Original Article

MTHFR Gene polymorphisms and susceptibility to Migraine Attacks

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Abstract

Background: Migraine consisting of migraine with aura (MA) and migraine without aura (MO) is a painful neurovascular disorder affecting approximately 16% of the general population. A combination of genetic and environmental factors is involved in the pathogenesis of migraine. The MTHFR enzyme is involved in homocysteine (Hcy) metabolism and it has been reported that 1298 A to C and 677 C to T mutations in the MTHFR gene are associated with an increased in plasma Hcy levels. Hcy is a highly reactive amino acid and causes endothelial injury. Because a plausible theory about vascular impairment in migraine, it is considered that mutations in MTHFR gene and folate metabolism are associated with migraine. **Materials and Methods:** In total, 75 patients with migraine (24 with MA and 51with MO) in accordance with the IHS criteria participated in this case-control study. Control group were 128 normal matched healthy subjecys who selected from same region without history of migraine or other neurologic disorder after interviewing and examining by a physician. Mean age at entry was 36.42±9.6 and 31.64±8.9 years old in migraine and control group respectively. MTHFR polymorphisms were investigated by PCR-RFLP. **Results:** Genotypic results indicated that the prevalence of the MTHFR 677TT genotype in migraine subjects was higher than control (17.3% and 3.1% respectively, P<0.001). Interestingly the risk of migraine was 6-fold higher in subjects possessing the MTHFR 677T homozygous variant (OR=6.5; CI95%: 2.03-20.76). No significant difference in the prevalence of MTHFR A1298C genotypes was observed in migraine group when compared to controls (P>0.001). **Conclusion:** It seems that MTHFR C677T is a potential genetic risk factor for migraine attacks, both in MA and MO subclasses in Iranian population. C677T and A1298C joint effect could amplify the potential influence of each SNPs.

Keywords: Genetic, MTHFR, Migraine, Iran

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Introduction

Migraine disorder is a common debilitating neurovascular disorder affecting approximately 16% of the population and is more frequent in women [1]. Unilateral headache, nausea, vomiting, photo- and phonophobia are the main features of disease

[1]. Common migraine ,migraine without aura (MO) and migraine with aura (MA) are two main subclasses of the disease and up to one-third of patients experience an aura prior or during the headache attacks [2].

Migraine is a complex disorder and both genetic susceptibility and environmental triggers could play a role in the pathogenesis of disease. Many genes has been considered as predisposing factors in initiating migraine attacks mainly in MA than in MO [1-3]. Several studies have indicated various susceptibility genes for common migraine including MTHFR and some other genes [2, 4, 5].

The MTHFR gene , located on chromosome 1 (1p36.3), is composed of 11 exons and 10 introns. MTHFR catalyzes the biosynthesis of 5-methyltetrahydrofolate and used for homocysteine remethylation to methionine. It has been demonstrated that hemocysteine could inhibit DNA-methyltransferase and causes DNA hypomethylation.Therefore, impaired enzyme activity of MTHFR was associated with dysregulation of folate metabolism [6, 7]. Among polymorphisms in MTHFR gene, the C677T and A1298C substitution have been investigated so far. The substitution of alanine for valine in the MTHFR protein resulted from C677T polymorphism which is located within the catalytic domain of the enzyme.

In this regard, the presence of T allele was associated with decrease in enzyme activity, thereby alteration in circulating levels of homocysteine and folate metabolites.

Similarly, Polymorphism A1298C affects the enzyme activity of MTHFR as well as folate concentrations, although less than those in C677T. Additionally, this polymorphism is located within the regulatory domain. Hence, the investigation of MTHFR polymorphism and its effect on metabolic pathway of folate has been considered as an interesting issue in recent years [8].

Considering involvement of vascular mechanisms in migraine pathogenesis, polymorphisms in methylenetetrahydrofolate reductase (MTHFR) gene appear to act as an interfering factor in folate metabolism and cause elevation in plasma homocysteine (Hcy) level [9] and ultimately endothelial injury [10, 11]. This could be in favor of the role of vascular malfunction in migraine etiology [6, 10].

Here we aimed to investigate the relationship between two MTHFR gene SNPs (rs1801133; C677T and rs1801131; A1298C) with susceptibility to migraine in Iranian patients with migraine attacks.

Methods

Patients were selected from those who referred to Neurology Clinics, Imam Khomeini, Rasoul Akram Hospitals and Aramesh Migraine Clinic, Tehran, Iran based on international headache society (HIS) criteria [12]. A complete demographic and medical history questionnaire was obtained from all cases. A brief neurologic examination was performed for each case. Healthy controls were selected from those without history of migraine or other neurologic disorder after interviewing and examining by a physician.

Venous blood sample was collected from all participant and genomic DNA was extracted from EDTA-treated whole-blood samples from white blood cells using salting out method [13]. Informed consent was obtained from all participants prior to the study. The project was approved by the Ethics Committee of Tehran University of Medical Sciences.

C677T and A1298C polymorphisms were analyzed by PCR-RFLP (Restriction Fragment Length Polymorphism) methods using primers (Table 1) obtained from previous studies with minor modification [14]. PCR amplification was performed in a final volume of 25 µl containing 2.5 µl 10X PCR buffer, 0.8 µl of 50mM Mgcl2, 0.5µl of each 10pmol forward and reverse primer, 0.6µl of 10mM dNTPs, 3µl of DNA template and 0.5µl of 5u/µl Tag DNA polymerase. Thermal cycling was performed in Eppendorf master cycler (Germany) with an initial denaturation of 3 min at 95°C, then 34 cycles of denaturation at 94°C for 55 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 50 seconds followed by a final extension at 72°C for 10 min. Amplified DNA were visualized and approved using gel electrophoresis on agarose 1% and staining.

PCR products were digested by *HinfI* and *MboII* restriction enzymes to identify specific alleles in C677T and A1298C polymorphisms respectively. The digestion reaction for each PCR product was carried out in a final volume of 15µL containing 1µL

buffer, 0.2µL restriction enzyme and 10µL PCR product. Then it was incubated at 37°C overnight. Digested products were visualized on 2.5% agarose gel stained with ethidium bromide.

C677T PCR product (198 bp) was digested into 175 and 23 bp fragments by *Hinf* I for T-allele. A1298C PCR product (256 bp) was digested into 204, 30 and 22 bp fragments by *Mbo* II for C-allele. DNA sequencing used to confirm PCR-RFLP results.

Analysis was carried out using SPSS 13.0 and P<0.05 was considered significant. Hardy–Weinberg equilibrium was evaluated using PowerMarkerV.325. Genotype and allele frequencies were tested using $\chi 2$-test.

We used $\chi 2$-test for evaluating genotype and allale frequencies. The risk of migraine for mutant allele carriers was reported as odds ratios (OR) and 95% confidence intervals between groups. Continuous variables were expressed as means ± SD and evaluated between groups by t-Test.

Results

Seventy-five migraineurs (57 women and 18 men) and 128 healthy people (85 women and 43 men) were recruited into the study after initial evaluation. Mean age at entry was 36.42±9.6 and 31.64±8.9 years old in migraine and control group respectively.

A-allele and C-allele were more frequent in A1298C and C677T SNPs as was shown in Table 2 and allele frequency of A1298C and C677T were in Hardy–Weinberg equilibrium among migraineurs and controls (P=0.74 and P=1.0, respectively).

A significantly higher frequency of T667-allele was observed in the migraineurs group as compared to controls that was associated with an increased risk of migraine (OR=1.47; CI95%: 1.10-1.97). A non-significantly higher frequency of C1298-allele was observed in the migraineurs group as compared to controls (Table 2).

A significantly higher frequency of TT667 genotype was observed in the migraineurs patient group as compared to controls (17.3% and 3.1% respectively, P< 0.001) (OR=6.5; CI95%: 2.03-20.76) and A1298C polymorphic genotype distribution did not show any statistically frequency difference among the migraineurs patient group as compared to controls (Table 3).

Table 1: Primer sequence for C677T and A1298C polymorphism detection.

SNP	Primer	Primer Sequence
C677T (rs1801133)	C677T R	5'-AGG ACG GTG CGG TGA GAG TG-3'
	C677T F	5'-TGA AGG AGA AGG TGT CTG CGGGA -3'
A1298C (rs1801131)	A1298C R	5'-CATGTCCACAGCATGGAG-3'
	A1298C F	5'-CTTCTACCTGAAGAGCAAGTC-3'

Table 2: Allele frequency of A1298C and C677T polymorphisms in cases and controls.

Polymorphism	**Allele**	**All samples (%)**	**Cases (%)**	**Controls (%)**
C677T		283 (69.70)	93(62)	190 (74.2)
		123 (30.30)	57(38)	66 (25.7)*
A1298C		237 (58.37)	86(57.3)	151 (58.9)
		169 (41.63)	64(42.6)	105 (41.02)

* P<0.001, Odd ratio=1.47; CI95%:1.10-1.97

Table 3: Genotypic frequency of A1298C and C677T polymorphisms in case and control groups.

Polymorphism	Genotype	Cases (%)	Controls (%)
C677T	CC	31 (41.3)	66 (51.6)
	CT	31 (41.3)	58 (45.3)
	TT	13 (17.3)	4 (3.1)*
A1298C	AA	25 (33.3)	44 (34.4)
	AC	36 (48.0)	63 (49.2)
	CC	14 (18.7)	21 (16.4)

* P<0.001, Odd ratio=6.5; CI95%:2.03-20.76

Based on comparing migrane subclasses, MA and MO with healthy controls, 677TT genotype was related to both types but A1298C genotype was not associated with MA or MO (Table 4).

When two MTHFR polymorphisms were analyzed simultaneously, allelic changes (677CT-1298CC or 677CC-1298AC) were more frequent in the migraineurse group as compared to controls (20% and 3.9% respectively, P< 0.0001) (Table 5) and TT677/AC1298 genotype was also more frequent in patients than controls (P<0.01) (Table 6).

Discussion

It has been suggested that MTHFR dysfunction

Table 4: Genotypic frequency of A1298C and C677T polymorphisms in MA, MO and control groups.
*P<0.0001.

Polymorphism	Genotype	Controls (%)	MA (%)	MO (%)
C677T MTHFR	CC	66 (51.6)	6 (25)*	25 (49)
	CT	58 (45.3)	14 (58.3)	17 (33.3)
	TT	4 (3.1)	4 (16.7)*	9 (17.6)*
A1298C MTHFR	AA	45 (35.2)	8 (33.3)	17 (33.3)
	AC	62 (48.4)	12 (50)	24 (47.1)
	CC	21 (16.4)	4 (16.7)	10 (19.6)

could lead to a wide range of vascular and neurological diseases. Therefore, investigation of functional MTHFR polymorphisms in disorders with the vascular pathology is an interesting topic.

Migraine is a common cause of headache disorder mostly in middle age women, affecting their productivity and health related quality of life. Its direct and indirect annual cost is more than 11 billion and 19.6 billion USD in USA [15].

As familial aggregation of migraine strengthens the possible inherited pattern of disease [16-18], many investigations were conducted to find candidate genes involving in migraine pathophysiology. However, this familial aggregation seems to be influenced by different factors like environmental triggers, susceptibility genotypes, mode of inheritance and penetrance. Because of multi-factorial and multigenic property of migraine, many candidate genes like MTHFR, ESR1, PGR, INSR, ACE genes were introduced as role-players in migraine attacks mainly in MA [1-5] .These genes are mostly related to neural pathways and molecular mechanisms affecting endothelial function as main points in migraine pathophysiology [19].

Since candidate genes using case-control study is a powerful method and direct strategy for recognition of low effect but predisposing genes for a disease [19], we compared MTHFR common polymorphisms in migraine patients with healthy individuals. Result showed that 677TT genotype in

Table 5: Frequency of allelic changes due to CT677/AC1298 polymorphisms in case and control groups.
* P<0.0001.

C677T	A1298C	Patients (%)	Controls (%)
CC	AA	9 (12)	22 (17.3)
CC	AC	26 (34.7)	44 (34.6)
CT	AA		
CC	CC	25 (33.3)	56 (44.1)
TT	AA		
CT	AC		
CT	CC	15 (20.0)	5 (3.9)*
TT	AC		
TT	CC	0 (0)	0 (0)

Table 6: Diplotype frequency from CT677/AC1298 polymorphisms in case and control groups.
* P<0.01.

C677T/A1298C Diplotype	Patients (%)	Controls (%)	Significance
CC/AA	9 (12)	23 (18)	N.S
CC/AC	13 (17.3)	27 (21.1)	N.S
CC/CC	9 (12)	16 (12.5)	N.S
CT/AA	13 (17.3)	17 (13.3)	N.S
CT/AC	13 (17.3)	36 (28.1)	N.S
CT/CC	5 (6.7)	5 (3.9)	N.S
TT/AA	3 (4)	4 (3.1)	N.S
TT/AC	10 (13.3)	0 (0)*	
TT/CC	0 (0)	0 (0)	

migraine patients is significantly more prevalent than normal people and also in both MO and MA subgroups than control group. These results are in compliance with some indicative findings about increase susceptibility to migraine and presence of 677T allele [10, 19-22] particularly when homozygote form is present and in patient with migraine with urea [10, 19, 21-24].

In this study, when A1298C genotype frequency in migraineurs was compared with healthy

group, no significant discrepancy was found which was in conflict with other perusal observing positive relevance between migraines and both 1298AC and 677TT genotypes. In contrast with our result, they also showed these alleles have higher genotype frequency in migraineurs in comparison with the control group [25, 26]. Different ethnicity and might be lower sample size particularly in MA subgroup might verified our opposite results.

However an extremely higher frequency of genotypes with 3 changes in both polymorphisms (677CT+1298CC and 677TT+1298AC), were observed in patient group. Furthermore a more significant susceptibility to migraine was also showed in subjects with 677TT/1298AC diplotype in comparison with 677CT/1298CC subjects.

Based on the fact that the C677T SNP takes places in the catalytic domain and the A1298C SNP occurs within the regulatory domain of the MTHFR enzyme, 677TT/1298CC genotype might cause quiet enzyme disability that could have lifelessness effect [25], as expected, subjects who had simultaneously homozygote form (677TT/1298CC) of two common MTHFR polymorphisms were not found in our study which was in agreement with other studies [25, 27].

In 2002 Kara's group study it was found that individuals with 677CC/1298CC and 677TT/1298AA diplotype are more susceptible to migraine attacks in comparison with subjects who have normal genotype of enzyme (1298AA/677CC) [25].

Finally while our study showed the positive and powerful effect of the simultaneous occurrence of two polymorphisms on decrease of MTHFR enzyme activity, some studies analyzed the effect of these polymorphisms on migraine together. Therefore, to get higher qualified data and better understanding about effects of MTHFR gene polymorphisms on migraine status, the incidence effects should be studied simultaneously.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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