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Analysis of dopamine beta hydroxylase gene polymorphisms in migraine

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ABSTRACT

Background: Migraine is a complex neurological disorder characterized by severe recurrent headache, nausea, vomiting, photophobia, and phonophobia. The frequency and duration of these symptoms varies among individuals. Dopaminergic systems are believed to be involved in migraine pathophysiology. We aimed to look for association of polymorphisms in dopaminergic genes in genetic susceptibility to migraine in Turkey population.

Methods: The present study was designed to explore possible association of three polymorphisms, (1021C>T (Rs1611115), +1603C>T (Rs6271; C535R) and +444G>A (rs1108580), of Dopamin Beta Hydroxylase gene in migraine patients.

200 migraine patients and 267 healthy controls were included in the study. Genomic DNA was extracted from blood and genotypes were analyzed using polymerase chain reaction–restriction fragment length polymorphism methods (PCR-RFLP).

Results: Statistical evaluation of data results showed a significant association for allelic and genotypic frequency distribution between the Dopamin Beta Hydroxylase gene +1603C>T polymorphism and migraine ($p = 0.000$, OR: 4.36, 95% CI: 2.73–7.16). There was no association observed between the −1021C>T and +444 G>A polymorphisms of the Dopamin Beta Hydroxylase gene and migraine ($p = 0.8731$ and $p = 0.7584$).

Conclusions: This study reflects that Dopamin Beta Hydroxylase gene +1603C>T polymorphism may be one of the many genetic factors for migraine susceptibility in the Turkish population.

1. Introduction

Migraine is a neurovascular disorder, which is characterized by recurrent headache attacks with associated symptoms of nausea and vomiting [1]. The most common forms of this disorder have been classified as migraine with aura (MA) and migraine without aura (MO) [2].

Monozygotic twins and dizygotic twins studies have also helped to elucidate the hereditary contribution to migraine. Genetic prediction varies 28–65% among studies; this rate may vary in different populations [3,4] The pathophysiology of migraine remains still unknown. However, migraine has a complex etiology determined by genetic and environmental factors, hormonal and neurotransmitter pathways [5,6,7]. Some of the neurotransmitters including serotonin, acetylcholine and catecholamine (dopamine and norepinephrine) is thought to be associated with migraine [7]. Several genetic association studies have research the possible role of the dopaminergic system in migraine [8,9]. A central dopaminergic hyperfunction and possible noradrenergic dysfunction may lead to migraine attacks. Migraine attacks is thought to be the cause of central a dopaminergic excessive activity and noradrenergic dysfunction be together. Hypersensitivity of dopaminergic system can cause such as nausea, sweating and yawning symptoms. There is this symptoms during migraine attacks [10].

Therefore, some of the candidate genes associated with migraine is related with the dopaminergic system [8,11,12]. Several dopaminergic genes have been investigated in different migraine study with varying results [8,10]. An imbalance in dopamine (DA) to norepinephrine (NE) ratio resulting in dopaminergic hypersensitivity leads to a higher susceptibility for migraine. Dopamine beta hydroxylase (DBH) enzyme catalyzes the conversion of DA to NE [1,11,13]. Serum levels of DBH enzyme are elevated in migrainous patients during the headache phase of an attack and this

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Table 1
The PCR primers, PCR programme, restriction enzymes for *DBH* +1603 C > T, *DBH* −1,021C > T and *DBH* +444 G > A polymorphisms.

Polymorfizm	primeer sequens	Restriction enzyme	Product size	Restriction product size	PCR programme
DBH + 1603 C > T	Sense: 5′CCAGGGACAGGACTCGAGTTG-3′ Antis ense: 5′AGCAGTTTGGAGTGCAGACCC-3′	Bst UI	352 bp	The T alleles did not digest, 184, 139 and 29 bp for C allel.	5 min 94 °C, 30 s 94 °C, 30 s 62 °C, 35 cycles 30 s 72 °C 10 min at 72 °C.
DBH − 1021 C > T	Sense: 5′-GGAGGGACAGCT TCT AGTCC-3′ Anti sense: 5′CACCTCTCCCTCCTGTCCTCTCGC-3′	HhaI	131 bp	The T alleles did not digest, 109 bp and 22 bp for C allel	5 min 94 °C, 30 s at 94 °C, 30 s at 60 °C, 35 cycles 30 s at 72 °C 10 min at 72 °C.
DBH +444 G > A	Sense: 5′-CCTGGAGCCCAGTGCTTGTC-3′ Antisense: 5′-ACGCCCTCCTGGGTACTCGC-3′.	EcoNI	207 bp	The A alleles did not digest, G alleles digested to give 169 and 38 bp fragments.	5 min at 94 °C 30 s at 94 °C, 30 s at 55 °C, 30 cycles 30 s at 72 °C 5 min at 72 °C

reaction is known that critically involved in the biosynthesis of catecholamines. The dopamine beta hydroxylase gene (DBH) is consequently plays an important role in the regulation of noradrenergic and adrenergic neurotransmission. DBH, the human gene encoding DBH, is located on chromosome 9q34. The DBH gene is composed of 12 exons and comprises a sequence of approximately 23 kb [11,14,15].

There are several polymorphisms in the DBH gene. One of the polymorphisms, +1603C > T (ref SNP database, rs6271) is a single nucleotide polymorphism (SNP) at position 1603 in exon 11 that was defined as the change in the C > T, encoding a non-conservative difference in primary amino acid sequence (arg535cys) [10,16]. İndividuals with the 1603 T (cys encoding) allele may have disulfide bridge formation among the four units constituting the DBH holoenzyme (tetramer). This status may affect homospecific activity. [10].

One other DBH gene polymorphism, −1021C > T, is to detect the transition from a C–T located in promotor of DBH and in 1021 bp upstream of translational start codon, ATG (ref SNP database, rs 1611115). This polymorphism showed that very-low plasma DBH activity individuals were homozygous for the T allele, while individuals homozygous for the C allele showed higher mean level [5,17].

Also showed that one other SNP, 444 G > A (ref SNP database, rs6271) polymorphism, located in exon 2 of DBH, associated with plasma DBH levels and with levels of DBH protein [Cubells et al., 2004]. Although DBH gene 444 G > A polymorphismi change the third base of a Glu codon, the primary structure of DBH protein does not change [18]. DBH 444 G allele were associated with higher plasma DBH activity [19].

In the study, we investigated the possible role of DBH gene +1603 (ref SNP database, rs6271), −1021 (ref SNP database, rs 1611115) and +444 (ref SNP database, rs1108580) polymorphisms to look for their association in genetic susceptibility to migraine as compared with healthy controls (HC) in a Turkish population.

2. Methods

2.1. Study populations

The study group consisted of 200 patients with migraine (24 male and 176 female; mean age: 36.49 ± 9.55 standard deviation [SD] years), and 267 (96 male and 171 female; mean age: 38.18 ± 8.42 SD years) healthy controls who had taken part in our previous study [6,20] with no previous or current history of migraine, all of whom live in Tokat, Turkey. All migraine patients were registered at the outpatient clinic of the Neurology Department at Gaziosmanpasa Medical Faculty, and they all fulfilled the International Headache Society criteria for classification) [2]. The control subjects matched for age and geographic area. The study protocol was approved by the ethics committee of Gaziosmanpasa Medical Faculty, and written informed consent was obtained from the study participants.

2.2. Blood samples and DNA isolation

Peripheral blood samples were collected from each subject and genomic DNA was isolationed from venous blood samples using a PureLinkTM Genomic DNA isolation Kit (Invitrogene) according to the manufacturer's directive.

2.3. Genotyping

DBH gene +1603C > T (rs6271; C535R), −1021C > T (rs1611115) and 444G > A (rs1108580) polymorphisms were genotyped by means of a polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method. The PCR analysis was also performed using a modification of a previously described study [8]. The PCR reactions (25 μl final volume) contained 17 nmol/L, 2.5 nmol/L buffer, 1.5 nmol/L $MgCl_2$, 0.3 nmol/L dNTPs, 0.8 mol/L of each primer, 1 U of Taq polymerase and approximately 2 μg of genomic DNA. The PCR primers, PCR programme, restriction enzymes are shown in Table 1. Restriction fragments of all three polymorphisms of *DBH* gene were electrophoresed on an 2% agarose and 1% nusieve agarose gel stained with ethidium bromide, and the genotypes were determined under ultraviolet (UV) illumination (Table 1).

2.4. Statistical analysis

Statistical analysis was performed by using PEPI 3.0 (available at: http://www.usdinc.com/pepi.html) and SPSS 15.0 (SPSS Inc., Chicago, IL, USA). The distribution of *DBH* genes polymorphisms between migraine patients and healthy controls were compared by using the c2 or Fisher's exact test. *P* values smaller than 0.05 were considered significant. Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated whenever c2 or Fisher's exact test was significant. Goodness of fit c2 test was used to check Hardy–Weinberg equilibrium in the control population, Arlequin software v. 2000 (University of Geneva, Geneva, Switzerland). Significant probability values obtained were corrected for multiple testing (Bonferroni correction; Pc). The correlation of mean age with groups was analyzed using the *t*-test for independent samples. The comparison of the categorical variables between the groups

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