The Homozygous C677T Mutation in the Methylenetetrahydrofolate Reductase Gene Is a Genetic Risk Factor for Migraine

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Increased homocysteine levels are associated with various pathological conditions in humans, including stroke and cardiovascular disorders. Homocysteine acts as an excitatory amino acid in vivo and may influence the threshold of migraine headache. Frosst et al. [1995] reported an association between the homozygous C677T mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene and serum homocysteine levels. This study was designed to determine the prevalence of the MTHFR mutation in Japanese patients with migraine and tension-type headache (TH). Seventy-four patients with migraine headaches (22 with aura and 52 without aura), 47 with THs, and 261 normal controls were recruited. Genotyping of MTHFR C677T polymorphism was performed by polymerase chain reaction-restriction fragment length polymorphism. We detected that the incidence of the homozygous transition (T/T) in migraine sufferers (20.3%) was significantly higher than that in controls (9.6%). Moreover, the frequency of the T/T genotype in individuals with migraine headaches with aura was remarkably high (40.9%). The MTHFR T allele was more frequent in the migraine group than in the control group. Our results support the conclusion that the MTHFR gene, causing mild hyperhomocysteinemia may be a genetic risk factor for migraine. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 96:762–764, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: association; headache; homocysteine; methylenetetrahydrofolate reductase (MTHFR); migraine; polymorphism

INTRODUCTION

The pathophysiology of migraine is not yet fully understood but may involve painful vasodilatation of cerebral blood vessels and/or the release of vasoactive neurotransmitters from the perivascular axons in the dura mater after activation of the trigeminovascular system [Moskowitz and Macfarlane, 1993]. The spreading depression phenomenon may explain clinical as well as experimental findings in migraine. Storer and Goadsby [1997] developed a model of craniovascular pain using cats and demonstrated that spontaneous trigeminal cell firing was accelerated by DL-homocysteic acid, the oxidized homocysteine derivative that mimics the effect of homocysteine on arteries and was suppressed by ergometrine and 5-HT1B/1D agonists. In humans, serum homocysteine levels are influenced by environmental as well as genetic factors. 5,10-methylenetetrahydrofolate reductase (MTHFR) is one of the key enzymes responsible for hyperhomocysteinemia. Individuals homozygous for the mutation C677T show reduced specific MTHFR activity and elevated homocysteine concentrations [Frosst et al., 1995]. This mutation may be a genetic risk factor for cerebrovascular disorders [Coull et al., 1990]. Migraine is in part associated with cerebral circulation. We assessed the possible contribution of this polymorphism in Japanese patients with migraine headaches and tension-type headaches (THs).
MATERIALS AND METHODS

Subjects

The study population consisted of 74 patients with migraine headaches and 47 with THs. First, we individually diagnosed the condition as migraine with aura (MWA), migraine without aura (MOA), or TH, then combined the migraine group (MWA + MOA) for statistical evaluation. The diagnosis of headache was made according to the criteria of International Headache Society [1988]. Two hundred sixty-one non-headache healthy volunteers comprised the control group. All the subjects were Japanese and gave their informed consent for participation in the study.

Genetic Analysis

Genotyping for MTHFR C677T polymorphism was performed on leukocyte genomic DNA samples by polymerase chain reaction-restriction fragment-length polymorphism analysis, as previously reported [Frosst et al., 1995]. The sense oligonucleotide primer was 5'-TGA AGG AGA AGG TGT CTG CGG GA-3', and the antisense primer was 5'-AGG ACG GTG CGG TGA GAG TG-3'. The cycle parameters were as follows: 3 min initial denaturation at 95°C, followed by 35 cycles of denaturation for 1 min at 94°C, primer annealing for 1 min at 65°C, and primer extension for 2 min at 72°C. A final extension step was performed for 10 min at 72°C. These primers generated a 198-bp fragment. The fragment derived from the C allele was not digested by HinfI, whereas the fragment of the same length from the T allele was digested by HinfI into 175- and 23-bp fragments. The HinfI-treated polymerase chain reaction fragments underwent electrophoresis in 3% agarose gels and was stained with ethidium bromide.

Statistical Analysis

The differences in the frequency of MTHFR alleles and genotypes between groups were evaluated by the gene-counting method, and comparison of groups was made using the $\chi^2$ test. The level of significance was set at $P < 0.05$. The risk of migraine for the MTHFR T/T genotype carriers was evaluated, calculating the odds ratios (OR) and 95% confidence intervals between subjects with the T/T genotype and subjects with the C/C or C/T genotype.

RESULTS

The distributions of the MTHFR genotype in headache patients and controls did not deviate significantly from the Hardy-Weinberg equilibrium. The incidence of the homozygous transition (T/T) in MWA patients (40.9%) was especially higher than that in the controls, in MOA patients, and in TH patients (Table I). The OR of MWA patients for the MTHFR T/T genotype was 6.5, which was significant (Table II). In the combined migraine group (MWA + MOA), the frequency of the T/T genotype was 20.3%, which was significantly higher than in the controls (Table I), giving an OR of 2.4 (Table II). The MTHFR T allele was more frequent in the MWA group than in the control, MOA, and TH groups. The T allele was more frequent in the combined migraine group than in the control group (Table I).

DISCUSSION

Migraine is a common form of chronic headache syndrome, characterized by pulsating headache, nausea, vomiting, photophobia, and phonophobia. Approximately 15 to 20% of the general population experiences migraine headaches in Western communities [Rasmussen, 1995]. In Japan, approximately 9% of the people experience migraine headaches [Sakai and Igarashi, 1997]. The pathogenesis of migraine still remains enigmatic. Moskowitz [1993] proposed the “trigemino-vascular theory” of migraine headache, which claims that neurogenic inflammations of meningeal blood vessels are evoked by excitation of trigeminovascular fibers.

Recently, migraine has been shown to have a partly genetic basis. Point mutations in the P/Q-type Ca$^{2+}$ channel alpha 1 subunit gene have been identified in familial hemiplegic migraine, which is linked to chromosome 19 [Ophoff et al., 1996]. Patients with mitochondrial encephalomyopathy such as mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) often suffer from migraine-like headaches. These two specific migraines are rare forms and

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<th>TABLE I. Genotype Distributions and Allele Frequencies of the MTHFR Gene</th>
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* $P < 0.01$, versus controls.
* $P < 0.01$, versus MOA and TH.
* $P < 0.05$, versus controls.
* $P < 0.01$, versus TH.
* $P < 0.05$, versus MOA.
are caused by mutations in single genes. On the other hand, it is unlikely that “normal” migraine is caused by a single gene abnormality. Rather, it is probably caused by multifactorial genetic factors and environmental factors, including foods and lifestyle.

The C677T allelic variations of MTHFR may be associated with coronary heart diseases and cerebrovascular diseases [Frosst et al. 1995]. Our data suggest that the homozygous C677T mutation in the \textit{MTHFR} gene is a genetic risk factor for migraine. This is the first report that demonstrates a clear association of a common \textit{MTHFR} mutation with migraine headache.

A few studies have investigated the relationship between homocysteine and headache. Evers and colleagues [1997] reported elevated serum levels of homocysteine in MWA patients, but not in MOA or TH patients. They postulated a possible comorbidity of mild hyperhomocysteinemia and vascular headache. Our data agreed with the genetic aspects of their results. Homocysteine is one of the excitatory amino acids. The results of Storer and Goadsby [1997], who used the cat model of trigeminal vascular nociceptive activation, suggested that hyperhomocysteinemia would sensitize the dura mater and/or cerebral arteries and that subjects with hyperhomocysteinemia would be predisposed to migraine.

In addition, hyperhomocysteinemia can cause alterations in the synthesis and metabolism of neurotransmitters such as serotonin, norepinephrine, and dopamine [Matthews and Kaufman, 1980]. It is well-known that patients with migraine have autonomic nervous system dysfunction and altered levels of neurotransmitters. The decrease of MTHFR activity due to the C677T mutation would result in decreased levels of the neurotransmitters and autonomic nervous system dysfunction. These states appear to be analogous to those found during migraine headache. We conclude that the homozygous C677T mutation in the \textit{MTHFR} gene is a genetic risk factor for migraine.

\textbf{REFERENCES}


