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ORIGINAL RESEARCH

The rs4846049 polymorphism in the 3′UTR region of the *MTHFR* gene increases the migraine susceptibility in an Iranian population

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Mohaddeseh Salehi[1,*]
Mona Amin-Beidokhti[2,*]
Behnam Safarpour Lima[3]
Milad Gholami[2]
Gholam-Reza Javadi[1]
Reza Mirfakhraie[2,4]

[1]Department of Biology, Islamic Azad University, Science and Research Branch, [2]Department of Medical Genetics, [3]Department of Neurology, School of Medicine, [4]Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

*These authors contributed equally to this work

Introduction: Migraine is a painful complex neurovascular disease characterized by recurrent moderate-to-severe headaches. Increased level of homocysteine is related to dilation of cerebral vessels and endothelial injury that could trigger migraine attacks. Functional polymorphisms in the MTHFR gene affect homocysteine metabolism and, therefore, play an important role in the etiology of the disease.

Objectives: We aimed to investigate the possible association between MTHFR gene rs4846049, C677T, and A1298C polymorphisms and the risk of migraine in Iranian population.

Methods: In this genetic association study, 498 individuals were enrolled, including 223 migraine patients and 275 healthy controls. Genotyping was performed using tetra-primer ARMS-PCR for rs4846049 and PCR-restriction fragment length polymorphism for C677T and A1298C polymorphisms.

Results: The association between rs4846049 and C677T polymorphisms and migraine was observed. For the rs4846049 polymorphism, the association was detected under a dominant model (P=0.007; odds ratio [OR] =0.60; 95% confidence interval [CI], 0.41–0.87), and for the C677T polymorphism, the TT genotype frequency was significantly different in the studied groups (P=0.009; OR =2.48; 95% CI, 1.25–4.92). No significant differences in the genotype or allele frequencies were found for the A1298C polymorphism between the migraineurs and controls.

Conclusion: Present data provide evidence for the association of rs4846049 and C677T polymorphisms in the MTHFR gene and migraine. Further studies are required to validate the significance of the studied genetic variations in diverse ethnic populations.

Keywords: migraine, genetic association study, MTHFR, single nucleotide polymorphism

Introduction

Migraine is a recurrent unilateral pulsatile headache with a significant negative effect on the quality of life. The clinical manifestations of migraine are nausea, vomiting, photophobia, and phonophobia and sometimes, transient neurologic attacks known as "aura".[1–3] Based on the presence or absence of aura, migraine is classified into two particular subtypes, such as migraine without aura (MO) and migraine with aura (MA).[4] Several factors including genetic, environment, hormonal factors, and neurotransmitters play roles in the disease etiology.[5–7] Among the genetic factors, MTHFR gene can be studied in more detail due to its effect on the plasma level of homocysteine. MTHFR is a key enzyme in folate metabolism, which converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate that is required for the remethylation of homocysteine to methionine.[8–11] Therefore, reduction in the MTHFR enzymatic activity results in

Correspondence: Reza Mirfakhraie
Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Koodakyar Street, Velenjak Avenue, Chamran Highway, Terhan 19395-4719, Iran
Tel/fax +98 21 2387 2572
Email reza_mirfakhraie@yahoo.com

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hyperhomocysteinemia that is usually accompanied by spontaneous trigeminal cell firing, one of the possible reasons for headache and pain in migraine.[12,13] Genetic polymorphisms in the MTHFR gene could lead to alteration in the enzyme activity. Previous studies have revealed that two common nonsynonymous single-nucleotide polymorphisms (SNPs), C677T (Ala > Val; rs1801133) and A1298C (Glu > Ala; rs1801131), affect the enzyme activity.[8] It is suggested that the 677TT and 1298CC genotypes are associated with higher plasma homocysteine level.[8,14] However, the results concerning the association between these polymorphisms and susceptibility to migraine were conflicting. Furthermore, SNPs in the 3′UTR region of the genes may affect mRNA-binding affinity for microRNAs, small molecules that play important roles in several complex diseases including cancer, inflammatory diseases, cardiovascular diseases, and atherosclerosis. Emerging data also suggest that miRNAs are regulators of key processes in the nervous and inflammatory systems and, therefore, are likely to be involved in pain signaling and migraine.[15] Therefore, the aim of this study was to investigate the association between the MTHFR SNPs including rs4846049 (located in the MTHFR 3′UTR region), C677T, and A1298C with the risk of migraine in Iranian patients.

Subjects and methods

Subject selection

In this case–control study, 498 individuals were enrolled including 223 patients (158 MO patients and 65 MA patients) who suffered from migraine as the case group and 275 healthy controls. Diagnosis of migraine was confirmed based on the results of neurological examination and brain imaging studies in accordance with the International Headache Society (HIS) criteria.[16] Control subjects had no personal history or family history of chronic headache and neurological diseases. The samples were obtained between October 2014 and December 2015 from Imam Hossain Hospital, Tehran, Iran. All of the subjects were of Iranian descent and shared common ethnogeographic origin. Patients and controls were matched in age and gender. The mean age of the patients and controls was 33.69±8.92 (range 18–57) and 34.5±10.03 (range 17–58) years, respectively. Written informed consent was obtained from all individual participants included in the study or their parents. The ethics committee of the Shahid Beheshti University of Medical Sciences (SBMU) approved the study protocol (code no: IR.SBMU.MSP.REC.1395.527).

Genotyping of rs4846049 polymorphism

Peripheral blood samples were collected from the study participants in ethylenediaminetetraacetic acid (EDTA) tubes, and genomic DNA was extracted by using the M&D DNA Extraction kit (SBMU, Tehran, Iran) according to the manufacturer's protocol. Primers were designed using the Primer1 online software for genotyping with tetra primer-amplification refractory mutation system-polymerase chain reaction method.[17] The primers used in the PCR included one pair of outer primers, FO 5′TATAACATCTCTTCTACGATGCCACCAGTG3′ and RO 5′ATATACTCTTTTGGTGGGGAGCACTTGC3′, and one pair of inner primers, Fi 5′TTTATATGTACTGCACGGGCTCCAGGT3′ and Ri 5′TATACTGGGACTCCCAGTGAACTTGCC3′. PCR amplification was carried out in a total volume of 25 µL containing genomic DNA (100 ng), 5 pmol/L of outer primers, 10 pmol/L of inner primers, and 12.5 µL of Taq DNA Polymerase 2X Master Mix RED (Ampliqon, Odense M, Denmark). Amplifications were carried out on a FlexCycler (Analytik Jena, Jena, Germany). The PCR program consisted of an initial denaturation step at 94°C for 5 minutes followed by 32 cycles including denaturation at 94°C for 30 seconds, annealing at 63°C for 1 minute, and extension at 72°C for 30 seconds. Finally, one cycle of extension at 72°C for 5 minutes was performed. PCR products were subjected to 2% agarose gel prepared in 0.5× tris/borate/EDTA. The 2572G allele generated a 101 bp band, the T allele generated a 177 bp band, and a common 224 bp band was amplified by the outer primers. Ten percent of the genotypes revealed by TP-ARMS-PCR were further confirmed by sequencing using an ABI 3730xl DNA analyzer (Macrogen, Seoul, Korea).

Genotyping of C677T (rs1801133) polymorphism

The C677T variant was determined by PCR-restriction fragment length polymorphism (RFLP) using the following primers: 677-forward 5′AGCTTTGAGGCTGACCTGAAG3′ and 677-reverse 5′AGGACGGTGCGGTGAGAGTG3′. PCR amplification was carried out in a total volume of 25 µL reaction containing genomic DNA (100 ng), 5 pmol/L of each primer, and 12.5 µL Taq DNA Polymerase 2X Master Mix RED. Amplifications were carried out on a FlexCycler. The PCR program consisted of an initial denaturation step at 94°C for 5 minutes, followed by 32 cycles including denaturation at 94°C for 30 seconds, annealing at 60°C for 1 minute, and extension at 72°C for 30 seconds. Finally, one cycle of extension at 72°C for 5 minutes was performed. PCR amplicons were digested with Hinf *I* restriction enzyme. Digested products were subjected to 2% agarose gel prepared in 0.5× TBE. The T variant was digested by the enzyme; therefore, wild-type homozygote (CC), heterozygote (CT), and mutant homozygote (TT) showed one band (223 bp), three bands

(223, 172, and 51 bp), and two bands (172 and 51 bp), respectively. For further confirmation, 10% of the samples were sequenced by using an ABI 3730xl DNA analyzer.

Genotyping of A1298C (rs1801131) polymorphism

For genotyping A1298C polymorphism, we performed PCR-RFLP by using proposed primers by Anna-Liisa Lorenz et al.[18] PCR amplification was carried out in a total volume of 25 µL reaction containing genomic DNA (100 ng), 5 pmol/L of each primer, and 12.5 µL of Taq DNA Polymerase 2X Master Mix RED. Amplifications were carried out on a Flex-Cycler. The PCR program was the same as the PCR program for genotyping C677T polymorphism with the exception that annealing temperature was 63°C. PCR amplicons were digested with Mbo*II* restriction enzyme. Digested products were subjected to 8% polyacrylamide gel electrophoresis (PAGE) prepared in 0.5×TBE and stained with silver nitrate. Three genotypes including AA, AC, and CC showed four bands (22, 28, 30, and 176 bp), five bands (22, 28, 30, 176, and 204 bp), and three bands (22, 30, and 204 bp), respectively. For further confirmation, 10% of the samples were sequenced by using an ABI 3730xl DNA analyzer.

Statistical analysis

Allele and genotype frequencies and the Hardy–Weinberg equilibrium were calculated by chi-squared test using the SNPStats online software available from http://bioinfo.iconcologia.net/SNPstats.[19] The association of all polymorphisms with migraine was studied using recessive and dominant models. The strength of association between selected polymorphisms and susceptibility to migraine was evaluated by odds ratios (ORs) and 95% confidence intervals (CIs). A *P*-value of <0.05 was considered statistically significant.

Results

The results obtained from genotyping using TP-ARMS-PCR and PCR-RFLP was in 100% concordance with the sequencing results. The genotype distribution in the patients and controls were in complete Hardy–Weinberg equilibrium for all studied polymorphisms. Table 1 lists the genotype and allele frequencies of the studied polymorphisms for the migraine patients

Table 1 Genotype and allele frequencies of the MTHFR gene rs4846049, C677T, and A1298C polymorphisms in case and control groups

Genotype	Case, n (%)	Control, n (%)	OR (95% CI)	*P*-value
rs4846049 polymorphism				
GG	88 (39.46)	77 (28.00)	1.00 (reference)	
GT	96 (43.05)	138 (50.18)	0.61 (0.41–0.91)	0.01
TT	39 (17.49)	60 (21.82)	0.57 (0.34–0.94)	0.02
GT + TT vs GG			0.60 (0.41–0.87)	0.007
TT vs GG + GT			0.76 (0.48–1.19)	0.23
Allele				
G	272 (60.99)	292 (53.09)	1.38 (1.07–1.78)	0.01
T	174 (39.01)	258 (46.91)	0.72 (0.56–0.93)	0.01
C677T polymorphism				
CC	107 (47.98)	159 (57.82)	1.00 (reference)	
CT	91 (40.81)	101 (36.73)	1.34 (0.92–1.95)	0.13
TT	25 (11.21)	15 (5.45)	2.48 (1.25–4.92)	0.009
TT + CT vs CC			1.49 (1.04–2.12)	0.03
TT vs CT + CC			2.19 (1.12–4.26)	0.02
Allele				
C	305 (68.39)	419 (76.18)	0.34 (0.24–0.47)	<0.0001
T	141 (31.61)	65 (23.82)	2.98 (2.14–4.14)	<0.0001
A1298C polymorphism				
AA	81 (36.32)	100 (36.36)	1.00	
AC	101 (45.29)	134 (48.73)	0.93 (0.63–1.38)	0.72
CC	41 (18.39)	41 (14.91)	1.23 (0.73–2.08)	0.43
CC + AC vs AA			1.29 (0.80–2.07)	0.30
CC vs AC + AA			1.42 (0.76–2.66)	0.28
Allele				
A	263 (58.97)	334 (60.73)	0.93 (0.72–1.20)	0.57
C	183 (41.03)	216 (39.27)	1.08 (0.83–1.39)	0.54

Abbreviations: CI, confidence interval; OR, odds ratio.

and the control group. For the rs4846049, the frequency of the G allele was significantly higher in the case group compared with the controls (P=0.01; OR =1.38; 95% CI, 1.07–1.78). The analysis of the rs4846049 genotype frequencies showed that the frequency of the GT and TT genotypes was significantly different between the studied groups (P=0.01; OR =0.61; 95% CI, 0.41–0.91 and P=0.02; OR =0.57; 95% CI, 0.34–0.94, respectively). Moreover, a significant difference under dominant model was observed between the studied case and control groups (P=0.007; OR =0.60; 95% CI, 0.41–0.87).

For the C677T polymorphism, the frequency of the T allele was significantly higher in the case group compared with the controls (P<0.0001; OR =2.98; 95% CI, 2.14–4.14). The frequency of the TT genotype was significantly different in the studied groups (P=0.009; OR =2.48; 95% CI, 1.25–4.92). Moreover, significant differences were observed under dominant and recessive models between the studied case and control groups (P=0.03; OR =1.49; 95% CI, 1.04–2.12 and P=0.02; OR =2.19; 95% CI, 1.12–4.26).

No significant differences were detected for A1298C genotype and allele frequencies or any of the genetic models between cases and controls (Table 1).

Discussion

In the present study, we investigated possible association between rs4846049, C677T, and A1298C polymorphisms in the MTHFR gene and migraine in Iranian patients. We observed that the rs4846049 polymorphism increases the susceptibility to migraine in the studied population. The rs4846049 polymorphism exists on the 3′UTR of the MTHFR gene and is a potential binding site for microRNA-149 (miR-149).[20,21] SNPs located in the 3′UTR region of the genes could change the genes' expression regulation due to their effect on the binding of miRNAs. According to the literature, the effectiveness of miR-149 binding to the rs4846049 T allele is more than wild-type G allele. Furthermore, the TT genotype has a reduced MTHFR protein level in peripheral blood mononuclear cells compared to the GG genotype, although interestingly, the MTHFR mRNA expression level is not significantly different between the mentioned genotypes.[22]

Several studies have proved the role of vascular dysfunction in migraine pathophysiology. For instance, the association of migraine with the risk of ischemic stroke was confirmed in two meta-analysis studies.[23,24] In addition, Wu et al[22] reported that rs4846049 polymorphism increases coronary heart disease risk through modifying miR-149 binding. Considering the results obtained from Wu et al and the present study, we may hypothesize that the regulatory role of miR-149 on MTHFR expression via rs4846049 polymorphism may explain the vascular dysfunction in migraine. However, further functional studies are required to confirm this hypothesis.

There are several association studies concerning the role of C677T MTHFR polymorphism in migraine that showed conflicting results. Most of the studies suggested the association of 677 T allele with increased risk of migraine, although some suggest no association.[8,25–28] In order to prove the exact role of C677T polymorphism in migraine, several meta-analyses were performed. Schurks[8] confirmed the positive association between C677T polymorphism and migraine among non-Caucasian populations. However, Rubino et al[29] reported no overall association of C677T polymorphism with migraine. The same result was obtained from the meta-analysis conducted by Liu et al[28] in Caucasian populations.

In this study, we found that the frequency of the TT genotype was significantly different in the studied groups (Table 1).

The 677T allele decreases MTHFR enzymatic function, and the reduction of enzymatic capacity was previously reported in TT genotypes.[9] As it was mentioned previously, failure of activity of MTHFR enzyme could led to hyperhomocysteinemia and reduction in the folate plasma levels that induce dilation of cerebral vessels. These alterations result in an increased risk for coronary heart disease, peripheral vascular and cerebrovascular disease, and inflammation in the meninges that could be involved in the migraine pain.[9,12,30] In a recent study in the Turkish population, it was shown that the CT genotype was significantly higher in the migraine patients.[26] Our finding is in concordance with the result of the Turkish study which may be explained by the similarity in the genetic background. An et al[25] also showed evidence of an association between MTHFR C677T polymorphism and risk of migraine. They observed significantly higher frequency of the T allele in the MO migraine patients than in the controls. The same association between the T allele and migraine was reported by Azimova et al.[27] They mentioned that TT and CT genotypes were significantly associated with sensitivity to migraine attack triggers. Furthermore, the TT genotype was accompanied by photophobia symptom more than in other genotypes.

Previous studies concerning the association of the A1298C polymorphism with migraine showed conflicting results.[16,31] Anna-Liisa Lorenz et al[18] showed no association between A1298C polymorphism and the risk of migraine. However, Kara et al[31] suggested that the 1298C allele was significantly higher in migraine patients than in controls. Our data were consistent with the negative association of A1298C polymorphism with migraine.

Conclusion

This study suggests the association of rs4846049 and C677T polymorphisms with the risk of migraine in Iranian population. To the best of our knowledge, this is the first study to investigate the association between rs4846049 polymorphism and migraine. Further studies are required to validate the significance of the studied genetic variations in diverse ethnic populations.

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Disclosure

The authors report no conflicts of interest in this work.

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