role of NOS in heart failure: lessons from murine genetic models". *Heart Fail Rev* **7** (4): 407–22
10. Chinje EC, Stratford IJ (1997). "Role of nitric oxide in growth of solid tumours: a balancing act". *Essays Biochem.* **32**: 61–72
11. Haut SR, Bigal ME, Lipton RB. (2005). Chronic disorders with episodic manifestations: focus on epilepsy and migraine. Lancet Neurol. 5(2):148–157. doi: 10.1016/S1474-4422(06)70348-9.
12. Silberstein SD. (2004). Migraine. Lancet. 363(9406):381–391. doi: 10.1016/S0140-6736(04)15440-8.
13. Bille B. A 40-year follow-up of school children with migraine. Cephalalgia. 1997;17(4):488–491. doi: 10.1046/j.1468-2982.1997.1704488.
14. Svensson DA, Larsson B, Waldenlind E, Pedersen NL. (2003).Shared rearing environment in migraine: results from twins reared apart and twins reared together. Headache. 43(3):235–244. doi: 10.1046/j.1526-4610.2003.03047.

15. Mulder EJ, Baal C, Gaist D, Kallela M, Kaprio J, Svensson DA, Nyholt DR, Martin NG, MacGregor AJ, Cherkas LF, Boomsma DI, Palotie A. Genetic and environmental influences on migraine: a twin study across six countries. Twin Res. 2003;6(5):422–431. doi: 10.1375/136905203770326420.
16. Ligthart L, Boomsma DI, Martin NG, Stubbe JH, Nyholt DR. (2006). Migraine with aura and migraine without aura are not distinct entities: further evidence from a large Dutch population study. Twin Res Hum Genet. 9(1):54–63. doi: 10.1375/twin.9.1.54.
17. Merikangas KR, Fenton BT, Cheng SH, Stolar MJ, Risch N. (1997).Association between migraine and stroke in a large-scale epidemiological study of the United States. Arch Neurol. 54:362–368.
18. Tzourio C, Tehindrazanarivelo A, Iglésias S, Alperovich A, Chedru F, d'Anglejan-Chatillon J, et al. (1995). Case-control study of migraine and risk of ischemic stroke in young women. BMJ.310:830–833.
19. Marini C, Carolei A, Roberts RS, Prencipe M, Gandolfo C, Inzitari D, et al. (1993). The National Research Council Study Group. Focal cerebral ischaemia in young adults: a collaborative case-control study. Neuroepidemiology.12:70–71.